Relating Imaging Indices of White Matter Integrity and Volume in Healthy Older Adults

Age-related alterations in white matter have the potential to profoundly affect cognitive functioning. In fact, magnetic resonance imaging (MRI) studies using fractional anisotropy (FA) to measure white matter integrity reveal a positive correlation between FA and behavioral performance in older adults. Confounding these results are imaging studies demonstrating age-related white matter atrophy in some areas displaying altered FA, suggesting changes in diffusion may be simply an epiphenomenon of tissue loss. In the current study, structural MRI techniques were used to identify the relationship between white matter integrity and decreased volume in healthy aging adults. The data demonstrated that white matter atrophy did in fact account for differences in some areas, but significant FA decreases remained across much of the white matter after adjusting for atrophy. Results suggest a complex relationship between changes in white matter integrity and volume. FA appears to be more sensitive than volume loss to changes in normal appearing tissue, and these FA changes may actually precede white matter atrophy in some brain areas. As such, the ability to detect early white matter alterations may facilitate development of targeted treatments that prevent or slow age-related white matter degradation and associated cognitive sequelae.

Keywords: aging, diffusion tensor imaging (DTI), fractional anisotropy (FA), voxel-based morphometry (VBM)

Introduction

Histological and imaging studies consistently demonstrate that the human brain loses gray and white matter tissue volume with age (Tang et al. 1997; Guttmann et al. 1998; Courchesne et al. 2000; Good et al. 2001; Marner et al. 2003; Pakkenberg et al. 2003; Resnick et al. 2003; Sowell et al. 2003; DeCarli et al. 2005; Lemaître et al. 2005; Raz et al. 2005; Walhovd et al. 2005 but also see Taki et al. 2004). This age-related atrophy typically manifests as sulcal deepening and widening, enlarged ventricles, and an increased ratio of cerebrospinal fluid (CSF) to gray and white matter (for review see Kemper 1994). However, it is now accepted that extensive neuronal loss is a feature of pathological rather than normal aging and, therefore, cannot fully account for the atrophy or cognitive deterioration observed in healthy older adults (for review see Wickelgren 1996; Peters and Rosene 2003). Thus, efforts to explain age-related cognitive changes increasingly focus on modifications in synaptic remodelling and white matter integrity (Bartzokis 2004).

Alterations in white matter and axonal properties have the potential to profoundly impact cognitive functioning. Animal studies have shown age-related changes in myelin composition and thickness (Peters and Sethares 2002; Sugiyama et al. 2002; Bartzokis 2004; Hinman et al. 2006), ion channel location and subtype (Hinman et al. 2006), and cytoskeletal components (Cash et al. 2003; Sato et al. 2005). Degradation of the timing of neural transmissions brought about by these changes could significantly alter cognitive processes such as memory and attention that require synchronization of neural firing in multiple brain areas (Peters et al. 2000; Peters and Sethares 2002; Bartzokis 2004). In support of this theory, myelin alterations in primates and white matter lesion load observed with $T_1$ and $T_2$-weighted magnetic resonance imaging (MRI) sequences in humans have been shown to be predictive of cognitive decline (Peters and Sethares 2002; O'Sullivan et al. 2004; Nordahl et al. 2005; Soderland et al. 2006). In addition, such breakdowns in tissue integrity may signify the beginnings of actual tissue loss, although the relationship between myelin degeneration and atrophy is not clearcut (Peters et al. 2000; Peters and Sethares 2002).

White matter has traditionally been characterized in human studies using conventional clinical MRI sequences, such as $T_1$ and $T_2$-weighted images. One of the most promising new technological advances for evaluating white matter structure with MRI is diffusion tensor imaging (DTI), which uses $T_2$ imaging with strong directional gradients to image the diffusional movement of water. Sensitivity to water movements on a microstructural level allows diffusion imaging to capture both gross diffusion changes, as in stroke, and subtle alterations in white matter that may actually appear normal on conventional $T_1$ or $T_2$-weighted images. Fractional anisotropy (FA) is a quantification of the directionality of water diffusion (Le Bihan and van Zijl 2002) that can be obtained from DTI data. Water that is relatively free to diffuse, as in CSF or gray matter, will diffuse equally (isotropically) in all directions, resulting in low FA values. In contrast, water constrained by myelin, axonal flow, and cytoskeletal structure in white matter has directional (anisotropic) flow parallel to the white matter fibers, which results in high FA values.

FA studies of healthy aging have demonstrated decreases in ordered diffusion subcortically in several different brain regions, such as temporal lobe (Head et al. 2004; Grieve et al. 2007), occipital lobe (Head et al. 2004; Madden et al. 2004), postcentral gyrus (Bhagat and Beaulieu 2004), and in white matter in the frontal lobes (Pfefferbaum et al. 2000; O'Sullivan et al. 2001; Bhagat and Beaulieu 2004; Head et al. 2004; Madden et al. 2004; Pfefferbaum et al. 2005; Salat et al. 2005; Sullivan et al. 2006; Grieve et al. 2007). FA decreases with aging are also evident in several white matter tracts, including the centrum semiovale (Pfefferbaum et al. 2000), anterior (Madden et al. 2004) and posterior (Bhagat and Beaulieu 2004; Salat et al. 2005) limbs of the internal capsule, anterior cingulum bundle (Pfefferbaum et al. 2005), corpus callosum (particularly the anterior corpus callosum) (Pfefferbaum et al. 2000; Pfefferbaum et al. 2005;
Bhagat and Beaulieu 2004; Head et al. 2004; Sullivan et al. 2006; Ota et al. 2006), and anterior forceps (Madden et al. 2004; Pfeiferbaum et al. 2005). These findings of reduced white matter integrity with age fit well with available histological data, as discussed above. Morphological changes such as fluid bubbles in the myelin sheath, changes in myelin membrane content, and loss of small myelinated fibers in the anterior corpus callosum would all be expected to interrupt the normally tight apposition of white matter tracts and alter water diffusion within and around them. Furthermore, lower FA values have been linked to declining performance on higher-order cognitive tasks (O’Sullivan et al. 2001; Madden et al. 2004; Sullivan et al. 2006; Tuch et al. 2005; Charlton et al. 2006; Persson et al. 2006; Grieve et al. 2007), suggesting that white matter coherence, as indexed by FA, may influence cognitive functioning in healthy aging.

Despite this apparent wealth of information about the characteristics of aging white matter, fundamental questions remain about the relationship between age-related atrophy and microstructural changes in white matter. Changes in FA could be independent from atrophy, where loss of tissue provides no information about the likely FA of a given region. This would be the case if decreased FA were observed in nonatrophied tracts, or tracts that have atrophied nonetheless retained their FA values. It seems likely, though, that FA and volume loss are related. For instance, if degradation of myelin occurred prior to atrophy, it would be expected that atrophic areas would have lower FA, as would regions that are deteriorating but have not yet lost tissue. Thus, it is possible that FA decreases may represent different processes occurring in different regions of the same brain at the same time.

Some of these questions were raised in a recent review article that correlated FA and VBM in regions of interest (ROIs) in anterior white matter (Salat et al. 2005). A positive correlation between FA and white matter volume was observed and interpreted as showing that FA may be a ‘microstructural index’ of volume loss; that is, regions of decreased FA represent decreased volume. In contrast, another recent study correlated diffusion and volume findings using whole-brain histograms (Benedetti et al. 2006) and found little relationship between them. However, this negative finding could result from the loss of spatial variability of both FA and volume decreases with age inherent in whole-brain averaging techniques. In order to capture this spatial variability, the relationship between these metrics must be made using a region-by-region (i.e., voxel-wise) analysis. The current study investigated the relationship between white matter microstructural changes and atrophy in healthy aging adults using a novel integrated analysis (Casanova, Ryali, et al. 2006) that accounted for the effects of volume loss on FA changes in a voxel-wise manner.

Materials and Methods

Participants

Healthy adult volunteers were recruited from the Wake Forest University Baptist Medical Center and the broader Winston-Salem area through flyers, community presentations, and word of mouth. Sixty-six participants ranged in age from 18 to 80 years old and were divided into 3 age groups: young (18–38 years old; 20 subjects, mean age = 28.30 ± 3.8, 12 female, 18 right handed), middle-aged (39–64 years old; 23 subjects, mean age = 47.57 ± 7.6, 12 female, 20 right handed), and older (65–90 years old; 23 subjects, mean age = 71.17 ± 4.3, 12 female, 23 right handed). All participants granted written, informed consent and were compensated for their time. All subject recruitment, informed consent, and data collection procedures were completed in accordance with the Wake Forest University School of Medicine Institutional Review Board, the Health Insurance Privacy and Portability Act, and the Declaration of Helsinki.

As these experiments were intended to study a healthy adult population, potential participants were screened and excluded for evidence of dementia, head injuries, or brain surgery, axis I psychiatric disorders, substance abuse, and medications or implants contraindicated for the MRI scanner. Due to requirements of the task used in a functional study (data reported elsewhere) as well as a desire to restrict the subjects to a healthy population, several sensory acuity measures were exclusionary. Subjects were excluded for moderate hearing loss, in the 1000–2000 Hz range, as tested with a digital audiometer (Digital Recordings, Halifax, Nova Scotia, Canada), audiometer (Welch Allyn, Skaneateles Falls, NY), or audiologists at the Wake Forest University School of Medicine Department of Speech and Hearing. Visual acuity of at least 20/40 was required and was tested with a modified Snellen visual acuity exam. Color-blindness measured as a score of less than 7 on the Concise Edition of Ishihara’s Test for Color-blindness (Kanekara and Co., Tokyo, Japan) was exclusionary as well. The Mini-Mental State Examination was used to evaluate and exclude potential subjects at risk for dementia based on age and education adjusted values (Bravo and Herbert 1997; mean score per participant group: young = 28.9 ± 1.1; middle-age = 29.1 ± 0.8; older = 28 ± 3.2). Potential participants were excluded for high risk of alcoholism, defined by a score greater than 10 on the Alcohol Use Disorders Identification Test (Bohm et al. 1995). In addition, a detailed self-report medical history was used to assess for contraindicated or exclusionary diagnoses, or medications consistent with them. All anatomical images were reviewed by a neuroradiologist (J.B.) for evidence of disease or abnormalities.

MRI Acquisition

High-resolution anatomical structural images and DT MRI data were acquired from 66 subjects. Results from 64 of the subjects are reported here (20 young, 21 middle-aged, 23 old). Data from one young subject were unusable due to acquisition errors, and DTI data were not collected for one middle-aged subject.

All data were acquired on a 1.5 T GE scanner with twin speed gradients and a quadrature birdcage head coil. High-resolution T1-weighted structural images were acquired using a spoiled-gradient inversion recovery (3DSPGR-IR) sequence with 128 contiguous slices covering the whole brain (time echo [TE] = 1.9 ms; inversion time [TI] = 600 ms; flip angle 20°; frequency = 256; phase = 256; slice thickness = 1.5 mm; in-plane resolution 0.938 × 0.938). To minimize the imaging distortion associated with eddy currents, DTI data was acquired using a dual spin-echo single shot echo-planar imaging (EPI) (Reese et al. 2003). Diffusion images were acquired along 15 non-collinear directions with a diffusion weighting of 1000 s/mm², which is commonly referred to as the b-value. In addition, a T2 weighted image with the diffusion gradients turned off (b = 0 s/mm²) was also acquired. Other imaging parameters for the DTI data are as follows: 45 contiguous slices with a slice thickness of 3 mm; echo time [TE] = 77.5 ms; repetition time [TR] = 12 400ms; field of view (FOV) = 28 × 22.4cm; acquisition matrix = 128 × 128; acquisition resolution = 2.18 × 1.75 × 3.0 mm; frequency direction was Right to Left; each diffusion direction was measured twice for a total scan time of 7m2s. The DTI images were zero-filled into the imaging plane to 256 × 256 for a final image resolution of 1.09 × 1.09 mm. Imaging data were transferred from the MRI scanner to Sun workstations for further processing. All images are presented in neurological space, with the right side of the brain on the right.

Voxel-Based Morphometry

Custom Template Creation

Voxel-based morphometry (VBM) was performed on high-resolution T1-weighted structural images using a study-specific with matter template based on the methods of Good et al. (2001; Senjem et al. 2005) using Statistical Parametric Mapping (SPM2) software from the Wellcome Department of Cognitive Neurology, London, UK (http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab (The Mathworks Inc., Sherborn, MA). Each subject’s data were initially segmented in native space using the Custom Template Creation process.
standard SPM2 segmentation processes, yielding 3 segmented tissue images in native space for each subject. Each subject’s segmented white matter image was then normalized to the SPM2 white matter prior probability map following standard procedure in SPM2. Parameters from this normalization were applied back to the participant’s T1 image in native space. These normalized T1 images and white matter images were averaged across all 64 subjects to yield a study-specific template and prior probability maps.

Image Normalization
The same iterative process used to generate the template was applied to normalize each subject’s data to the study-specific template. Each subject’s high-resolution structural image was segmented based on the custom prior probability maps after an affine transformation. The segmented white matter image was then normalized to the custom white matter probability map and the parameters from this normalization were applied to the high-resolution T1-weighted image.

Estimation of Volume
Volume in VBM analyses is indexed in 2 ways. Systematic failures of normalization in one population relative to another suggest volume differences so great that they could not be corrected by the warping algorithm (Ashburner and Friston 2000). This metric is captured in simple group comparisons of normalized white matter segments, commonly referred to as unmodulated VBM. If normalization is successful, white matter segments match perfectly between populations. Therefore, volume loss is not detectable by simply comparing successfully normalized regions. Rather, localized volumetric differences in successfully warped areas are captured by scaling intensity on a voxel-wise basis with the Jacobian determinants calculated from the normalization (Ashburner and Friston 2000; Good et al. 2001), commonly termed modulated VBM. These measures were used together in the following analyses in order to reflect both kinds of volumetric variance associated with atrophy. The use of both modulated and unmodulated VBM is referred to hereafter as combined VBM.

All images were resampled to 1 x 1 x 1 mm and smoothed with an 8 x 8 x 8 mm FWHM isotropic Gaussian smoothing kernel. When group comparisons were made, an absolute threshold of 0.15 was implemented to eliminate voxels with a low probability of being white matter. As with all analyses in this paper, results were thresholded at P < 0.05 using family-wise error (FWE) as implemented in SPM2 to correct for multiple comparisons. All volumetric analyses also included intracranial volume (ICV) as a covariate of no interest to account for the potential effects of cranial vault size on brain volume.

DTI Analyses
DTI data were processed using the FMRIB Software Library (FSL) program, and brain extraction was accomplished using the FSL Brain Extraction Tool (Smith 2002; Smith and Ratcliff 2004). DTI data were aligned with a reference scan using an affine registration to correct for eddy current distortions. The first, second, and third eigenvectors and eigenvalues of the diffusion tensors were calculated on a voxel-wise basis using the DTIFit tool in FSL (Basser et al. 1994; Pierpaoli and Basser 1996). This information was used to construct maps of FA.

In order to make whole-brain comparisons, DTI data were normalized to a white matter study-specific template. Optimal methods to perform whole-brain analyses on DTI data are currently a topic of active interest and debate. For instance, the appropriate threshold to minimize the effects of partial volume averaging (e.g., Pfefferbaum and Sullivan 2003; Salat et al. 2005) and the optimal smoothing kernel (Jones et al. 2005) are still under investigation. For this study, a method of normalizing DTI data to a template was chosen that has recently been validated against ROI analyses (Toosy et al. 2004; Salat et al. 2005). The study-specific template was generated in a manner similar to the process described for the T1 data. Rather than using T1 images and templates, the b0 image was normalized to the SPM2 EPI template. Like the other DTI images, the b0 image is an EPI image, but unlike the other DTI images, it is acquired without any gradients applied. Therefore, it can be normalized to the SPM2 template and is also in the same space as the other DTI images. After creation of the custom template, each subject’s b0 image and segmented tissue images were normalized to the custom template and prior probability maps. DTI images were normalized by applying the parameters obtained from warping the white matter segments of the b0 image to the template white matter prior probability map. All images were smoothed with an 8 x 8 x 8 mm FWHM Gaussian kernel. As mentioned above, the optimal smoothing kernel for DTI images is still under investigation (Jones et al. 2005), but the 8 x 8 x 8 mm kernel was selected for all analyses in this study for compatibility with further analyses (for details, see Biological parametric mapping section below). Age group comparisons were made on FA maps warped into common template space and using an absolute threshold of 0.15. Examples of raw FA maps and the smoothed, normalized maps from a representative older and younger subject are shown in Figure 1.

Biological Parametric Mapping
Biological parametric mapping (BPM) combines information from different imaging modalities on a voxel-wise basis using the general linear model (GLM) (Casanova, Srivastan et al. 2007). Imaging variables are integrated on a voxel-wise basis, so each voxel has a unique regression design that includes the value of each imaging modality for that voxel. A BPM multiple regression was used for 3 analyses. The relationship of age to atrophy was assessed by regressing the scalar volume measure with combined VBM measures. Similarly, the relationship of FA to combined VBM measures was reflected in a regression with FA as the dependent variable and VBM measures as independent variables. Finally, the relationship of FA with age after accounting for volumetric change was investigated with an analysis including FA as the dependent variable, age as the covariate of interest, and combined VBM measures as covariates of no interest. ICV was included in all analyses as a covariate of no interest to account for the influence of cranial vault size on brain volume. ICV was calculated by summing the gray matter, white matter, and CSF volumes generated for each subject by the VBM procedure.

The BPM analysis performs a unique regression at each voxel. Therefore, the voxel size and smoothness must be consistent across imaging modalities. To meet this requirement, both VBM and FA data were resampled to the same voxel size (1 x 1 x 1 mm) and smoothed equally with an 8 x 8 x 8 mm FWHM Gaussian kernel. The T maps generated in the BPM program were then exported to the SPM environment where they were thresholded at P < 0.05 using FWE as implemented in SPM2 to correct for multiple comparisons. A previous study (Jones et al. 2005) raised concerns that DTI analyses may not meet the parametric assumptions necessary for GLM analyses. The data presented here were analyzed using both parametric and nonparametric methods. Results of the nonparametric analysis reiterated the findings reported here in the present study (Hayasaka et al. 2006). Parametric analyses are used here because parametric procedures are required at the present time to complete the voxelwise integrated analyses described above (Casanova, Srivastan et al. 2007).

Supplemental Analyses
Age group comparisons (e.g., younger vs. older, middle-aged vs. younger, middle-aged vs. older) of DTI and VBM were made using analysis of covariance in SPM2 and BPM in order to facilitate interpretation of the data within the context of previous research. Additional analyses in SP2 also explored the potential impact of hypertension and leukoaraiosis, which were found to be negligible. Results of these analyses are reported in the Supplementary Materials.

Regression plots for ROIs were generated to illustrate the relationship shown in whole-brain maps in a representative manner. Five-millimeter spheres were placed in the anterior white matter and genu and splenium of the corpus callosum using the Wake Forest University Pickatlas tool (Maldjian et al. 2003). The ROIs were positioned to minimize inclusion of gray matter or CSF, and all ROIs were located on the sagittal section illustrated throughout the paper (Montreal Neurology Institute [MINI] x = -11). In MINI coordinates, the anterior white matter ROI was located at x = -11, y = 49, z = 29; genu ROI at x = -11, y = 28, z = 0; and splenium ROI at x = -11, y = -38, z = 12. A figure illustrating the placement of ROIs is included in the Supplementary Materials.
Results

Regression of Age with FA

As discussed in the introduction, multiple studies have consistently demonstrated that FA is reduced in older adults. Age-related declines in FA were evident in raw data in individual subjects and in correlations with age in specific ROIs (Fig. 1). Whole-brain analysis revealed these apparent decreases in FA were robust and significant ($P < 0.05$, $T > 5.22$) in a large contiguous area in anterior white matter that included the genu and anterior midbody of the corpus callosum, and anterior forceps (Fig. 2A). Declines in FA in deep frontal white matter extended anteriorly into the frontal pole and rostrocaudally from the edge of the superior frontal gyrus (MNI $z = 43$) including the centrum semiovale to the inferior frontal gyrus nearly reaching orbitofrontal white matter (MNI $z = -11$). Decreases in FA were also observed in the optic radiations and bilaterally in the external capsule. Although medial aspects of the thalamus did exhibit age-related decreases in FA, there was a notable absence of FA change in the internal capsule.

Regression of Age and VBM

Figure 2B illustrates areas where combined VBM measures of white matter atrophy are related with age ($P < 0.05$, $F > 20.32$). Age was significantly associated with VBM bilaterally in the genu of the corpus callosum extending into the posterior midbody on the right side. In addition, white matter changes with age were observed in deep anterior white matter on the right side evident ventrally in orbitofrontal cortex and dorsally in the middle frontal gyrus. Atrophy was also noted in the right optic radiations.

Correlation between White Matter Volume and FA

It is clear that FA and white matter atrophy are each related to age and occur in overlapping regions. However, correlation with age does not address the potential for an age-independent relationship between microstructure and volume. In order to investigate the relationship between microstructural integrity and white matter volume, a whole-brain regression was performed examining the relationship between FA and combined VBM on a voxel-by-voxel basis while adjusting for the effects of age and ICV (Fig. 3A). Results of this analysis revealed significant ($P < 0.05$, $F > 19.96$) relationships between FA and combined VBM in many areas of white matter, including the length of the corpus callosum, temporal and parietal regions of the corona radiata, and centrum semiovale. More limited association between VBM and FA was also observed in some regions of the white matter of the middle and inferior frontal gyri.

Age-Related Changes in FA after Accounting for Decreased White Matter Volume

The significant relationships between VBM and FA measures suggest that decreased FA may represent atrophied white matter, at least in some regions. To examine this further, the relationship between FA and age was evaluated ($P < 0.05$, $F > 20.32$), with significant decreases in FA with age in anterior white matter ($x = -11$, $y = 49$, $z = 29$; $R^2 = 0.29$) and genu of the corpus callosum ($x = -11$, $y = 28$, $z = 0$; $R^2 = 0.59$), and splenium of the corpus callosum ($x = -11$, $y = -38$, $z = 12$; $R^2 = 0.18$). A figure showing the location of the ROIs used to generate the correlation plots is included in the Supplementary Materials.
T > 5.59) while accounting for changes in combined VBM and ICV using a voxel-wise regression model (Fig. 3B). Accounting for VBM results substantially changed the observed relationship between FA and age. The relationship of FA and age was reduced to insignificance in the most anterior and ventral portion of the genu of the corpus callosum, the external capsule, and parts of the anterior forceps, particularly on the right side. Including VBM measures as covariates also limited the relationship of FA and age along the length of the corpus callosum and in the centrum semiovale.

Many important age-related decreases in FA remained statistically significant, however, even after correcting for decreases in white matter volume. These areas include the deep anterior white matter, dorsal and rostral genu of the corpus callosum, portions of the corticospinal tracts, and a focal area in the right optic radiation. Interestingly, regressing out white matter volume actually enhanced the extent of age-related FA change in some small areas of the corona radiata where no significant volume loss was noted in the VBM analysis.

**Discussion**

The observation that the directionality of water diffusion is changing throughout the white matter after adjusting for atrophy suggests some decreases in FA reflect alterations in white matter that are not predicted by tissue loss. Accounting
for volume substantially changes the relationship between FA and age in other regions, suggesting a regionally specific association between FA and atrophy. Together these findings suggest that FA may reflect multiple processes, acting as a sensitive marker that detects microstructural changes in white matter both prior to atrophy and in atrophied tissue. Clinically, the ability to detect subtle neurobiological changes presaging the death of white matter offers a window of opportunity to rescue the tissue and the functions it serves. Although longitudinal and clinical studies are needed to further explore the relationship of tissue volume and structure, target critical points of change, and identify potential interventions, the data presented here suggest that FA may be an early and sensitive marker for white matter disease. In addition, the need to integrate information from different imaging modalities to fully characterize decreases in FA is evident from these results.

Spatial Normalization in the Aging Brain

Whole-brain comparisons and normalization to a common brain space are widely used techniques, as they eliminate subjective identification of specific brain regions by individual raters, improve the speed and consistency of data processing, and do not rely solely on a priori hypotheses (Shen and Davatzikos 2003). Normalization procedures have improved rapidly, but are still do not always succeed in fully warping regions with significant atrophy, such as the ventricles in aging adults (Mechelli et al. 2005; Salat et al. 2005). An alternative explanation for age-related decreases of FA in a study where whole-brain comparisons are made on spatially normalized images is that systematic failures in warping resulted in the FA of white matter in one population being compared to the FA of another tissue type in the other population. One method to try to correct for errors of this kind is to raise the absolute threshold for inclusion in the FA analysis, for instance 0.25 instead of 0.15 (Pfefferbaum and Sullivan 2003; Salat et al. 2005). The more stringent threshold excludes voxels with low FA values, and is therefore likely to exclude voxels representing other tissue types, or voxels containing more than one tissue type (partial volume averaging). However, such a high threshold may also discard regions where white matter microstructure has significantly degraded, the exact areas targeted for study. For instance, in this data set, applying a threshold of 0.25 removed findings of significant decreases of FA in the thalamus and in the area of the genu, regions where age group comparisons of white matter segments suggested that warping in older brains was suboptimal. These regions were also removed when unmodulated VBM was included as a covariate in the integrated analysis, suggesting that the group differences indeed reflect normalization errors. However, the higher threshold also removed significant decreases in parts of the deep anterior white matter. Examination of structural images confirms these areas of deep frontal white matter are definitely white matter in both young and older adults, so employing a more stringent threshold would obscure genuine age-related changes. We attempted to capture variance related to atrophy by including a combined VBM measure.
reflecting these minor failures in warping as well as alterations in successfully normalized regions. This is an effective stop-gap solution, but ideally in the future, more sophisticated warping algorithms will resolve this concern altogether.

**The Relationship between Volume Loss and FA**

The association between atrophy and FA was apparent both in the direct correlation of FA and volumetric measures, and in changes in the relationship of FA and age when accounting for VBM measures. The idea that FA may be a microstructural representation of atrophy is supported in regions where inclusion of VBM measures attenuates the relationship between FA and age. However, many FA decreases throughout the brain remained when volume was included, suggesting that in these areas, FA decreases are occurring without volume loss. In fact, it may be that FA is a sensitive marker that identifies alterations in white matter occurring prior to tissue loss in addition to identifying areas that have already atrophied.

If it is true that FA changes precede volume loss, it would be expected that comparisons of young and middle-aged adults would show FA decreases in areas exhibiting atrophy when middle-aged and older adults are compared. In other words, regions that exhibit FA changes in middle age should be expected to be the same areas that exhibit volume loss in older age. Although in our current study no significant FA changes were observed between young and middle-aged adults, trends were evident that FA decreased in middle-aged adults in the same areas of the genu of the corpus callosum where atrophy was observed direct comparison of middle-age and older (Fig. 4). Decreased FA in middle-aged adults when no correction for multiple comparisons was applied has also been reported by Salat et al. (2005). Future studies employing a longitudinal design and/or including a larger number of subjects are needed to further explore these subtle, yet intriguing, findings.

The relationship of volume and FA in anterior white matter is quite interesting in terms of functionality, as evidence indicates that functions served by frontal regions may decline with age (e.g. Cabeza et al. 2004; Persson et al. 2006). The robust relationship of FA and age in the anterior white matter was attenuated, but not abolished, by accounting for atrophy. This suggests that some FA decreases in frontal white matter are a microstructural indicator of atrophy, but that many FA decreases reflect other potentially related aging processes. Curiously, given the strength of the association between age and FA, studies demonstrating a relationship between FA and cognitive decline have typically not implicated frontal areas (O’Sullivan et al. 2001; Madden et al. 2004; Sullivan et al. 2006; Charlton et al. 2006), although Persson and colleagues (Persson et al. 2006) noted greater FA decreases in anterior corpus callosum associated with cognitive decline. The intricacies of the relationship between functional evidence of frontal cortical decline and the substantial change in frontal FA even after accounting for volume loss clearly warrants further study.

**Aging Processes in Axonal Membranes and Myelin may Underlie Decreases in FA**

The biological basis of the FA signal is not completely understood, but is thought to result primarily from constraints of the many tightly packed membrane barriers in white matter, whether myelinated or not (Beaulieu 2002; Nair et al. 2005). There is evidence that changes occur in axons and myelin during the course of normal aging that create a more permissive microstructural environment. One reason myelinated fibers come into such close apposition is their extracellular membranes contain a high proportion of cholesterol, a hydrophobic molecule. The amount of cholesterol in aging myelin is decreased (Bartzokis 2004), permitting more water between myelinated fibers. Furthermore, histological studies of primates and rats reveal extensive aging related changes in myelin, including splits along the intraperiod line causing fluid bubbles within the myelin sheath and double myelin sheaths, where one ring of myelin is separated from another by a fluid-filled space (Peters et al. 2000; Peters and Sethares 2002; Sugiyama et al. 2002; Sandell and Peters 2003). Importantly, myelin changes are apparently taking place in functioning axons, that is, prior to frank loss of the white matter fibers. Therefore, these changes might result in white matter that appears normal on conventional T1- and T2-weighted images, but shows altered diffusion using DTI.

**Developmental Links to Spatially Specific Vulnerability of Aging White Matter**

This study as well as others demonstrated that FA does not decrease uniformly across the brain (Pakkenberg et al. 2003; Sowell et al. 2004; Pfefferbaum et al. 2005; Salat et al. 2005). The spatially specific vulnerability is believed to be rooted in development; the same areas whose shared characteristics cause them to develop together may also produce shared vulnerabilities. In humans, heavily myelinated connections serving primary sensory and motor functions seem to complete myelination first, are heavily myelinated, and have a high ratio of oligodendrocytes to myelinated fibers. In contrast, association areas of the brain, such as portions of the frontal and temporal cortices, have thinner myelination, one oligodendrocyte may myelinate multiple small fibers, and have a longer period of myelin formation thought to extend into middle age (Yakovelet al. 1964; Sampaio and Truwit 2001; Bartzokis 2004). In fact, while gray matter volume in humans peaks in the teenage years and declines thereafter, white matter volume appears to peak in middle age (Courchesne et al. 2000; Sowell et al. 2004;
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Fotenos et al. 2005). As in this study, many imaging studies of humans do not observe significant declines in white matter volume until people reach around 60 years of age or older (Jernigan et al. 1991; Courchesne et al. 2000; DeCarli et al. 2005; Wallhovd et al. 2005). The data presented here suggest that diffusion imaging may be an excellent tool for observing such changes.

White Matter Structure Directly Impacts Total Brain Function

Structural alterations in a functioning axon, such as those suggested by decreased FA, fundamentally alter neural communication by changing the speed and temporal fidelity of action potential propagation. For instance, around the nodes of Ranvier, there is evidence of both fluid inclusions and degradation of myelin (Sugiyama et al. 2002; Sandell and Peters 2003), as well as reorganization of ion channels (Hinnan et al. 2006) in the aging brain. If myelin begins to degrade, exposing areas of axon that do not contain sufficient ion channels, propagation of the action potential would be sluggish until either the integrity of the myelin were restored or the segment of axon were supplied with more ion channels. Recent research indicates that action potentials may actually increase myelinization (Demerens et al. 1996; Bengtsson et al. 2005) through the release of cytokine trophic factors that support oligodendrocytes (Demerens et al. 1996; Bengtsson et al. 2005; Ishibashi et al. 2006). Therefore, changes in myelination or axonal flow that degrade the action potential may reinforce demyelination by depriving oligodendrocytes of needed trophic support. Conversely, interventions that increase neuronal activity may actually stimulate myelination. Moreover, age-related white matter changes are intimately related to gray matter changes. In fact, the predominant loss of smaller rather than larger axons (Tang et al. 1997; Marner et al. 2003; Pakkenberg et al. 2003) neatly complements the loss of synapses and supporting glia believed to comprise the bulk of gray matter loss (Markham and Greenough 2004).

Limitations and Future Directions

As discussed earlier, whole-brain analyses have significant benefits, but also caveats that must be considered when interpreting outcomes, such as taking into consideration the potential for suboptimal normalization. Another consideration is that VBM analyses categorize tissue types based on MR signal intensity. In the aging brain, changes in white matter that result in signal intensities closer to that of gray matter potentially lessen the accuracy of tissue segmentations. Certain areas of the brain also possess tissue characteristics that complicate segmentation. For instance, the thalamus is commonly included in the white matter segment, as occurred in our data. Because it is a highly structured, heterogenous tissue that contains both gray and white matter, it is not surprising that segmentation in this area is not straightforward. In the future, methods of volumetric assessment that do not rely so heavily on signal intensity may address some of these concerns.

In addition, this study targeted aging processes in a cohort of older adults that have achieved what is commonly referred to as “successful aging.” It is likely that a more inclusive sample of people between 65 and 80 years old would contain a higher disease burden that might affect white matter differently. The current study also employed a cross-sectional design. Future studies utilizing a longitudinal design will provide better evidence for and further explore the important and multifaceted relationship between white matter volume and microstructure.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Notes

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