Brain Imaging and Behavior
DOI 10.1007/s11682-015-9383-7

ORIGINAL RESEARCH

Regional age differences in gray matter diffusivity among healthy older adults

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Abstract Aging is associated with microstructural changes in brain tissue that can be visualized using diffusion tensor imaging (DTI). While previous studies have established age-related changes in white matter (WM) diffusion using DTI, the impact of age on gray matter (GM) diffusion remains unclear. The present study utilized DTI metrics of mean diffusivity (MD) to identify age differences in GM/WM microstructure in a sample of healthy older adults (N=60). A secondary aim was to determine the functional significance of whole-brain GM/WM MD on global cognitive function using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Participants were divided into three age brackets (ages 50–59, 60–69, and 70+) to examine differences in MD and cognition by decade. MD was examined bilaterally in the frontal, temporal, parietal, and occipital lobes for the primary analyses and an aggregate measure of whole-brain MD was used to test relationships with cognition. Significantly higher MD was observed in bilateral GM of the temporal and parietal lobes, and in right hemisphere WM of the frontal and temporal lobes of older individuals. The most robust differences in MD were between the 50–59 and 70+ age groups. Higher whole-brain GM MD was associated with poorer RBANS performance in the 60–69 age group. Results suggest that aging has a significant and differential impact on GM/WM diffusion in healthy older adults, which may explain a modest degree of cognitive variability at specific time points during older adulthood.

Keywords Gray matter · White matter · DTI · Diffusivity · Aging · Cognition

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Introduction

Brain aging can be visualized in vivo using numerous imaging modalities that are differentially sensitive to pathogenic processes (Bartrés-Faz and Arenaza-Urquijo 2011; Good et al. 2001; Rossini et al. 2007; Westlye et al. 2010). While earlier studies focused on volumetric changes using structural magnetic resonance imaging (MRI) (Good et al. 2001), there is increased interest in examining microstructural characteristics of aging brain tissue using diffusion tensor imaging (DTI). DTI can detect subtle changes in cellular microstructure by measuring patterns of water diffusion that cannot be quantified using traditional MRI sequences (Basser and Pierpaoli 1996; Westlye et al. 2010). As a result, DTI has been shown to be useful for identifying early signs of degeneration that precede gross changes in brain anatomy (Bosch et al. 2012; Jahng et al. 2011; Wang et al. 2011).

Mean diffusivity (MD) is a commonly used DTI metric that measures the rate of water diffusion within an image voxel (Basser and Pierpaoli 1996; Bosch et al. 2012). MD can be used to characterize structural integrity under the assumption that cerebral diffusion is based on two components (Jespersen

Published online: 12 April 2015

et al. 2007). The first component is that diffusion in cylindrical structures is relatively slow due to membrane barriers that restrain the movement of water molecules (e.g., myelinated axon fibers). This type of diffusion is indicated by low MD values and is characteristic of white matter (WM) (Jespersen et al. 2007; Sundgren et al. 2004). The second component accounts for diffusion in other brain structures (particularly somas, glial cells, and extracellular space), and can be described by the random displacement of water molecules that occurs quickly and equally in all directions. This pattern of Brownian motion is associated with high MD values on DTI scans and is characteristic of gray matter (GM) (Sundgren et al. 2004).

Until recently, most DTI studies had focused on WM diffusivity because GM is sensitive to partial volume effects that overestimate MD (Koo et al. 2009; Ma et al. 2004; McNab et al. 2013; Wald 2012). However, advancements in scanner technology and artifact correction methods have significantly reduced inaccuracies in MD measurements (Sundgren et al. 2004; Wald 2012) and studies have begun to acknowledge the importance of MD as an index of microstructural GM integrity in the aging brain (Bhagat and Beaulieu 2004; Ni et al. 2010; Pfefferbaum et al. 2010). Recent evidence suggests that older age is associated with increased diffusivity in deep GM structures (Bhagat and Beaulieu 2004; Pfefferbaum et al. 2010) and cortical GM of the frontal lobe (Bhagat and Beaulieu 2004; Ni et al. 2010). Age-related increases in GM MD are consistent with diffusion abnormalities in GM of individuals with mild cognitive impairment (MCI), early stage Alzheimer's disease, Parkinson's disease, multiple sclerosis, and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Eriksson et al. 2001; Jacobs et al. 2013; Kim et al. 2013; Molko et al. 2001; Ray et al. 2006). In each of these studies, higher MD was associated with more severe clinical impairment, which is consistent with MD changes that have been observed in degenerative WM (Madden et al. 2009; Mielke et al. 2009; Sexton et al. 2011). Despite these associations, the impact of age on GM diffusivity in regions beyond the frontal lobe remains unclear, and no studies have directly compared GM/WM MD within a confined lobe in a non-clinical sample. In addition, the significance of age-related differences in GM/WM diffusion has yet to be defined. Investigation of these relationships will further our understanding of variability associated with brain aging and identification of diffusion abnormalities between tissues may reveal early biomarkers of neurodegeneration.

The present study utilized DTI to examine GM/WM MD across three different age ranges of healthy older adults: 50–59, 60–69, and 70+. WM indices were examined to compare aging effects between tissues. Secondary analyses were completed to examine the functional significance of age differences in MD using a global measure of cognition and whole-brain GM/WM MD. We hypothesized that older individuals would demonstrate higher MD in both GM and WM compared to younger individuals, and that higher MD would be inversely related to cognitive performance. Consistent with previous studies of brain aging (Naganawa et al. 2003; Pal et al. 2011; Sexton et al. 2011; Yoshiura et al. 2010), we further hypothesized that diffusion abnormalities would be most robust in the frontal and temporal lobes of the right hemisphere.

Methods

Participants

Data were analyzed from 60 older individuals (male n=22, female n=38) who were participating in a longitudinal study of healthy aging. Recruitment occurred through local print and radio advertisements and from the Research Participant Registry of the Washington University Institute of Clinical and Translational Sciences. All individuals were required to be English-speaking adults over the age of 50 and demonstrate ability to complete basic daily activities based on the Lawton & Brody Activities of Daily Living Scale (Lawton and Brody 1969). Screening procedures included a comprehensive evaluation of self-reported medical and psychiatric history for each participant, and medical records were obtained when necessary. Exclusion criteria included history of one or more significant medical conditions (e.g., thyroid disease) or a neurological disorder (e.g., Huntington's disease) capable of impacting cognition, all Axis I and II disorders (with the exception of treated depression), substance abuse disorders, history of head injury (defined by a loss of consciousness> 30 min.), treatment-dependent diabetes, and contraindications for MRI (e.g., metal implants). In this study, no individuals indicated past or current depression and therefore "treatment effects" were not an issue. A physician examined all initial MRI scans for gross radiological abnormalities and individuals with abnormal scans were excluded from participation.

Participants were evaluated for dementia using the Mini Mental State Examination (MMSE). Those who scored<24 were excluded from the study. While scores between 24 and 27 may indicate mild cognitive impairment (MCI), the present study leveraged previous studies demonstrating that the sensitivity of the MMSE to detect MCI is restricted, yet the scale remains useful for detecting dementia (Nasreddine et al. 2005; Trenkle et al. 2007). Since our study was focused on identifying age-related relationships to imaging indices, we targeted the exclusion of dementia versus milder age-related cognitive impairments that do not meet criteria for dementia.

All individuals provided informed consent and were financially compensated for their time. All study procedures were approved by the local institutional review board (IRB).

Imaging protocol

Neuroimaging procedures were completed using a head-only Magnetom Allegra 3 Tesla scanner at Washington University in St. Louis, MO (Siemens Healthcare, Erlangen, Germany). This high performance scanner has gradients with a maximum strength of 40 m/T/m in a 100 μs rise time and a slew rate of 400/T/m/s. Imaging acquisition parameters were designed for whole-brain coverage, high signal-to-noise ratio (SNR), and minimal artifact. Subject head movement was restrained through application of surgical tape across the forehead and specialized foam pads, and by limiting the total scan time to less than 1 hour. Each scanning session began with the collection of a scout scan consisting of three orthogonal planes to confirm head positioning. Daily quality assurance tests were also completed to ensure consistent MRI performance and data fidelity.

Structural whole-brain scans were obtained using a T1-weighted magnetization-prepared rapid-acquisition gradient echo (MP-RAGE) sequence (Mugler and Brookeman 1990, 1991), a double-echo proton-density (PD)/T2-weighted turbo spin echo (TSE) sequence, and a T2-weighted fluid-attenuated inversion-recovery (FLAIR) TSE sequence (Hijnal et al. 1992). Magnetic inhomogeneities were adjusted through automated high-order shimming. A more detailed description of acquisition protocols can be found in Paul et al. (2011).

DWI acquisition

DWI acquisition included a customized single-shot multi-slice echo-planar tensor-encoded sequence with diffusion gradients applied in 31 non-collinear diffusion directions and 24 main directions (diffusion weighting of b=996 s/mm^2). A "core" of tetrahedral-perpendicular directions (Conturo et al. 1996) (b= 1412 and 680 s/mm^2) with 5 I_0 acquisitions (b≈0) was also used to maximize SNR and directional coverage. We acquired 64 contiguous slices per contrast with an acquisition matrix of 128 x128 and a 256 x 256 mm field of view (FOV; isotropic 2.0 x 2.0 2.0 mm voxels). Using a full-Fourier transform, TR= 7.82 s and TE=86.2 ms. Seventy-two total acquisitions were averaged over 2 scan repeats. Raw neuroimaging data were saved to a CD and backed up on the operating system, and a SunFire V880 computer server was used to reconstruct floating-point DWIs.

Image analysis

Each diffusion-weighted volume was corrected for subject motion and imaging artifacts by affine registration to the B0 volume (reference scan) using FSL FLIRT (Oxford Centre for Functional MRI of the Brain (FMRIB) Linear Image Registration Tool; Jenkinson and Smith 2001) with mutual information. The orientations of the gradient encoding directions were corrected by the rotation induced by these registrations, and brain tissue was extracted using FSL BET (brain extraction tools; FMRIB, version 5.0). Freesurfer version 5.1.0 was used to segment the high-resolution T1-weighted volume, producing lobe regions-of-interest that included bilateral frontal, temporal, parietal, and occipital areas for both GM and WM. The T1-weighted image was then registered to the diffusion volume using FSL FLIRT with mutual information. Each region was then resampled to diffusion space with nearest-neighbor interpolation, and the average MD within the region was computed. Whole-brain MD was computed separately for GM and WM using the average MD across the frontal, temporal, parietal, and occipital lobes.

Secondary analysis: re-computation of GM MD

To minimize the possibility of CSF contamination, MD was re-computed for each ROI using a highly conservative acquisition approach. We computed isotropic diffusion-weighted images (isoDWIs) by taking the geometric mean of the three perpendicular images (isoDWIs at b=680.0), of the 24 intermediate images (isoDWIs at b=996.451), and of the four tetrahedral images (isoDWIs at b=1412.137). This procedure removes direction dependence in the signal that might occur due to subtle anisotropic diffusion. We also calculated the mean of the I_0 images (b~0). All ROIs in the study were applied to the isoDWI and I_0 images.

We analyzed the signal decay curves by graphing the log of intensity vs. b. The log signal of the b~0 data point significantly deviated from the log signal of the three isoDWI data points. The latter three points were observed to fall on a straight line for all lobar regions and nearly all smaller ROIs (e.g., caudate). The linear relation of the three b>0 points indicated that the b=680.0 data demonstrated negligible CSF contamination in nearly all cases (indicating a mono-exponential decay). We further tested this by first performing non-linear least squares fitting of a mono-exponential to a representative sample of four contiguous gray matter voxels and then assessing the goodness-of-fit with a X^2 test. We found the data supported the mono-exponential model, with a X^2 of 1.051, df=9, and p=0.9993. Distortion in the shape of gyri across the different diffusion weightings was not a major issue, as eddy current effects were minimal. This was evidenced by the lack of need to apply higher-order transformations to co-register the different diffusion-weighted images and slices in this study.

Assessment of global cognition

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph et al. 1998) is a commonly used screening tool for detecting cognitive decline in aging populations (Duff et al. 2003; Gontkovsky

et al. 2004). The RBANS contains a series of subtests that evaluate performance in five cognitive domains: immediate memory, visuospatial skills, language, attention, and delayed memory. Raw scores for each subtest are scaled to create index scores for each domain and then summed and converted to a total scaled score (provided in 10-year age bands). Total scaled scores were used as a proxy measure of global cognitive status in this study.

Statistical analyses

Participants were grouped into three age brackets to determine whether significant differences in MD existed by decade: 50–59 (n=25), 60–69 (n=22), and 70+ (n=13). These age brackets have been used in previous studies to characterize structural and cognitive changes associated with advanced age (Ge et al. 2002; Mortamet et al. 2005; Plumet et al. 2005). Demographic variables such as gender, ethnicity, and years of education did not differ significantly between groups (Table 1).

A series of multivariate analyses of variance (MANOVAs) were computed to examine the impact of age on GM/WM MD. Age bracket served as the independent variable and MD for the frontal, temporal, parietal, and occipital lobes served as the dependent variables in each MANOVA. Dependent variables for each MANOVA were grouped by the type of brain tissue (e.g., GM or WM) and by cerebral hemisphere. We examined MD separately for each hemisphere to allow for comparison to previous studies that have examined brain integrity bilaterally and reported lateralization of aging effects (Bosch et al. 2012; Dolcos et al. 2002; Li et al. 2009; Naganawa et al. 2003; Rathi et al. 2013; Westlye et al. 2010; Yoshiura et al. 2010). A Tukey HSD post hoc analysis was computed for significant multivariate main effects to examine the nature of significant differences between groups.

To establish global aging effects in MD, an additional MANOVA was computed with age bracket serving as the independent variable and whole-brain GM/WM MD serving as the dependent variables. Bivariate correlations were then computed between RBANS total scores and DTI metrics to examine MD relationships to global cognition. Correlations were run separately for each age group to examine associations between MD and cognitive performance by decade. A Sidak correction for multiple comparisons was used for all multivariate analyses.

Results

Relationships between age and WM MD

A significant multivariate effect of MD was observed in right hemisphere MD (Wilks' Λ=0.674, F(8108)=2.94, p=0.005, partial eta^2=0.179), with significant univariate effects in the frontal (F(2,57)=5.53, p=0.006) and temporal (F(2,57)=5.32, p=0.008) lobes (Table 2). MD in the right frontal and temporal lobes was significantly higher in the 70+ age bracket compared to the 50–59 age bracket. Although higher MD was observed in the right temporal lobe of the 60–69 age bracket compared to the 50–59 age bracket (p=0.066), and in the right frontal lobe of the 70+ age bracket compared to the 60–69 age bracket (p=0.074), these differences did not reach statistical significance. No significant differences were observed in regions of the left hemisphere.

Relationships between age and GM MD

Results revealed a significant multivariate effect of MD in both hemispheres (left: Wilks' Λ=0.651, F(8108)=3.23, p=0.002, partial eta^2=0.193; right: Wilks' Λ=0.481, F(8108)=5.97, p<0.001, partial eta^2=0.307) (Figs. 1 and 2).

Significant bilateral effects were observed for MD in temporal (right: F(2,57)=16.72, p<0.001; left: F(2,57)=9.94, p<0.001) and parietal (right: F(2,57)=6.827, p=0.002; left: F(2,57)=6.74, p=0.002) GM (Figs. 1 and 2). In the right temporal lobe, the 70+ age bracket demonstrated significantly higher MD than 60–69 age bracket (p=0.002) and the 50–59 age bracket (p<0.001). In the left temporal lobe, the

Table 1 Demographic variables between groups

Age Group	Age (*M, SD*)	Education[a] (*M, SD*)	Gender (*n*) (Males, Females)	Ethnicity (*n*) (C, AA, A, H)[b]
50–59	54.6±2.60	15.6±2.43	8, 17	16, 8, 1, 0
60–69	64.5±2.82	15.5±2.30	7, 15	17, 2, 0, 3
70+	75.00±3.37	15.0±3.00	7, 6	12, 1, 0, 0

No significant group differences were observed between any of the demographic variables (p>.05)

[a] Education was measured as the number of years of the highest level of completed education. Trade school credits and continuing education completed beyond a terminal degree were not included. [b] C = Caucasian, AA = African American, A = Asian, H = Hispanic

Table 2 White matter MD[a] in the right hemisphere

	50–59	60–69	70±	*F*	*p* value	partial eta^2
Frontal	6.80±1.75	6.87±1.99	7.02±2.08	5.532	0.006*	0.163
Temporal	6.96±1.78	7.12±2.69	7.20±2.27	5.321	0.008*	0.157
Parietal	7.00±1.99	7.08±2.37	7.18±2.55	2.701	0.076	0.087
Occipital	7.12±1.75	7.18±1.99	7.11±2.08	0.239	0.788	0.008

*$p<0.05$ Statistically significant

[a] Mean values were multiplied by 10^{-4}. Standard deviation values were multiplied by 10^{-5}

70+ age bracket demonstrated significantly higher MD compared to the 50–59 age bracket ($p<0.001$). Bilateral differences were observed for higher MD with each subsequent increase in age bracket (50–59 vs. 60–69, $p=0.053$; 60–69 vs. 70+, $p=0.062$), yet these differences did not reach statistical significance. In the parietal lobe, the 70+ and 60–69 age brackets demonstrated significantly higher MD than the 50–59 age bracket in both hemispheres (right: $p=0.045$ and $p=0.002$, respectively; left: $p=0.011$ and $p=0.007$, respectively). No significant differences were observed between the 60–69 and 70+ age brackets in the parietal lobe of either hemisphere.

Results of the MANOVA examining left hemisphere GM also revealed significant group differences for frontal lobe MD ($F(2,57)=3.64$, $p=0.033$), yet these differences did not reach statistical significance when examined between each age bracket.

Relationships between age and whole-brain MD

The MANOVA examining age differences in whole-brain MD was significant (Wilks' $\Lambda=0.791$, $F(4112)=3.49$, $p=0.010$, partial eta$^2=0.111$) in both GM ($F(2, 57)=6.39$, $p=0.003$) and WM ($F(2, 57)=3.46$, $p=0.038$). Whole-brain WM MD was significantly higher in the 70+ age bracket compared to the 50–59 age bracket ($p=0.032$). Group differences were not observed in consecutive decades. Whole-brain GM MD was significantly higher in the 70+ and 60–69 age brackets compared to the 50–59 age bracket ($p=0.006$ and $p=0.021$, respectively), though the former age groups did not differ significantly from one another.

Relationships between whole-brain MD and global cognition

Correlational analyses revealed a moderately strong negative relationship between whole-brain GM MD and RBANS total scores ($r=-.568$) in the 60–69 age bracket. No significant relationships were observed in other age groups or with whole-brain WM MD.

Secondary analysis of GM MD

A significant multivariate effect of MD was observed in right hemisphere MD (Wilks' $\Lambda=0.744$, $F(8104)=2.07$, $p=0.046$, partial eta$^2=0.137$), with a significant univariate effect in the temporal lobe ($F(2,55)=6.09$, $p=0.004$). Post hoc analyses indicated that the 70+ age bracket demonstrated significantly higher MD than the 60–69 age bracket ($p=0.033$) and the 50–59 age bracket ($p=0.003$). No significant group differences were observed in GM regions of the left hemisphere (Wilks' $\Lambda=0.819$, $F(8104)=1.36$, $p=0.223$, partial eta$^2=0.095$) or whole-brain GM MD (Wilks' $\Lambda=0.863$, $F(4108)=2.059$, $p=0.091$, partial eta$^2=0.071$).

Consistent with the primary analysis, a moderately strong negative relationship was observed between whole-brain GM MD and RBANS total scores ($r=-.511$) in the 60–69 age bracket. No significant correlations were observed in the other age groups.

Discussion

Results revealed a region-specific impact of advanced age on GM/WM MD. Age differences in GM MD were more

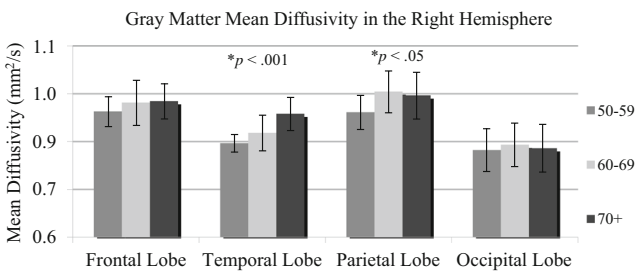

Fig. 1 Gray matter mean diffusivity in the right hemisphere

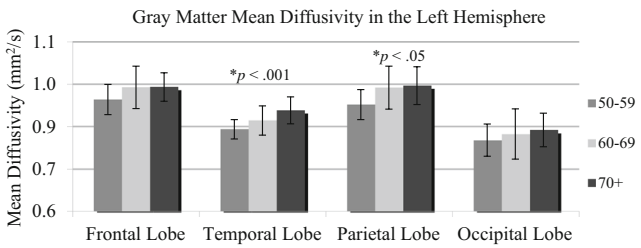

Fig. 2 Gray matter mean diffusivity in the left hemisphere

pervasive and posteriorly located than in WM, with older adults demonstrating higher MD in temporal and parietal GM of both cerebral hemispheres. By contrast, advanced age was associated with higher MD in frontal and temporal WM of the right hemisphere. Secondary analyses revealed a similar pattern of age differences in whole-brain MD that was negatively associated with global cognition among individuals between ages 60–69. However, these relationships were specific to GM and not observed in other age groups. Collectively our results suggest that age differentially impacts the microstructural integrity of GM and WM during older adulthood, and that these differences may underline global changes in overall cognitive function.

Previous research has identified significant WM changes with advanced age that begin in the frontal lobes and progress to posterior brain regions (Freeman et al. 2008; Madden et al. 2009; Sullivan and Pfefferbbaum 2006; Tamnes et al. 2013; Westlye et al. 2010). DTI studies have revealed higher MD in aging WM, which is believed to represent chronic myelin damage and/or axonal loss (Westlye et al. 2010; Bosch et al. 2012). Our results support the findings of previous studies by revealing a significant impact of age on MD in frontal and temporal WM between the 50–59 and 70+ age groups.

Analysis of GM revealed a significant effect of advanced age on higher MD in the temporal and parietal lobes. Although cortical diffusivity has not been examined by lobe in healthy aging populations, previous studies have reported other brain abnormalities (e.g., gray matter volume loss, metabolic abnormalities, etc.) within temporal and parietal lobes among healthy older individuals (Resnick et al. 2003) and those with neurodegenerative disease (Barnes et al. 2007; Baron et al. 2001; Chetelat et al 2003; Dukart et al. 2003). Results of the present study revealed higher MD in frontal GM of older individuals, yet group differences did not reach statistical significance.

It is unclear why significant aging effects were observed in GM of the temporal and parietal lobes but not the frontal lobe. Previous research has indicated that the temporal and parietal lobes are sensitive to age-related changes in blood flow (De Jong et al. 1997, 1999; De la Torre 2000; de Toledo Ferraz Alves et al. 2011) and compromised vasculature has been associated with GM loss in the temporal-parietal cortices (De la Torre 2000). Although our sample consisted of generally healthy older adults, cerebrovascular disease is evident in the majority of individuals over the age of 65 and many of these individuals appear clinically asymptomatic (De la Torre 2000; Paul et al. 2005; Shaw et al. 1984). This is consistent with a previous study of the same participants in which 49 individuals demonstrated mild cerebrovascular disease in the absence of cognitive dysfunction (Salminen et al. 2014). It is possible that cortical diffusion in temporal-parietal areas is particularly sensitive to age-related changes in cerebral circulation, though the physiological mechanism behind this relationship is unclear. More studies are needed to determine the stability of this finding among a larger sample of healthy older adults.

Previous research utilizing DTI to examine GM diffusion has revealed abnormalities in frontal lobe diffusivity among healthy older individuals (Ni et al. 2010), particularly in deep GM structures of the frontal-subcortical circuits (Pfefferbaum et al. 2010). Pfefferbaum et al. (2010) reported increased MD in the caudate and putamen of older adults (ages 65–79) compared to younger adults (ages 22–37). Our study revealed a significant univariate effect for age-related changes in frontal lobe MD, but this effect did not reach statistical significance when examined by age bracket. A distinction of the present study from Pfefferbaum et al. (2010) is the examination of cortical versus subcortical GM structures. It is possible that more pronounced alterations in diffusion occur in the subcortical versus the cortical GM, or precede changes in the cortical GM as a function of the aging process. Research has demonstrated that subcortical diffusion metrics are highly influenced by localized alterations in iron accumulation that are specific to the subcortical GM regions of the brain among older individuals (Bartzokis et al. 2007; Bilgic et al. 2012; Pfefferbaum et al. 2009, 2010). Since iron accumulation was not measured in the present study, the possibility that subcortical-cortical differentiation of diffusion metrics is associated with iron accumulation remains conjecture. Future studies are needed to explore this potential interpretation of the results described herein.

The laterality of diffusion outcomes in this study is noteworthy. Aging has been associated with reduced hemispheric asymmetry and various "hemi-aging models" have been proposed to explain lateralized brain changes with advanced age (Dolcos et al. 2002). There is some evidence to support a right hemi-aging model that describes higher heterogeneity of brain aging in the right hemisphere compared to the left (Bosch et al. 2012; Dolcos et al. 2002). Although most studies of hemi-aging have focused on functional outcomes of asymmetry, there have been previous reports of increased WM diffusivity (Naganawa et al. 2003; Yoshiura et al. 2010) and reduced functional connectivity (Dolcos et al. 2002; Li et al. 2009) in the right hemisphere of older individuals. Accordingly, results of the present study revealed significant differences in WM MD between age groups that were localized to the right hemisphere. Lateralized differences in MD were not observed in GM, however, which may reflect enhanced tissue dissimilarity with advanced age (Rathi et al. 2013). It is also possible that hemispheric changes in GM and WM occur at different time points during older adulthood, therefore investigation of hemispheric diffusion patterns between tissues is an important direction for future research.

Similar to the primary analyses, results of the secondary analyses revealed higher MD in whole-brain GM and WM among older individuals, yet these differences were only

related to lower global cognitive function in GM between ages 60–69. The limited number of individuals in the 70+ group may have reduced power to detect a significant relationship between cognition and GM MD after age 70. Studies employing a larger number of individuals in this age group are needed to determine the plausibility of this explanation.

The distinct pathological mechanisms of age-related differences in GM/WM diffusion are unclear. The coherent alignment of WM microstructure facilitates directional restriction of water diffusion along the length of myelinated axons (Koo et al. 2009). Thus, changes in WM diffusion are highly suggestive of microstructural decline (Bosch et al. 2012; Westlye et al. 2010). GM, however, is highly isotropic and alterations in diffusivity may be a secondary effect of decreased neuronal size and macrostructural damage (Bartzokis et al. 2007; Freeman et al. 2008; Sundgren et al. 2004). Reduced neuronal size is associated with an increase in extracellular space that may lead to increased diffusivity (Pfefferbaum et al. 2010). The compositional complexity of GM structures also decreases with age (Bartzokis et al. 2007; Freeman et al. 2008), and fewer neuropil boundaries may contribute to age-related increases in GM diffusivity. GM diffusion may also be sensitive to age differences in CNS inflammation and future studies are needed that investigate potential relationships between DTI metrics and inflammatory biomarkers.

An important element of this study was the re-computation of GM MD. Although some variations in MD across subjects are due to physiologic factors in GM such as water content (Alexander et al. 2007), another main cause of GM MD variation across subjects is partial volume averaging with CSF. This effect is an artifact, rather than a microstructural feature of GM and is a source of "multi-exponential decay" that is not a good biological approximation of integrity. The solution to this problem is to suppress the CSF signal by excluding the b~0 data from the analysis and re-calculating the diffusion tensor and MD. As indicated by our secondary analyses, the CSF effect on the resulting MD measurements was reduced to negligible levels for almost all brain regions and the underlying signal measures a straightforward, mono-exponential decay. Implementing this conservative approach eliminated some of the findings presented in the primary analyses and this as an important design consideration for future research investigations of GM MD.

Some limitations of this study should be noted. First, the cell sizes of each age bracket may have limited our ability to detect additional differences in MD and cognition between age groups. Several outcomes observed in this study were just above the .05 alpha level and it is possible that these results would achieve statistical significance in a larger sample of older adults. Second, we cannot comment on the impact of WM lesions on age-related DTI alterations, as presence of WM lesions was not an exclusionary factor in this study. WM lesions are recognized as a consequence of the aging process for many individuals, and therefore exclusion of nondemented individuals with WM lesions would have eliminated age-related effects that the grouping variables were intended to capture. Third, this cohort consisted primarily of highly educated Caucasian and African American individuals and results may not be generalizable to a more diverse sample of older adults. Finally, we cannot infer patterns of intraindividual diffusion changes by age bracket, as this study was restricted to cross-sectional data. Longitudinal studies are needed to determine the evolution of diffusion changes in both GM and WM regions among older individuals.

Conclusion

The present study revealed distinct differences in GM/WM diffusivity across older adulthood. The most robust differences were observed between the 50–59 and 70+ age groups in both GM and WM, with the oldest age group demonstrating significantly greater diffusion abnormalities compared to the youngest age group. Age differences in GM MD were observed bilaterally in the temporal and parietal lobes, while differences in WM MD were specific to the right frontal and temporal lobes. Correlational analyses revealed a modest relationship between whole-brain GM MD and global cognitive performance between the ages of 60–69. Re-calculation of the diffusion tensor and MD and revealed a positive relationship between age and GM MD in the right temporal lobe, and this approach represents an important design consideration for future research. Studies utilizing larger sample sizes within each age range are needed to confirm the results of the present study, particularly among a more diverse sample of older adults. Longitudinal examination of tissue diffusion will further our understanding of intraindividual changes between GM and WM microstructure in older adulthood.

Acknowledgments This work was supported by the National Institutes of Health/National Institute of Neurological Disorders and Stroke [grant numbers R01NS052470, R01NS039538]; the National Institutes of Health/National Institute of Mental Health [grant number R21MH090494]; and the Australian National Health and Medical Research Council [grant number 1037196]. Recruitment database searches were supported in part by the National Institutes of Health/National Center for Research Resources [grant number UL1 TR000448].

Conflict of interest L. Salminen, T. Conturo, D. Laidlaw, R. Cabeen, E. Akbudak, E. Lane, J. Heaps, J. Bolzenius, L. Baker, S. Cooley, S. Scott, L. Cagle, S. Phillips, and R. Paul declare no conflicts of interest.

Informed Consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all patients for being included in the study.

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