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NeuroImage

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NeuroImage 34 (2007) 61 – 73

Diffusion tensor imaging of the corpus callosum in Autism

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Received 5 April 2006; revised 10 August 2006; accepted 13 August 2006
Available online 4 October 2006

The corpus callosum is the largest commissural white matter pathway that connects the hemispheres of the human brain. In this study, diffusion tensor imaging (DTI) was performed on subject groups with high-functioning autism and controls matched for age, handedness, IQ, and head size. DTI and volumetric measurements of the total corpus callosum and subregions (genu, body and splenium) were made and compared between groups. The results showed that there were significant differences in volume, fractional anisotropy, mean diffusivity, and radial diffusivity between groups. These group differences appeared to be driven by a subgroup of the autism group that had small corpus callosum volumes, high mean diffusivity, low anisotropy, and increased radial diffusivity. This subgroup had significantly lower performance IQ measures than either the other individuals with autism or the control subjects. Measurements of radial diffusivity also appeared to be correlated with processing speed measured during the performance IQ tests. The subgroup of autism subjects with high mean diffusivity and low fractional anisotropy appeared to cluster with the highest radial diffusivities and slowest processing speeds. These results suggest that the microstructure of the corpus callosum is affected in autism, which may be related to nonverbal cognitive performance.

Keywords: Autism; Diffusion tensor imaging; Corpus callosum; Radial diffusivity; Performance IQ

Introduction

The neuropathology of autism appears to be complex (Lainhart, 2006). Several lines of evidence suggest that abnormalities of the corpus callosum are involved. Complex information processing, which requires cortico-cortical interhemispheric as well as intrahemispheric transfer of information, has been found to be deficient across multiple domains in autism (Minshew et al., 1997). Abnormalities of corpus callosum mid-sagittal area and white matter density have been found (Chung et al., 2004; Egaas et al., 1995; Filipek, 1996; Hardan et al., 2000; Manes et al., 1999; Piven et al., 1997; Vidal et al., 2006; Waiter et al., 2005). Interhemispheric functional underconnectivity has been suggested by several recent fMRI studies of autism in language processing and working memory (Just et al., 2004; Koshino et al., 2005, respectively). Postmortem studies have found thinning of the corpus callosum in some cases (Bailey et al., 1998). A better understanding of corpus callosum white matter differences between autistic individuals and controls, in general and throughout development, may help identify potential neuroanatomical markers of autism and important neurobiological subtypes of the disorder.

The corpus callosum is responsible for conduction of signals between homologous and heterotopic cortical regions and is an essential component for brain lateralization and interhemispheric communication (Innocenti, 1986; Pandya and Seltzer, 1986; Zaidel and Iacoboni, 2003). The most rostral region of the corpus callosum, the genu and the rostrum (hereafter referred to as the genu), has connections between prefrontal brain regions (Witelson, 1989). The most caudal region, the splenium, contains connections between occipital, temporal and parietal regions (Witelson, 1989).

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.neuroimage.2006.08.032

The midsections between the genu and splenium are the body and isthmus (hereafter referred to as the body). Methods for subdividing the corpus callosum into subregions have been developed although there is no clear consensus regarding approaches for the divisions (de Lacoste et al., 1985; Witelson, 1989; Clarke and Zaidel, 1994). Recently Huang et al. (2005) used diffusion tensor imaging (DTI) and white matter tractography to parcellate the corpus callosum into regions that connect to specific cortical areas.

Neuroimaging techniques have provided insight into general corpus callosum development and more localized changes that occur with age (see reviews in Brambilla et al., 2003; Lainhart et al., 2005). In typically developing individuals, overall area of the corpus callosum increases during childhood and adolescence. The greatest increase occurs in the posterior regions of the corpus callosum and during the childhood years (Giedd et al., 1999, 1996; Keshavan et al., 2002). Overall callosal area may continue to increase into the twenties (Pujol et al., 1993), but the anterior corpus callosum may reach adult size well before then (Giedd et al., 1996).

Studies investigating the development of the corpus callosum in autism have provided mixed results. Although a decrease in the area of the anterior corpus callosum has been noted (Hardan et al., 2000), a recent study (Rice et al., 2005) found no differences in area, shape, or contour of the corpus callosum between autistic participants with macrocephaly and normal participants with benign macrocephaly. These findings suggest that group differences in head size may have influenced previous results. In addition to area, another type of measure may be necessary to identify abnormalities in the corpus callosum in autism.

Diffusion tensor imaging (DTI) is a non-invasive method for mapping the diffusion properties of tissue water (Basser and Pierpaoli, 1996). DTI is extremely sensitive to subtle differences in the architecture of white matter at the microstructural level. The white matter tracts of the corpus callosum are highly coherent which makes them well suited for study with DTI. The diffusion tensor defines the magnitude, anisotropy (variation of the diffusion properties with direction) and orientation of anisotropic water diffusion in biological tissues. The diffusion tensor may be decomposed into three principal eigenvalues with corresponding eigenvectors. The major eigenvector ($\mathbf{e}_1$), corresponding to the largest eigenvalue (λ_1), also referred to as the axial diffusivity D_a, is the direction of fastest diffusivity and is generally assumed to be parallel to the direction of axon bundles in white matter. The medium and smallest eigenvalues (λ_2 and λ_3, respectively) are assumed to be perpendicular to the white matter tracts. A measure of diffusivity in the perpendicular plane is the radial diffusivity, $D_r=(\lambda_2+\lambda_3)/2$ (Song et al., 2002). The average of the three eigenvalues is referred to as the mean diffusivity (MD). A commonly used measure of diffusion anisotropy is the fractional anisotropy (FA), which is a normalized (ranges between 0 and 1) version of the eigenvalue standard deviation (Pierpaoli and Basser, 1996).

To date, the only published study examining DTI in autistic children showed lower FA values in the genu and rostral body in seven high-functioning autistic male children and adolescents compared to controls (Barnea-Goraly et al., 2004). Filippi et al. (2003) noted significantly lower FA and higher MD values in both the genu and splenium of developmentally delayed children compared to controls, although autistic participants were excluded from their study. Thus, the present study was designed to elucidate some of the developmental changes that occur in the corpus callosum in autism.

In this study, diffusion tensor measurements (MD, FA, D_a and D_r) in corpus callosum were investigated in a large group of high-functioning autistic male children, adolescents, and young adults compared to matched controls. Relationships between age, volume of the corpus callosum, FA, and MD measures were explored. Potential functional correlates of white matter organization, such as IQ and social functioning, were also examined.

Materials and methods

All subjects were ascertained, assessed, and scanned at the University of Utah. Image processing and analysis were done at the University of Wisconsin.

Subjects and assessment

Ascertainment

Autism and typically developing subjects were recruited during a 3-year period (2002–2005) from community sources, including parent support groups, youth groups, and schools, and from clinic social skills groups. After complete description of the study to subjects and parents, written informed consent was obtained.

Diagnosis

Autism was rigorously diagnosed. Autism spectrum subjects were classified into 4 categories according to the Collaborative Programs of Excellence in Autism (CPEA) diagnostic criteria, permitting standardized diagnostic classification of subjects (described in Lainhart et al., under review). The CPEA criteria use the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994), the Autism Diagnostic Observation Schedule-Generic (ADOS-G) (Lord et al., 2000), age, and IQ. The ADI-R is an investigator-based parent interview about the individual's early childhood and current social and communication development and stereotyped, repetitive behaviors and interests. The ADI-R has good reliability and validity (Lord et al., 1994). The ADOS-G is a semi-structured interactive observation session that involves play and activities for young children and activities and an interview for older, verbal subjects. Individuals are tested with one of four different modules depending on their age and verbal ability. The ADOS-G is designed to elicit signs of autism if they are present. In order to meet CPEA diagnostic criteria for an ASD, subjects also had to meet DSM-IV (American Psychiatric Association, 1994) and ICD-10 (World Health Organization Staff, 1993) criteria for the disorder. History, observation, Fragile-X gene testing, and karyotype were used to exclude medical causes of autism.

Autism spectrum disorders were diagnosed in a hierarchical fashion. First, subjects were considered for a diagnosis of autism. Subjects who did not meet criteria for autism were considered for a possible diagnosis of Asperger's syndrome, and barring that, PDD-NOS. A final classification of "broad Autism Spectrum Disorder" (*broad ASD*) took into consideration the fact that all of the data necessary for a specific ASD diagnosis might not be available for all subjects. Subjects classified as having *broad* ASD included individuals who may have met criteria for autism, Asperger's, or PDD-NOS in the past or if additional data were available. Based on these criteria, lifetime diagnosis and diagnosis at the time of DTI scanning were determined. Psychiatric

comorbidity was assessed in all ASD and normal control subjects using the Autism Comorbidity Interview (Leyfer et al., in press). Forty percent of the ASD sample had no psychiatric comorbidity or history of psychotropic medication use. The rate of signs of other psychiatric conditions was comparable to the rate reported in other community ascertained autism samples (Leyfer et al., in press). Features of ADHD were present in 18.5%, anxiety disorder in 9.3%, OCD in 14%, and depression in 30%. Twenty-one percent of the ASD subjects were taking a stimulant medication, 46% a serotonin reuptake inhibitor, 9% valproic acid, and 5% an atypical neuroleptic. Signs of other psychiatric conditions are frequently occurring associated features of autism as we know it today. Effects of comorbidity and psychotropic medication use on corpus callosum findings were considered in the analyses and are discussed at the end of the Results section.

Typically developing subjects had no history of learning, developmental, cognitive, neurological, or neuropsychiatric problems. All of them had extensive testing, including the ADOS, IQ, language, and psychiatric testing, to confirm that they were typically developing. None had a history of psychotropic medication use.

IQ

IQ was measured in the autism and control subjects with the Differential Abilities Scale, WISC-III, or for adults, the WAIS-III (Elliott, 1990; Wechsler, 1991, 1997). For those receiving either the WISC-III or WAIS-III, the Symbol-Coding and Symbol Search subtests form the Processing Speed Index, which is a composite measure of perceptual processing, working memory and psychomotor speed (Kennedy et al., 2003) and requires integration of the two hemispheres to effectively complete the tasks (Mathias et al., 2004). Shorter and more accurate processing times reflect better (higher) Processing Index Scores, where the mean is 100 and the standard deviation is 15.

Handedness

Handedness was measured using the Edinburgh Handedness Inventory (Oldfield, 1971). A score of 100 signifies complete right handedness and −100 indicates complete left handedness.

Head circumference

Maximal occipital–frontal head circumference was measured. Reliability for head circumference was established. The intra-class correlation coefficient was ≥.90, including inter-rater and test–retest reliability. Head circumference is an index of maximal brain volume in development and is significantly correlated with current brain volume (r=.67 in adolescents and adults) (Hazlett et al., 2005; Lainhart et al., 2005; Piven et al., 1996).

Social impairment

The degree of impairment in social reciprocity at the time of DTI examination was measured in autism subjects with the ADOS-G (Lord et al., 2000) and in autism and control subjects with the Social Reciprocity/Responsiveness Scale (SRS) (Constantino and Todd, 2003). The SRS is a quantitative, dimensional measure of social functioning across the entire distribution from normal to severely impaired functioning. The ADOS-G qualitative impairments in reciprocal social interaction and combined communication+social interaction algorithm scores and the SRS total scores were used.

Neuroimaging

Magnetic resonance (MR) images were acquired on a Siemens Trio 3.0 Tesla Scanner. In addition to DTI, a wide range of pulse sequences (3D MP-RAGE, 2D proton-density- and T2-weighted, and 2D FLAIR) and image contrasts were collected for clinical review but were not used in the current study. An 8-channel, receive-only, RF head coil was used for the imaging experiments. DTI was performed using a product single-shot, spin-echo, echo planar imaging (EPI) pulse sequence with diffusion-weighting, which was performed using bipolar gradients with dual-echo refocusing to reduce eddy currents (Reese et al., 2003). For each slice, a single non-diffusion-weighted (b=0) reference image and twelve diffusion-weighted images with unique non-collinear diffusion encodings and b=1000 s/mm^2 were obtained. Parallel imaging was employed with a geometric reduction factor of 2 to reduce the distortion caused by susceptibility differences at tissue interfaces. Sixty contiguous, 2.5-mm-thick, axial slices were acquired covering the cerebrum and cerebellum. The acquisition matrix was 128×128 and the field of view was 256 mm, resulting in 2-mm isotropic in-plane resolution. Other imaging parameters were TR/TE=7000/84 ms, pixel bandwidth=1346 Hz, and 4 averages. A pair of 2D gradient echo images with different echo times (TE1/TE2=7/10 ms) was obtained for field mapping at the same slice locations as the DTI acquisition (Jezzard and Balaban, 1995). The acquisition times for the DTI study and field map were 6:55, and 3:34 min, respectively.

Five young subjects with autism received sedation for scanning. Sedation, using a combination of remifentanil and propofol, followed a strict clinical protocol approved by the institutional review board by the University of Utah and performed by an onsite faculty anesthesiologist (J.L.). No complications occurred in the subjects who underwent sedation. In several cases, rehearsal was used to 'practice' lying in the scanner. In all cases written, informed consent was obtained prior to any imaging. No complications or untoward effects were encountered.

Diffusion tensor image analysis

Image misregistration from eddy currents and head movements were first corrected using an affine registration software program (AIR–http://bishopw.loni.ucla.edu/AIR5/). Distortions from magnetic field (B0) inhomogeneities were corrected using a field map derived from the phase difference image obtained from the gradient echo image data at two echo times. Field map correction was performed using methods described by Jezzard and Balaban (1995) with custom software. The corrected raw images were interpolated from 2×2×2.5-mm to 2×2×2-mm isotropic voxels, and the 3×3 diffusion tensor was estimated for each voxel location. The interpolation to isotropic voxels was performed to minimize the effects of voxel grid orientation relative to the signal heterogeneity in the corpus callosum regions. From the diffusion tensor, the eigenvalues (λ_1, λ_2, λ_3) and eigenvectors ($\mathbf{e}_1$, $\mathbf{e}_2$, $\mathbf{e}_3$) were estimated. Maps of the mean diffusivity (MD—average of the eigenvalues), the fractional anisotropy (FA), and the axial and radial diffusivities ($D_a=\lambda_1$ and $D_r=(\lambda_2+\lambda_3)/2$, respectively) were generated from the eigenvalues.

In this study, the corpus callosum was extracted in two steps. First, the white matter of the brain was segmented using FAST (Zhang et al., 2001) in the FMRIB software library (http://www.fmrib.ox.ac.uk/fsl/) with maps of the largest and smallest eigenvalues (λ_1 and λ_3, respectively) as the inputs (see Fig. 1 for representative example). Visual inspection of the white matter

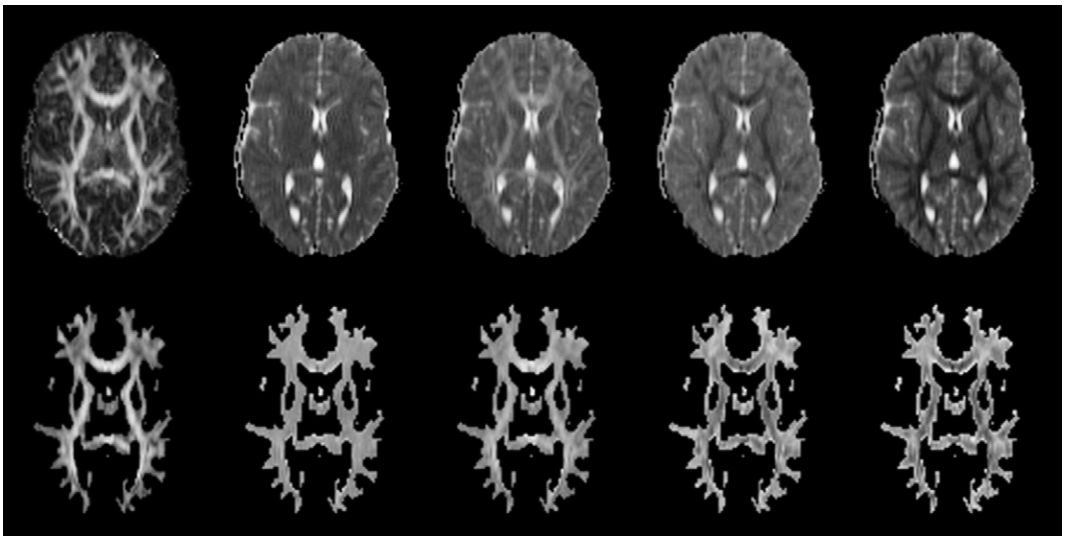

Fig. 1. Example of the WM segmentation achieved by putting the smallest and largest eigenvalues (λ_1 and λ_3) into a two-channel segmentation algorithm (mFAST). The top row shows the images before segmentation and the images on the bottom row depict the images after white matter segmentation. From left to right, the images are the fractional anisotropy (FA), the mean diffusivity (MD), the major eigenvalue (λ_1), the medium eigenvalue (λ_2) and the minor eigenvalue (λ_3).

masks created using this combination of eigenvalues appeared to provide the most consistent segmentation with white matter regions that were apparent on the DTI maps (compared with any single eigenvalue, MD, FA or any other combination). However, the approach was not rigorously evaluated in comparison with a gold-standard segmentation. The example in Fig. 1 does illustrate that the approach is quite good, although not perfect. For example, regions of the thalamus were incorrectly labeled as white matter, although this is not an issue with respect to this study, which focuses only on the corpus callosum. This segmentation approach did consistently segment the corpus callosum in all subjects from the surrounding non-white matter tissues and thus was a good starting point for more specific segmentation.

The mean and standard deviation of the white matter MD over the entire brain was computed and voxels that were above 2 standard deviations from the mean were excluded. This removed voxels that were bordering regions between white matter and CSF, and contained significant partial volume averaging between tissue groups. The corpus callosum was then extracted by computing maps of the "X" (right/left) component of the major eigenvector ($e_{1x}=\mathbf{e}_1\cdot\mathbf{x}$, where $\mathbf{x}$ is a unit vector in the x direction) multiplied by FA with a minimum FA * e_{1x} threshold of .2. The head positions in all cases appeared to be straight (e.g., the brain sagittal midline was very close to the y-axis of the axial images). The entire corpus callosum was then extracted manually between the intersection with the left and right centrum semiovale (Fig. 2). The segmented corpus callosum was then used as a mask to extract mean values of MD, FA, λ_1, λ_2, and λ_3 in the corpus callosum. More regionally specific measurements were obtained by increasing the FA * e_{1x} threshold to .4 and then placing $18\times18\times18$ mm cubic regions ($9\times9\times9$ voxels) centered on the genu, splenium, and mid-body in the mid-sagittal plane. The genu and splenium points were defined using the approximate centers of anterior and posterior 'bulbs' in the mid-sagittal plane. The body was defined as the half length point also in the mid-sagittal plane. The intersection of the segmented corpus callosum and the cubic region was used to

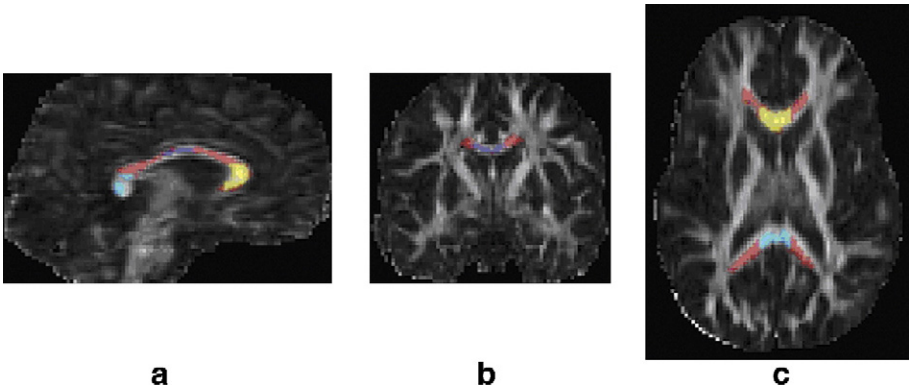

Fig. 2. Global and regional corpus callosum regions of interest for one subject with autism in (a) the mid-sagittal plane, (b) a coronal plane through the mid-body, and (c) the axial plane depicting regions of the genu and splenium. The total corpus callosum is shown in red, genu in yellow, body in purple and splenium in blue.

define subregions within the corpus callosum, which were used also as regions-of-interest for extracting the mean MD, FA, D_a, and D_r measurements in these areas. The slightly more conservative threshold for the more specific regions was used to further minimize any partial volume averaging effects in the smaller volumes. It should be noted that the statistical thresholding approach used in this paper will likely underestimate the true volume of the corpus callosum and subregions although the volume trends should be preserved. The same person defined the corpus callosum regions in all image sets. The segmentation of the corpus callosum and subregions was repeated twice (spaced by one week) in 10 subjects. The voxel reproducibility was very high (correlation $r>0.98$ for all regions).

Statistical analysis

A one-way analysis of variance (ANOVA) was used to measure group differences in age, intelligence, handedness, head circumference, and social and communication scores. Between group differences in the corpus callosum volumes were also analyzed with a one-way ANOVA with volumes of the genu, body, splenium and total corpus callosum, as dependent variables, and group as the independent variable. The DTI measurements of FA, MD, D_a, and D_r were compared with a one-way ANOVA using group as the independent variable and mean FA, MD, D_a, and D_r within the genu, body, and splenium as dependent measures.

The relationships between DTI measures and either age or volume were examined using bivariate correlation analysis. Finally, bivariate correlations were used to examine relationships between DTI measures and both IQ and social impairment scores.

Results

Demographics

The autism spectrum group (hereafter referred to as the autism group) consisted of 43 participants (lifetime diagnosis: 38 autism, 5 PDD-NOS; diagnosis at the time of DTI: 30 autism, 6 PDD-NOS, 7 Broad ASD). As shown in Table 1, the autism and control subjects were group-matched on age, IQ, handedness, and head circumference. The control group performed slightly better than the autism group on performance IQ. The difference failed to reach significance but the effect size was 0.42. Performance IQ was therefore used as a covariate in some of the analyses.

Corpus callosum volume differences

The autism group exhibited significantly smaller total and regional corpus callosum volumes (see Table 2). In addition, consistently higher standard deviations were found for the autism group relative to controls, demonstrating greater variability in the volumes of the corpus callosum for the autism group. When pIQ was used as a covariate, the effect of diagnostic group remained significant ($F=9.114$, df 1, $p=0.003$ for mean total CC volume).

Group differences in DTI measures

DTI measurements (FA, MD, D_a and D_r) obtained for the genu, body, splenium, and total corpus callosum are summarized in Table 3. Significantly lower mean FA values were found in the total corpus callosum, the genu and splenium in the autism group compared to controls. Mean FA values in the body were also lower in the autism group, although this comparison did not quite reach significance ($p=0.058$). The MD measurements also showed significant differences between groups in the total corpus callosum, genu, and body with the autism group exhibiting higher mean MD relative to controls. Comparisons of the axial diffusivities, D_a, did not reveal significant differences between the groups. However, the radial diffusivities, D_r, were significantly larger in the autism group for all regions and the total corpus callosum. Consequently, it appears that the radial diffusivities are driving the group differences in FA and MD. Similar to the volumetric findings, the autism group shows larger standard deviations for all DTI measures compared to controls. Diagnostic group continued to have a significant effect on D_r when pIQ was used as a covariate ($F=14.09$, df 1, $p<0.001$ for mean total CC D_r).

Relationship between volume and DTI measures

Correlations between corpus callosum volume and the DTI measures are presented in Table 4. The autistic group exhibited a significantly positive correlation between FA and volume in the total corpus callosum and within each subregion. Positive correlations between volume and FA were also found in the control group, but were significant in the total corpus callosum, genu, and body only. In contrast, a different pattern of correlations between groups was found for the MD and volume correlations. The small negative correlations exhibited by the control group differ from the highly negative correlations found in the autism group (see Table 4). Radial diffusivities demonstrated strong negative correlations with volume in all regions in the autism group and only in the genu and body regions for the control group. Small positive correlations were observed for the axial diffusivity in the body and total corpus callosum for controls. Because of the correlation between total and regional corpus callosum volume and FA and MD, we repeated the case-control analysis of FA and MD controlling for volume. After controlling for volume, group differences in total corpus callosum were still significant for MD ($p=0.018$) whereas differences in FA were not quite significant ($p=0.058$). After controlling for both volume and pIQ, the results were similar for the DTI measures (mean total CC DTI measures: MD: $F=6.564$, df 1, $p=0.012$; FA: $F=2.914$, $p=0.092$; D_r: $F=6.086$, df 1, $p=0.016$).

Table 1
Demographic variables and group comparisons

	Autism $n=43$			Normal Control $n=34$			Group comparisons	
	Mean	SD	Range	Mean	SD	Range	F	Significance
Age (years)	16.23	6.70	7–33	16.44	5.97	8–29	0.02	0.89
Performance IQ	107.49	13.04	85–135	112.79	12.08	90–134	3.35	0.07
Handedness	80.81	21.69	13–100	72.79	29.01	0–100	1.93	0.17
Head circumference	56.56	2.30	52.2–60.75	56.05	2.03	52.5–59.3	0.29	0.34

Table 2
Comparison of corpus callosum volumes (in voxels)

	Autism (n=43)			Control (n=34)			Group comparison	
	Mean	SD	Range	Mean	SD	Range	F	Significance
Genu	173.3	63.5	50–306	215.8	53.4	98–318	9.80	0.002**
Body	116.5	36.2	23–213	136.6	27.5	78–175	7.17	0.009**
Splenium	201.8	51.4	99–317	230.1	48.8	136–340	6.03	0.016*
Total CC	2681.6	608.7	1435–4369	3116.3	471.1	2160–4239	11.76	0.001**

ANOVA=analysis of variance.
Voxel volume was 8 mm^3 (=2×2×2 mm).
*Significant at the 0.05 level; **Significant at the 0.01 level.

Relationship between FA and MD

Fig. 3 shows the relationship between FA and MD values in the total corpus callosum. Although FA and MD were negatively correlated for both groups, a stronger correlation was found in the autism group (r=−0.600, p<0.0001) than the control group (r=−0.339, p=0.05). The higher correlation appeared to be driven by a subgroup of the autism sample that had low total corpus callosum FA and high MD.

Autism subgroups

When the scatterplot of FA and MD (Fig. 3) was examined, it became apparent that the autism subjects clustered into two subgroups. One autism subgroup had significantly lower FA and higher MD compared to controls. The other autism group had FA and MD similar to controls. The DTI, volume, cognitive, behavioral and physical measures for these autism subgroups are summarized in Table 5. The two subgroups did not differ in mean age, puberty stage, head circumference, rate of macrocephaly, handedness, severity of core social features of autism (ADI-R, ADOS-G), or SRS score. However, mean verbal, performance, and full scale IQ were significantly lower in the autism subgroup with low FA and high MD (all p values ≤0.003). Radial diffusivity measurements were significantly higher in the low FA/high MD group; whereas the axial diffusivities were relatively constant for all groups. In all regions, the measured volumes were also significantly lower in the low FA/high MD subgroup(all p values <0.0001).

Relationship between age and changes in the corpus callosum

Table 6 summarizes the correlations between age and corpus callosum volumetric and DTI measures. Although age was significantly positively correlated with increased total corpus callosum volume in control participants, age was not correlated with overall volumetric changes in the corpus callosum in the

Table 3
Group comparison of anisotropy and diffusivities in the corpus callosum determined by one-way ANOVA

	Autism (n=43)			Control (n=34)			Group comparison	
	Mean	SD	Range	Mean	SD	Range	F	Significance
Fractional anisotropy								
Genu	0.663	0.042	0.56–0.74	0.692	0.037	0.56–0.75	10.01	0.002**
Body	0.664	0.045	0.56–0.74	0.683	0.038	0.62–0.77	3.71	0.058
Splenium	0.693	0.045	0.61–0.77	0.720	0.032	0.67–0.81	8.22	0.005**
Total corpus callosum	0.552	0.037	0.46–0.62	0.579	0.024	0.53–0.63	13.45	<0.001***
Mean diffusivity (10^{-3} mm^2/s)								
Genu	0.842	0.048	0.75–0.96	0.820	0.034	0.76–0.91	5.13	0.026*
Body	0.869	0.041	0.81–1.01	0.840	0.041	0.80–0.88	13.69	<0.001***
Splenium	0.832	0.050	0.75–0.95	0.818	0.026	0.76–0.87	2.21	0.141
Total corpus callosum	0.833	0.037	0.78–0.95	0.807	0.023	0.77–0.85	13.01	0.001**
Axial diffusivity (10^{-3} mm^2/s)								
Genu	1.61	0.074	1.41–1.79	1.62	0.067	1.42–1.74	0.33	0.569
Body	1.67	0.077	1.49–1.85	1.65	0.067	1.54–1.84	1.74	0.191
Splenium	1.64	0.091	1.44–1.78	1.67	0.071	1.52–1.80	1.30	0.257
Total corpus callosum	1.43	0.054	1.32–1.57	1.43	0.043	1.34–1.54	0.11	0.747
Radial diffusivity (10^{-3} mm^2/s)								
Genu	0.457	0.057	0.36–0.60	0.420	0.043	0.34–0.58	10.51	0.002**
Body	0.466	0.057	0.37–0.63	0.434	0.039	0.34–0.50	8.12	0.006**
Splenium	0.426	0.060	0.33–0.57	0.394	0.036	0.29–0.45	7.57	0.007**
Total corpus callosum	0.533	0.045	0.43–0.63	0.495	0.027	0.44–0.56	18.01	<0.001***

ANOVA=analysis of variance
*Significant at the 0.05 level; **Significant at the 0.01 level; ***Significant at the 0.001 level.

Table 4
Summary of correlations between DTI measures as a function of volume (in voxels)

	Autism (n=43)		Controls (n=34)	
	r	Significance	r	Significance
Fractional anisotropy				
Genu	0.755	<0.001***	0.573	<0.001***
Body	0.664	<0.001***	0.505	0.002**
Splenium	0.537	<0.001***	0.305	0.079
Total corpus callosum	0.637	<0.001***	0.408	0.017*
Mean diffusivity				
Genu	−0.570	<0.001***	−0.247	0.159
Body	−0.544	<0.001***	−0.061	0.731
Splenium	−0.538	<0.001***	−0.219	0.214
Total corpus callosum	−0.467	0.002**	0.032	0.858
Axial diffusivity				
Genu	0.021	0.894	0.249	0.155
Body	0.142	0.364	0.436	0.010**
Splenium	−0.080	0.609	0.090	0.611
Total corpus callosum	0.077	0.623	0.341	0.048*
Radial diffusivity				
Genu	−0.742	<0.001***	−0.494	0.003**
Body	−0.693	<0.001***	−0.427	0.012*
Splenium	−0.619	<0.001***	−0.325	0.061
Total corpus callosum	−0.615	<0.001***	−0.232	0.186

*Significant at the 0.05 level (two-tailed); **Significant at the 0.01 level; ***Significant at the 0.001 level.

autism group. Additionally, FA was positively correlated (and D_r was similarly negatively correlated) with age in the control group in the total corpus callosum and within each subregion but was only significantly related to age in the splenium of the autism participants. In both groups, measurements of MD were strongly negatively correlated with age in the total corpus callosum and splenium region. The axial diffusivity was negatively correlated with age in the total corpus callosum for the autistic group, and was positively correlated in the body region of controls.

Because of the correlations with age in the control group, analyses of corpus callosum volumes, FA, and MD were also performed controlling for age using a general linear model. Multivariate tests of between-subject effects were performed with volume, FA and MD entered as independent variables, group as the fixed factor, and age as a covariate. Volume, FA and MD group differences were analyzed for the total corpus callosum and also the genu, body and splenium. Controlling for age did not affect the results (see Table 7).

Correlations between DTI and cognitive and behavioral measures

Significant relationships were found between IQ and DTI measures in the autism group only. As shown in Table 8, FA was positively correlated (and D_r was negatively correlated) with PIQ (performance IQ), whereas MD was negatively correlated with PIQ in the total corpus callosum and within each subregion for the autistic participant group. In a subset of subjects (21 autism and 19 controls), measurements of the Weschler Processing Speed Index was obtained as part of the IQ assessment. The Weschler Processing Speed Indices were positively correlated with FA in the genu and negatively correlated with MD in the splenium and total corpus callosum in the autism group. Within the autism group, total and regional FAs and MDs were not significantly correlated with ADOS-G social or SRS scores.

Because it is important to understand relationships not only with case and control groups but across the entire distribution of individuals from normal to severe autism, the relationship between total corpus callosum DTI measures and cognitive (pIQ and processing speed) and the behavioral measure of social reciprocity/responsiveness were examined in the combined autism and control samples. In this distribution, performance IQ and SRS scores were significantly related with total corpus callosum mean volume, FA, MD, and D_r (pIQ: r=0.273 to 0.370, p=0.016 to 0.001; SRS: r=−0.272 to −0.317, p=0.039 to 0.015). Processing speed was significantly correlated with total corpus callosum FA, MD, and D_r (FA r=0.494, p=0.001; MD r=−0.494, p=0.001; D_r r=−0.534, p<0.0001) but not volume. Figs. 4 and 5 show the relationships between the radial diffusivity and processing speed and SRS score, respectively. The Wechsler Processing Speed Index was robustly related to radial diffusivity, where slow processing speed was associated with the highest radial diffusivity scores. Across the distribution of social reciprocity/responsiveness scores, as individuals were more impaired (higher SRS scores), mean total corpus callosum D_r and variance increased. Strikingly, there are some autism individuals with severe degrees of difficulties in social reciprocity (high SRS scores) who have values of D_r similar to normal controls (low SRS scores). The findings were similar for FA and MD.

Effects of psychiatric comorbidity and psychotropic medication use

Independent t-tests were used to compare behavioral, cognitive, volumetric, and diffusion tensor measurements between autism

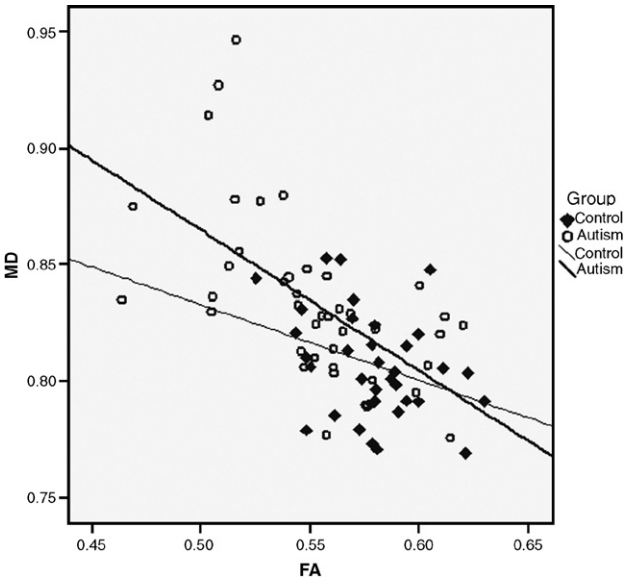

Fig. 3. Scatter plot of MD (in 10^{-3} mm^2/s) versus FA measurements in the total corpus callosum for all subjects. Note that there is considerable overlap between groups in the region with FA >0.52 and MD <0.86×10^{-3} mm^2/s. However, there is a subset of 12 autism subjects with high mean diffusivity and low FA that do not overlap. Best fit lines: Controls R^2=0.115; Autism R^2=0.36.

Table 5
Autistic subgroups compared to controls—(Low FA/High MD subgroup includes the 12 outliers in the autism group from Fig. 3)

	Autism (n=12) Low FA/High MD	Autism (n=31) High FA/Low MD	Control (n=34)
Volume (mean voxels)			
Total CC	2022.5 ***	2936.7	3116.3
Genu	122.7 ***	192.8	215.8
Body	78.0 ***	131.4	136.6
Splenium	153.1 ***	220.7	230.2
Mean FA			
Total CC	0.506 ***	0.570	0.579
Genu	0.621 ***	0.680	0.692
Body	0.613 ***	0.684	0.683
Splenium	0.639 ***	0.714	0.720
Mean MD (10^{-3} mm^2/s)			
Total CC	0.875 ***	0.817	0.807
Genu	0.891 ***	0.823	0.820
Body	0.914 ***	0.851	0.840
Splenium	0.883 ***	0.812	0.818
Mean axial diffusivity (10^{-3} mm^2/s)			
Total CC	1.44	1.43	1.43
Genu	1.63	1.60	1.62
Body	1.67	1.68	1.65
Splenium	1.65	1.64	1.67
Mean radial diffusivity (10^{-3} mm^2/s)			
Total CC	0.593 ***	0.509 ****	0.495
Genu	0.520 ***	0.433	0.420
Body	0.537 ***	0.439	0.434
Splenium	0.502 ***	0.396	0.394
Mean age, cognitive, behavioral, and physical measures			
Age (SD) [range]	14.25 (8.6) [7–33]	17.00 (5.8) [9–28]	16.44 (6.0) [8–29]
PIQ	96.67 ***	111.68	112.79
Processing speed	83.40 *	96.00	107.11
Head circumference	55.0	57.1	56.0
ADOS social	9.11	8.26	
ADOS ComSoc	14.22	12.61	
SRS score	108.18 **	94.59 **	17.44
Prevalence of psychiatric comorbidity/psychotropic medication			
Comorbidity [a]	41.7%	64.5%	0%
Medication [b]	41.7%	67.7%	0%

[a] Features of psychiatric comorbidity in Low FA/High MD group: ADHD 16.6%, depression 25%, OCD 8.3%; in High FA/Low MD group: ADHD 25.8%, depression 38.7%, OCD 16.1%, anxiety disorder 9.6%, other mood disorder 6.4%.

[b] Types of psychotropic medication use in Low FA/High MD group: SSRI 25%, stimulants 16.6%, valproic acid 16.6%, atypical neuroleptic 8.3%, taking 2 or more types of medication 16.6%; in High FA/Low MD group: SSRI 51.6%, stimulants 22.6%, valproic acid 3.2%, atypical neuroleptic 3.2%, taking 2 or more types of medication 19.3%.

* Significantly different from Controls at $p<0.01$.

** Significantly different from Controls at $p<0.001$.

*** Significantly different from Autism High FA/Low MD and Controls at $p<0.001$.

**** Significantly different from Controls at $p<0.05$.

Table 6
Summary of correlations between age and corpus callosum volume and DTI measures

	Autism (n=43)		Controls (n=34)	
	r	Significance	r	Significance
Volume				
Genu	−0.002	0.990	0.171	0.332
Body	0.017	0.913	0.199	0.258
Splenium	0.006	0.970	0.334	0.054
Total corpus callosum	0.067	0.668	0.346	0.045*
Fractional anisotropy				
Genu	0.252	0.104	0.456	0.007**
Body	0.103	0.511	0.500	0.003**
Splenium	0.345	0.023*	0.365	0.034*
Total corpus callosum	0.042	0.791	0.467	0.005**
Mean diffusivity				
Genu	−0.293	0.057	−0.276	0.114
Body	−0.248	0.108	−0.172	0.332
Splenium	−0.421	0.005**	−0.464	0.006**
Total corpus callosum	−0.402	0.008**	−0.457	0.007**
Axial diffusivity				
Genu	−0.113	0.472	0.160	0.367
Body	−0.128	0.414	0.381	0.026*
Splenium	−0.134	0.391	−0.052	0.770
Total corpus callosum	−0.417	0.005**	−0.038	0.829
Radial diffusivity				
Genu	−0.301	0.050	−0.458	0.007**
Body	−0.185	0.234	−0.470	0.005**
Splenium	−0.431	0.004**	−0.453	0.007**
Total corpus callosum	−0.244	0.115	−0.557	0.001**

*Significant at the 0.05 level (two-tailed); **Significant at the 0.01 level.

Table 7
Between group comparison of corpus callosum volume and DTI measures with and without controlling for age

	Not controlling for age		Age entered as covariate	
	F	p	F	p
Volume (voxels)				
Genu	9.80	0.002**	9.65	0.003**
Body	7.17	0.009**	7.06	0.010*
Splenium	6.03	0.016*	5.96	0.017*
Total CC	11.76	0.001**	11.76	0.001**
Fractional anisotropy				
Genu	10.01	0.002**	10.74	0.002**
Body	3.71	0.058	3.76	0.056
Splenium	8.22	0.005**	8.92	0.004**
Total CC	13.45	<0.001***	13.49	<0.001***
Mean diffusivity				
Genu	5.13	0.026*	5.32	0.024*
Body	13.69	<0.001***	13.96	<0.001***
Splenium	2.21	0.141	2.44	0.122
Total CC	13.01	0.001**	14.97	<0.001***

*Significant at the $p<0.05$ level; **Significant at the $p<0.01$ level; ***Significant at the $p<0.001$ level.

Table 8
Correlation between Wechsler performance IQ, processing speed, and DTI measures

Performance IQ	Autism (n=43)		Controls (n=34)	
	r	Significance	r	Significance
Fractional anisotropy				
Genu	0.285	0.064	0.197	0.263
Body	0.307	0.045*	−0.098	0.580
Splenium	0.399	0.008**	−0.079	0.658
Total corpus callosum	0.396	0.009**	0.146	0.412
Mean diffusivity				
Genu	−0.263	0.089	−0.125	0.483
Body	−0.330	0.031*	−0.032	0.855
Splenium	−0.320	0.037*	0.048	0.786
Total corpus callosum	−0.327	0.032*	−0.068	0.701
Axial diffusivity				
Genu	−0.064	0.684	0.057	0.748
Body	−0.007	0.966	−0.127	0.475
Splenium	0.035	0.824	−0.045	0.801
Total corpus callosum	0.016	0.921	0.051	0.773
Radial diffusivity				
Genu	−0.294	0.055	−0.194	0.271
Body	−0.358	0.018*	0.083	0.640
Splenium	−0.431	0.004**	0.096	0.589
Total corpus callosum	−0.408	0.007**	−0.129	0.467
Processing speed	Autism (n=21)		Controls (n=19)	
Fractional anisotropy				
Genu	0.526	0.014*	0.273	0.257
Body	0.360	0.109	−0.202	0.409
Splenium	0.324	0.152	0.317	0.186
Total corpus callosum	0.379	0.090	0.432	0.065
Mean diffusivity				
Genu	−0.400	0.072	−0.421	0.073
Body	−0.423	0.056	−0.170	0.485
Splenium	−0.470	0.032*	−0.170	0.487
Total corpus callosum	−0.481	0.027*	−0.301	0.211

*Significant at the $p<0.05$ level (two-tailed); **Significant at the $p<0.01$ level.

participants with and without psychiatric comorbidity and a history of psychotropic medication use. The subgroups were compared on mean performance IQ, processing speed index, ADOS-G communication, social and total algorithm scores, and SRS scores. Only performance IQ significantly differed between the subgroups; the subgroup *with* psychiatric comorbidity had a higher mean pIQ than the subgroup without comorbidity (mean pIQ=111.2 and 102.3, respectively; t=2.31, p=0.026). No significant differences were found between the autistic subgroups on volumetric or diffusion tensor measurements of the corpus callosum. A possible confounding effect of psychiatric comorbidity and psychotropic medication use on the relationship between DTI measures and pIQ and processing speed was also examined. Partial correlations between DTI measures and either pIQ or processing speed were calculated while controlling for comorbidity and psychotropic medication use. The pattern of partial correlations was very similar to the correlations shown in Table 8. While controlling for psychiatric comorbidity, strong positive correlations were found between pIQ and FA in all regions in the autism group and the correlations remained significant for the splenium and total corpus callosum. A similar pattern of correlations between pIQ and FA was found when medication use was controlled. Performance IQ and MD correlations remained negative and significant in the body and total corpus callosum while controlling for comorbidity, and significant in the body, splenium, and total corpus callosum when controlling for medication use. Radial diffusivity measures continued to show strong and significant negative correlations with pIQ while controlling for both comorbidity and medications.

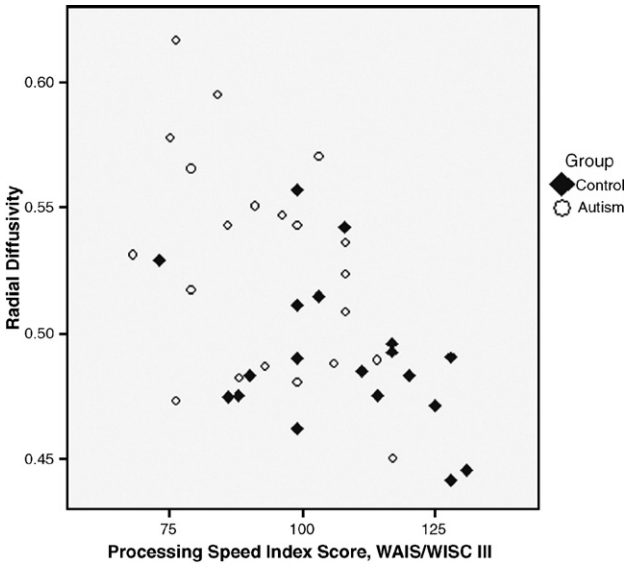

Fig. 4. Scatter plot of the relationship between radial diffusivity of the total corpus callosum and the Wechsler Processing Speed Index score in the combined autism and control samples.

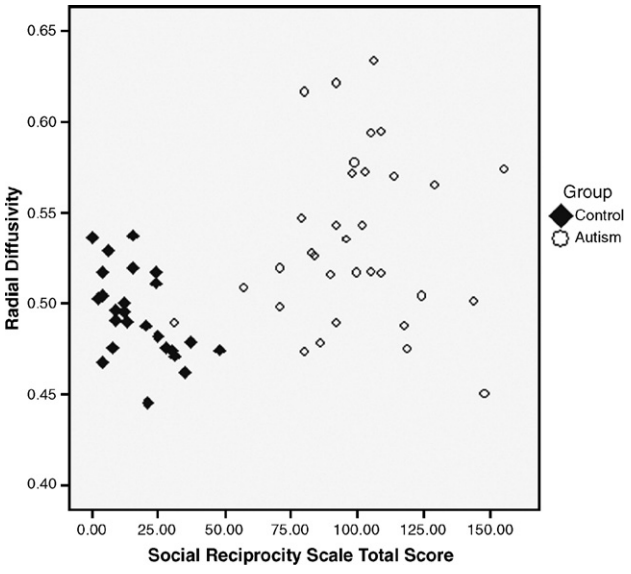

Fig. 5. Scatter plot of the relationship between the mean radial diffusivity of the total corpus callosum and the Social Reciprocity/Responsiveness Score (SRS) in the combined sample of autism and normal control subjects.

Axial diffusivity correlations with pIQ and correlations between processing speed and FA and MD measures were unaffected when comorbidity and medication use were controlled. Linear regression was also conducted, with corpus callosum DTI parameters entered as the dependent variable and the cognitive measure (pIQ or processing speed), comorbidity, and psychotropic medication use entered as independent variables. Neither comorbidity or medication use had an independent effect on the DTI measures.

The low FA/high MD and high FA/low MD subgroups were also compared in regard to comorbidity and psychotropic medication use. The rates, shown at the bottom of Table 5, were not statistically different in the subgroups ($z=0.20$, $p=0.42$).

Discussion

This study demonstrates that both the morphology and microstructure of the corpus callosum appear to be affected in autism. Volumetric measurements demonstrated that the corpus callosum volume both globally and regionally is reduced relative to typically developing controls, which is consistent with observations by several other research groups (Chung et al., 2004; Egaas et al., 1995; Filipek, 1996; Hardan et al., 2000; Manes et al., 1999; Piven et al., 1997; Vidal et al., 2006; Waiter et al., 2005). The fractional anisotropy of the genu, splenium and total corpus callosum was significantly reduced in autism. The mean diffusivity of the genu, body and total corpus callosum was significantly increased; however, the splenium was not for this measure. Further investigation of the diffusion properties revealed that the differences in both FA and MD were caused by significantly higher radial diffusion as represented by the medium and minor eigenvalues (λ_2 and λ_3, respectively), whereas the axial diffusivity defined by the major eigenvalue (λ_1) is relatively unaffected in the corpus callosum in individuals with autism. Psychiatric comorbidity and psychotropic medication use did not have independent effects on any of the DTI measures.

The specific mechanisms for the differences in the diffusion tensor measures are unclear. Increased radial diffusivity may reflect differences in myelination, axonal diameter or packing density, and/or glial densities. In a mouse model of dysmyelination (Song et al., 2002), observed that the radial diffusivity was increased in the absence of myelin, whereas the axial diffusivity was unaffected. As the diffusion signal is averaged over both the intra- and extra-axonal spaces, changes in either compartment may also cause the radial diffusivity to change. To date, differences in myelination or axonal structure have not been reported in autism. In the future, complimentary quantitative imaging methods may provide additional and complimentary information about the properties of the corpus callosum. For example quantitative T2 myelin water fraction (MacKay et al., 1994) and magnetization transfer (e.g., Henkelman et al., 1993; Sled and Pike, 2000) measurements may provide more specific information about the myelination of the corpus callosum. Recently, Hermoye et al. (2006) used the $b=0$ signal in white matter (normalized by the signal in CSF) from the DTI study as an indirect measure of T2 to study myelination changes with typical brain development. This technique was not applied in the current study because the sensitivity of the 8-channel receiver head coil was not uniform.

One interesting observation is that the diffusion differences appear to be driven largely by a subgroup of autistic subjects with low FA and high MD (Fig. 3) relative to the other subjects with autism, who did not have significantly different diffusion tensor or volume properties in the corpus callosum relative to controls. These low FA/high MD subjects were generally characterized by smaller corpus callosum volumes, and lower (but normal) performance IQ measures. However, the groups did not differ in ADOS social, social+communication scores, or WAIS performance.

Using DTI, researchers have identified changes in corpus callosum microstructural organization during normal development. Several DTI studies of white matter (Barnea-Goraly et al., 2005; Mori et al., 2006; Mukherjee et al., 2001; Partridge et al., 2005; Snook et al., 2005) have found significant reductions in mean diffusivity and increases in diffusion anisotropy with age. However, after 4 or 5 years up until the mid-twenties, the age-related changes appear more gradual (Mukherjee et al., 2001; Snook et al., 2005). Conversely, in older subjects (>20 years) corpus callosum regions (genu and splenium) appear to show a gradual decrease in FA with age and gradual increase in mean diffusivity with number of years (Abe et al., 2002; Madden et al., 2004; Pfefferbaum et al., 2000).

In this study, the age-related changes to DTI measures appeared to be generally different between the two main groups (Table 5). One exception was the splenium region, which demonstrated similar relative changes with age in both autism and controls. However, the differences in the age dependencies of these measures between regions and groups could cause the results to differ if the age distribution of the sample was different as was recently demonstrated in a DTI study of schizophrenia (Jones et al., 2006). Using age as a covariate in the analysis of volume, MD and FA data did not seem to affect the group differences (Table 6). This means that it is likely that age related factors are not driving the group differences in this study.

A few studies have explored the relationship between DTI measures of the corpus callosum and interhemispheric processing performance (Madden et al., 2004; Schulte et al., 2005). Reaction time measured during a visual target detection task appeared related to FA values in the splenium in young adults (Madden et al., 2004), an area thought to underlie attentional circuits. In another study of interhemispheric transfer measures (visuomotor interhemispheric transfer, and parallel processing of visual information presented to each cerebral hemisphere), mean diffusivity and fractional anisotropy of the genu were found to predict interhemispheric performance in both healthy controls and alcoholics (Schulte et al., 2005). Conversely, another study utilizing a self-paced choice reaction time task did not find any correlation between FA values in the corpus callosum and performance (Tuch et al., 2005). Despite this apparent contradiction, Tuch et al. did find that FA values in the visual and parietal white matter were related to performance and used these results to support the idea that interhemispheric communication through the corpus callosum was not involved in the visuospatial attention network necessary for their task. The importance of callosal trajectories is even more evident when reviewing deficits found in patient populations with isolated damage to the corpus callosum (i.e., agenesis, schizophrenia, seizures, etc.) (Paul et al., 2003; Bigler et al., 1988; Suzuki et al., 1998).

Abnormal neocortical circuitry in the autistic brain has been suggested in research using functional magnetic resonance imaging (fMRI) and voxel-based morphometry. Studies show that autistic individuals exhibit abnormalities in the neural areas thought to underlie the ‘social’ brain, language comprehension, and also

higher executive functions. Compared to controls, autistic children show reduced correlations between morphometry in interconnected frontal and parieto-temporal cortices (McAlonan et al., 2005), a finding that is complemented by abnormalities in the 'mirror neuron system' of autistic individuals recorded during fMRI (Dapretto et al., 2005). An elegant fMRI study of functional connectivity by Just et al. (2004) found apparent underconnectivity between interhemispheric as well as intrahemispheric cortical areas important for sentence comprehension. high-functioning autistic individuals displayed a lower degree of synchronization in activation between left and right frontal areas and between left parietal and right frontal areas compared to controls. This finding in combination with our finding of reduced callosal volume and abnormalities of the physical properties of callosal white matter supports a possible role for the corpus callosum in previous fMRI studies that have found differences in cortical activation in autistic individuals compared to controls during tasks of language processing and working memory (Just et al., 2004; Koshino et al., 2005; Luna et al., 2002). Thus, cognitive, behavioral, and social impairments displayed by autistic individuals may be related to callosal white matter organization and ultimately disordered interhemispheric communication. The recently reported abnormalities of cingulate cortex deactivation may be relevant to our findings because if the cingulate cortex was damaged early in development, it could affect corpus callosum development (Kennedy et al., 2006; Rash and Richards, 2001).

The neuropsychological tasks that make up the Processing Speed Index on the Wechsler scales require bi-hemispheric integration to perform them fast and efficiently, with the implication being that such functions are dependent on an intact corpus callosum (Bigler and Clement, 1997; Zaidel and Iacoboni, 2003). As shown in Fig. 4, increased radial diffusivity was associated with slower processing speed and worse overall cognitive performance on these tasks. The very fastest Processing Speed performers were those with the lowest radial diffusivity. Numerous studies have implicated slow processing speed in autism, involving diverse cognitive functions (Behrmann et al., 2006; Dawson et al., 2005; Scheuffgen et al., 2000). In the current study, the highest radial diffusivity scores were consistently related to slowest processing speed, certainly implicating delayed interhemispheric transmission via the CC.

The IQ-DTI correlations in the autism group are more difficult to explain. Spencer et al. (2005) found that reduced CC size was related to intellectual impairments, but because the CC contains interconnecting pathways, and not the terminus of the pathway, the overall correlation of CC size to IQ is weak. It is thought that any correlation of IQ with CC size, is actually a reflection of the CC relationships to projecting regions of the cerebral cortex and not the CC itself (Nosarti et al., 2004). Schmithorst et al. (2005) showed that FA correlated positively with IQ in various brain regions, however, MD did not, but the CC was not specially investigated. Others using voxel-based morphometry methods have shown diverse cortical areas that relate to IQ as well (Haier et al., 2005).

There are several potential limitations associated with this study. The first is the coarse spatial resolution of the DTI data, which increases the partial volume signal averaging between corpus callosum and CSF signals. In this study, we used a statistical approach to exclude these voxels although this also excludes potentially relevant regions from the analysis. Consequently, it may be advisable to use CSF suppression techniques like FLAIR in future DTI studies of the corpus callosum (Bhagat and Beaulieu, 2004; Papadakis et al., 2002). Another limitation is that the Wechsler Processing Speed measurements were not obtained from all subjects. Further, this measure is a relatively indirect measure of interhemispheric signal processing. It may be desirable in future studies to obtain more direct measures of interhemispheric transfer rates (Madden et al., 2004; Schulte et al., 2005; Tuch et al., 2005). Finally, the relationship between DTI measures and specific changes to the white matter structure are still unclear. Group-based differences in the radial diffusivity may be caused by any combination of factors related to myelination, axonal dimensions, and axonal density. Histopathological studies from autopsy specimens may be necessary to clarify the specific changes in the corpus callosum in autism. Finally, although we ruled out an independent effect of psychiatric comorbidity and psychotropic medication in general on the findings of our study, we were not able to examine effects of specific comorbid psychiatric disorders or psychotropic medications.

In summary, this study demonstrates that the average DTI properties of the corpus callosum in high-functioning autism are significantly different from typically developing control subjects. These differences in the DTI measures appear driven mainly by a subgroup of autism subjects with significantly elevated radial diffusivity. This group was also characterized by smaller corpus callosum volumes and decreased performance IQ measurements. The radial diffusivity also appears to be correlated with task processing speed over all subjects. Future studies are necessary to determine the specificity of the microstructural changes in the tissue and the relationship to interhemispheric processing.

Acknowledgments

This work was supported by the NICHD U19 HD35476 (University of Utah), the NICHD/NIDCD Collaborative Programs of Excellence in Autism (CPEA), the NIH Mental Retardation/Developmental Disabilities Research Center (MRDDRC–Waisman Center), NIMH 62015 (ALA), NIDA15879 (ALA), the Autism Society of Southwestern Wisconsin, and the National Alliance for Research in Schizophrenia and Affective Disorders (NARSAD–ML). We thank the other members of the Utah Autism Research Program (Jed Elison, Michael Johnson, Jubel Morgan, Lori Krasny, Megan Farley, Heidi Block, Lindsey Warner, Barbara Young, Tami Elsner, Kelly Pelzel, Logan Griffall and Dr. Hilary Coon) and the University of Utah Center for Advanced Imaging Research (Henry Buswell) for their contributions. We express our sincere gratitude to the children and adults who participated in this CPEA study and their families.

References

Abe, O., Aoki, S., Hayashi, N., Yamada, H., Kunimatsu, A., Mori, H., Yoshikawa, T., Okubo, T., Ohtomo, K., 2002. Normal aging in the central nervous system: quantitative MR diffusion-tensor analysis. Neurobiol. Aging 23 (3), 433–441.

American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders: DSM-IV, 4th ed. American Psychiatric Association, Washington, DC.

Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M., Rutter, M., Lantos, P., 1998. A clinicopathological study of autism. Brain 121 (Pt 5), 889–905.

Barnea-Goraly, N., Kwon, H., Menon, V., Eliez, S., Lotspeich, L., Reiss, A.L., 2004. White matter structure in autism: preliminary evidence from diffusion tensor imaging. Biol. Psychiatry 55 (3), 323–326.

Barnea-Goraly, N., Menon, V., Eckert, M., Tamm, L., Bammer, R., Karchemskiy, A., Dant, C.C., Reiss, A.L., 2005. White matter development during childhood and adolescence: a cross-sectional diffusion tensor imaging study. Cereb. Cortex 15 (12), 1848–1854.

Basser, P.J., Pierpaoli, C., 1996. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. J. Magn. Reson., B 111 (3), 209–219.

Behrmann, M., Avidan, G., Leonard, G.L., Kimchi, R., Luna, B., Humphreys, K., Minshew, N., 2006. Configural processing in autism and its relationship to face processing. Neuropsychologia 44 (1), 110–129.

Bhagat, Y.A., Beaulieu, C., 2004. Diffusion anisotropy in subcortical white matter and cortical gray matter: changes with aging and the role of CSF-suppression. J. Magn. Reson. Imaging 20 (2), 216–227.

Bigler, E.D., Clement, P.F., 1997. Diagnostic Clinical Neuropsychology. University of Texas Press, Austin, TX.

Bigler, E.D., Rosenstein, L.D., Roman, M., Nussbaum, N.L., 1988. The clinical significance of congenital agenesis of the corpus callosum. Arch. Clin. Neuropsychol. 3 (2), 189–200.

Brambilla, P., Hardan, A., di Nemi, S.U., Perez, J., Soares, J.C., Barale, F., 2003. Brain anatomy and development in autism: review of structural MRI studies. Brain Res. Bull. 61, 557–569.

Clarke, J.M., Zaidel, E., 1994. Anatomical–behavioral relationships: corpus callosum morphometry and hemispheric specialization. Behav. Brain Res. 64, 185–202.

Chung, M.K., Dalton, K.M., Alexander, A.L., Davidson, R.J., 2004. Less white matter concentration in autism: 2D voxel-based morphometry. NeuroImage 23 (1), 242–251.

Constantino, J.N., Todd, R.D., 2003. Autistic traits in the general population: a twin study. Arch. Gen. Psychiatry 60 (5), 524–530.

Dapretto, M., Davies, M.S., Pfeifer, J.H., Scott, A.A., Sigman, M., Bookheimer, S.Y., Iacoboni, M., 2005. Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. Nat. Neurosci. 9 (1), 28–30.

Dawson, G., Webb, S.J., McPartland, J., 2005. Understanding the nature of face processing impairment in autism: insights from behavioral and electrophysiological studies. Dev. Neuropsychol. 27 (3), 403–424.

de Lacoste, M.C., Kirkpatrick, J.B., Ross, E.D., 1985. Topography of the human corpus callosum. J. Neuropathol. Exp. Neurol. 44 (6), 578–591.

Egaas, B., Courchesne, E., Saitoh, O., 1995. Reduced size of corpus callosum in autism. Arch. Neurol. 52 (8), 794–801.

Elliott, C.D., 1990. Differential Ability Scales: Introductory and Technical Handbook. The Psychological Corporation, New York.

Filipek, P.A., 1996. Brief report: neuroimaging in autism: the state of the science 1995. J. Autism. Dev. Disord. 26 (2), 211–215.

Filippi, C.G., Lin, D.D., Tsiouris, A.J., Watts, R., Packard, A.M., Heier, L.A., Ulug, A.M., 2003. Diffusion-tensor MR imaging in children with developmental delay: preliminary findings. Radiology 229 (1), 44–50.

Giedd, J.N., Rumsey, J.M., Castellanos, F.X., Rajapakse, J.C., Kaysen, D., Vaituzis, A.C., Vauss, Y.C., Hamburger, S.D., Rapoport, J.L., 1996. A quantitative MRI study of the corpus callosum in children and adolescents. Brain Res. Dev. Brain Res. 91 (2), 274–280.

Giedd, J.N., Blumenthal, J., Jeffries, N.O., Rajapakse, J.C., Vaituzis, A.C., Liu, H., Berry, Y.C., Tobin, M., Nelson, J., Castellanos, F.X., 1999. Development of the human corpus callosum during childhood and adolescence: a longitudinal MRI study. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 23 (4), 571–588.

Haier, R.J., Jung, R.E., Yeo, R.A., Head, K., Alkire, M.T., 2005. Structural brain variation, age, and response time. Cogn. Affect. Behav. Neurosci. 5 (2), 246–251.

Hardan, A.Y., Minshew, N.J., Keshavan, M.S., 2000. Corpus callosum size in autism. Neurology 55 (7), 1033–1036.

Hazlett, H.C., Poe, M.D., Gerig, G., Smith, R.G., Provenzale, J., Ross, A., Gilmore, J., Piven, J., 2005. Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. Arch. Gen. Psychiatry 62 (12), 1366–1376.

Henkelman, R.M., Huang, X., Xiang, Q.S., Stanisz, G.J., Swanson, S.D., Bronskill, M.J., 1993. Quantitative interpretation of magnetization transfer. Magn. Reson. Med. 29 (6), 759–766.

Hermoye, L., Saint-Martin, C., Cosnard, G., Lee, S.K., Kim, J., Nassogne, M.C., Menten, R., Clapuyt, P., Donohue, P.K., Hua, K., Wakana, S., Jiang, H., van Zijl, P.C., Mori, S., 2006. Pediatric diffusion tensor imaging: normal database and observation of the white matter maturation in early childhood. NeuroImage 29 (2), 493–504.

Huang, H., Zhang, J., Jiang, H., Wakana, S., Poetscher, L., Miller, M.I., van Zijl, P.C., Hillis, A.E., Wytik, R., Mori, S., 2005. DTI tractography based parcellation of white matter: application to the mid-sagittal morphology of corpus callosum. NeuroImage 26 (1), 195–205.

Innocenti, G.M., 1986. General organization of callosal connections in the cerebral cortex. In: Jones, E.G., Peters, A.J.E. (Eds.), Cerebral Cortex, vol. 5. Plenum, New York, pp. 291–353.

Jezzard, P., Balaban, R.S., 1995. Correction for geometric distortion in echo planar images from B0 field variations. Magn. Reson. Med. 34 (1), 65–73.

Jones, D.K., Catani, M., Pierpaoli, C., Reeves, S.J., Shergill, S.S., O'Sullivan, M., Golesworthy, P., McGuire, P., Horsfield, M.A., Simmons, A., Williams, S.C., Howard, R.J., 2006. Age effects on diffusion tensor magnetic resonance imaging tractography measures of frontal cortex connections in schizophrenia. Hum. Brain Mapp. 27 (3), 230–238.

Just, M.A., Cherkassky, V.L., Keller, T.A., Minshew, N.J., 2004. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain 127 (Pt 8), 1811–1821.

Kennedy, J.E., Clement, P.F., Curtiss, G., 2003. WAIS-III processing speed index scores after TBI: the influence of working memory, psychomotor speed and perceptual processing. Clin. Neuropsychol. 17 (3), 303–307.

Kennedy, D.P., Redcay, E., Courchesne, E., 2006. Failing to deactivate: resting functional abnormalities in autism. Proc. Natl. Acad. Sci. U. S. A. 103 (21), 8275–8280.

Keshavan, M.S., Diwadkar, V.A., DeBellis, M., Dick, E., Kotwal, R., Rosenberg, D.R., Sweeney, J.A., Minshew, N., Pettegrew, J.W., 2002. Development of the corpus callosum in childhood, adolescence and early adulthood. Life Sci. 70 (16), 1909–1922.

Koshino, H., Carpenter, P.A., Minshew, N.J., Cherkassky, V.L., Keller, T.A., Just, M.A., 2005. Functional connectivity in an fMRI working memory task in high-functioning autism. NeuroImage 24 (3), 810–821.

Lainhart, J.E., 2006. Advances in autism neuroimaging research for the clinician and geneticist. Am. J. Med. Genet. C Semin. Med. Genet. 142 (1), 33–39.

Lainhart, J.E., Lazar, M., Bigler, E.D., Alexander, A.L., 2005. The brain during life in autism: advances in neuroimaging research. In: Casanova, M.F. (Ed.), Recent Developments in Autism Research. Nova Science Publishers, Inc.

Leyfer, O.T., Folstein, S.E., Bacalman, S., Davis, N.O., Dinh, E., Morgan, J., Tager-Flusberg, H., Lainhart, J.E., in press. Comorbid psychiatric disorders in children with autism: Interview development and rates of disorders. J. Autism Dev. Disord. (Jul 15; Electronic publication ahead of print).

Lord, C., Rutter, M., Le Couteur, A., 1994. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J. Autism Dev. Disord. 24 (5), 659–685.

Lord, C., Risi, S., Lambrecht, L., Cook Jr., E.H., Leventhal, B.L., DiLavore, P.C., Pickles, A., Rutter, M., 2000. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J. Autism Dev. Disord. 30 (3), 205–223.

Luna, B., Minshew, N.J., Garver, K.E., Lazar, N.A., Thulborn, K.R., Eddy, W.F., Sweeney, J.A., 2002. Neocortical system abnormalities in autism: an fMRI study of spatial working memory. Neurology 59 (6), 834–840.

MacKay, A., Whittall, K., Adler, J., Li, D., Paty, D., Graeb, D., 1994. In vivo visualization of myelin water in brain by magnetic resonance. Magn. Reson. Med. 31 (6), 673–677.

Madden, D.J., Whiting, W.L., Huettel, S.A., White, L.E., MacFall, J.R., Provenzale, J.M., 2004. Diffusion tensor imaging of adult age differences in cerebral white matter: relation to response time. NeuroImage 21 (3), 1174–1181.

Manes, F., Piven, J., Vrancic, D., Nanclares, V., Plebst, C., Starkstein, S.E., 1999. An MRI study of the corpus callosum and cerebellum in mentally retarded autistic individuals. J. Neuropsychiatry Clin. Neurosci. 11 (4), 470–474.

Mathias, J.L., Bigler, E.D., Jones, N.R., Bowden, S.C., Barrett-Woodbridge, M., Brown, G.C., Taylor, D.J., 2004. Neuropsychological and information processing performance and its relationship to white matter changes following moderate and severe traumatic brain injury: a preliminary study. Appl. Neuropsychol. 11 (3), 134–152.

McAlonan, G.M., Cheung, V., Cheung, C., Suckling, J., Lam, G.Y., Tai, K.S., Yip, L., Murphy, D.G., Chua, S.E., 2005. Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. Brain 128 (Pt 2), 268–276.

Minshew, N.J., Goldstein, G., Siegel, D.J., 1997. Neuropsychologic functioning in autism: profile of a complex information processing disorder. J. Int. Neuropsychol. Soc. 3 (4), 303–316.

Mori, S., Zhang, J., Bulte, J.W., 2006. Magnetic resonance microscopy of mouse brain development. Methods Mol. Med. 124, 129–147.

Mukherjee, P., Miller, J.H., Shimony, J.S., Conturo, T.E., Lee, B.C., Almli, C.R., McKinstry, R.C., 2001. Normal brain maturation during childhood: developmental trends characterized with diffusion-tensor MR imaging. Radiology 221 (2), 349–358.

Nosarti, C., Rushe, T.M., Woodruff, P.W., Stewart, A.L., Rifkin, L., Murray, R.M., 2004. Corpus callosum size and very preterm birth: relationship to neuropsychological outcome. Brain 127 (Pt 9), 2080–2089.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9 (1), 97–113.

Papadakis, N.G., Martin, K.M., Mustafa, M.H., Wilkinson, I.D., Griffiths, P.D., Huang, C.L., Woodruff, P.W., 2002. Study of the effect of CSF suppression on white matter diffusion anisotropy mapping of healthy human brain. Magn. Reson. Med. 48 (2), 394–398.

Pandya, D.N., Seltzer, B., 1986. The topography of commissural fibers. In: Lepore, F., Ptito, F., Jasper, H. (Eds.), Two Hemispheres–One Brain: Function of the Corpus Callosum, vol. 17. Alan Liss, New York, pp. 47–73.

Partridge, S.C., Mukherjee, P., Berman, J.I., Henry, R.G., Miller, S.P., Lu, Y., Glenn, O.A., Ferriero, D.M., Barkovich, A.J., Vigneron, D.B., 2005. Tractography-based quantitation of diffusion tensor imaging parameters in white matter tracts of preterm newborns. J. Magn. Reson. Imaging 22 (4), 467–474.

Paul, L.K., Van Lancker-Sidtis, D., Schieffer, B., Dietrich, R., Brown, W.S., 2003. Communicative deficits in agenesis of the corpus callosum: nonliteral language and affective prosody. Brain Lang. 85 (2), 313–324.

Pfefferbaum, A., Sullivan, E.V., Hedehus, M., Lim, K.O., Adalsteinsson, E., Moseley, M., 2000. Age-related decline in brain white matter anisotropy measured with spatially corrected echo-planar diffusion tensor imaging. Magn. Reson. Med. 44 (2), 259–268.

Pierpaoli, C., Basser, P.J., 1996. Toward a quantitative assessment of diffusion anisotropy. Magn. Reson. Med. 36 (6), 893–906.

Piven, J., Arndt, S., Bailey, J., Andreasen, N., 1996. Regional brain enlargement in autism: a magnetic resonance imaging study. J. Am. Acad. Child Adolesc. Psych. 35 (4), 530–536.

Piven, J., Bailey, J., Ranson, B.J., Arndt, S., 1997. An MRI study of the corpus callosum in autism. Am. J. Psychiatry 154 (8), 1051–1056.

Pujol, J., Vendrell, P., Junque, C., Marti-Vilalta, J.L., Capdevila, A., 1993. When does human brain development end? Evidence of corpus callosum growth up to adulthood. Ann. Neurol. 34 (1), 71–75.

Rash, B.G., Richards, L.J., 2001. A role for cingulate pioneering axons in the development of the corpus callosum. J. Comp. Neurol. 434 (2), 147–157.

Reese, T.G., Heid, O., Weisskoff, R.M., Wedeen, V.J., 2003. Reduction of eddy-current-induced distortion in diffusion MRI using a twice-refocused spin echo. Magn. Reson. Med. 49, 177–182.

Rice, S.A., Bigler, E.D., Cleavinger, H.B., Tate, D.F., Sayer, J., McMahon, W., Ozonoff, S., Lu, J., Lainhart, J.E., 2005. Macrocephaly, corpus callosum morphology, and autism. J. Child. Neurol. 20 (1), 34–41.

Scheuffgen, K., Happe, F., Anderson, M., Frith, U., 2000. High "intelligence", "low IQ"? Speed of processing and measured IQ in children with autism. Dev. Psychopathol. 12 (1), 83–90.

Schmithorst, V.J., Wilke, M., Dardzinski, B.J., Holland, S.K., 2005. Cognitive functions correlate with white matter architecture in a normal pediatric population: a diffusion tensor MRI study. Hum. Brain Mapp. 26 (2), 139–147.

Schulte, T., Sullivan, E.V., Muller-Oehring, E.M., Adalsteinsson, E., Pfefferbaum, A., 2005. Corpus callosal microstructural integrity influences interhemispheric processing: a diffusion tensor imaging study. Cereb. Cortex 15 (9), 1384–1392.

Sled, J.G., Pike, G.B., 2000. Quantitative interpretation of magnetization transfer in spoiled gradient echo MRI sequences. J. Magn. Reson. 145 (1), 24–36.

Snook, L., Paulson, L.A., Roy, D., Phillips, L., Beaulieu, C., 2005. Diffusion tensor imaging of neurodevelopment in children and young adults. NeuroImage 26 (4), 1164–1173.

Song, S.K., Sun, S.W., Ramsbottom, M.J., Chang, C., Russell, J., Cross, A.H., 2002. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. NeuroImage 17 (3), 1429–1436.

Spencer, M.D., Gibson, R.J., Moorhead, T.W., Keston, P.M., Hoare, P., Best, J.J., Lawrie, S.M., Johnstone, E.C., 2005. Qualitative assessment of brain anomalies in adolescents with mental retardation. AJNR Am. J. Neuroradiol. 26 (10), 2691–2697.

Suzuki, K., Yamadori, A., Endo, K., Fujii, T., Ezura, M., Takahashi, A., 1998. Dissociation of letter and picture naming resulting from callosal disconnection. Neurology 51 (5), 1390–1394.

Tuch, D.S., Salat, D.H., Wisco, J.J., Zaleta, A.K., Hevelone, N.D., Rosas, H.D., 2005. Choice reaction time performance correlates with diffusion anisotropy in white matter pathways supporting visuospatial attention. Proc. Natl. Acad. Sci. U. S. A. 102 (34), 12212–12217.

Vidal, C.N., Nicolson, R., Devito, T.J., Hayashi, K.M., Geaga, J.A., Drost, D.J., Williamson, P.C., Rajakumar, N., Sui, Y., Dutton, R.A., et al., 2006. Mapping corpus callosum deficits in autism: an index of aberrant cortical connectivity. Biol. Psychiatry 60 (3), 218–225.

Waiter, G.D., Williams, J.H., Murray, A.D., Gilchrist, A., Perrett, D.I., Whiten, A., 2005. Structural white matter deficits in high-functioning individuals with autistic spectrum disorder: a voxel-based investigation. NeuroImage 24 (2), 455–461.

Wechsler, D., 1991. Wechsler Intelligence Scale for Children—Third Edition (WISC-III). The Psychological Corporation, San Antonio, TX.

Wechsler, D., 1997. Wechsler Adult Intelligence Scale—Third Edition (WAIS-III). The Psychological Corporation, San Antonio, TX.

Witelson, S.F., 1989. Hand and sex differences in the isthmus and genu of the human corpus callosum. A postmortem morphological study. Brain 112 (Pt 3), 799–835.

World Health Organization Staff, 1993. ICD-10: International Statistical Classification of Diseases and Related Health Problems: Instruction Manual, vol. 2. American Psychiatric Publishing, Incorporated.

Zaidel, E., Iacoboni, M., 2003. The Parallel Brain: The Cognitive Neuroscience of the Corpus Callosum. MIT Press, Cambridge, MA.

Zhang, Y., Brady, M., Smith, S., 2001. Segmentation of brain MR images through a hidden Markov random field model and the expectation maximization algorithm. IEEE Trans. Med. Imag. 20 (1), 45–57.