Diffusion Tensor Imaging in the Corpus Callosum in Children after Moderate to Severe Traumatic Brain Injury

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ABSTRACT

Diffusion tensor imaging (DTI) is a recent imaging technique that assesses the microstructure of the cerebral white matter (WM) based on anisotropic diffusion (i.e., water molecules move faster in parallel to nerve fibers than perpendicular to them). Fractional anisotropy (FA), which ranges from 0 to 1.0, increases with myelination of WM tracts and is sensitive to diffuse axonal injury (DAI) in adults with traumatic brain injury (TBI). However, previous DTI studies of pediatric TBI were case reports without detailed outcome measures. Using mean FA derived from DTI fiber tractography, we compared DTI findings of the corpus callosum for 16 children who were at least 1 year (mean 3.1 years) post-severe TBI and individually matched, uninjured children. Interexaminer and intraexaminer reliability in measuring FA was satisfactory. FA was significantly lower in the patients for the genu, body, and splenium of the corpus callosum. Higher FA was related to increased cognitive processing speed and faster interference resolution on an inhibition task. In the TBI patients, higher FA was related to better functional outcome as measured by the dichotomized Glasgow Outcome Scale (GOS). FA also increased as a function of the area of specific regions of the corpus callosum such as the genu and splenium, and FA in the splenium was reduced with greater volume of lesions in this region. DTI may be useful in identifying biomarkers related to DAI and outcome of TBI in children.

Key words: child; corpus callosum; diffuse axonal injury; diffusion tensor imaging; fiber tracking; morphometry; traumatic brain injury

INTRODUCTION

Traumatic brain injury (TBI) is the most frequent cause of death in children in the United States and often results in disability secondary to cognitive deficit, behavioral disturbance, and psychosocial maladjustment (Kraus et al., 1990). The Centers for Disease Control and Prevention estimates that there are approximately a half million traumatic brain injuries annually among children 0–14 years of age (Langlois et al., 2003). Since these in-
Neuropathologic and imaging studies of TBI have emphasized damage to white matter (Tomaiuolo et al., 2004; Tasker et al., 2005; Wilde et al., 2005) and have shown that TBI alters brain tissue microstructure, including a widening of extracellular space secondary to glial cell shrinkage (Rugg-Gunn et al., 2001; Goetz et al., 2004) and Wallerian degeneration which results in axonal collapse, breakdown of myelin, and ostensibly disconnection effects (Povlishock and Katz, 2005). However, an impediment in linking injury mechanisms and neuropathological changes to pediatric TBI outcomes is the insensitivity of conventional magnetic resonance imaging (MRI) to traumatic axonal injury (Rugg-Gunn et al., 2001; Arfanakis et al., 2002; Goetz et al., 2004).

Diffusion tensor imaging (DTI) is a relatively new imaging technique that may be especially useful in extending knowledge about the consequences of head injury to the white matter in vivo. DTI assesses the microstructure of the cerebral white matter, and is based on the characteristics of myelin sheaths and cell membranes of white matter tracts that restrict the movement of water molecules. Consequently, water molecules tend to move faster in parallel to nerve fibers rather than perpendicular to them. This characteristic, which is referred to as anisotropic diffusion and is measured by fractional anisotropy (FA), is determined by factors including the thickness of the myelin sheath and of the axon and axon membrane, axon packing, relative membrane permeability to water, internal axon structure, and the amount of water in the extracellular space. FA ranges from 0 to 1, where 0 represents maximal isotropic diffusion (e.g., free diffusion in perfect sphere) and 1 represents maximal anisotropic diffusion, that is, diffusion in one direction (e.g., a long cylinder of minimal diameter). Diffusion anisotropy varies across white matter regions, putatively reflecting differences in fiber myelination, fiber diameter, and directionality (Pierpaoli and Basser, 1996). Pathological processes that alter the microstructure such as loss or disorganization of fibers associated with breakdown of myelin and downstream nerve terminals (Povlishock and Katz, 2005), neuronal swelling or shrinkage, and increased or decreased extracellular space, could affect diffusion or anisotropy (Ducorre et al., 2005).

Initial studies using DTI in adult TBI patients have indicated that FA is reduced in the first week after injury despite normal-appearing white matter on MRI (Arfanakis et al., 2002; Huisman et al., 2004). Robust reductions in FA in adults following TBI have been reported for the anterior and posterior corpus callosum, posterior limb of the internal capsule, and the external capsule (Arfanakis et al., 2002; Huisman et al., 2004). Reduction in corpus callosum FA has also been reported in adults with TBI as late as 18 months postinjury (Rugg-Gunn et al., 2001; Chan et al., 2003). By contrast, changes in FA were minimal in thalamus and putamen, thus implicating white matter specificity. The validity of DTI in adult TBI is supported by positive correlation of FA in the internal capsule and splenium with the Glasgow Coma Scale (GCS) (Teasdale and Jennett, 1974) score, i.e., milder impairment of consciousness heralded less severe injury to the white matter (Huisman et al., 2004). In contrast, low FA was related to worse outcome upon hospital discharge (Huisman et al., 2004). Fiber tractography, a technique of DTI to study three-dimensional white matter connectivity among brain regions, has been used recently to elucidate traumatic axonal injury (Naganawa et al., 2004; Le et al., 2005) and white matter maturation (Glenn et al., 2003). However, investigation of DTI in pediatric TBI is limited to small samples of patients or case reports (Lee et al., 2003) without detailed assessment of outcome. In summary, while a few clinical studies of adult TBI patients provide preliminary support for the potential use of DTI as a biomarker of traumatic white matter injury, little is known regarding diffusivity changes over the course of recovery from TBI in children or the relation of FA to cognitive and functional outcome.

The largest white matter tract, the corpus callosum, has long been considered especially vulnerable to TBI (Adams et al., 1980; Gorrie et al., 2001) due to its unique location and composition. Focal lesions produced by shear-strain mechanisms are particularly common in the splenium (Gennarelli et al., 1982; Gentry et al., 1988; Mendelsohn et al., 1992a), and long-term diffuse atrophy of the corpus callosum following TBI has been well-documented in both adults (Levin et al., 1990; Anderson and Bigler, 1994; Gale et al., 1995) and children (Benavidez et al., 1999; Levin et al., 2000) using MRI. Indeed, some imaging studies have used atrophy of the corpus callosum as a surrogate measure of overall white matter atrophy (Gentry et al., 1988; Takaoka et al., 2002; Mathias et al., 2004; Tomaiuolo et al., 2004).

The location, conspicuity, and composition of the corpus callosum and its vulnerability to TBI are conducive to examination by DTI with fiber tracking, an emerging technique which may provide valuable insights into white matter pathology (Inglese et al., 2005). First, the size and central location of the corpus callosum reduce the possibility of susceptibility artifacts that have caused concern in DTI (Hasan et al., 2005). As the most discernible structure of the brain on DTI, the corpus callosum’s fiber bun-
Corpus callosum injury is a concern because of this structure’s role in the interhemispheric transfer of auditory, visual, sensory and motor information relevant to multiple cognitive processes (Benavidez et al., 1999; Mathias et al., 2004; Pollmann et al., 2004; Schulte et al., 2005). Following severe TBI, children exhibit disconnection effects on tasks involving interhemispheric transfer of information, thus implicating compromise of the corpus callosum (Benavidez et al., 1999). Additionally, myelinated fibers throughout the brain, such as those which primarily compose the corpus callosum are considered to play a central role in basic processing speed and reaction time.

In this report, we performed DTI with fiber tracking to characterize long-term changes in the FA of corpus callosum fiber systems following moderate to severe TBI in children relative to a demographically matched, typically developing comparison group. In addition, we examined the relation between mean FA in fiber systems coursing through the corpus callosum and outcome measures, including cognitive processing speed and the Glasgow Outcome Scale (GOS) (Jennett and Bond, 1975) score. We also investigated the relation between white matter area obtained by established volumetric methods and FA utilizing DTI, for identical ROIs. Finally, we examined the relation between lesion volume and FA in the posterior aspect of the corpus callosum using DTI.

METHODS

Subjects

Sixteen children (eight boys, eight girls) aged 9–16 years (mean = 12.9 ± 2.5) who had sustained closed head trauma due to motor vehicle-related or pedestrian-motor vehicle injuries at least 1 year prior to imaging comprised the TBI group. Postresuscitation GCS (Teasdale and Jennett, 1974) scores recorded in the emergency center ranged from 3 to 11, with a mean of 5.7 ± 2.8. Eligibility criteria for TBI patients included a score less than 4 on an Abbreviated Injury Scale (AIS) (Committee on Injury Scaling, 1998) for areas of the body other than the head and absence of post-resuscitation hypoxia or hypotension exceeding 30 min in duration. The comparison group comprised 16 typically developing children recruited from the local community and selected to individually match the TBI patients on age (within 6 months), gender, handedness, and maternal education. All children included in the study were English-speaking and had no pre-existing head injury, neurologic disorder associated with cerebral dysfunction and/or cognitive deficit (e.g., cerebral palsy, mental retardation, epilepsy), diagnosed learning disability, psychiatric disorder, or history of child abuse. Demographic and injury-related characteristics for each pair—including age at testing and scanning, ethnicity, gender, handedness, maternal education, age at injury, time post-injury, and injury severity as measured by GCS and GOS scores—appear in Table 1.

Magnetic Resonance Imaging Acquisition

All subjects underwent MRI without sedation on Philips 1.5-T Intera scanners (Philips, Best, The Netherlands) at either Texas Children’s Hospital, Houston, or at the Rogers MRI Center, University of Texas Southwestern Medical Center, Dallas, using identical protocols.

Area analysis. Three-dimensional (3D) fast field echo (FFE) T1-weighted (T1-w; 15 msec TR, 4.6 msec TE, 10-degree flip angle, 1.0-mm slices) and 3D turbo spin echo (TSE) T2-weighted (T2-w; 3500 msec TR, 114 msec TE, 1.5-mm slices) sagittal acquisition series were used. A 256-mm field of view (FOV) was used for these series with a reconstructed voxel size of 1 × 1 × 1 mm for the T1-w images and a reconstructed voxel size of 1 × 1 × 1.5 mm for the T2-w series.

Lesion analysis. A coronal T2-w fluid-attenuated inversion recovery (FLAIR) sequence was used (1100 msec TR, 140 msec TE, 5.0-mm slices). For this sequence, a 220-mm FOV was used with a reconstructed voxel size of 0.86 × 0.86 × 5.0 mm.

DTI fiber tracking analysis. Transverse multislice spin echo, single shot, echo planar imaging (EPI) sequences were used (10,150.5 msec TR, 90 msec TE, 2.7-mm slices, 0-mm gap). A 256 FOV (RFOV = 100%) was used with a measured voxel size of 2.67 × 2.69 × 2.70 mm. Diffusion was measured along 15 directions (number of b-value = 2, low b-value = 0, and high b-value = 860 sec/mm²). To improve the SNR, each high b-value im-
age was acquired twice and averaged, but the low b image was only acquired once. The acquisition time was approximately 5:45 min, and 55 slices were acquired.

**Post-Processing**

**Area analysis.** Each data set was imported into Analyze 6.0 (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN), converted into a 3D volume, and re-sliced to create 1-mm isovoxels. T1-w and T2-w image sets were co-registered after realignment to the anterior to posterior commissure (AC-PC) line and the interhemispheric fissure. Next, interactive manual segmentation of gray matter, white matter, and cerebrospinal fluid was performed using the Analyze multispectral tool, using both the T1- and the T2-w images as previously described (Blatter et al., 1995). Manual tracing of the corpus callosum ROIs was performed with Analyze trace and editing tools on the classified image, using T1- and T2-w images to cross-check boundaries as necessary. Finally, areas (cm²) for each ROI were calculated by summing pixels designated as WM within the ROI. Generally, lesions within the corpus callosum were small enough (less than 0.5 cc in total volume across all slices).

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**Table 1. Demographic and Injury Characteristics of TBI and Typically Developing Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Pair</th>
<th>Gender</th>
<th>Hand</th>
<th>Race</th>
<th>Age at test</th>
<th>Maternal education</th>
<th>Time since injury</th>
<th>Age at injury</th>
<th>GCS</th>
<th>GOS</th>
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<tbody>
<tr>
<td>Control 1</td>
<td>F</td>
<td>R</td>
<td>H</td>
<td>8.99</td>
<td>12</td>
<td>1.04</td>
<td>8.01</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>R</td>
<td>C</td>
<td>9.80</td>
<td>13</td>
<td>3.01</td>
<td>6.89</td>
<td>8</td>
<td>1</td>
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</tr>
<tr>
<td>3</td>
<td>F</td>
<td>R</td>
<td>H</td>
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<td>3.90</td>
<td>6.03</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>R</td>
<td>C</td>
<td>11.38</td>
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<td>2.72</td>
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<tr>
<td>5</td>
<td>F</td>
<td>R</td>
<td>H</td>
<td>11.52</td>
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<td>0.96</td>
<td>10.07</td>
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<td>3</td>
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<td>6</td>
<td>F</td>
<td>R</td>
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<td>R</td>
<td>C</td>
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<td>12.17</td>
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<td>R</td>
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<td>0.98</td>
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<td>16</td>
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<td>16</td>
<td>3.03</td>
<td>13.76</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**a**Age at test, maternal education, age at injury, and time since injury are represented in years.

GCS, Glasgow Coma Scale Score; GOS, Glasgow Outcome Scale score; TBI, traumatic brain injury. For gender, F = female, M = male. For handedness, R = right-handed, L = left-handed. For race, H = Hispanic, C = Caucasian, AA = African American, A = Asian. For GOS, 1 = good recovery, 2 = moderate disability, 3 = severe disability.
that they remained classified as white matter within the corpus callosum rather than as cerebrospinal fluid (CSF)–filled spaces on the slice used for area measurement. In addition to being small, the entire lesion volume and some corpus callosum lesions were not evident on the midsagittal image (e.g., some lesions were lateral to the midsagittal image); therefore, these lesions had minimal impact upon corpus callosum area measurement. Rather, decreased area appeared to be related primarily to generalized, atrophic changes in the corpus callosum.

Lesion analysis. Areas of signal abnormality were identified and traced by a board-certified neuroradiologist (J.V.H.) using FLAIR imaging as previously described (Wilde et al., 2005). All corpus callosum lesions visible on MR were within the posterior aspect (posterior body and splenium). Four of the 16 children with TBI had corpus callosal lesions apparent on FLAIR imaging; lesion volumes ranged from 0.211 to 0.569 cm³ (mean = 0.097 ± 0.186). (All 16 TBI patients had extracallosal lesions, and no lesions were identified in the typically developing children.)

DTI analysis. Before calculating FA maps, the Philips PRIDE-registration tool (Netsch, 2001) was used to remove shear and eddy current distortion and head motion. No ghosting artifact was observed in the b > 0 images, and b = 0 images had approximately 6% ghosting artifact. Philips fiber tracking 4.1V3 Beta 2 software was used to calculate FA maps. ROIs were drawn manually on the mid-sagittal image using the protocol described below. After drawing the ROIs, automated Philips 3D fiber tracking tool was utilized to determine fiber tracks passing through ROIs in the corpus callosum; mean FA of the fiber systems identified using these fiber tracking results was used as the quantitative measure for all DTI variables. For example, Figure 1 illustrates the fiber system that emerged when the total corpus callosum was used as an ROI. The algorithm for fiber tracking is based upon the fiber assignment by continuous tracking (FACT) method (Mori et al., 1999). Tracking terminated if the FA in the voxels decreased below 0.2 or if the angle between adjacent voxels along the track was larger than 41.4 degrees. Philips software generates an automatic mean FA for each of the ROIs.

Corpus Callosum Regions of Interest

In delineating ROIs in both area measures and DTI fiber tracking regions originating from the corpus callosum, we followed a well-established protocol with seven subdivisions of the corpus callosum (Witelson, 1989). The ROIs delineated in this protocol have been successfully applied to both volumetric (Benavidez et al., 1999; Levin et al., 2000; Rice et al., 2005) and DTI studies (Sackheim et al., 1990; Hasan et al., 2005) of the corpus callosum in other patient populations. ROIs for the area analysis were drawn on segmented images derived from midsagittal T1- and T2-w images, using the T1- and T2-w images to confirm boundaries. ROIs for the DTI data were drawn on FA color maps in the sagittal plane, using co-registered b = 0 images to cross check boundaries as necessary. To reduce the number of comparisons in this relatively small sample, but at the same time to maintain some of the known differences in fiber structure in different parts of the corpus callosum, we combined areas of the original Witelson protocol to create three simplified regions. Areas 1–2 were combined for the genu, and areas 4–5 were combined for the body of the corpus callosum. Area 7 was used for the splenium region of the corpus callosum (Fig. 2).

Interoperator Reliability

A single rater performed area analysis of the corpus callosum, but had previously demonstrated satisfactory intrarater reliability (>0.90) with other raters in the laboratory on this protocol. For DTI analysis, two raters supervised by the project neuroradiologist (J.V.H.) independently measured FA four times for each ROI in a subset of three TBI patients and three uninjured children. Intra- and inter-rater reliabilities were calculated using Shrout-Fleiss reliability statistics to obtain intraclass correlation coefficients (ICCs), which were all above 0.90 (range, 0.901–0.999; mean = 0.968).

Flanker Task

A variation of the Eriksen Flanker Task (Eriksen and Eriksen, 1974; Bunge et al., 2002) was selected to measure effects relating to reaction time and resistance to visual interference. In this paradigm, a competing response to irrelevant stimuli presented adjacent to a target must be inhibited. The general findings for this task are that reaction times are slower in the presence of irrelevant, but conflicting stimuli, as compared to neutral (baseline) stimuli.

In our version of the flanker task, each child was told that a central arrow pointing to the left or to the right would appear with other symbols next to it, and the goal was to press the key on the side of the keyboard corresponding to the direction of the central arrow as quickly and accurately as possible except when the central arrow had an X next to it, in which case no key was to be pressed. There were 112 randomized trials, comprising 28 trials of four conditions. In the present study, we utilized reaction time under the interference condition (in
which the two flanker arrows pointed in the direction opposite to the central arrow) and the baseline condition (in which a central arrow was flanked by two dashes). The dependent variables analyzed in relation to the DTI findings were baseline reaction time and interference, which we calculated as the difference between reaction time under the interference condition and the baseline reaction time. We selected these two variables because our previous work indicated susceptibility to interference on the flanker task is increased following severe TBI in children (Levin et al., 2004), the relevance of these two variables to the current investigation (Hillary et al., 2006), and an attempt to limit the number of statistical analyses in this small sample. For this study, we subtracted the reaction time in the baseline condition from the reaction time in the interference condition to isolate the effect of interference from simple cognitive slowing.

Assessment of Outcome

The GOS (Jennett and Bond, 1975), modified for pediatric use (Wilde et al., 2005), was utilized to assess functional outcome. The GOS is a five-point categorical scale scored as (1) good recovery, (2) moderate disability, (3) severe disability, (4) persistent vegetative state, and (5) death. The GOS was graded after a mean post-injury interval of 830.75 days (SD = 688.53 days; range, 151–2845 days) by an experienced examiner based on in-
formation obtained in an interview with the primary caregiver, available cognitive test data in relation to estimated pre-injury functioning, and neurologic sequelae. Examiners grading the GOS had no knowledge of the brain imaging findings. Since a GOS score of 3 or less was a prerequisite for children to provide assent to participate and undergo cognitive testing, we dichotomized GOS scores into two categories: (a) good recovery and (b) moderate/severe disability.

Statistical Analysis

Simple t-tests were used to examine group differences on area measurement for the three corpus callosum ROIs as well as performance on cognitive variables. Then a general linear model analysis approach was used to examine group differences in mean FA in the total corpus callosum as well as the genu, body, and splenium; the relation of mean FA in these regions to performance on the flanker task (baseline reaction time and resistance to interference); and the relation of mean FA to area measurement of the same ROIs. Critical assumptions of general linear model analysis were examined, and no violations were noted. Age was included as a covariate in the model when significant (e.g., for performance on flanker task variables). Simple correlations were used to examine the relation between mean FA and lesion volume in the posterior aspect of the corpus callosum of the TBI group. The analysis of lesions was confined to the posterior portions of the corpus callosum because there were no anterior lesions present in this sample of patients. To further examine the impact of corpus callosum lesions on regional FA, we examined differences between children with corpus callosum lesions versus those without lesions using additional t-tests. Finally, we utilized a multinomial logistic regression model to examine whether mean FA could predict outcome as measured by GOS score. We performed this analysis first with all children in the TBI group and then using only children without focal injury in the corpus callosum. In these analyses, we used the interval between injury and administration of the GOS as a covariate. We then also used a nonparametric, Wilcoxon one-tailed test to further examine TBI patients with good recovery on the GOS versus patients with moderate or severe disability.

RESULTS

Demographic Characteristics

The TBI group and the typically developing children did not significantly differ in gender, age or maternal education. However, the groups differed in race/ethnicity (Fisher’s exact p = 0.02), with equal numbers of Caucasian children in both groups, but a higher number of Hispanic and Asian children in the typically developing group, and a higher number of African-American children in the TBI group.

Group Differences in Diffusion Tensor Imaging of the Corpus Callosum

General linear model analyses revealed significant group differences in mean FA for fibers systems emanating from the genu (F(1,29) = 10.36, p = 0.003), body (F(1,29) = 43.11, p < 0.001), splenium (F(1,29) = 26.20, p < 0.001), and total corpus callosum (F(1,29) = 33.52, p = 0.003). (Corresponding means and standard deviations by region and group are presented in Table 2.)

Relation between Processing Speed (Baseline Reaction Time) and Interference and Diffusion Tensor Imaging in the Corpus Callosum

Differences on cognitive measures between the TBI and typically developing groups were examined using t-tests.

<table>
<thead>
<tr>
<th>Corpus callosum region</th>
<th>TBI group</th>
<th>TD group</th>
<th>p-value</th>
<th>TBI group</th>
<th>TD group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genu (areas 1–2)</td>
<td>0.504 (0.036)</td>
<td>0.533 (0.013)</td>
<td>0.0063</td>
<td>1.995 (0.264)</td>
<td>2.344 (0.261)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Body (areas 4–5)</td>
<td>0.472 (0.025)</td>
<td>0.522 (0.015)</td>
<td>&lt;0.0001</td>
<td>1.313 (0.217)</td>
<td>1.446 (0.223)</td>
<td>0.0970</td>
</tr>
<tr>
<td>Splenium (area 7)</td>
<td>0.558 (0.029)</td>
<td>0.602 (0.017)</td>
<td>&lt;0.0001</td>
<td>1.413 (0.317)</td>
<td>1.770 (0.299)</td>
<td>0.0026</td>
</tr>
<tr>
<td>Total (areas 1–7)</td>
<td>0.514 (0.018)</td>
<td>0.547 (0.012)</td>
<td>&lt;0.0001</td>
<td>5.291 (0.738)</td>
<td>6.269 (0.819)</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

*For delineation of areas included in the regions of interest, see Figure 2.

The p-values are derived from t-tests to examine group differences on the variables listed (degrees of freedom for each = 30).

FA, fractional anisotropy; SD, standard deviation; TBI, traumatic brain injury; TD, typically developing.
There was a marginally significant group difference on baseline reaction time ($t(30) = -1.93, p = 0.063$) and a significant difference between the groups on the interference variable ($t(30) = 2.46, p = 0.020$), with the TBI group demonstrating the expected longer reaction times.

On a measure of basic processing speed (flanker task baseline reaction time), general linear model analyses revealed that this variable was significantly related to mean FA in fibers passing through the splenium of the corpus callosum ($F(1,28) = 4.51, p = 0.043$, Cohen’s $f^2 = 0.22$: moderate-to-large effect size), and marginally related to mean FA in fibers passing through the corpus callosum body ($F(1,28) = 3.82, p = 0.061$, Cohen’s $f^2 = 0.23$: moderate-to-large effect size). Higher FA was related to decreased reaction time, reflecting more efficient processing.

There were no group interaction effects, indicating a similar relationship between mean FA and reaction time in both groups (Fig. 3). (However, we acknowledge the presence of an outlier in the TBI group whose FA fell below 0.48 and whose baseline reaction time was longer than 1000 msec.) Mean FA in fibers passing through the corpus callosum genu was not related to basic processing speed.

To examine the contribution of lesions in the splenium of the corpus callosum to the relationship with flanker baseline reaction time, we excluded the four children with lesions and the matched typically developing children (Table 3). This repeat analysis of the relation between baseline reaction time and FA in the splenium reduced the significance of this effect ($F(1,20) = 2.80, p = 0.1096$, Cohen’s $f^2 = 0.14$: moderate effect size).

Analyses of a reaction time measure of interference (flanker interference reaction time minus baseline reaction time) revealed a marginally significant relation to mean FA in fibers passing through the corpus callosum body ($F(1,28) = 3.82, p = 0.060$, Cohen’s $f^2 = 0.22$: moderate-to-large effect size), again with higher FA being associated with reduced susceptibility to interference as reflected by decreased reaction times. Again, no group interaction was evident, indicating a similar pattern of results for both TBI and typically developing children. Mean FA in fibers passing through the genu and splenium was not related to susceptibility to interference.

**Relation between Global Outcome and Diffusion Tensor Imaging in the Corpus Callosum**

Logistic regression demonstrated a significant relation between increased mean FA in the total corpus callosum and the increased probability of good recovery ($\chi^2(1) = 5.34, p = 0.021$) and decreased probability of poor outcome (moderate or severe disability) on the GOS as shown in Figure 4. Comparison of raw means indicated that typically developing children had higher FA than TBI patients with good recovery as measured by GOS, and that TBI patients with good recovery had higher FA than TBI patients with a GOS classification of moderate or severe disability. Using a nonparametric Wilcoxon one-tailed test, results showed that typically developing children had higher FA than TBI patients with good recovery as measured by GOS.

**Table 3. Mean FA for TBI Children with and without Focal Lesions in the Splenium of the Corpus Callosum**

<table>
<thead>
<tr>
<th>Corpus callosum region</th>
<th>Lesion group ($n = 4$)</th>
<th>No lesion group ($n = 12$)</th>
<th>$t$-statistic</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genu</td>
<td>0.488 (0.029)</td>
<td>0.051 (0.038)</td>
<td>−0.93$^a$</td>
<td>0.366</td>
</tr>
<tr>
<td>Body</td>
<td>0.466 (0.036)</td>
<td>0.477 (0.022)</td>
<td>−0.76$^b$</td>
<td>0.460</td>
</tr>
<tr>
<td>Splenium</td>
<td>0.518 (0.031)</td>
<td>0.571 (0.013)</td>
<td>−3.34$^b$</td>
<td>0.038</td>
</tr>
<tr>
<td>Total</td>
<td>0.493 (0.011)</td>
<td>0.522 (0.015)</td>
<td>−3.56$^b$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$Pooled $t$-test for equal variances

$^b$Satterthwaite $t$-test for unequal variance

Standard deviations are listed in parentheses following the means.

FA, fractional anisotropy; TBI, traumatic brain injury.
FIG. 4. Probability curve reflecting the percentage chance of obtaining either a favorable GOS outcome (GOS of 1 = good recovery) or poor outcome (GOS of 2 = moderate disability; GOS of 3 = severe disability) based upon mean FA for a fiber system emanating from the total corpus callosum.

Children had significantly higher FA in the total corpus callosum ($p = 0.019$) than TBI patients with good recovery. However, TBI patients with good recovery did not significantly differ from patients with moderate or severe disability on mean FA in the total corpus callosum.

To examine whether the relation between mean FA and GOS was impacted by focal injury in the corpus callosum, we repeated the multinomial logistic regression analysis after excluding children with corpus callosum lesions. The result of this analysis indicated that the relation was no longer significant ($\chi^2(1) = 2.28$, $p = 0.131$); however, the effect size was large ($w = 0.513$), indicating that a significant result would be likely with a larger sample size.

**Relation between Fractional Anisotropy and White Matter Area in Corpus Callosum Regions of Interest**

Groups significantly differed on mean area for the genu ($t(1,30) = 3.77$, $p = 0.001$) and splenium ($t(1,30) = 3.28$, $p = 0.003$) ROIs using simple t-tests, and a trend was noted for group differences on the body ROI ($t(1,30) = 1.71$, $p = 0.097$). General linear model analyses revealed a near significant ($F(1,28) = 3.17$, $p = 0.085$; Cohen’s $f^2 = 0.11$; moderate effect size) group by genu area interaction whereby the area of the corpus callosum genu and the mean FA of fibers passing through this region were related in the TBI children ($t(1,28) = 2.11$, $p = 0.044$), with greater genu area related to higher mean FA. A near-significant ($F(1,28) = 3.88$, $p = 0.059$; Cohen’s $f^2 = 0.14$; moderate effect size) group by splenium interaction was found such that splenium area and mean FA of the fibers passing through the splenium were also positively related in the children with TBI ($t(1,28) = 2.61$, $p = 0.014$). Although both the mean FA and the area of the body of the corpus callosum were greater in the typically developing children, there was no significant relation between the two variables ($F(1,28) = 1.31$, $p = 0.263$) using this model.

**Relation between Lesion Volumes and Diffusion Tensor Imaging**

For the splenium of the corpus callosum, we found a strong negative correlation between lesion volume and mean FA in the TBI group ($r = -0.903$, $p = <0.0001$), indicating that larger lesion volumes were associated with decreased mean FA. This exploratory analysis, which was based on only four patients, was limited to the splenium because of the absence of lesions in more anterior regions of the corpus callosum. Figure 5 illustrates the loss of fiber tracking in the splenium of a child with a lesion in that area.

Examination of children in the TBI group with lesions in the splenium of the corpus callosum versus those children in the TBI group without a splenial lesion revealed a significant difference ($t(3) = -3.34$, $p = 0.038$; Satterthwaite $t$-test), where the children with lesions had significantly decreased FA in the splenium region on DTI. However, these two groups did not significantly differ for FA in the corpus callosum genu and body. Table 2 presents mean FA and standard deviations for these regions for each group.

**DISCUSSION**

Consistent with previous DTI studies reporting decreased anisotropy in the corpus callosum of adult TBI patients (Chan et al., 2003; Huisman et al., 2004), we found that mean FA in corpus callosum fiber systems was lower in children imaged at least 1 year after sustaining moderate to severe TBI relative to typically developing children matched on several demographic variables. Our results also confirm previous case reports suggesting decreased FA in children with TBI (Lee et al., 2003), and extend these findings to a larger cohort of pediatric patients as compared to DTI data obtained from typically developing children. In addition, we demonstrate that mean FA derived from DTI with fiber tracking is related to midsagittal area and lesion volume, two well-established imaging indices of injury, in children with TBI. Finally, our study is the first to examine the relation between FA in the corpus callosum in chronic pediatric survivors of TBI and indicators of clinical recovery including the GOS score and measures of cognitive processing speed and susceptibility to interference.
Group Differences in Diffusion Tensor Imaging

Based on the neuropathology of TBI in children (Povlishock and Katz, 2005), DAI is the most parsimonious mechanism to explain the group differences in DTI findings. This view is consistent with the high velocity mechanism of injury such as motor vehicle crashes in all of our TBI patients (Adams et al., 1982). Despite the presence of focal lesions in the splenium of 25% of children with TBI, which could have contributed to decreased FA, we note that every TBI patient exhibited decreased mean FA in the posterior regions of the corpus callosum in relation to his or her matched control, regardless of the presence or location of specific callosal or extra-callosal focal injury. This pattern of reduced FA in TBI subjects was also present in anterior regions of the corpus callosum despite the absence of focal lesions. These findings further support the likelihood of diffuse injury mechanisms affecting the microstructural integrity of these corpus callosum fibers. In the early phases following injury, reductions in anisotropy in DAI have been attributed to misalignment of the cytoskeletal network or axonal membranes, and then later impairment of axoplasmic transport and local accumulation of organelles, causing local expansion of the axonal cylinder (Arfanakis et al., 2002). Later changes in anisotropy may be related to disconnection and subsequent death of the distal and proximal neuron segments leading to membrane degeneration or an increase in permeability in injured axons, both of which may lead to increased diffusivity (reduced anisotropy) in directions perpendicular to the axons (Christman et al., 1994; Pettus et al., 1994; Povlishock and Christman, 1995; Gennarelli, 1997).

Cognitive Functioning and Outcome in Relation to Diffusion Tensor Imaging

Developmental cognitive research has shown that cognitive processing speed increases with age in children (Kail, 1993; Fry and Hale, 1996), a finding that has been attributed to myelination and pruning of synapses (Gogtay et al., 2004). Consequently, it is plausible that reduced FA reflecting loss and possibly reduced growth of white matter is the mechanism mediating the slowed processing speed and greater susceptibility to interference on the flanker task observed in our patients.

While the groups differed in FA throughout the corpus callosum, this difference did not appear to differentially influence performance on the flanker task. In both groups, posterior regions of the corpus callosum showed the strongest relation of mean FA to basic reaction time in our study. One possibility for the larger effect in the more posterior regions may be related to the known heterogeneity in fiber distribution within the corpus callosum (Witelson, 1989; Aboitiz et al., 1992). Consistent with other studies (Chepuri et al., 2002; Madden et al., 2004), we observed higher FA in the splenium than in other regions of the corpus callosum, which has been attributed to several possible variables including a larger axon diameter, tighter packing of axons, less permeable myelin sheaths, and fewer obliquely oriented axons in the splenium (Chepuri et al., 2002), factors which could im-

FIG. 5. (A) Focal lesion in the splenium of the corpus callosum for a 10.8-year-old boy with TBI on conventional T1-weighted MRI midsagittal image. (B) Fiber systems projecting from the corpus callosum using DTI with fiber tracking. Note the absence of identifiable fiber tracks in the posterior regions corresponding to the focal lesion evident on the midsagittal T1 slice as well as other more lateral intercallosal posterior body abnormalities visible on conventional imaging (not shown here). (C) Corpus callosum fiber system using DTI with fiber tracking of a demographically matched, uninjured child. Interestingly, the TBI patient had no focal lesions or obvious white matter atrophy in the frontal, temporal, or anterior parietal regions on conventional imaging, which also corresponds to the normal appearing fiber tracking of regions coursing through the anterior and mid regions of the corpus callosum.
impacts the reliability and accuracy of FA calculation in DTI with fiber tracking. In addition, we note that the flanker task involves processing visual information, and the splenium has known projections to the occipital areas of the brain prerequisite for performance on visually based tasks. We did observe the relationship between splenium FA and reaction time in both the TBI group and the typically developing children. Although we acknowledge that the presence of an outlier with extremely low FA in the TBI group was contributory to the relation between splenium FA and basic processing time (Fig. 3), our finding is consistent with a previous report using DTI in young healthy adults which also revealed the corpus callosum splenium to be a better predictor than the genu of reaction time on a visual target detection test with an attentional component (Madden et al., 2004). In a second study, increased FA in the splenium, rather than the genu of the corpus callosum, was again associated with decreased reaction times on an object recognition task (Baird et al., 2005).

The corpus callosum body FA (Fig. 2, regions 4 and 5) was marginally related to resistance to interference in our sample. In one study, patients with posterior body corpus callosum lesions demonstrated decreased performance on a visual target detection task (Pollmann et al., 2004). The authors concluded that disruption in commissural pathways between the temporoparietal junction areas played a role in the performance of these patients. Our DTI fiber tracking maps of the corpus callosum body ROI consistently indicated pathways between its posterior aspect and the temporoparietal junction areas, which have also been implicated in visual search and attention.

Callosal body projections on our DTI fiber tracking maps are also consistent with areas reported to activate in fMRI studies during the interference condition of the flanker task, including frontal, cingulate, and parietal areas. Using a flanker task to measure resistance to interference, Hazeltine et al. (2000) reported activation in right inferior prefrontal cortex, left supplementary motor cortex, and left inferior parietal cortex. Bunge et al. (2002), whose task we replicated, found activation in children and adults in ventrolateral prefrontal cortex and inferior parietal lobule. Mean FA in the genu of the corpus callosum, which projects to the ventrolateral prefrontal cortex, had no significant relation to task performance in our preliminary study. However, the activation noted by Bunge et al. (2002) in the parietal region is consistent with our finding of a relation between improved performance on the task and increased FA in the corpus callosum body and its projections to the parietal lobes.

Our finding that mean FA in the total corpus callosum was predictive of functional outcome as measured by the GOS is consistent with previous studies demonstrating that total corpus callosum volume was related to GOS-defined outcome of TBI in children (Levin et al., 2000) and adults (Takaoka et al., 2002) with TBI. In a subsequent analysis of TBI subjects classified with good recovery versus those with moderate to severe disability, we did not find a significant difference in total corpus callosum FA between these groups, possibly due to our limited sample size. However, examination of raw means reflects a consistent pattern whereby typically developing children had higher total corpus callosum mean FA than TBI subjects with good recovery, and TBI subjects with good recovery had higher mean FA than subjects with moderate to severe disability.

Relation of Diffusion Tensor Imaging to Volumetric Measures and Lesion Volumes

The posterior aspect of the corpus callosum has been identified on conventional and high-field-strength T2*-weighted MRI as a site with a predilection for shear injuries (Gentry et al., 1988; Mendelsohn et al., 1992b; Scheid et al., 2003), and consistent with this, the only visible shear lesions in the corpus callosum of our TBI patients on conventional MRI were located in the posterior aspect (posterior body and splenium). This pattern of lesion location is consistent with previous reports indicating that callosal lesions in the genu are comparatively less common (Gentry et al., 1988; Benavidez et al., 1999). Four of our children with TBI (25% of the TBI sample) had notable lesions in the splenium, and this is also generally consistent with the previously reported incidence of callosal lesions associated with severe TBI in adults (Gentry et al., 1988; Takaoka et al., 2002). In addition to mechanical injury forces being greater in the splenium due to its proximity to the falx, histological markers for axonal damage such as amyloid precursor protein accumulation in the corpus callosum have also revealed a rostro-caudal profile, with highest accumulations in the splenium (Leclercq et al., 2000). In this study, the FA in all three corpus callosum ROIs significantly differed between groups, with the TBI group consistently exhibiting decreased EA relative to TD children (for group means, see Table 2). The area measures were also significantly different between groups for the genu (t(30) = 3.77, p = 0.0007) and splenium (t(30) = 3.28, p = 0.003) (for group means, see Table 2). Levin et al. (2000) found similar results in a previous report of area corpus callosum measures in children with TBI using the same protocol where genu and splenium were the most affected regions of the corpus callosum in children with TBI. Therefore, it is not surprising that these two areas also demonstrated the strongest relationship between mean FA and area measurement for the TBI.

WILDE ET AL.
children. To our knowledge, one previous study in healthy adults has compared DTI and area measurement in three corpus callosum ROIs (Westerhausen et al., 2004). This study reported the absence of a significant correlation between relative anisotropy (RA) and an index of mean diffusion (MD) and area in the anterior and posterior ROIs for the overall group, but did find significant or near-significant associations between posterior RA and posterior area in a subgroup analysis. The relationship between these two measures may have been more evident in TBI children due to the increased range in area measures and mean FA. Histological studies have found that fiber density is related to area in the corpus callosum (Aboitiz et al., 1992), and our findings suggest that decreased FA may also be related to decreased callosal area, a surrogate for fiber density.

Limitations and Future Directions

This initial report was limited to the corpus callosum given its vulnerability for TBI-related diffuse axonal injury, its central role in interhemispheric transfer of information and preprocessing speed, and characteristics that enhance its suitability for DTI analysis (Basser and Pajevic, 2000). However, the effects of white matter injury are clearly not restricted to this region, and an investigation is underway to examine FA in other prominent regions of the brain using DTI in a larger, longitudinal study with less heterogeneity in age, age at injury, and post-injury interval. In addition, the relation of DTI in the corpus callosum to other injury parameters and cognitive domains more specific to the structure may be examined in future studies. Caution is advised in the interpretation of these initial findings given the small sample of patients who were heterogeneous in chronicity of TBI and age at injury. Assets of our study include an age-matched comparison group, analysis of established morphometric MRI measures in relation to FA for the same ROIs, and assessment of functional and cognitive measures in relation to FA.

DTI remains an evolving technology where artifacts and potential limitations must be recognized and carefully addressed (Basser and Pajevic, 2000; Le Bihan et al., 2001; Mori et al., 2002; Mori and van Zijl, 2002; Masutani et al., 2003; Pfefferbaum et al., 2003; Wakana et al., 2004). In this study, we attempted to address known artifacts in DTI (i.e., eddy current, ghosting, and susceptibility), and utilize imaging parameters (e.g., near-isotropic voxels) and ROIs that would optimize analysis. We also demonstrated the reliability of our DTI methodology by consistently following an established protocol to delineate the ROIs. Clearly, moderate to severe TBI during childhood results in persistent, deleterious effects on white matter integrity which may be better understood with evolving imaging techniques such as DTI. Despite its limitations, DTI may become a tool that elucidates the effects of TBI on the microstructure of the brain, and it may have future applications as a biomarker for injury severity and a prognostic tool (Huisman et al., 2004; Le et al., 2005), particularly with respect to DAI.

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