Abstract: Mild traumatic brain injury (TBI) is a common source of morbidity from the wars in Iraq and Afghanistan. With no overt lesions on structural MRI, diagnosis of chronic mild TBI in military veterans relies on obtaining an accurate history and assessment of behavioral symptoms that are also associated with frequent comorbid disorders, particularly posttraumatic stress disorder (PTSD) and depression. Military veterans from Iraq and Afghanistan with mild TBI (n=30) with comorbid PTSD and depression and non-TBI participants from primary (n=42) and confirmatory (n=28) control groups were assessed with high angular resolution diffusion imaging (HARDI). White matter-specific registration followed by whole-brain voxelwise analysis of crossing fibers provided separate partial volume fractions reflecting the integrity of primary fibers and secondary (crossing) fibers. Loss of white matter integrity in primary fibers (P<0.05; corrected) was associated with chronic mild TBI in a widely distributed pattern of major fiber bundles and smaller peripheral tracts including the corpus callosum (genu, body, and splenium), forceps minor, forceps major, superior and posterior corona radiata, internal capsule, superior longitudinal fasciculus, and others. Distributed loss of white matter integrity correlated with duration of loss of consciousness and most notably with "feeling dazed or confused," but not diagnosis of PTSD or depressive symptoms. This widespread spatial extent of white matter damage has typically been reported in moderate to severe TBI. The diffuse loss of white matter integrity appears consistent with systemic mechanisms of damage shared by blast- and
INTRODUCTION

Deployment-related head trauma is a signature injury of the wars in Iraq and Afghanistan and constitutes a major health concern in military personnel and veterans [Hoge et al., 2008]. Chronic mild traumatic brain injury (TBI) does not present with overt lesions on structural MRI. Diagnosis has relied upon accurate history taking and assessing a constellation of behavioral symptoms that show significant overlap with symptoms of posttraumatic stress disorder (PTSD) and depression, which are frequently comorbid with mild TBI [Stein and McAllister, 2009]. Mild TBI is characterized by brief loss of consciousness (LOC), altered mental status such as feeling “dazed or confused,” and anterograde amnesia. Thus, the identification of clinical measures associated with closed head injury, and comorbid psychiatric conditions that are predictors of white matter integrity are of great interest.

Mild TBI in military personnel may result from several mechanisms including blast (primary), projectiles (secondary), and acceleration forces (tertiary). Exposure to TBI from explosives is a pervasive threat to military personnel deployed to Afghanistan and Iraq accounting for up to 75% of casualties [Hoge et al., 2008]. Mild TBI resulting from a concussive injury commonly co-occurs with blast. However, concussive injuries more similar to civilian and sports injuries also occur at high rates in military personnel independently of blast-related injury. Military personnel may experience multiple injuries during their deployments [Cernak and Noble-Haeusslein, 2010], but the severity of injuries is often not considered sufficient to warrant removal from duty. Therefore, many soldiers return to combat following a minimum of one and an average of three light-duty days [Gondusky and Reiter, 2005], increasing the likelihood of reinjury. Successive brain traumas may increase risk for more serious injury and poorer clinical outcomes [Cernak and Noble-Haeusslein, 2010]. While a growing body of evidence supports the presence of primary blast-induced neurotrauma in animal models [Cernak et al., 2011], studies of the effect of primary blast injury on the human brain are scarce in humans [Hayes et al., 2011; Sponheim et al., 2011].

Loss of white matter integrity is widely considered to play a major role in the clinical phenomenology of mild TBI [Arfanakis et al., 2002] and is usually not observed with conventional T1 or T2-weighted MRI [Hughes et al., 2004]. However, diffusion tensor imaging (DTI) provides qualitative information about white matter integrity. Several studies (see Table I) reported the presence and location of white matter injury in TBI, but were limited by methodological and clinical concerns. For instance, individuals with mild TBI also have high rates of comorbid PTSD, major depression, and alcohol and substance use [Hoge et al., 2008]. However, there is scant literature on the effect of PTSD on white matter integrity [Schuff et al., 2011], particularly when PTSD is comorbid with TBI. Of the 29 TBI studies in Table I, only one study incorporated PTSD in the analysis of its DTI data [Levin et al., 2010]. Many prior studies were limited to specific regions or tracts [e.g., MacDonald et al., 2011], and thus potentially more pervasive effects of mild TBI on the whole brain were not explored or reported. Finally, conclusions about the tissue microstructure available from the tensor model were largely unexplored.

One can confidently state the direction of axons is coincident with the principal diffusion direction only when all axons in a voxel are coherently oriented [Pierpaoli et al., 2001]. An extension of the tensor model permits the detection of distinct populations of fiber bundles with distinct orientations in a single imaging voxel [Behrens et al., 2007; Hosey et al., 2005]. Modeling crossing fibers from high angular resolution diffusion images (HARDI) has identified a number of tracts that are well known from postmortem dissection of the human brain but were not visualized using conventional diffusion tractography [Behrens et al., 2007]. Consequently, we chose this crossing fiber methodology for our study because of its potential advantages in assessing mild TBI, and in helping reconcile some inconsistent and negative findings in the literature [Levin et al., 2010] as highlighted in Table I. While a few prior studies acquired high angular resolution diffusion images from mild TBI [Levin et al., 2010] and also used to analyze crossing fibers.

In our analysis, PTSD diagnosis, LOC, feeling dazed or confused, number of TBIs, and age were included as regressors in the whole brain voxelwise analyses using a white matter-specific registration method with subsequent tensor modeling capable of discerning the contribution of crossing fibers that were quantified as partial volume fractions of the primary fiber (f1) and the crossing fiber (f2). We hypothesized that mild TBI and associated variables such as LOC, feeling dazed or confused, and the number of TBI events would influence the white matter integrity of primary or crossing fibers.

METHODS

Subject Data and Recruitment

Patients with mild TBI (n = 30) were compared to a primary control group (n = 42). As our results (below)
### TABLE I. Group comparison diffusion imaging studies of TBI

<table>
<thead>
<tr>
<th>Reference</th>
<th>Mean age or age range</th>
<th>Severity</th>
<th>N (case, cntrl)</th>
<th>Brain Regions with lower FA in TBI</th>
<th>DTI Analysis method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arfanakis, 2002</td>
<td>35.6 ± 14.8</td>
<td>mild</td>
<td>5, 10</td>
<td>CC, IC</td>
<td>ROI, no normalization</td>
</tr>
<tr>
<td>Bazarian, 2007</td>
<td>18–31</td>
<td>mild</td>
<td>6, 6</td>
<td>pCC, AIC</td>
<td>VBM on DTI some ROIs</td>
</tr>
<tr>
<td>Bendlin, 2008</td>
<td>30.5 ± 11.4</td>
<td>mod/seq</td>
<td>35, 36</td>
<td>CC, CB, SLF, ILF, UF, and BS</td>
<td>FSL preprocessing, SPM normalization, and whole brain analysis</td>
</tr>
<tr>
<td>Cubon, 2011</td>
<td>19.7 (mild), 27 (seq)</td>
<td>mild; seq</td>
<td>13, 13</td>
<td>SS, ILF, and IFO</td>
<td>FSL TBSS</td>
</tr>
<tr>
<td>Greenberg, 2008</td>
<td>18–80</td>
<td>mod/seq</td>
<td>13, 0</td>
<td>RFT, LFT, RTT, and LTT</td>
<td>ROI</td>
</tr>
<tr>
<td>Huisman, 2004</td>
<td>31 ± 10</td>
<td>not reported</td>
<td>20, 15</td>
<td>sCC and IC</td>
<td>ROI</td>
</tr>
<tr>
<td>Inglese, 2005</td>
<td>18–58</td>
<td>mild</td>
<td>46, 29</td>
<td>CC, IC, and CS</td>
<td>ROI</td>
</tr>
<tr>
<td>Kennedy, 2009</td>
<td>39.1 ± 12.4</td>
<td>severe</td>
<td>8, 8</td>
<td>CS, SPF, INF</td>
<td>FSL preprocessing and ROI</td>
</tr>
<tr>
<td>Kinnunen, 2011</td>
<td>38.9 ± 12.2</td>
<td>mild; mod/seq</td>
<td>28, 26</td>
<td>sCC, gCC, bCC, UF, ILF, SLF, and IFO</td>
<td>FSL preprocessing and ROI; normalized ROI; no correction for multiple comparisons (13 ROIs)</td>
</tr>
<tr>
<td>Kraus, 2007</td>
<td>35.9; mild, 34.9; mod/seq</td>
<td>all</td>
<td>37, 18</td>
<td>CST, SS, SLF (mild-TBI), various (mod/seq)</td>
<td>fiber tracking and ROI</td>
</tr>
<tr>
<td>Levin, 2008</td>
<td>&lt;18</td>
<td>mod/seq</td>
<td>32, 36</td>
<td>CC, bCC, gCC, sCC, and IC</td>
<td>fiber tracking and ROI</td>
</tr>
<tr>
<td>Levin, 2010</td>
<td>31.5 ± 7.2</td>
<td>mild/mod</td>
<td>37, 15</td>
<td>none</td>
<td>whole brain SPM2, fiber tracking, and ROI</td>
</tr>
<tr>
<td>Lipton, 2008</td>
<td>26–70</td>
<td>mild</td>
<td>17, 10</td>
<td>CC, subcortical WM, IC</td>
<td>ART registration and whole brain</td>
</tr>
<tr>
<td>Lipton, 2009</td>
<td>21–50</td>
<td>mild</td>
<td>20, 20</td>
<td>dIPFC</td>
<td>ART registration and whole brain</td>
</tr>
<tr>
<td>Lo, 2009</td>
<td>44 ± 10.9</td>
<td>mild</td>
<td>10, 10</td>
<td>CC, IC (higher FA)</td>
<td>ROI</td>
</tr>
<tr>
<td>MacDonald, 2011</td>
<td>19–58</td>
<td>mild</td>
<td>63, 21</td>
<td>CB, UF, aIC, CC, pIC</td>
<td>Analyze software and ROI</td>
</tr>
<tr>
<td>Mamere, 2009</td>
<td>39.1 ± 12.4</td>
<td>mod/seq</td>
<td>9, 9</td>
<td>WM, CC</td>
<td>ROI</td>
</tr>
<tr>
<td>Messé, 2011</td>
<td>18–65</td>
<td>mild</td>
<td>23, 23</td>
<td>fMaj, fMin, ILF, and IFF</td>
<td>FSL preprocessing and FSL TBSS</td>
</tr>
<tr>
<td>Nakayama, 2006</td>
<td>27.4 ± 12.1</td>
<td>not reported</td>
<td>23, 23</td>
<td>gCC, bCC, sCC, and fornix</td>
<td>whole brain and ROI (2 mm round)</td>
</tr>
<tr>
<td>Newcombe, 2007</td>
<td>16–66</td>
<td>mod/seq</td>
<td>33, 28</td>
<td>ACR, UF, gCC, ILF, and CB</td>
<td>ROI</td>
</tr>
<tr>
<td>Niogi, 2008a</td>
<td>37.4, 16–61</td>
<td>mild</td>
<td>34, 26</td>
<td>whole-brain WM</td>
<td>Native ROI</td>
</tr>
<tr>
<td>Niogi, 2008b</td>
<td>32.4, 17–61</td>
<td>mild</td>
<td>43, 23</td>
<td>left ACR and UF</td>
<td>Native ROI</td>
</tr>
<tr>
<td>Palacios, 2011</td>
<td>23.6, 18–32</td>
<td>severe</td>
<td>15, 16</td>
<td>CC, SLF, ILF, SFO, UF, and CB</td>
<td>FSL TBSS</td>
</tr>
<tr>
<td>Rutgers, 2008a</td>
<td>32 ± 9</td>
<td>mild</td>
<td>21, 11</td>
<td>cerebral lobar WM, CB, and CC</td>
<td>fiber tracking, DPTools normalization, and whole brain</td>
</tr>
<tr>
<td>Rutgers, 2008b</td>
<td>34 ± 12</td>
<td>all</td>
<td>39, 10</td>
<td>gCC and sCC</td>
<td>fiber tracking, DPTools normalization, and whole brain</td>
</tr>
<tr>
<td>Smits, 2010</td>
<td>28 ± 12</td>
<td>mild</td>
<td>19, 12</td>
<td>IFO, UF, pIC, and CC</td>
<td>FSL preprocessing and SPM whole brain</td>
</tr>
<tr>
<td>Wilde, 2008</td>
<td>14–19</td>
<td>mild</td>
<td>10, 10</td>
<td>CC</td>
<td>Fiber tracking and ROI</td>
</tr>
<tr>
<td>Xu, 2007</td>
<td>26.4, 21–36</td>
<td>severe</td>
<td>9, 11</td>
<td>CC, IC, EC, SLF, ILF, and fornix</td>
<td>FSL preprocess; SPM normalize, whole brain, and ROI</td>
</tr>
<tr>
<td>Yuan, 2007</td>
<td>7.89 ± 1</td>
<td>mod/seq</td>
<td>9, 12</td>
<td>gCC, PIC, SLF, and CS</td>
<td>SPM normalization, whole brain, and ROI</td>
</tr>
</tbody>
</table>

ACR, anterior corona radiate; aIC, anterior internal capsule; bCC, body of corpus callosum; BS, brain stem; CB, cingulum bundle; CC: corpus callosum; CS, centrum semiovale; CST, cortico-spinal tracts; dIPFC, dorsolateral prefrontal cortex; EC, external capsule; fMaj, forceps major; fMin, forceps minor; gCC, genu of corpus callosum; IC, internal capsule; IFO, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; IPF, inferior frontal; LFT, left frontal tract; LTT, left temporal tract; pCC, posterior corpus callosum; PCR, posterior corona radiate; pIC, posterior internal capsule; RFT, right frontal tract; RTT, right temporal tract; sCC, splenium of corpus callosum; SFO, superior fronto-occipital fasciculus; SLF, superior longitudinal fasciculus; SPF, superior frontal; SS, sagittal stratum; UF, uncinate fasciculus; WM, white matter; ART, automatic registration toolbox.
TABLE II. Demographic and clinical characteristics of participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-TBI n = 42 (primary)</th>
<th>Non-TBI n = 28 (confirmatory)</th>
<th>TBI n = 30</th>
<th>Group Comparison (primary)</th>
<th>Group Comparison (confirmatory)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (SD)</td>
<td>36.8 (9.9)</td>
<td>37.5 (11.3)</td>
<td>39.6 (10.8)</td>
<td>t(70)=1.1, P = 0.26</td>
<td>t(56) = 0.7, P = 0.49</td>
</tr>
<tr>
<td>Gender, no. (%) of females</td>
<td></td>
<td>10 (23.8)</td>
<td>2 (7.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race, no. (%) of Caucasian</td>
<td></td>
<td>36 (85.7)</td>
<td>25 (89.3)</td>
<td>X² (1) = 5.7, P &lt; 0.02</td>
<td>X² (1) = 0.43, P = 0.51</td>
</tr>
<tr>
<td>right-handed</td>
<td></td>
<td></td>
<td>20 (66.7)</td>
<td>X² (2) = 4.3, P &lt; 0.12</td>
<td>X² (2) = 4.5, P = 0.11</td>
</tr>
<tr>
<td>Diagnosis of PTSD, no. (%)</td>
<td></td>
<td></td>
<td>22 (52.4)</td>
<td>X² (1) = 3.4, P &lt; 0.19</td>
<td>X² (1) = 1.1, P = 0.58</td>
</tr>
<tr>
<td>Education (years), (SD)</td>
<td>14.6 (2.2)</td>
<td>12.6 (4.1)</td>
<td>13.5 (1.5)</td>
<td>t(70) = 2.3, P &lt; 0.05</td>
<td>t(56) = 1.1, P = 0.29</td>
</tr>
<tr>
<td>Number of TBIs (SD)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years between TBI and scan (SD)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>9.7 (10.8)</td>
<td></td>
</tr>
<tr>
<td>Blast + impact TBI (%)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (36.7)</td>
<td></td>
</tr>
<tr>
<td>Impact only TBI (%)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>19 (63.3)</td>
<td></td>
</tr>
<tr>
<td>Premorbid injury (%)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>13 (43.3)</td>
<td></td>
</tr>
<tr>
<td>LOC 1–20 min, no. (%)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>9 (30.0)</td>
<td></td>
</tr>
<tr>
<td>LOC 21–59 min, no. (%)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (6.7)^d</td>
<td></td>
</tr>
<tr>
<td>Dazed and confused, no. (%)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>17 (56.7)</td>
<td></td>
</tr>
<tr>
<td>Anterograde amnesia, no. (%)</td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Combat Exposure Scale (SD)</td>
<td>6.2 (8.7)</td>
<td>7.7 (8.7)</td>
<td>16.8 (10.2)</td>
<td>t(69) = 4.7, P &lt; 0.0001</td>
<td>t(55) = 3.6, P &lt; 0.0001</td>
</tr>
<tr>
<td>PTSD Scale (CAPS) (SD)</td>
<td>17.7 (16.4)^c</td>
<td>11.9 (11.4)</td>
<td>75.8 (35.8)</td>
<td>t(70) = 9.1, P &lt; 0.0001</td>
<td>t(56) = 6.9, P &lt; 0.0001</td>
</tr>
<tr>
<td>Diagnosis of PTSD, no. (%)</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>19 (63)</td>
<td>X² (1) = 118, P &lt; 0.0001</td>
<td>X² (1) = 90.7, P &lt; 0.0001</td>
</tr>
<tr>
<td>Beck Depression Inventory (SD)</td>
<td>4.0 (3.9)</td>
<td>4.8 (5.5)</td>
<td>19.0 (11.2)</td>
<td>t(69) = 8.0, P &lt; 0.0001</td>
<td>t(55) = 6.0, P &lt; 0.0001</td>
</tr>
<tr>
<td>Alcohol Use Disorders</td>
<td>2.3 (2.2)</td>
<td>3.2 (4.6)</td>
<td>4.4 (5.3)</td>
<td>t(69) = 2.3, P &lt; 0.05</td>
<td>t(55) = 0.9, P = 0.36</td>
</tr>
<tr>
<td>Identification Test (SD)</td>
<td>0.5 (0.7)</td>
<td>0.7 (0.9)</td>
<td>1.7 (3.4)</td>
<td>t(69) = 2.3, P &lt; 0.05</td>
<td>t(55) = 1.4, P = 0.15</td>
</tr>
</tbody>
</table>

^aData values represent means except where indicated otherwise.
^bCAPS unavailable for three participants; Davidson Trauma Scale (DTS) substituted.
^cCAPS unavailable for four participants; DTS substituted.
^dOne subject reported LOC < 30 min at follow-up contact; the second reported LOC between 30 and 60 min with no focal neurological signs; both consistent with ACRM criteria.

showed extensive differences in white matter integrity between the TBI and primary control group, we acquired a second non-TBI control group for further analysis. Given prior reports of white matter differences associated with alcohol [Yeh et al., 2009], gender [Hsu et al., 2008], substance use [Xu et al., 2010], and possibly education [Scholz et al., 2009], this second, confirmatory, non-TBI control group (n = 28) was matched on alcohol and drug use, as well as gender and education (see Table II). All subjects were recruited from a large registry of military service members and veterans who served in Iraq and Afghanistan [Dedert et al., 2009]. Enrollment was based on inclusion and exclusion criteria that were consistent with the American Congress of Rehabilitation Medicine (ACRM) criteria for mild TBI [Kay et al., 1993]. Specifically, subjects had to endorse a closed head injury with a description of the event and endorse one or more of the following (inclusion criteria) to fit the mild TBI diagnosis: need for hospitalization, retrograde amnesia, anterograde (posttraumatic) amnesia, LOC, feeling dazed, or confused. Exclusion criteria consisted of penetrating head injury, LOC of 1 h or greater, history of neurological disorders (seizure, stroke, multiple sclerosis, chronic encephalitis, cerebrovascular disease, ischemic or hypoxic brain injury, and CNS neoplasm), severe chronic medical conditions (e.g., uncontrolled diabetes, liver failure, HIV, renal failure, heart failure, and severe metabolic disturbance), neurosurgery, Axis I psychiatric disorders other than PTSD, and comorbid major depression. Current or lifetime diagnosis of substance dependence was excluded. Current diagnosis of substance abuse was excluded but history of past substance abuse was permitted.

Subjects were recruited through telephone contact by study coordinators and provided written informed consent to participate in procedures approved by the Institutional Review Boards at Duke University and the Durham Veterans Affairs Medical Center. The TBI group was enrolled into the study between October 12, 2007 and April 30, 2010, with 4 subjects in 2007, 8 in 2008, 14 in 2009, and 4 in 2010 (n = 30). Subjects in the primary control group were enrolled between December 12, 2007 and May 10, 2010 with 3 subjects in 2007, 16 in 2008, 16 in 2009, and 7 in 2010 (n = 42). Subjects in the confirmatory control group were enrolled between January 17, 2008 and October 6, 2010 with 4 in 2008, 4 in 2009, and 20 in 2010 (n = 28). All subjects completed a computerized version of the Ivins TBI questionnaire [Ivins et al., 2003] and were evaluated for psychiatric illness with the Structured Clinical...
Interview for DSM-IV (SCID) and the Clinician Administered PTSD Scale (CAPS). The Ivins TBI questionnaire, which was developed in consultation with the Defense and Veterans Brain Injury Center (DVBIIC), assessed the number of lifetime “head injuries,” mechanism, date, need for hospitalization, presence of altered mental status, LOC (ordinal increments of 1–20 min, 21–59 min, and >59 min), retrograde amnesia (ordinal increments of <24 h, 1–7 days, and >7 days), anterograde amnesia for the injury (none, do not know, or recovered memory in <1 h, 1–24 h, 24 h–7 days, >7 days), and “feeling dazed or confused” separately for each injury. All images were clinically evaluated by a neuroradiologist (DM) to assess for structural brain abnormalities.

**Image Acquisition**

Images were acquired on a GE 3.0 Tesla EXCITE scanner with an 8-channel headcoil. All participants underwent (i) DTI with $2 \times 2 \times 2$-mm voxel size automatically resampled to $1 \times 1 \times 2$-mm, FOV $240 \times 240$-mm, flip angle $90^\circ$, TR = 17,000-ms, TE = 76-ms, 1 average, 55 non-collinear directions (diffusion gradients), nonzero b-value = 1,000 s/mm$^2$, scanning time = 11-min, and (ii) high resolution T1-weighted 3D-FSPGR images with 1-mm isotropic voxels (TR/TE/flip angle = 7.484-ms/2.984-ms/12°, 256-mm FOV, 166 slices, and 1 excitation). All diffusion-weighted and T1-weighted images were visually inspected for quality and no scans were rejected.

**Analysis of Diffusion Imaging Data**

Standard preprocessing steps to correct motion and eddy current distortion were performed with the FMRIB Diffusion Tool Box (FDT; FMRIB Centre, University of Oxford, UK). The three principal eigenvalues were calculated from the diffusion tensor of the DTI data. Fractional anisotropy (FA), a scalar metric that serves as a measure of white matter integrity was calculated from the orientational coherence of the diffusion compartments within each voxel. The voxelwise analytic approach of whole brain data was based on white matter alignment with a non-linear intermediate degrees-of-freedom registration [Smith et al., 2006]. This robust white matter specific registration and subsequent voxelwise analyses were conducted on skeleton voxels using the Tract-Based Spatial Statistics method (TBSS; FMRIB Centre, University of Oxford, UK). The DTI data was registered to a $1 \times 1 \times 1$ mm standard space (MNI) high-resolution average of 58 well-aligned good quality FA images (FMRIB58_FA template) from male and female subjects aged 20–50 years (see http://www.fmrib.ox.ac.uk/fsl/). In TBSS, a mean FA skeleton was created as lines and surfaces that pass through the centers of white matter tracts in the mean FA image. After thresholding the skeleton to exclude low FA values (<0.2) indicative of nonwhite matter, each subject’s aligned FA image was projected onto the mean FA skeleton.

**Crossing Fiber Analysis**

A major limitation of FA is the assumption that water diffusion has a Gaussian profile. More complex tensor models, made possible with high angular resolution imaging, provide diffusion measurements that can be used to infer the underlying tissue microstructure such as orientation of crossing fibers. The partial volume fractions for primary (f1) and secondary/crossing (f2) fibers, modeled with second-order tensor modeling using TBSS-X (X to indicate crossing fiber), were the primary metrics of white matter integrity in the crossing fiber analysis [Jbabdi et al., 2010]. Crossing fiber analysis was performed with Bayesian Estimation of Diffusion Parameters Obtained with Sampling Techniques (BEDPOSTX), which determines the proportion of primary and crossing fibers at each voxel based on the tensor model [Behrens et al., 2007]. Each volume is partitioned into two volume fractions associated with primary and crossing fiber populations, and two scalar measures (f1 and f2) are calculated that represent the magnitude of these two volumes, respectively. The diffusion signal for each compartment is modeled as the weighted sum of signals from two compartments. The partial volume fraction approach for crossing fibers is briefly summarized here but a detailed description is available in Jbabdi et al. [2010].

For each anisotropic volume, the model estimates independent weights of the signals (f1 and f2) and independent orientations, which represent independent anisotropies. Thus no anisotropy is inferred, rather these are pre-specified in the model. Essentially, f1 and f2 are scalar measures that represent the volume fraction of a given voxel that can be attributed to the primary and crossing fiber populations respectively and are independent of the angle between them. Any loss of white matter integrity, regardless of which fiber population is affected, is going to alter f1 and f2. Thus, FA and f1, f2 are different diffusion coefficients that are extracted from different diffusion models: FA is a measure from the diffusion tensor model and f1 and f2 come from the partial volume model [Behrens et al., 2003a,b]. When considering f1 and f2 measures obtained from a group of subjects, f1 and f2 may be assigned to different fibers depending on the relative size of each fiber in the subjects. Thus the assignment primary fiber and crossing fiber is arbitrary with the larger quantity consistently assigned to f1. The TBSS-X algorithm sorts the consistent labeling of f1 and f2 to the same fiber across subjects, which requires f1 and f2 to be swapped for a subset of voxels in each subject [Jbabdi et al., 2010].

**Permutation Testing**

Sample specific assumptions about the distribution of DTI data (e.g., gaussianness) were avoided with permutation testing to make inferences about group differences and associations with clinical regressor variables. This nonparametric analysis was conducted using randomise.
(FMRIB Centre, University of Oxford, UK), an implementation of permutation testing for whole brain voxelwise analyses. Simple permutation testing is designed to determine if the observed difference between sample means is large enough to reject the null hypothesis that the two groups have an identical probability distribution. A variant of this simple permutation test, the t-test with covariates, was used with clinical regressors to establish a significance level for every skeleton voxel from a distribution generated by 5,000 permutations. This approximate exact permutation test becomes more accurate with a growing sample size assuming a fixed number of regressors. For details about permutation testing with regressors see Kennedy [1995] or Anderson and Robinson [2001].

Clinical Regressors

The analysis was performed with nine covariates in a whole brain analysis on the dependent variables f1, f2, and FA. Nine covariates included (i) age, based on FA reduction with aging [Madden et al., 2004], (ii) PTSD, given it was more prevalent in the TBI group, (iii) number of events contributing to TBI, (iv) duration of LOC, (v) presence of feeling dazed or confused, (vi) presence of anterograde amnesia, (vii) Beck Depression Inventory (BDI), (viii) Alcohol Use Disorder Identification Test (AUDIT), and (ix) Drug Abuse Screening Test (DAST). Intermediate results showed that the last four covariates (anterograde amnesia, BDI, AUDIT, and DAST) were not correlated with the dependent variables and their inclusion in the model did not appreciably change the outcome. Therefore, results of only the first five covariates are presented. The confirmatory control group (n = 28) matched on the (i) AUDIT, (ii) DAST, (iii) gender, and (iv) education (summarized in Table II), and was compared with the TBI group using identical analyses.

Correction for Multiple Comparisons

The results of permutation testing were followed up with correction for multiple comparisons using threshold free cluster enhancement (TFCE) as an alternative to the overly conservative corrections such as Bonferroni that have poor control of Type II error [Smith and Nichols, 2009]. Rather than requiring the selection of an arbitrary initial clustering threshold for subsequent computation of P-values based on Gaussian Random Field theory (cluster-level-correction), TFCE accounts for “cluster-like local spatial support,” that is, intensity and extent of the test statistic. Recommended (default) TFCE parameters for cluster height (H = 2), cluster extent (E = 1), and cluster connectivity C (C = 26) were used with randomise.

Visualization of Results

The significance maps (P < 0.05; corrected) were superimposed on the normalized group skeleton of the FMRIB58_FA template brain. The TBSS-fill feature was applied to all significance maps to enhance the visualization of TBSS results and is commonly used in similar studies [Cubon et al., 2011; Messe et al., 2011; Zhuang et al., 2010]. Tracts are reported according to nomenclature of the Johns Hopkins University White-Matter Tractography Atlas [Mori et al., 2005].

RESULTS

Clinical Measures

The TBI group experienced an average of 2.2 (SD = 2.1) TBI events; 30% suffered LOC between 1 and 20 min, 6.7% suffered LOC between 21 and 59 min; 56.7% experienced feeling dazed or confused; 56.7% experienced blast-related TBI, ostensibly with concomitant impact TBI. The PTSD symptom scores were much greater in the TBI than the non-TBI group [t(70) = 9.1, P < 0.0001] and PTSD symptom scores were highly correlated with BDI scores (r = 0.82, P < 0.0001) and the AUDIT (r = 0.40, P < 0.06). Detailed demographic and clinical characteristics of the three participant groups are provided in Table II.

The neuroradiologist’s examination of conventional T1 MRI scans revealed normal brains with no structural abnormalities, no encephalomalacia (e.g., from stroke or contusion), no focal cortical volume loss, and no cortical lobar atrophy.

Group Differences?

A whole brain voxelwise comparison between TBI and primary as well as confirmatory non-TBI control subjects of the partial volume fraction associated with the primary fiber (f1) covarying for age, severity of PTSD, number of TBI events, duration of LOC, and feeling dazed or confused are summarized in Figure 1. These results show a significantly lower primary fiber partial volume fraction in the TBI group in diffuse cortical and subcortical tracts (P < 0.05; corrected). Both major fiber bundles and smaller peripheral tracts are represented. These include the body of the corpus callosum, genu of the corpus callosum, splenium of the corpus callosum, forceps minor, forceps major, superior corona radiata (L,R), posterior corona radiata (L,R), posterior limb of internal capsule (L), posterior thalamic radiation (L,R), retrolenticular part of internal capsule, superior longitudinal fasciculus (L), and the tapetum (R).

Clinical Correlations with White Matter Integrity

Duration of LOC was significantly correlated with lower f1 (see Fig. 2) in a distribution that overlapped with the
between-group analysis. A correlation with duration of LOC was observed in the following tracts: inferior fronto-occipital fasciculus (L,R), anterior thalamic radiation (L,R), forceps minor (L,R), uncinate fasciculus (L,R), genu of corpus callosum, anterior corona radiata (L,R), external capsule (L,R), inferior longitudinal fasciculus (L,R), cingulum (L), posterior limb of the internal capsule, (not cingulate), superior longitudinal fasciculus (L,R), sagittal stratum (L,R), retrolenticular part of the internal capsule (L,R).

Presence of feeling dazed and confused was significantly correlated with lower f1 (see Fig. 3) that was more widespread than the correlation of f1 with LOC. These voxels extended further into the periphery, particularly posteriorly into the occipital cortex, as well as in the posterior corona radiata (L,R), fornix/stria terminalis (L,R), superior longitudinal fasciculus temporal part (L,R), cerebral peduncle (R), and posterior thalamic radiations (L,R).

There were no correlations detected between f1 and the remaining regressors: age, PTSD, and the number of TBIs. Results of analyses using the confirmatory control group were consistent with those using the primary control group at the between-group level as well as the results of correlations with LOC and feeling dazed and confused.

**Comparison with FA**

Given the lack of previous DTI studies based on assessment, we compared results of partial volume fractions with FA, a well-established measure of white matter integrity. The results from f1 and FA showed an overall consistent pattern of abnormalities in the mild TBI group. However, notable differences were also apparent. In particular, the f1 results showed no voxels in the brainstem...
where the FA results showed significant difference (see Fig. 4). There were no significant findings for f2.

**DISCUSSION**

This study examined white matter integrity in mild TBI and comorbid PTSD in recent military veterans of the wars in Iraq and Afghanistan. White matter integrity was assessed with HARDI and whole brain voxelwise crossing fiber analyses performed to separately assess the integrity of primary and crossing fibers. Our results show significant loss of white matter integrity in patients with mild TBI in a distributed network of tracts consisting of the corpus callosum (genu, body, and splenium), forceps minor and major, superior and posterior corona radiata, internal capsule, superior longitudinal fasciculus, and several others. Significant loss of white matter integrity was correlated with duration of LOC, and more widely distributed loss of white matter integrity was correlated with feeling dazed or confused, two clinical measures frequently employed in the diagnosis and clinical evaluation of mild TBI. Importantly, PTSD, a common comorbid condition that displays partially overlapping symptoms with mild TBI, showed no association with loss in white matter integrity.

In simple terms, partial volume fractions (f1 and f2) can be considered as the contribution of each fiber population to the diffusion MR signal, which is related to the amount of space occupied by each fiber population. Thus, f1 is lower in mild TBI compared to non-TBI controls and a lower f1
was associated with duration of LOC and feeling dazed or confused. Validation studies conducted in aging [Jbabdi et al., 2010] and imaging of well-known anatomical tracts [Jones et al., 1999] from postmortem dissection [Behrens et al., 2007] suggest that f1 and f2 obtained from high angular resolution provides less sensitivity but greater specificity than FA. Our FA results also show a more widespread pattern than the between group differences in the analysis using the primary control group (top) and the confirmatory control group (bottom). These disrupted tracts extended further into the periphery, particularly posteriorly into the occipital cortex, as well as in the posterior corona radiata, fornix/stria terminalis, superior longitudinal fasciculus temporal part, cerebral peduncle, and posterior thalamic radiation. Skeleton voxels are highlighted in green and correlated voxels are in pink. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 3.

A significant correlation showed lower partial volume fraction (P < 0.05; corrected TFCE) in the primary fibers (f1) and feeling dazed or confused in a distribution that was more widespread than the between group differences in the analysis using the primary control group (top) and the confirmatory control group (bottom). These disrupted tracts extended further into the periphery, particularly posteriorly into the occipital cortex, as well as in the posterior corona radiata, fornix/stria terminalis, superior longitudinal fasciculus temporal part, cerebral peduncle, and posterior thalamic radiation. Skeleton voxels are highlighted in green and correlated voxels are in pink. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Effects of TBI on White Matter Integrity

was associated with duration of LOC and feeling dazed or confused. Validation studies conducted in aging [Jbabdi et al., 2010] and imaging of well-known anatomical tracts [Jones et al., 1999] from postmortem dissection [Behrens et al., 2007] suggest that f1 and f2 obtained from high angular resolution provides less sensitivity but greater specificity than FA. Our FA results also show a more widespread pattern than the f1 pattern, from which we infer that f1 findings are more specific to mild TBI. Comparison of f1 with FA (see Fig. 4) shows many areas of discrepancy. Two factors may have influenced the discrepant finding between the FA and crossing fiber approach in the corpus callosum and the internal capsule often thought to contain high directional coherence. The first is the repeatability and precision of anisotropy estimates obtained from diffusion imaging. The cone of uncertainty in fiber orientation in the internal capsule and the corpus callosum has been measured by Jones [2003]. While the cone of uncertainty is lower in the corpus callosum (∆3.5°) than the internal capsule (∆8.2°) and the frontal white matter (∆10°), it results in imprecise FA measurements that might differentially affect standard FA relative to partial volume fractions. Second, while fiber direction is far more uniform in the corpus callosum than other brain regions, the presence of crossing fibers in the corpus callosum is well established using HARDI in conjunction with intravoxel modeling [Tuch et al., 2002], as well as electron microscopy of postmortem human brains [Aboitiz et al., 1992].
The widely distributed pattern of loss of white matter integrity found between the mild TBI and the non-TBI control group is particularly striking, and in contrast to previous findings in mild TBI that were generally limited to either large fiber bundles, such as the corpus callosum and the internal capsule [Kraus et al., 2007] or very focal areas [Lipton et al., 2009]. Primary blast induced neurotrauma and nonblast (impact) trauma have grossly differing mechanisms of imparting damage to neural tissue [Cernak, 2010]. The latter is transmitted by rapid acceleration and deceleration mechanics leading to coup-counter-coup injury [Weber, 2007]. Blast trauma, informed by animal models, is thought to be transmitted by (i) direct interaction of the blast wave with the head where the blast wave passes through the skull and causes acceleration and/or rotation of the brain and (ii) transmission of highly concentrated kinetic energy from the overpressure wave via large blood vessels in the abdomen/chest to the central nervous system [Cernak, 2010]. The latter is transmitted by rapid acceleration and deceleration mechanisms leading to coup-counter-coup injury [Weber, 2007]. Blast trauma, informed by animal models, is thought to be transmitted by (i) direct interaction of the blast wave with the head where the blast wave passes through the skull and causes acceleration and/or rotation of the brain and (ii) transmission of highly concentrated kinetic energy from the overpressure wave via large blood vessels in the abdomen/chest to the central nervous system [Cernak, 2010]. Furthermore, blast injury may involve concomitant acceleration-deceleration and/or concussive injury, which could increase the complexity of primary blast-induced neurotrauma [Taber et al., 2006]. Despite these differences, both nonblast and blast injury lead to damage at the cellular level via several biochemical pathways including free radical generation, disruption of calcium homeostasis, and release of inflammatory mediators [Cernak, 2010]. The earlier model of impact TBI that emphasized focal or multifocal injury that evolves from a pericontusional process has been supplanted with a model that also includes diffuse mechanical forces of injury that may trigger noncontusion cell death cascades [Buki and Povlishock, 2006]. This hypothesis for primary blast-induced neurotrauma involves the production of nitric oxide synthase and glial activation as well as immune-mediated and/or glutamate-mediated cell damage that results in apoptosis and necrosis of glial cells responsible for maintaining homeostasis, formation of myelin, and support of neurons [Cernak, 2010]. Our results appear to be consistent with loss of white matter integrity that may be proximally explained by factors associated with the magnitude of the mechanical forces, but its diffuse pattern is consistent with evidence of damage from a downstream cascade of neurochemical and neurotoxic processes [Buki and Povlishock, 2006].

After experiencing sufficient trauma to result in head injury, it is reasonable to expect a concomitant psychological trauma with elevated risk for developing PTSD, particularly in military and veteran groups [Marx et al., 2009; Schneiderman et al., 2008]. The onset of PTSD, in turn, is associated with higher rates of depression, alcohol and drug use [O’Donnell et al., 2004]. With a few exceptions [Levin et al., 2010; Lipton et al., 2009] these psychiatric comorbidities have been ignored or excluded in neuroimaging studies [Stein and McAllister, 2009]. In one recent study that included depression, anxiety, and “stress scores,” these measures were not associated with FA or mean diffusivity [Lipton et al., 2009]. Nevertheless, the relationship between mild TBI and PTSD has been the focus of intense debate and speculation. Among returning veterans from Iraq and Afghanistan, mild TBI incurred during combat approximately doubled the risk for PTSD. Even when overlapping symptoms between PTSD and

**Figure 4.**

The differences in results between the conventional approach (FA; top panel) and the crossing fiber approach (f1; bottom panel) show grossly consistent abnormalities, however notable differences in methods are apparent. In particular, no voxels were detected in the brainstem for the primary fiber (bottom row) but large clusters were present there with conventional FA (top row). Skeleton voxels are highlighted in green and between group differences are in red/yellow. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
mild TBI were removed, the association remained strong [Stein and McAllister, 2009]. Large-scale longitudinal studies have specifically examined the role of mild TBI and PTSD on persistent postconcussive symptoms and psychosocial outcomes. The reports conclude that the reduction in psychosocial function and persistent postconcussive symptoms are not independently affected by history of mild TBI [Polusny et al., 2011; Schneiderman et al., 2008]. In fact, they found that after accounting for PTSD, mild TBI alone was not associated with postconcussive symptoms, depression, alcohol abuse, nonspecific somatic complaints, social adjustment, or quality of life. However, it is clear from our study and several prior studies (see Table I) that mild TBI results in objective disruption of white matter. Perhaps the combination of mild TBI and PTSD leads to a significant increase in postconcussive symptoms and decline in psychosocial function that is not observed with PTSD alone or with mild TBI alone. Thus, the biological mechanisms that predispose poor outcome to TBI may also predispose individuals to PTSD and these two ostensibly unique illnesses may not be fully dissociated.

Unfortunately, our study did not explicitly measure functional or psychosocial outcomes. Conversely, neuroimaging and other biologically based markers demonstrate clear evidence of loss of white matter integrity explained by injury from blast [Hayes et al., 2011; Sponheim et al., 2011] or from acceleration-deceleration injury, but have not systematically examined the contribution of PTSD [Stein and McAllister, 2009]. This study fails to show an association between PTSD and DTI-based loss of white matter integrity associated with mild TBI. Our findings are not necessarily inconsistent with reports of FA differences associated with PTSD [Schuff et al., 2011] because PTSD may not specifically modulate the effect of TBI on white matter integrity. Moreover, it is unclear whether these findings implicating mild TBI would be explained by PTSD, given that the loss of white matter integrity in this study is far more extensive than reported in PTSD. In addition, the complaints of feeling dazed or confused might be explained not only by an “altered state of consciousness or psychological stress,” but by systemic effects caused by the activation of the parasympathetic nervous system and other factors common to both blast and impact TBI [Cernak, 2010]. While we have established LOC and feeling dazed and confused are associated with widespread white matter disruption, this white matter disruption does not appear to be associated with PTSD, but its association with persistent postconcussive symptoms is yet unknown.

The spatial extent of loss of white matter integrity that we have demonstrated has typically been associated with moderate to severe TBI. For instance, Kraus et al. [2007] found FA differences in 11 of 13 ROIs tested were associated with moderate to severe TBI group, but only three ROIs showed reduced FA in mild TBI. Likewise, Bendlin et al. [2008] performed whole brain analyses as part of a longitudinal study and found a widespread distribution showing reduced white matter integrity despite improvements in neuropsychological performance at 12 months compared with 2 months. These changes, consistent with findings from other groups [Levin et al., 2008], were investigated in moderate to severe TBI (GCS < 13).

Until recently, the spatial extent of FA differences in mild TBI has been fairly circumscribed most frequently reported in subdivisions of the corpus callosum and internal capsule [Huisman et al., 2004; Lipton et al., 2008; Niogi et al., 2008a]. Aside from these regions, the remaining reports lack consistency, with most studies reporting a unique region(s) and occasional reports of overlap (see Table I). However, the most recent studies show initial evidence in support of our findings of widespread loss of white matter integrity in mild TBI [Kinnunen et al., 2011; MacDonald et al., 2011; Messe et al., 2011; Moore, 2009; Yang et al., 2011] with the pattern of damage from blast described as a pepper-spray pattern [Moore, 2009]. There is evidence of this widespread pattern appearing in the chronic stage initially at 1 month and extending further at 1 year following mild TBI [Yang et al., 2011]. It is possible that various enhancements in acquisition and analysis, such as white matter specific registration, crossing fiber analyses, and whole brain analyses provided enhanced sensitivity to detecting FA reductions.

Earlier studies have been conducted with conventional FA, and therefore the crossing fiber approach is certain to improve analyses of any regions/voxels where there is a rich population of crossing fibers (lack of orientational coherence) particularly in the most peripheral tracts where there is inherently lower signal-to-noise ratio (SNR) [Jones, 2003]. This may be an additional reason that our analysis reveals differences in many peripheral tracts not previously associated with mild TBI. Findings from ROI analyses are likely to have diluted between-group differences due to variance from voxels within a given ROI resulting in Type II errors or an artifactual reduction in effect size. It is conceivable that feeling dazed and confused may be the most strongly correlated, because patients have the best recollection and therefore the most accurate reporting of experiencing this symptom. Memory for other symptoms such as LOC and posttraumatic amnesia is less reliable for obvious reasons, and consequently underreported.

This is the first study focused on TBI to show differences using high angular resolution DTI and crossing fiber analyses. This widely accessible tool provides a satisfactory solution to aligning FA images from multiple subjects enabling valid conclusions of subsequent voxelwise analysis [Smith et al., 2006], whereas prior studies have been compromised by the use of standard registration algorithms developed for T1 acquisitions. TBSS uses a carefully tuned nonlinear registration designed to preserve the underlying intersubject variability in diffusion measures, followed by projection onto an alignment-invariant tract representation referred to as a “mean FA skeleton” [Smith et al., 2006]. Indeed, the TBSS approach has been adopted in assessing white matter integrity in bipolar disorder,
mild cognitive impairment, and schizophrenia, as well as mild [Messe et al., 2011] and moderate-severe TBI [Kinnunen et al., 2011; Palacios et al., 2011]. The ultimate success of these studies will rely on understanding the relationship of diffusion abnormalities with localizing information from neuropsychological testing [Kraus et al., 2007; Lipton et al., 2009; Niogi et al., 2008a]. However, the diffuse pattern of loss of white matter integrity revealed in this study poses a major challenge in identifying a consistent repertoire of neuropsychological impairments and associated white matter tracts.

Caveats and Limitations

A few limitations deserve mention. First, our approach does not segregate white matter injury according to its cause. Thus, multiple antecedent events leading to TBI (e.g., football injury + improvised explosive device + motor vehicle crash) could result in cumulative effects and potentially similar endpoints on DTI measures. However, this cannot be confirmed definitively given the present sample composed of patients with heterogeneous sources of injury. Second, while subjects were selected according to ACRM criteria [Kay et al., 1993] for mild TBI, we expect that assessment by retrospective self-report resulted in limited accuracy compared with clinician assessment immediately following injury. Relatedly, the assessment lacked accurate information on the time elapsed since the TBI, however all cases were chronic. Higher rates of PTSD in the TBI group than the control group may present a confound in detecting effects of PTSD on partial volume fractions. Assessment of structural abnormalities was made with T1-weighted imaging, but subjects were not assessed with gradient echo T2* imaging capable of detecting microbleeds in the acute stage and T2-weighted FLAIR capable of detecting white matter loss. Lack of neuropsychological data was a limitation; this data would enable clinicobehavioral correlations with objective neuromarkers. Finally, our analysis and that of two recent studies were unable to find an association between psychotropic medications and white matter tracts.

CONCLUSIONS

Assessment of crossing fibers coupled with methodological advances for robust whole brain analyses confirm the presence of a diffuse pattern of loss of white matter integrity associated with mild TBI and particularly with LOC and feeling dazed and confused but not the presence of PTSD. These findings of diffuse injury in mild TBI appear consistent with a systemic mechanism of damage shared by blast- and impact-related TBI that involves a cascade of inflammatory and neurochemical events.

REFERENCES

Effects of TBI on White Matter Integrity


