Combining Whole-Brain Voxel-Wise Analysis with *In Vivo* Tractography of Diffusion Behavior after Sports-Related Concussion in Adolescents: A Preliminary Report

Michael Borich,1 Nadia Makan,1 Lara Boyd,1 and Naznin Virji-Babul1,2

Abstract

We have previously shown that sports-related concussion in adolescents is associated with changes in whole-brain properties of white-matter pathways. Here, we assess local changes within these pathways. Twelve adolescents with a clinical diagnosis of subacute concussion and 10 healthy adolescents matched for age, gender, and physical activity completed magnetic resonance imaging scanning. Voxel-wise tract-based spatial statistics and tractography were performed to assess local changes in diffusion-based measures of microstructural properties of white-matter pathways (fractional anisotropy, mean diffusivity, radial diffusivity, and axial diffusivity) between the two groups. Fractional anisotropy values were higher for the concussed group in multiple cluster regions using tract-based spatial statistics, primarily in frontal white-matter regions, including the anterior corona radiata bilaterally. Using these regions of altered diffusion characteristics to seed fiber tractography, significantly reduced axial diffusivity in tracts passing through these areas were detected in the concussed group (*p* = 0.04). A trend toward reduced mean and radial diffusivity in the concussed group was also observed within the same reconstructed tracts. Diffusion behavior within these tracts was significantly correlated with an assessment of concussion status (Sports Concussion Assessment Tool 2). Fractional anisotropy within the reconstructed tracts was not significantly different between the two groups. These results suggest that subacute concussion in adolescents is associated with altered diffusion properties within regional white-matter tissue and along reconstructed fiber pathways. Combining voxel-wise analysis with fiber tractography provides an alternative objective approach to evaluate and identify subtle changes in white-matter fiber integrity after concussion.

Key words: adolescents; concussion; diffusion tensor imaging, mild traumatic brain injury; tract-based spatial statistics, tractography

Introduction

*MILD TRAUMATIC BRAIN INJURY* (mTBI) is a major public health concern and is presently one of the least understood neurological injuries, according to the Centers for Disease Control and Prevention. mTBI results in a complex cascade of events that affects brain physiology, including diffuse axonal injury, altered cerebral blood flow, neural inflammation, as well as autonomic and metabolic changes.1 Current neuropsychological, behavioral, and standard neuroimaging tools [e.g., computed tomography (CT) and magnetic resonance imaging (MRI)] are not sensitive to detect subtle changes in brain structure and function in the majority of cases, thus making an initial diagnosis of concussion, as well as evaluating functional recovery, difficult. Clinicians typically rely on observable physical symptoms; however, these symptoms may not accurately reflect the complex underlying pathophysiology of mTBI. This is of particular concern in adolescents because an increasing number of studies show that the pattern of recovery in children and adolescents is not the same. Though most adults appear to recover from a sports-related concussion/mTBI within 2–10 days,2–4 14% of children over the age of 6 remain symptomatic 3 months after injury, and for most children with mTBI, it can take up to 1 year for symptoms to resolve.5

In mTBI, there is evidence of an enhanced inflammatory response in pediatric populations, in comparison to adults. Although the pathophysiology of this inflammatory response is not clear, differences in glutamate receptor expression, increased vulnerability to oxidative stress, and altered regulation of cerebral blood flow all play a role in the extent of injury.6–8 Diffuse axonal injury may be the primary mechanism of injury in concussion and, most

1Department of Physical Therapy, University of British Columbia, Vancouver, British Columbia, Canada.
2Child and Family Research Institute, Vancouver, British Columbia, Canada.
commonly, affects regions of white matter within the brain. \textsuperscript{10} Diffusion tensor imaging (DTI) has been shown to be sensitive to changes in microstructural properties of white matter and is increasingly being used to study effects of concussion and mTBI. \textsuperscript{11-14} The primary DTI-derived measures used to quantify white-matter microstructural status are fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). FA is a scalar value commonly used to quantify the directionality and magnitude of water diffusion, ranging from zero to one. FA is influenced by microstructural properties of white-matter tracts, including, but not limited to, fiber organization, axon diameter, and myelination. \textsuperscript{15} MD is an overall index of the rate of diffusion averaged over all directions. AD reflects diffusion along the principal axis of the tensor and may be related to axonal health, whereas RD is associated with diffusion behavior orthogonal to the principal direction of diffusion and may potential reflect aspects of myelin status. \textsuperscript{16}

Concussion/mTBI has been associated with both increases and decreases in all four of these measures and appears to be sensitive to phase of recovery and patient populations. \textsuperscript{13,14,17} In addition, there are a wide range of scanning parameters, analysis approaches, and processing techniques that have been applied to DTI data. We recently employed a deterministic streamline tractography approach to whole-brain DTI data and observed increased FA and decreased MD values in adolescents after sports-related concussion, compared to matched controls. \textsuperscript{18} These values were associated with total scores on the Sports Concussion Assessment Tool 2 (SCAT2), with lower scores indicating greater observable impairment associated with higher FA and lower MD values. These findings demonstrated subtle, global differences in diffusion behavior that were associated with a behavioral assessment, but did not provide specific insights into potentially common sites of injury after concussion in adolescents.

Overall, there has been a great deal of variability reported in the spatial locations of the diffusion abnormalities using voxel-based techniques. Concussion may result in changes in multiple areas of the brain, and this factor, combined with variable experimental procedures, has led to apparent discrepancies on the nature of diffusion abnormalities in the concussion literature. In particular, \textit{a priori} investigations are a significant limitation of voxel-based techniques, given that changes in white-matter integrity may occur anywhere in the brain. Another approach to DTI data analysis that does not rely on fiber-tract reconstruction is tract-based spatial statistics (TBSS), which relies on voxel-wise analyses of white-matter tracts and is not dependent on \textit{a priori} hypotheses regarding loci of white-matter changes. \textsuperscript{19} This voxel-wise analysis approach can detect regional differences in DTI-based measures of water diffusion in clusters of voxels throughout the brain. However, TBSS relies on a skeletonized map of white-matter anatomy based on diffusion information from the tract center. This technique is also unable to provide insights into long-range fiber-tract integrity and structural connectivity between brain regions, thus it is difficult to extend cluster attributes to the status of specific fiber pathways. The tractography-based approach mentioned above can generate three-dimensional fiber-tract reconstructions of pathways between brain regions of interest (ROIs). Therefore, the aim of the current study was to combine these two approaches to DTI data analysis in adolescents after sports-related concussion and healthy matched controls. Combining tractography with TBSS that is independent of \textit{a priori} hypotheses of injury location provides the opportunity to extend the understanding of global changes in diffusion behavior in whole-brain white-matter tracts to determine whether (1) there are common regions of white-matter changes associated with sports-related concussion in adolescents and (2) fiber-tract pathways passing through these regions are also affected by concussion.

**Methods**

Twelve adolescents with a clinical diagnosis of subacute (≤2 months before) sports-related concussion and 10 healthy, physically active adolescents matched for age, gender, and physical activity level (Table 1) participated in this study. Adolescents with other focal neurologic deficits, pathology, and/or those on prescription medications for neurological or psychiatric conditions were excluded. Recruitment occurred through the BC Hockey, Minor Hockey division. Players were recruited from the Bantam (13–14 year olds) and Midget (15–17 year olds) teams. All participants were right handed. The team coach and/or physician made the diagnosis of concussion. Parents signed an informed consent form that was approved by the University of British Columbia (Vancouver, British Columbia, Canada), and all participants provided assent. We have previously published results using data from the same sample. \textsuperscript{14} These previous results described differences between diffusion-based metrics of whole-brain reconstructed fiber tracts between individuals with concussion and control participants.

Whole-brain high-angular resolution diffusion imaging (HARDI) was conducted at the University of British Columbia 3T Research Facility on a Philips Achieva 3.0T MRI scanner (Phillips Healthcare, Andover, MD) using an eight-channel sensitivity encoding head coil (SENSE factor = 2.4) and parallel imaging. Two diffusion-weighted scans were performed with a single-shot echoplanar imaging (EPI) sequence [repeat time (TR) = 7013 ms; echo time (TE) = 60 ms; field of view (FOV) = 224 × 224 mm; 70 slices; voxel dimension = 2.23 mm; scan time = 7 min/scan]. Diffusion weighting was performed across 60 different noncollinear orientations (b = 700 s/mm\textsuperscript{2}). Ten minimally weighted (b = 0) diffusion images were also acquired.

The software package, ExploreDTI, \textsuperscript{20} was used for diffusion-imaging data preprocessing and analysis. Initially, diffusion-weighted images were visually inspected before subject motion and eddy-current–induced geometric distortion correction. Tensor estimation was performed using the RESTORE approach, and the data were rigidly transformed to standard Montreal Neurologic Institute (MNI) space for subsequent tractography analyses. Motion- and eddy-current–corrected diffusion-weighted images were used for voxel-wise analysis using tract-based spatial statistics.

To investigate local changes in white-matter structure, voxel-wise analysis of the FA data was carried out using the TBSS\textsuperscript{21} part

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, years (SD)</th>
<th>Gender</th>
<th>Time since concussion days (SD)</th>
<th>Number of concussions (n)</th>
<th>SCAT2 score (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concussion</td>
<td>15.5 (1.2)</td>
<td>10M, 2F</td>
<td>35.6 (15.0)</td>
<td>1 (4), 2(3)</td>
<td>83.5 (7.7)</td>
</tr>
<tr>
<td>Control</td>
<td>15.7 (0.9)</td>
<td>9M, 1F</td>
<td></td>
<td>3(4), 4(1)</td>
<td>88.7 (7.6)</td>
</tr>
</tbody>
</table>

SD, standard deviation; M, male; F, female.
of FMRIB Software Library (FSL).22 TBSS projects all subjects’ FA data onto a mean FA tract skeleton. Between-group statistical analyses of the skeletonized data using FSL’s randomise tool were conducted to measure voxel-wise differences in FA between concussed and control participants. Threshold-free cluster enhancement23 and multiple comparison correction were carried out, and bidirectional group contrasts were applied (concussion FA > control FA and control FA < concussion FA). The loci of intergroup differences in the skeletonized FA data were identified anatomically in MNI space using a standard white-matter atlas.24 These ROIs were then used to seed subsequent tractography-based analyses to examine long-range structural connectivity between these regions and other areas of the brain.

Whole-brain tractography was first performed using a deterministic streamline approach.18 FA thresholds for initiating and continuing tracking were set to 0.2, and the tract-turning angle threshold was set to 30 degrees. Fiber-tract pathways of interest were extracted for each subject individually using a seed mask containing the significant voxel clusters from TBSS group analysis. No other constraints were placed on fiber-tract pathway reconstruction. Properties of white-matter microstructure were represented by FA, MD, AD, and RD values averaged across each pixel comprising the reconstructed tracts of interest.

A multivariate analysis of variance (MANOVA) was performed to evaluate group differences in DTI-based measures of white-matter integrity (FA, MD, AD, and RD) in the reconstructed tracts of interest. Number of pixels reconstructed was also included to provide an index of tract integrity. Individual one-way (group) analyses of variance ANOVAs were then conducted for each measure. For all tests, \( p < 0.05 \) was considered statistically significant. A parametric correlation analysis using Pearson’s \( r \) was performed to evaluate relationships between DTI-derived measurements (FA, MD, AD, and RD) within the reconstructed fiber tracts of interest and characteristics of participants from both groups, including SCAT2 score, number of concussions sustained, and time since last diagnosed concussion. A critical \( \alpha \) of

\[
\begin{array}{|c|c|c|c|}
\hline
\text{Cluster index} & \text{Volume (mm}^3\text{)} & \text{Minimum } p \text{ value} & \text{Anatomic location of MNI coordinates of center of gravity} \\
\hline
1 & 754 & 0.046 & \text{Right anterior corona radiata, inferior fronto-occipital fasciculus, anterior thalamic radiation, uncinate fasciculus} \\
2 & 709 & 0.042 & \text{Left anterior corona radiata, forceps minor, anterior thalamic radiation} \\
3 & 610 & 0.048 & \text{Right anterior thalamic radiation} \\
4 & 285 & 0.048 & \text{Right forceps minor, anterior thalamic radiation, inferior fronto-occipital fasciculus} \\
5 & 148 & 0.050 & \text{Right inferior longitudinal fasciculus} \\
6 & 146 & 0.050 & \text{Left anterior thalamic radiation} \\
7 & 91 & 0.050 & \text{Fornix} \\
8 & 50 & 0.050 & \text{Left anterior thalamic radiation} \\
9 & 43 & 0.050 & \text{Left inferior fronto-occipital fasciculus, anterior thalamic radiation, uncinate fasciculus} \\
10 & 36 & 0.050 & \text{Left subcallosal cortex} \\
11 & 35 & 0.050 & \text{Right anterior thalamic radiation} \\
12 & 22 & 0.050 & \text{Right anterior thalamic radiation, forceps minor, inferior fronto-occipital fasciculus, uncinate fasciculus} \\
13 & 16 & 0.050 & \text{Right anterior thalamic radiation} \\
14 & 12 & 0.050 & \text{Left anterior thalamic radiation, inferior fronto-occipital fasciculus, uncinate fasciculus} \\
15 & 9 & 0.050 & \text{Right thalamus} \\
16 & 3 & 0.050 & \text{Right forceps minor, genu of corpus callosum} \\
\hline
\end{array}
\]

FA, fractional anisotropy.

FIG. 1. Results from tract-based spatial statistics analysis showing clusters of voxels within the white-matter skeleton with significantly elevated fractional anisotropy values in the concussed group (corrected \( p < 0.05 \); Table 2). For visualization purposes, the skeletonized results were thickened. Clusters were primarily located in anterior associative and descending motor output tracts.
p < 0.05 was considered significant without adjusting for multiple comparisons.

Results

Results from TBSS analysis between the concussed and control groups generated multiple clusters of voxels with significantly elevated FA values (concussed > control contrast) within the white-matter skeleton. These results are summarized in Table 2 (corrected p < 0.05). Clusters were primarily located in anterior associative and descending motor output tracts (Fig. 1). No significant clusters were identified with reduced FA values in the concussion group with permutation testing (concussed < control contrast).

Results from TBSS-guided deterministic fiber tractography produced robust fiber-tract reconstruction for each subject, regardless of group designation (Fig. 2A). The MANOVA demonstrated a trend for group differences across DTI-derived measures for the reconstructed tracts of interest (Pillai’s trace = 0.452; F(5,16) = 2.64; p = 0.063). Subsequent results from the univariate ANOVAs demonstrated group differences in AD (F(1,20) = 4.83; uncorrected p = 0.040), with reductions in the concussion group, compared to controls. Trends for group differences were also observed for MD (p = 0.052) and RD (p = 0.096), with lower values in the concussion group. Significant group differences in FA or number of pixels retained were not observed (p = 0.487 and 0.161, respectively).

Table 3 summarizes the results of the correlation analysis. All DTI measures were significantly associated with SCAT2 score, with the exception of mean FA values. The relationship between AD and SCAT2 score is depicted graphically in Figure 3. The number of concussions reported also correlated with SCAT2 score; time since last concussion was not significantly related to diffusion behavior in the reconstructed tracts or correlated with SCAT2 scores.

Discussion

In this preliminary study, we investigated whole-brain changes in white-matter integrity using voxel-wise tract-specific analysis of DTI data and subsequently applied the results to seed fiber

![FIG. 2. Representative example of from tract-based spatial statistics-guided deterministic fiber tractography for a subject from the concussion group (A). Group-averaged differences between the concussed and control athletes for the reconstructed tracts of interest for axial diffusivity (B), mean diffusivity (C), radial diffusivity (D), fractional anisotropy (E), and number of reconstructed pixels (F). Error bars represent standard deviation.](image)

**Table 3. Results of Correlation Analysis**

<table>
<thead>
<tr>
<th>No. of concussions</th>
<th>SCAT2 score</th>
<th>FA</th>
<th>MD</th>
<th>AD</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time since last concussion</td>
<td>Pearson’s r</td>
<td>−0.251</td>
<td>0.179</td>
<td>0.129</td>
<td>0.277</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.502</td>
<td>0.431</td>
<td>0.577</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>No. of concussions</td>
<td>Pearson’s r</td>
<td>−0.523*</td>
<td>0.062</td>
<td>−0.353</td>
<td>−0.407</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.012</td>
<td>0.784</td>
<td>0.107</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>SCAT2 score</td>
<td>Pearson’s r</td>
<td>−0.256</td>
<td>0.489*</td>
<td>0.465*</td>
<td>0.457*</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.251</td>
<td>0.021</td>
<td>0.029</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (two-tailed).

Values in bold represent significant correlations observed (*p < 0.05).

FA, fractional anisotropy; MD, mean diffusivity; AD, axial diffusivity; RD, radial diffusivity.
tissue may be assessed and that could potentially explain the lack of TBSS analysis, a substantially larger, more heterogenous amount of fusion behavior of the reconstructed tracts using the results of the are extracted for the entire pathway(s). When evaluating the differences in a priori independent of a priori hypotheses regarding location of injury. This multimodal diffusion-imaging analysis approach is well suited to investigate white-matter abnormalities after concussion based on the diffuse, subtle, and heterogeneous nature of this type of brain injury. Using this approach, we were able to detect the locations of common sites of FA changes after concussion in adolescents, compared to peers, with similar activity levels.

We would like to emphasize that TBSS evaluates diffusion behavior on a voxel-wise basis for the FA skeleton of major white-matter pathways. This restricts the analysis to the center of white-matter pathways. In contrast, fiber-tract reconstruction uses a deterministic approach to evaluate the diffusion characteristics of every voxel to perform fiber-tract reconstruction. Once the tracts are reconstructed, mean values for each diffusion metric of interest are extracted for the entire pathway(s). When evaluating the diffusion behavior of the reconstructed tracts using the results of the TBSS analysis, a substantially larger, more heterogeneous amount of tissue may be assessed and that could potentially explain the lack of between-group differences in reconstructed tract mean FA values that were observed in significant clusters of the skeletonized FA data produced by the TBSS analysis.

The two primary areas that showed significant differences between the two groups were the corona radiata and the genu of the corpus callosum. The corpus callosum has been implicated in several concussion studies, and is thought to be more susceptible to mechanical injury and may, in fact, be a common point of injury. Similarly, the anterior corona radiata has been hypothesized to be vulnerable in concussion/mTBI. Our main finding of reduced AD in the tracts passing through these regions is consistent with both animal models of TBI and recent work in adults. In addition, we also found that AD, MD, and RD significantly correlated with SCAT2 scores. To our knowledge, this relationship has not been previously reported. Though FA and MD are the most frequent reported measures and are often associated with increased symptoms and decreased clinical function, these markers may indicate general inflammation or edema within the cell and may be less sensitive to specific axonal and myelin pathology (see Shenton and colleagues for review). Decreased AD may reflect structural changes within the axon, which may have a greater effect on overall function, as reflected by the lower SCAT2 scores in the concussed group at a given time point. We should point out that this relationship was observed when evaluating both groups, but not noted when looking at just the concussion group alone. This may be a power issue because of the limited sample size collected in this preliminary investigation. It could also relate to the characteristics of the SCAT2 that may be insensitive to subtle differences between individuals with concussion and healthy controls in the subacute phase of recovery. Last, it could indicate that although subjects in the control group had no history of diagnosed concussion, each individual was involved in a contact sport at the time of assessment, so there is the potential that subtle subclinical changes in diffusion and SCAT2 score present in the control group that contributed to the observed relationship. We believe the addition of Figure 3 provides readers with information to evaluate the potential mechanisms underlying this statistical relationship.

Recently, Wilde and colleagues performed serial DTI assessments in a small sample of mTBI patients over an 8-day period. Interestingly, their results showed that whereas there were...
significant changes in all DTI-derived measures of white-matter microstructure, these changes were characterized by complex trajectories with significant fluctuations in all measures over time. This study highlights the need for sensitive and objective measures to characterize the complex, heterogeneous nature of mTBI.

Combining a voxel-wise analysis approach with in vivo fiber tractography provides a two-step objective technique to evaluate and identify subtle, but potentially meaningful, changes in white-matter microstructural status after concussion. This article presents preliminary findings and has several limitations. The diagnosis of concussion is not based on objective clinical criteria, and thus we have limited information about the extent of the brain injury and the recovery of function that may be taking place within individual participants. We employed a commonly administered concussion assessment tool (SCAT2) that has not yet been validated in the subacute recovery phase or in adolescent populations. However, it does provide an indication of symptomology, cognitive function, and motor control (i.e., balance and coordination). In addition, we had a relatively small number of participants. Despite this, we were able to identify common sites of change in diffusion behavior with a voxel-wise approach, yet we were unable to assess subject-specific changes that may be meaningful based on the heterogeneous location and mechanism of sports-related concussion. We also chose not to impose fiber-tract reconstruction constraints from the seed clusters to other specific brain regions. This limits our ability to identify changes in specific fiber-tract pathways, but does provide an overall indication of the status of white-matter fiber pathways passing through these regions. There are also limitations to fiber-tract reconstruction using DTI data, especially in regions with kissing or crossing fibers, and future work may benefit from incorporating multi-tensor or tensor-free analysis approaches. Despite these potential limitations, our results are consistent with recent reports of the changes in white-matter status after concussion during the subacute phase of recovery and provide an important contribution on the role of a synergistic pairing of two diffusion-based, DTI-based analysis approaches to provide insights into potential common sites of change in white-matter diffusion behavior associated with concussion and the influence of these changes on long-range fiber-tract structural connectivity. Future work could combine other neuroimaging techniques to further enhance the implementation of multi-modal imaging approaches to study the pathophysiology of concussion.

Acknowledgments

This research was supported by the Martha Piper Research Fund from the University of British Columbia, and the Brain Research Center at the University of British Columbia. The authors thank BC Hockey for their support and all the participants, trainers, and coaches who took part in this study. The authors also thank Dr. Alex Rauscher for his assistance with the MRI protocol.

Author Disclosure Statement

No competing financial interests exist.

References


