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Longitudinal assessment of brain structure and function in sports-related concussion.

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A. Original aims of this project

The recovery of cognitive function after mild traumatic brain injury (mTBI) is an important public health issue and the economic impact of mTBI is substantial and accounts for approximately 44% of the $56 billion annual cost of TBI in the United States (Thurman, 2001). Although it is clear that most individuals who sustained a concussion present primarily with acute cognitive deficits and some suffer in addition from physical and emotional symptoms, the nature and course of the structural changes in the brain during the acute and subacute phase of recovery remain an area of intense controversy. Most patients recover within the first two weeks post-injury, but approx. 20% suffer from persistent symptoms resulting in a post-concussion syndrome (Binder, 1997; Alexander, 1995; Rimel, 1981). Results of clinical magnetic resonance imaging (MRI) and CT-scans are within normal range in the majority of patients with mTBI despite the fact that the same patient's neuropsychological tests are abnormal and differences in brain activation patterns have been shown in functional magnetic resonance (fMRI) studies as well (Chen et al., 2004, 2008). Diffuse axonal injury in mTBI has also been reported in autopsy studies (Povlishock et al., 1995) and more recently in preliminary diffusion tensor imaging studies (Niogi et al., 2008b). However, the course of structural recovery (extending from 24-72 hours until 2 months post-injury) and its effect on functional activation patterns, in particular in the prefrontal lobe, has so far not been investigated. Here we propose a longitudinal study on a group of athletes with sports-related concussion to define the neural correlates of the early phase (first 2 weeks) as well as the later phase of structural and functional recovery.

<u>In particular, we are asking to what extent structural changes in the deep white matter during the early phase of recovery predicts long-term outcome in sports-related concussion</u>.

This will be tested through a series of structural and functional scans assessing the relationship between structural and functional changes during the early and late phase of recovery. Specifically, we propose to conduct a longitudinal series of combined diffusion tensor imaging (DTI) and functional MRI experiments on concussed athletes and healthy controls and to relate measures of structural changes to measures of brain activation as well as neuropsychological test scores during the two months following a concussion.

Specific hypotheses to be tested are the following:

1. Over the three time points assessing recovery, structural measures of white matter fiber tract integrity will be different between the concussed and normal control subjects immediately after injury (acute phase) but will approach similar values in the later stages of recovery.
2. Structural measures of prefrontal and posterior periventricular white matter within each phase will correlate with measures of cognitive function (neuropsychological tests, behavioral and brain activation measures of the n-back task, and SAT scores).
3. Mean diffusivity (MD) is more sensitive than fractional anisotropy (FA) at reliably measuring structural abnormalities during recovery of function.
4. The subset of concussed individuals with persistent symptoms will continue to demonstrate structural differences 2 weeks and 2 months post-injury.

Testing the first three hypotheses is particularly crucial to answer the underlined question above. This work will guide our future research beyond the scope of this proposal. The present study is a necessary starting point to define brain mechanisms responsible for structural and functional recovery after mTBI in the short and longer term. A deeper understanding of the structural and functional changes in the brain during the first two months after injury can eventually be used to inform treatment strategies for individual patients, and to assess adjuncts such as pharmacological interventions that could improve outcomes. Results of this study will provide a deeper understanding of the structural changes due to mTBI in sports and can in the future be used as a point of reference in the assessment of mTBI injuries that occur in other settings (civilian and military).

B. Project successes

1) Publications

The work accomplished in this project has so far resulted in two manuscripts, poster presentations as well as invitations for oral presentations at several conferences (see below). One of the two articles has been published and the second one has been submitted for publication.

The first article, entitled: "Persistent Differences in Patterns of Brain Activation after Sports-Related Concussion: A Longitudinal Functional Magnetic Resonance Imaging Study", (J Neurotrauma. 2014 Jan 15; 31(2): 180-8. doi: 10.1089/neu.2013.2983. Epub 2013 Oct 17), presents the results of the fMRI component of this project. The major findings of this project component suggest that functional brain activation differences persist at 2 months after injury in concussed athletes, despite the fact that their performance on a standard working memory task is comparable to normal controls and normalization of clinical and NP test results. These results might indicate a delay between neural and behaviorally assessed recovery after sports related concussion (see appendix for further details).

The second manuscript entitled: "A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports related concussion", was submitted to J. Neurotrauma in January 2014 and is currently under review. In this article we present the results of the DTI data of this project, demonstrating alterations in the microstructure of the deep white matter within 72 hours post injury, followed by a recovery process that may extend beyond 2 weeks (see appendix for further details).

We are currently assessing correlations between brain measures (fMRI and DTI) and cognitive test scores and plan to submit a third manuscript for publication in the next two months.

2) Oral presentations

"Neuroimaging and sports-related concussion"
Concussion Summit of the Athletic Trainers' Society of New Jersey, Princeton NJ, August 2011.

"New insights from advanced neuroimaging into mild traumatic brain injury and repetitive brain trauma: fMRI, DTI and MRS." CIC/Big Ten/Ivy League Traumatic Brain Injury Summit, Chicago IL, July 18th, 2013.

"Women and concussion"
Rutgers-Robert Wood Johnson Medical School Grand Rounds, Department of Obstetrics & Gynecology and Reproductive Sciences, New Brunswick NJ, Sept. 11th, 2013.

3) Poster presentations:

"Persistent differences in patterns of brain activation after sports-related concussion: a longitudinal fMRI study", A Dettwiler, M Murugavel, M Putukian, R Echemendia, V Cubon, J Furtado, D Osherson, Forth International Consensus on Conference on Concussion in Sport, November 1-2, Zurich, Switzerland.

"A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports related concussion", M Murugavel, V Cubon, M Putukian, R Echemendia, D Osherson, A Dettwiler, Society for Neuroscience annual meeting, San Diego, CA, 2013.

C. Project challenges

The most important challenges that we encountered in this project were the technical difficulties with the DTI pulse sequence, given the fact that the arrival of the new Siemens 3-TESLA (skyra) scanner coincided with the beginning of this study. As a result, consistent participation of subjects in all three scanning sessions, for the DTI component of the project was somewhat reduced. We made every attempt to address this problem, by increasing subject enrollment for the DTI component of this study.

D. Implications for future research and/or clinical treatment

Implications for future research are as follows:

1) Given the persistent differences in patterns of brain activation observed in this project, future longitudinal studies should be extended beyond the 2 months post injury time frame. Ideally, such research would involve a multimodal neuroimaging approach and the use of biomarkers that can be correlated to brain structure and function.

2) Based on the lack of recovery of brain measures after two weeks post injury when most athletes tested neurocognitively within normal range and hence were sent back to play, future studies should include pre/post season MRI scans to assess the effects of repetitive sub-concussive hits on brain structure and function.

3) Only three concussed females were enrolled in this study and hence, no gender specific analyses were possible. In addition, inspection of individual trajectories of recovery revealed a noticeable difference between female and male athletes. Future

longitudinal studies are needed to address gender differences, and to specifically assess patterns of neural recovery in female athletes.

Implications for clinical treatment:

1) Results of this study suggest an extension of the recommendation by the American Pediatric Association to college age athletes, proposing that 'return to play' should be delayed at least to 1-month post injury for high school student athletes with sports related concussion.

2) The area in the brain identified in the current and previous study (Cubon et al. 2011) both carried out by our group, might indeed be more vulnerable to the type of injury experienced in sports-related concussion. This particular finding may in the future be of diagnostic value.

E. Plans to continue the research, including applications submitted to other sources for ongoing support.

We are planning to extend this line of research to a project assessing the effects of concussion on the microstructure in the white matter of the adolescent female brain (15-18 years of age). Sports-related traumatic brain injuries in female adolescents remain under investigated and no published studies to date have assessed concussed and normal female adolescent brain structure in conjunction with progesterone levels, neuropsychological test results and menstrual cycle phase (luteal vs. follicular). With Title IX of the Education Amendments Act of 1972, many barriers to contact sports participation among young women have been removed and an increase of mTBI in the adolescent females (15-18 years of age) should therefore be expected. Quantifying the effect of progesterone on structural recovery might in the future inform pharmacological treatment of mTBI. Results of this type of study would not only elucidate the effects of sports related concussion in the adolescent female population, but help lay the ground work to uncover gender specific characteristics of sports-related concussion in the future.

A proposal for this project has been submitted to the NJCBIR for an individual grant.

We are also planning on submitting this proposal to NINDS Brain Initiative.

F. Explain how you have leveraged NJCBIR funding to obtain additional federal or other support for brain injury research and list the appropriate funding organizations:

Neuroimaging data collected for the project sponsored by the NJCBIR was included as preliminary data in the proposals presented to the organizations listed below and helped provide evidence that our group has the necessary expertise to bring the proposed project to fruition.

Funding Organizations:

1) The National Operating Committee on Standards for Athletic Equipment (NOCSAE) for a project entitled: "Multimodal neuroimaging: Diffusion Tensor Imaging, MR Spectroscopy and Susceptibility weighted Imaging in association with clinical measures to assess sports-related concussion in varsity level college athletes." Funding period from: 02/01/2011-02/01/2013.

2) Donation from the Goldstein family fund.

F. List of all publications emerging from this research

Publications:

1. "Persistent differences in patterns of brain activation after sports-related concussion: a longitudinal fMRI study", A Dettwiler, M Murugavel, M Putukian, V Cubon, J Furtado, D Osherson. J Neurotrauma. 2014 Jan 15; 31(2): 180-8. doi: 10.1089/neu.2013.2983. Epub 2013 Oct 17.

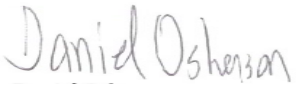
2. "A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports related concussion", M Murugavel, V Cubon, M Putukian, R Echemendia, J Cabrera, D Osherson, A Dettwiler. Submitted to J Neurotrauma, January 2014.

Abstracts:

3. "Persistent differences in patterns of brain activation after sports-related concussion: a longitudinal fMRI study", A Dettwiler, M Murugavel, M Putukian, R Echemendia, V Cubon, J Furtado, D Osherson, Society for Neuroscience annual meeting, New Orleans (2012).

4. "A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports related concussion", M Murugavel, V Cubon, M Putukian, R Echemendia, D Osherson, A Dettwiler, Society for Neuroscience annual meeting, San Diego, CA, 2013.

Submitted by:



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Appendix

Persistent Differences in Patterns of Brain Activation after Sports-Related Concussion: A Longitudinal Functional Magnetic Resonance Imaging Study

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Abstract

Avoiding recurrent injury in sports-related concussion (SRC) requires understanding the neural mechanisms involved during the time of recovery after injury. The decision for return-to-play is one of the most difficult responsibilities facing the physician, and so far this decision has been based primarily on neurological examination, symptom checklists, and neuropsychological (NP) testing. Functional magnetic resonance imaging (fMRI) may be an additional, more objective tool to assess the severity and recovery of function after concussion. The purpose of this study was to define neural correlates of SRC during the 2 months after injury in varsity contact sport athletes who suffered a SRC. All athletes were scanned as they performed an *n*-back task, for $n = 1, 2, 3$. Subjects were scanned within 72 hours (session one), at 2 weeks (session two), and 2 months (session three) post-injury. Compared with age and sex matched normal controls, concussed subjects demonstrated persistent, significantly increased activation for the 2 minus 1 *n*-back contrast in bilateral dorsolateral prefrontal cortex (DLPFC) in all three sessions and in the inferior parietal lobe in session one and two ($\alpha \leq 0.01$ corrected). Measures of task performance revealed no significant differences between concussed versus control groups at any of the three time points with respect to any of the three *n*-back tasks. These findings suggest that functional brain activation differences persist at 2 months after injury in concussed athletes, despite the fact that their performance on a standard working memory task is comparable to normal controls and normalization of clinical and NP test results. These results might indicate a delay between neural and behaviorally assessed recovery after SRC.

Key words: concussion; DLPFC; fMRI; *n*-back task; working memory

Introduction

The Centers for Disease Control and Prevention estimate that 300,000 sports-related concussions (SRC) occur annually in the United States.[1] The study included only concussions for persons who sustained loss of consciousness (LOC), which has been reported to occur in just 8%[2] or 19.2%[3] of sports-related concussions. Given the fact that athletes tend not to report their injury, a more accurate estimate may be that between 1.6 and 3.8 million SRC occur each year in the United States including injuries for which no medical treatment is sought.[4] At the same time, the annual rate of diagnosed concussions over the past 10 years in high school sports rose by 16.5%,[5] which may partly reflect an increased awareness by parents and coaches through increased media attention to concussive injury in sports, as well as better diagnosis and detection by clinicians. Nevertheless, the huge incidence of SRC in adolescents and young adults calls for a full understanding of the neural correlates and consequences of this condition.

According to the most recent consensus statement,[6] concussion is considered a brain injury (caused by a direct blow to the head, neck, or face), involving a complex pathophysiological process, induced by biomechanical forces, typically resulting in the rapid onset of short-lived impairment of neurological function that resolves spontaneously. The authors of this statement affirm that: ''a concussion may result in neuropathologic injury, but the acute clinical symptoms largely reflect a functional disturbance rather than structural injury.''

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This definition is questionable in light of recent neuroimaging research results. Although clinical and cognitive symptoms may subside after approximately 2 weeks in most athletes who sustain a concussion, alteration of physiological brain measures persist. For example, magnetic resonance spectroscopy (MRS) studies have demonstrated neurometabolic alterations lasting up to 1 month post-injury.[7–9] Structural changes from repetitive concussive head impacts have been reported in ice hockey players over the course of a single season,[10] in athletes with prolonged symptoms,[11] as well as in adolescents exhibiting close to normal Sports Concussion Assessment Tool (SCAT) 2 scores at ≤ 2 months postinjury.[12]

One methodology for evaluating the neural consequences of mild traumatic brain injury (mTBI) is blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI), which allows non-invasive evaluation of brain activity based on hemodynamic response to task demands[13–15] The imaging contrast in fMRI results from the higher ratio of oxy- to deoxyhemoglobin in local draining veins that accompanies neuronal activation, which in turn changes local magnetic susceptibility because of properties of hemoglobin.[13,16] fMRI can reveal brain pathology, often enjoying more power than standard clinical measures.[17–19] This makes fMRI an attractive marker for recovery of function after mTBI. fMRI can also be repeated safely, allowing successive measurements.[20,21]

Recent fMRI studies have revealed alterations of the BOLD signal after mTBI when subjects perform working memory, sensory-motor, attention, and other neurocognitive tasks. Specifically, differences in brain activation under varying cognitive load (0, 1, 2, and 3-back conditions in the *n-back* task) were identified in subjects with mTBI.[21] During the first month after injury, patients with mTBI, presenting with a Glasgow Coma Scale (GCS) score of 13–15, demonstrated a significant increase of activation in bilateral frontal and parietal regions during 1-back and 2-back tasks. An fMRI study[22] using a finger sequencing task in four football players with concussion and four uninjured player controls revealed significantly increased activity in lateral frontal, superior, and inferior parietal, and bilateral cerebellar regions within the week after injury. No significant differences in NP test performance were reported between pre-injury and 1 week post-injury conditions. Similarly, larger cluster sizes in the parietal cortex and right dorsolateral prefrontal cortex (DLPFC) and significantly larger BOLD signal percent change in the right hippocampus were identified in athletes with concussion as they performed a spatial memory navigation task in a virtual environment.[23]

On the other hand, fewer task-related activations in athletes with mTBI who had persistent symptoms were found compared with normal controls, when they performed a verbal memory task at one month after injury. In particular, working memory task-related BOLD signal changes were significantly decreased in the DLPFC in all athletes with concussion who had persistent symptoms.[24] A subsequent study[25] reported increased activation peaks in the left temporal lobe in response to a verbal memory task when comparing athletes with concussion and persistent symptoms (at 1 month post-injury) with normal elite athletes; this is in addition to decreased activity in the prefrontal areas.

The present study investigates the neural correlates of functional recovery after SRC during the 2 months after injury in male and female varsity level collegiate athletes. Specifically, we track changes of cortical activation at 2 days, 2 weeks, and 2 months post-injury in contact sports athletes who had sustained a concussion without LOC. The first time point (2 days post-injury) was chosen to assess brain function during the acute phase of concussive injury. Whereas neurometabolic changes during the first 3–6 days post-injury are well documented,[8,9] much less is known about neural function in the acute phase. The 2-week time point was chosen to assess functional brain activation at a time of recovery when the majority of concussions (80–90%) are thought to resolve (cognitive function returning to normal).[6] The third time point (2 months post-injury) provided the opportunity to track patterns of recovery in subjects whose brain activation continued to be atypical at 2 weeks (compared with normal control subjects) and whose NP test results had not returned to baseline at 2 weeks post-injury.

Based on the studies discussed previously (suggesting alterations in DLPFC, parietal, and temporal areas), we designed a working memory task inspired by Hockey and associates,[26] that allows assessment of the frontoparietal network during the 2 months after injury. We asked whether atypical brain activation in response to memory load persisted after persons with SRC were symptom free and tested neuropsychologically within normal range. Our results provide information about the appropriate timing of return to play and substantiate the idea that fMRI is a useful tool for the assessment and management of concussion.

Methods

Subjects

Participants in this study included 15 varsity level college students who sustained a SRC (12 male, 3 female; mean age 19.8, standard deviation [SD] 0.94 years). The concussions of all 15 athletes were diagnosed by University Athletic Medicine personnel using the third International Consensus Conference definition.[27] History of concussion was obtained through self-report. It should be noted that it is difficult to evaluate number of previous concussions objectively in contact sport athletes, given the limitations and subjectivity of self-report. On this basis, eight subjects had no history of concussion. Five subjects had sustained one previous concussion, one subject had two, and the remaining subject had sustained three previous concussions (mean time since last concussion 2.94, SD 1.99 years). Based on review of medical history and physical examinations performed by University Athletic Medicine personnel, no subject had a history of medical, genetic, or psychiatric disorder.

All subjects with concussion were enrolled in the Princeton University Concussion Program for high-risk sports. This program includes baseline NP testing using Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT)[28] and SCAT 2,[27] history, and physical examination as well as post-injury evaluation and testing. Post-injury testing included SCAT 2, hybrid NP tests from ImPACT and paper/pencil tests administered within 24–48 hours of injury; test results were interpreted by a consulting neuropsychologist. The following tests were included in the paper pencil tests: (1) Brief Visuospatial Memory Test-Revised,[29] (2) Hopkins Verbal Learning Test-Revised,[30] (3) Symbol Digit Modalities Test,[31] (4) Digit Span Test,[32] (5) Trail Making Test,[33] (6) The Stroop Test,[34] (7) Patient Health Questionnaire,[35] and (8) Generalized Anxiety Disorder Test.[36]

After their most recent SRC, subjects included in this study were evaluated by a certified athletic trainer and team physician. None of the subjects demonstrated symptomatology warranting further assessment by the GCS[37] or the use of computed tomography (CT) scan or any other clinical imaging. All subjects tested abnormally on paper/pencil and computerized ImPACT tests[28] administered immediately after injury (within 24–48 hours). A given athlete's degree of abnormality was determined through the comparison of post-injury NP test scores to that athlete's baseline scores. In particular, abnormality of ImPACT clinical composites was based on Reliable Change Indices at the 0.8 confidence interval.[28,38] After concussion, individualized management and return-to-play decisions were made by the team physician. Typically, athletes are kept out of activity until their symptoms resolve and their balance and

Table 1. Subject Demographics of Athletes with Concussion and Matched Controls

Concussed								Controls		
Subject	*Sex*	*Age*	*Sport*	*#Previous concussions*	*NP normal*	*Symptom free*	*Return to play*	*Sex*	*Age*	*Sport*
1	F	19	Field hockey	2	13 days	12	No return to play	F	19	Volleyball
2	M	19	Football	0	2 mo[a]	14	No return to play	M	19	Crew Heavyweight
3	M	19	Water polo	0	6 days	6	24 days	M	19	Volleyball
4	M	20	Football	1	24 days[b]	17 (1st), 77(2nd)	31 days	M	21	Crew lightweight
5	F	21	Basketball	1	9 days	6	9 days	F	21	Volleyball
6	F	18	Rugby	0	17days[c]	23	No return to play	F	18	Swimming
7	M	21	Ice hockey	0	3 days	5	12 days	M	21	Volleyball
8	M	20	Football	0	6 days	5	15 days	M	18	Swimming
9	M	20	Basketball	1	9 days	6	12 days	M	18	Squash
10	M	19	Football	0	18 days	10	22 days	M	18	Crew heavyweight
11	M	21	Ice hockey	3	15 days	162	No return to play	M	22	Cross country
12	M	20	Lacrosse	0	17 days	10	23 days	M	22	Track
13	M	20	Wrestling	0	11 days	3	18 days	M	22	Track
14	M	19	Ice hockey	1	2 days	4	23 days	M	18	Crew heavyweight
15	M	21	Sprint foot ball	1	13 days	7	16 days	M	22	Track

[a]Not normal at 2 weeks, not repeated until 2 months since season over.
[b]Returned to play after 1st injury at 31 days, sustained a 2nd concussion and decided not to return to play.
[c]No return to play, season was over.

NP testing return to pre-injury levels, at which time they are allowed to initiate a return to play progression and resume an exertional program that gradually increases both their level of exertion as well as their risk for contact following the First International Consensus Conference on Concussion guidelines.[39] Furthermore, the return to play progression is also adapted to each individual athlete, taking into consideration where he or she is in the competitive season and hence to what type of activities he or she might return.

All athletes with concussion agreed to participate in scanning sessions (fMRI) and repeated SCAT 2 and NP testing assessments within 2 days (session 1), 2 weeks (session 2), and 2 months (session 3) post-injury. Additional NP testing, however, was administered between these time points to determine the return to play for each athlete. Healthy control subjects included 15 sex and age matched non-contact varsity athletes (12 male, 3 female; mean age 19.8, SD 1.73 years). They were in good physical condition with no history of head trauma, psychiatric, neurological, or developmental disorder. For controls, data were analyzed from a single scanning session. Concussion and control subject demographics are presented in Table 1. All participants gave written consent to participate in the study, which was approved by the Princeton University Institutional Review Panel for Human Subjects Research.

Working memory paradigm

Subjects performed a working memory task inspired by Hockey and colleagues[26] during the three fMRI sessions. The task was ''*n*-back'' for $n = 1, 2, 3$. Specifically, subjects viewed successive presentations of one of four letters (B, G, Q, and R) chosen randomly in either upper or lower case, appearing at one of the four

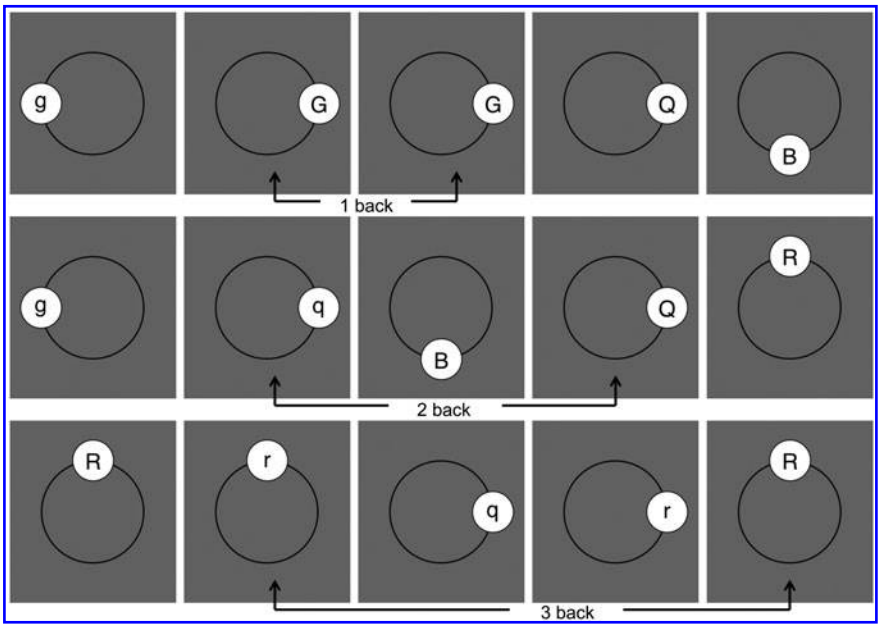

FIG. 1. Illustration of 1-back, 2-back, 3-back tasks.

compass points (Fig. 1). For 1-back, subjects were asked to press a button on a button box when successive images were identical (ignoring case). The 2-back task was the same except that matching images had to be separated by one non-matching image; for 3-back the matching images had to be separated by two non-matching images. Note that matches required that the corresponding letters appear at the same compass point (Fig. 1).

Subjects performed 10 blocks for each of 1-back, 2-back, and 3-back. The 30 blocks were individually randomized and divided into three runs of 10 blocks. Runs were separated by a few minutes. At the start of each block, subjects viewed a screen displaying the upcoming *n*-back task for 2 sec, followed by a blank screen for an additional 2 sec. They then viewed 20 images, each presented for 500 ms, followed by an interstimulus interval of 1200 ms. Between each block, there was a 18.5 sec rest period.

fMRI image acquisition

MR images were acquired on a 3T Siemens (Erlangen, Germany) Skyra whole body scanner with a 16 channel, phase array coil (Siemens). Siemens Skyra EPI PACE sequence was used for fMRI data acquisition with the following parameters: 36 axial slices, $3 \times 3 \times 3\ mm^3$ voxel resolution, repetition time (TR) = 2020 ms, echo time (TE) = 30 ms, flip angle = 76 degrees, field of view = 192 mm.

A single contiguous run comprised 284 volumes with an acquisition time of 9 min and 34 sec incorporating 10 randomized blocks of *n*-back trials with 18.5 sec rest intervals. Three volumes (3 TRs) were incorporated into the task paradigm to ensure syncing with the scanner pulse trigger. Before triggering the start of the run acquisition, additional hidden dummy volumes (3 TRs) were acquired by the scanner for magnetic stabilization. A total of three such runs comprised one fMRI imaging session. The *n*-back trials were programmed in a MATLAB (Mathworks, Natick, MA) environment using Psychophysics Toolbox extensions.[40–42] To support downstream image registration to the MNI (Montreal Neurological Institute) brain atlas and to align functional data across subjects and sessions, a high resolution T1-weighted MPRAGE image was acquired at the end of each fMRI session: 192 sagittal slices, $0.90 \times 0.94 \times 0.94\ mm^3$ voxel resolution, TR = 1900 ms, TE = 2.13 ms, flip angle = 9 degrees, field of view = 240 mm, and a total anatomical scan time of 4 min, 26 sec.

A total of 175 volumes of resting state images with identical imaging parameters as in the working memory task were also acquired before the fMRI *n*-back task sequence. During image acquisition (5 min, 54 sec), subjects were asked to stay awake but keep their eyes closed. Resting state data were acquired for all 15 subjects with concussion at 2 weeks and 2 months. For the 2 days post-injury time point, however, only 12 subjects completed their resting state scan. All 15 controls completed the resting state protocol.

fMRI image data analysis

All imaging data were processed using standard, General Linear Modeling (GLM) based block design routines in FSL-FEAT[43] (version 4.1.9). Preprocessing steps included spatial and temporal smoothening with a full width at half maximum filter (FWHM) of 6 mm (2 voxel spacing), high pass filter cutoff set to 110 sec, and motion correction via the included MCFLIRT algorithm. ''First Level'' analyses GLM used a double-gamma hemodynamic response function to model the three original *n*-back task conditions (1 to 3-back, with respect to the 15 sec rest intervals). Additional contrasts (2-1, 3-1, with respect to the 1-back task) were set up based on the original task conditions for each fMRI run comprising 10 blocks. Individual fMRI runs were coregistered to the MNI 152 standard brain atlas via a 12 degrees of freedom (DOF) linear registration search in FEAT, using the associated high resolution MPRAGE volume. A ''Second Level'' analysis via a ''Fixed Effects'' model was used to collapse the three discrete runs in each session, resulting in beta volumes for the 2-1 and 3-1 contrasts. *Post-hoc* analyses were run on the derived contrasts in the AFNI[44] environment, using 3d analysis of variance (3dANOVA3). All analyses were conducted in fMRI space.

To reduce the number of multiple comparisons, a gray matter mask of the imaged volume was obtained through binary thresholding[45] of the average 152 gray matter T1 volume (included in the FSL suite version 4.1.9) of the co-registered MNI 152 atlas. The ventricles were additionally masked (mask included in the FSL suite 4.1.9). Noise in the fMRI dataset was computed from the First Level residuals of the GLM. AFNI subroutines 3dFWHMx and 3dClustSim were used to compute cluster size thresholds to address the issue of multiple comparisons correction. For this dataset, the cluster size threshold was 31 voxels (in fMRI resolution) with a corresponding p value threshold of 0.005 for an alpha correction of 0.01. In this article, we report results of the between-group 2-1 contrast.

Amplitude of Low Frequency Fluctuation (ALFF) was chosen as a measure of resting state neuronal activity.[46–48] Whole brain ALFF was computed using the acquired resting state data, via AFNI and FSL 4.1.9 functions with a script adapted from www.nitrc.org/projects/fcon_1000. The T1-weighted MPRAGE volume served as the anatomical reference for each subject. The FWHM was set to 6 mm, the frequency band of interest was selected to range from 0.01 to 0.1 Hz, and the standard space was the 3 mm, MNI 152 T1 volume matching the spatial resolution of the resting scan data.

Results

Behavioral data

Each subject (concussed and control) was scored on the three *n*-back tasks via the accuracy statistic: number of correct detections (hits) minus number of incorrect responses (false alarms). The mean accuracy for each group at each of the three time points on each of the three *n*-back tasks is presented in Table 2. The t tests revealed no significant differences between the two groups (concussed vs. control) at any of the three time points with respect to any of the three *n*-back tasks.

For each group individually, 3-back was more difficult than 2-back, which was more difficult than 1-back (all comparisons by paired t test, $p < 0.05$ in all cases). These results suggest that concussed and control subjects were comparably challenged by the *n*-back paradigm. Of the 15 subjects with concussion, all but two tested in the normal range on the ImPACT and paper/pencil tasks by the date of their second scanning session, or a few days later (see column 6 in Table 1).

Imaging

For the imaging analysis, in each group we computed for each voxel the difference in beta values in response to the 2-back minus 1-back tasks (using the latter as baseline). We did not use 3-back for this purpose because of its relatively low performance scores and high variability in both groups. We identified all brain areas that

Table 2. Mean Accuracy (Hits Minus False Alarms) for Controls and Subjects with Concussion during 1-Back, 2-Back, and 3-Back Tasks

	1-back *M (SD)*	*2-back* *M (SD)*	*3-back* *M (SD)*
Controls	35.20 (2.88)	28.73 (4.61)	13.33 (5.12)
Concussed Session 1	36.07 (3.15)	26.53 (5.94)	12.40 (4.00)
Concussed Session 2	35.07 (3.43)	28.93 (7.58)	16.64 (7.08)
Concussed Session 3	35.21 (4.41)	28.36 (8.24)	16.36 (8.34)

SD, standard deviation.

Table 3. MNI Coordinates of the Voxel with the Minimum *P* Value for Each Significant Cluster (between Group Comparison for the 2 Minus 1 *N*-Back Contrast, $\alpha \leq 0.01$, Corrected)*

ROI	*Cluster size voxel count* (*1 voxel = 3×3×3 mm³*)	*MNI coordinates, min.* p *value*		
		x	*y*	*z*
Controls vs. concussed session 1				
Left inferior frontal gyrus, BA10 extending into BA 46	253	−36	57	0
Left cingulate gyrus BA31	212	0	−24	42
Left inferior parietal (supramarginal) gyrus BA40	166	−36	−66	51
Left medial frontal/cingulate gyrus BA6, BA9	140	0	24	51
Left middle frontal gyrus BA8	76	−33	18	57
Left thalamus	58	−3	−6	6
Right inferior parietal (angular) gyrus, BA39	55	39	−69	42
Right postcentral gyrus BA3	35	24	−39	66
Right superior frontal gyrus BA6	34	27	12	63
Right superior frontal gyrus BA11	33	27	60	−12
Right inferior frontal gyrus BA46	32	48	33	18
Controls vs. concussed session 2				
Left inferior frontal gyrus BA10 extending into BA46/44	339	−39	51	15
Left inferior parietal (supramarginal) gyrus BA40	134	−42	−54	54
Right inferior frontal gyrus BA46	46	48	33	18
Left caudate	43	−12	6	12
Right caudate	42	15	15	6
Left medial frontal gyrus BA8	41	−3	24	42
Controls vs. concussed session 3				
Left cingulate gyrus BA31	105	0	−24	42
Right inferior frontal gyrus, BA46	96	45	39	12
Right precentral BA4	87	33	−24	63
Left inferior frontal gyrus BA46	60	−48	33	12
Left parahippocampal (lentiform, putamen)	47	−18	−3	−18
Left precentral	33	−63	−21	24

*The corresponding cluster voxel count is also listed.
ROI, region of interest; MNI, Montreal Neurological Institute.

manifested significant between-group differences in each of the three sessions for the 2-back minus 1-back contrast (3dClustSim corrected $\alpha \leq 0.01$). In every one of these areas, subjects with concussion demonstrated significantly increased activity compared with their age and sex matched controls.

Turning to activation contrasts in particular sessions, subjects with concussion demonstrated significantly higher activation in 11 clusters in session 1, compared with only six clusters in session two and session three (Table 3). Thus, when performing a working memory task at a comparable level of performance to normal controls, subjects with concussion significantly increased their activation in a greater number of brain areas immediately after injury as compared with 2 weeks and 2 months post-injury. The areas showing group differences in activation for all three sessions were limited to left and right prefrontal areas (BA46, extending into BA10 in the left hemisphere). Restricting attention to just sessions one and two, there were significant group differences in the left inferior parietal (supramarginal) gyrus (BA40) as well (Fig. 2). These findings suggest that subjects with concussion demonstrated persistent significantly increased activation not only in the first phase of recovery, but also at 2 weeks and 2 months post-injury.

To study individual differences, we proceeded as follows. We computed the average percent signal change of the voxels in a given subject's brain that were mapped to the left BA46/BA10 cluster that was revealed by the between-group contrast (thresholded at $\alpha \leq 0.01$, corrected). Figure 3 shows the trajectory of these averages for each subject with concussion over the three sessions; the median value of the corresponding averages for control subjects is shown as well. The figure reveals the variability in recovery for subjects with concussion along with a suggestion of overall nonlinearity (increased hyperactivity from session one to two followed by decline). Group differences, however, were swamped by the variability in activation from session one to three; indeed, there was no main effect of session number for any of the neural areas revealed by the 2-back minus 1-back contrast.

It is important to note that by 2 weeks from injury or shortly thereafter, most athletes were symptom free and tested neuropsychologically within normal range. Most subjects returned to play between 9 and 31 days after injury, with the exception of four subjects who did not return to play either because the season ended too soon or because the subject decided not to resume the sport.

To test whether altered resting state activity might contribute to the observed persistent increased activity in the concussed compared with the normal control group, we assessed mean ALFF values in bilateral DLPFC. The session-specific left BA46 mask from the 2-1 between-group contrast served as the primary region of interest for between-group comparisons. The mask was mirrored for each session to create a BA46 mask for the right hemisphere. The mean ALFF values were examined for both left and right BA46 masks for each session. Pooled *t* tests were used to investigate between-group differences of the left minus right BA46 as well as between-group differences for each hemisphere separately, using the mean ALFF measures for each session.

None of these *t* tests produced significant results. That is, we found no between-group differences in resting state in the comparison between left and right BA46; likewise, no differences in

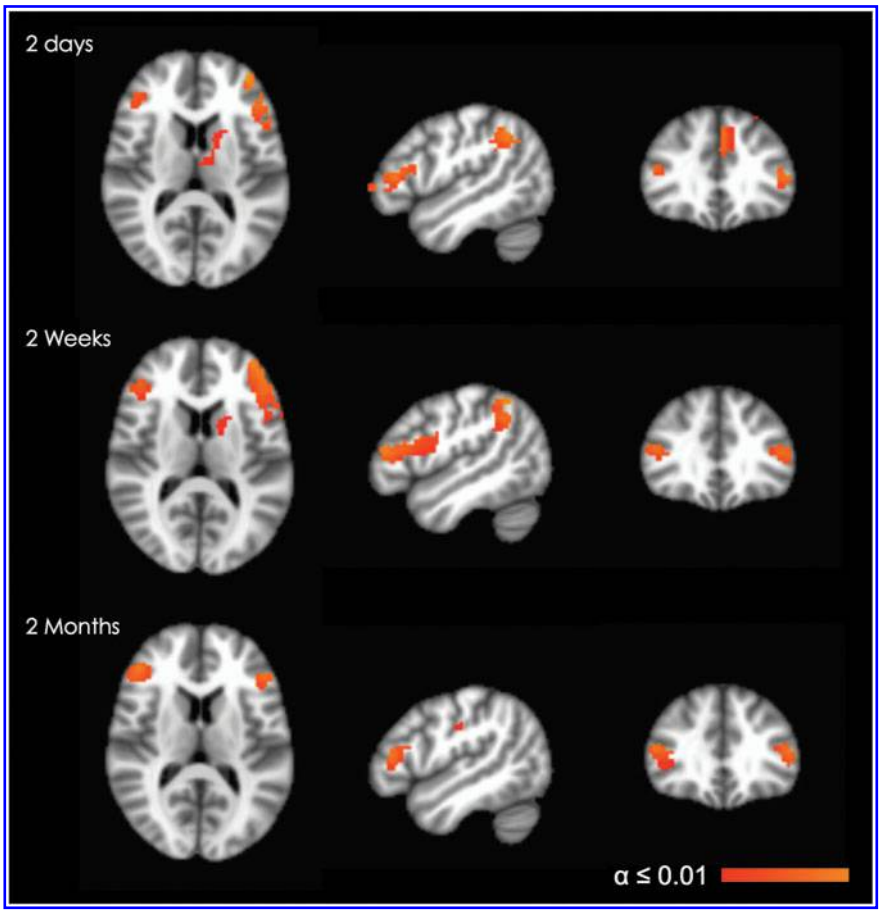

FIG. 2. Between group comparison (concussed–controls), for the 2 minus 1 *n*-back contrast, demonstrated persistent, significantly increased ($\alpha \leq 0.01$, corrected) activity in bilateral, dorsolateral prefrontal areas throughout all three time points and in the left inferior parietal area within 72 hours and at 2 weeks post-injury. Images are shown in radiological convention (right = subject's left) for slice coordinates: x = −48 mm, y = 32 mm, z = 12 mm. Color image is available online at www.liebertpub.com/neu

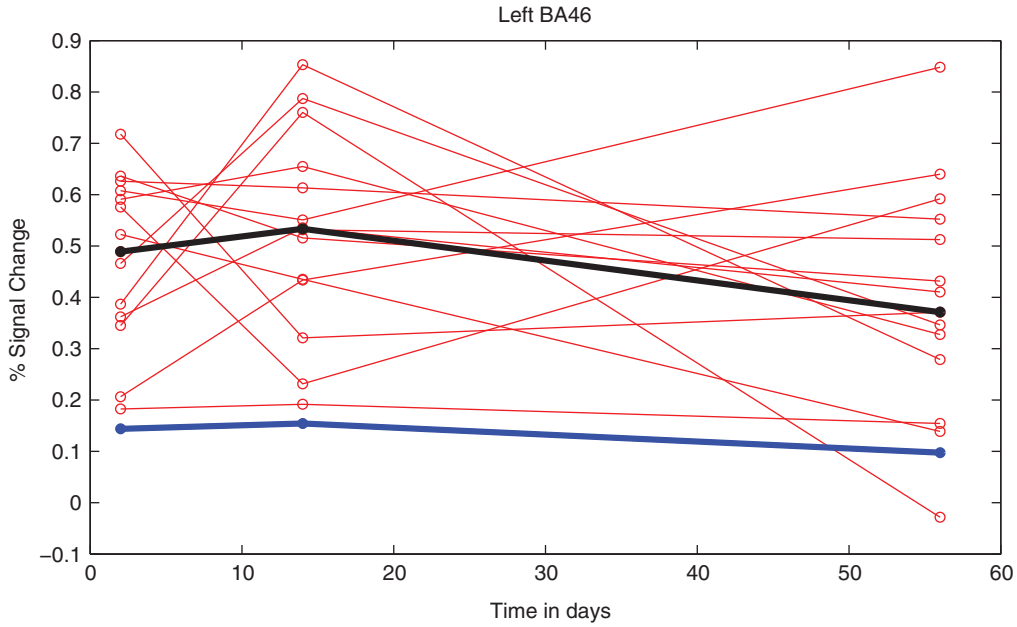

FIG. 3. Individual trajectories (red) of the average percent signal change derived for each subject with concussion in each of the three sessions for the cluster in left BA46 (mask derived from the 2 minus 1 contrast, $\alpha \leq 0.01$ for each session). The median of the concussed (black) and controls (blue) are overlaid. Color image is available online at www.liebertpub.com/neu

mean ALFF reached significance in tests within each hemisphere in any session. These negative results suggest that altered resting state activity does not drive the persistent BOLD signal differences identified in the DLPFC between concussed and normal groups. Rather, it appears that the increased BOLD signal identified in the whole brain analysis for the 2-1 *n*-back contrast in the participants with concussion can be attributed to a task-specific response to working memory load.

Discussion

The present study examined brain activation, clinical symptoms, and cognitive function based on NP testing in a sample of athletes with concussion within 2 days of injury, then at 2 weeks and 2 months later. Previous fMRI experiments have typically investigated changes in brain activation at just one time point, either during the first weeks after injury[49] or at a later stage of recovery.[24,25] Exceptions include a study[22] in which athletes with concussion who had previously participated in a baseline scan were scanned again within 1 week after injury and another experiment[50] in which athletes with concussion were scanned at 1 week after injury and at full clinical recovery. To our knowledge, no previous study has compared brain activation between normal and concussed brains at three temporal landmarks after injury.

Compared with normal control subjects, in response to a verbal/spatial working memory task (*n*-back), our subjects with concussion manifested significantly higher activity in left and right DLPFC (persisting up to 2 months) and in the left inferior parietal area (persisting up to 2 weeks post-injury). In contrast, the subjects with concussion performed the working memory task at a level of performance that is comparable to that of normal controls. Subjects with concussion also demonstrated abnormally high activation in brain regions beyond DLPFC and the inferior parietal area. The number of hyperactive brain regions was greater in the days after injury than at 2 weeks and 2 months.

In scanning sessions one and two, the largest clusters of activation demonstrating significant between-group differences were located in the left frontoparietal network, with hyperactivation persisting bilaterally in the DLPFC through session three. The hyperactive regions we observed in subjects with concussion are typically activated in the normal population only for high load conditions in the *n*-back task.[51,52]

The hyperactivation in bilateral DLPFC and inferior parietal areas is in close agreement with the results of previous working memory fMRI studies in athletes with SRC.[22,49,50] It also corresponds to findings involving subjects with mTBI who had a GCS score of 13–15.[21] It should be mentioned, however, that significantly decreased BOLD signal (hypoactivation) was reported in the DLPFC when athletes, diagnosed with post-concussive syndrome, performed a working memory task.[24,25] These opposite findings are likely because of methodological differences between the two studies. The studies differed in the choice of working memory tasks, timing of fMRI scan (the average was 4–5 months after injury), and history of multiple concussions.[24,25] Moreover, the subjects[24] exhibited prolonged symptoms for several months after injury, whereas in our sample, only one subject was symptomatic beyond 2 months.

Beyond SRC, the majority of imaging studies on working memory report differences in brain activation (increased activation in prefrontal regions and elsewhere) when comparing healthy controls with clinical populations with severe TBI or other neurological disorders.[53–56] In cases of mild brain dysfunction, where performance on the working memory task may not differ between the experimental and control groups, additional recruitment of neural resources has usually been interpreted as neural compensation in the face of cognitive deficits.[21,53,57,58] This hypothesis posits transient alteration of brain function to support task performance without permanent alteration of the underlying brain structure.

Neural compensation may be contrasted with the hypothesis of brain reorganization, which assumes that additional DLPFC recruitment reflects structural alteration and changes in functional networks associated with working memory.[54,59–61] Consistent with neural reorganization, DTI studies provide preliminary evidence of alteration in white matter fiber tracts in persons with SRC. These studies involved athletes with prolonged symptoms[11] among college varsity football players[62] and male ice hockey players.[10] The latter study was prospective, assessing brain structure in players in a pre- and post-season DTI scan.[10]

Yet another interpretation has been articulated, termed latent support hypothesis.[63] According to this idea, the hyperactivation of the prefrontal cortex observed in mTBI is representative of the engagement of additional cognitive and attentional resources to meet the task demands in a challenged neural system.[64,65] Neither structural alteration nor bolstering cognitive functioning is posited. Instead, the latent support hypothesis[63] suggests that the hyperactivation of the prefrontal cortex and (depending on the task demands) in the inferior parietal areas[26,51,56,66] is from increased cognitive and attention control similar to what is observed in load manipulations with healthy controls.[51,52,67]

Returning to the data presented here, the fact that no between-group differences in BOLD signal were found in bilateral DLPFC cortex during the resting state suggests that hyperactivation of the DLPFC is task driven. In turn, this discourages the thesis of structural brain reorganization, consistent instead with either neural compensation or latent support. Obviously, the relative merits of the foregoing hypotheses (specifically, the extent of structural brain reorganization after concussion) can only be resolved through future longitudinal studies based on both fMRI and DTI. Special attention should be devoted to possible structural alterations in the deep white matter.[10,11,62]

In the present study, the marked variability in the individual athlete's pattern of change in brain activation was unexpected, and its cause remains unclear. Still, most subjects demonstrated a non-linear, altered pattern of brain activation compared with their normal controls with a trend toward increased hyperactivity in the DLPFC at two weeks followed by a decline toward 2 months post-injury. In contrast, with one exception, all athletes with concussion tested neuropsychologically within normal range between 2–3 weeks after injury. Ten subjects returned to play between 2–3 weeks, and one subject returned to play at 1 month after injury. For three of the remaining four subjects, the season ended before being allowed to return to play. The remaining subject decided to discontinue her sport because of prolonged symptoms. The persistence of brain hyperactivation despite the fact that the athletes have returned to the normal range in NP testing (and often resumed play) raises the concern that athletes might be exposed to further concussive (and sub-concussive) shocks before the brain is fully recovered.

The discrepancy between normal NP test results at 2 weeks and hyperactivation in the DLPFC up to 2 months after injury might be explained by lack of sensitivity of the NP testing battery (ImPACT, paper pencil tests). Along with the resolution of symptoms and normalization of balance deficits, NP testing, especially hybrid testing protocols, are currently the best practices available for determining when athletes have recovered from SRC. Of course, the persistent hyperactivation in DLPFC until 2 months post-injury

raises the question of whether brain activation will ever normalize. The clarification of this issue seems vital for deciding when it is safe to return to play. In particular, correlations of NP test results with associated brain measures should be investigated further. The issue is especially pressing given recent results of neuropathological studies, indicating that repetitive injury might result in neurodegenerative changes later in life.[68,69] Given the millions of amateur and professional athletes, as well as military personnel, who are exposed to repetitive concussions, it seems imperative to further elucidate when and if concussion-induced changes in brain activation might remit.

Conclusion

The longitudinal nature of this study advances our understanding of the neural correlates of SRC by demonstrating alteration of brain activation subsequent to a return to normal scores on NP tests. Future research should include longitudinal experimental designs based on larger samples and scanning beyond 2 months post-injury. Ideally, such research would involve a multimodal neuroimaging approach and the use of biomarkers that can be correlated to brain structure and function.

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Author Disclosure Statement

No competing financial interests exist.

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A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports related concussion

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A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports related concussion

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Running title: A longitudinal DTI study of WM tracts after SRC

Abstract

The extent of structural injury in sports related concussion (SRC) is central to the course of recovery, long-term effects, and the decision to return to play. In the present longitudinal study, we used diffusion tensor imaging (DTI) to assess white matter (WM) fiber tract integrity within two days, two weeks and two months of concussive injury. Participants were right-handed male varsity contact-sport athletes (20.2 ± 1.0 years of age), with a medically diagnosed SRC (no loss of consciousness). They were compared to right-handed male varsity non-contact-sport athletes serving as controls (19.9 ± 1.7 years). We found significantly increased radial diffusivity (RD) in concussed athletes ($n = 12$, paired t-test, tract based spatial statistics, $p < 0.025$) at two days when compared to the two week post injury time point. The increase was found in a cluster of right hemisphere voxels, spanning the posterior limb of the internal capsule (IC), the retrolenticular part of the IC; the inferior longitudinal fasciculus, the inferior fronto-occipital fasciculus (sagittal stratum) and the anterior thalamic radiation. Post hoc, univariate, between group (controls vs. concussed) mixed effects analysis of the cluster showed significantly higher RD at two days ($p = 0.002$) as compared to the controls; with a trend in the same direction at two months ($p = 0.11$). Results for fractional anisotropy (FA) in the same cluster showed a similar, but inverted pattern; FA was decreased at two days and at two months post injury when compared to normal controls. At two weeks post injury no statistical differences between concussed and control athletes were found with regard to

either RD or FA. These results support the hypothesis of increased RD and reduced FA within 72 hours post injury, followed by recovery that may extend beyond 2 weeks. RD appears to be a sensitive measure of concussive injury.

Keywords Sports related concussion, mTBI, diffusion tensor imaging, radial diffusivity, longitudinal study

Introduction

The diagnosis of mild traumatic brain injury (mTBI) is often hindered by exclusive reliance on neurocognitive and clinical symptoms based on patient self-report. A more promising approach is to exploit radiological evidence from Magnetic Resonance Imaging (MRI) and computed tomography. Conventional clinical imaging techniques used to exclude intracranial hemorrhage or skull fracture, do not have the sensitivity to identify alterations in the neural microstructure resulting from mTBI. Advanced neuroimaging techniques, in particular, diffusion tensor imaging (DTI) are therefore worth exploring. The present study reports the use of DTI to assess White Matter (WM) fiber tract integrity in the brains of college athletes who sustained a Sports Related Concussion (SRC), one source of mTBI.

Sports are indeed a major cause of mTBI (often called “concussions”). A study by the Centers for Disease Control and Prevention estimates that 300,000 SRC’s occur annually in the United States.[1] However, this study only included concussions for which the person reported loss of consciousness, which is thought to characterize only a fraction of SRCs.[2,3] Given that athletes often do not report their injury, a more accurate approximation may be that 1.6 to 3.8 million SRCs occur each year including concussions for which no medical treatment is sought.[4]

According to the most recent consensus,[5] typical concussive injury results in the rapid onset of short-lived impairment of neurological function that resolves spontaneously. The authors of this statement affirm that: ‘‘a concussion may result in neuropathologic injury, but the acute clinical symptoms largely reflect a functional disturbance rather than structural injury’’. This claim is questionable in light of recent neuroimaging research. Although clinical and cognitive symptoms may subside after approximately two weeks in most concussed athletes, neurological alterations can persist. For example, magnetic resonance spectroscopy (MRS) studies have demonstrated neurometabolic changes after SRC lasting up to 1 month post-injury.[6–9] Similarly, in a functional magnetic resonance imaging study, hyperactivation of the dorsolateral prefrontal cortex was found to persist beyond two months post injury in athletes whose symptoms subsided at two weeks after injury.[10] DTI studies demonstrating structural changes from repetitive concussive head impacts have been reported in ice hockey players over the course of a single season,[11] in athletes with prolonged symptoms[12] as well as in adolescents exhibiting close-to-normal scores on the Sports Concussion Assessment Tool (SCAT2)[13] at two months post injury.[14]

Compared to standard MRI, DTI offers a more sensitive assessment of focal ischemic lesions and diffuse axonal damage.[15] Specifically, DTI provides information about the WM microstructure and fiber tract integrity by measuring the Brownian motion of water molecules in the brain.[16–18] Diffusion properties of

water in tissue can be either isotropic or anisotropic. In tissues with isotropic diffusion, water molecules diffuse equally in all directions. Isotropic diffusion is typically found in the gray matter of the brain. In the anisotropic case water has a preferred direction of diffusion. Anisotropic diffusion is typically found in tissue with strong directional organization such as the deep WM where axons form tightly packed fiber bundles. In such tissue, diffusion is normally highly restricted along the fiber membranes. Measures of anisotropy thus provide information about the WM microstructure and WM fiber tract integrity,[16–18] which is undetectable by conventional MRI methods.

DTI allows information about multiple diffusion gradients in a given tissue to be combined. A derived measure known as “fractional anisotropy” (FA) can then be used to quantify the degree of preferred diffusion direction in each voxel.[19] Overall diffusion in a tissue is measured by “mean diffusivity” (MD), which is calculated as the mean of the three eigenvalues of the diffusion tensor.[20] The eigenvalues of each directional vector can also be examined independently. The eigenvalue of the first eigenvector (also referred to as parallel diffusivity) was selectively altered in the presence of acute axonal damage in retinal ischemia in mice.[21] Similarly, “radial diffusivity” (RD), the mean of the second and third eigenvalues[18] may be selectively sensitive to alterations of the myelin sheath, as demonstrated in an animal model[22] and more recently in optic neuritis in humans.[23] These findings lend support to the sensitivity of diffusion measures with regard to specific pathologies.

Decreased FA has been reported in mTBI patients with a Glasgow coma score (GCS) of 13-15 within 24 hours post injury.[24] WM abnormalities have also been shown[25] in patients with mTBI exhibiting persistent cognitive impairment (eight months to three years post injury). The latter investigators demonstrated decreased FA and increased MD in the corpus callosum, bilateral capsula interna and other subcortical WM structures. Significant correlations between decreased FA (corpus callosum, capsula interna, and centrum semiovale) within 10 days post injury and neuropsychological (NP) test scores obtained at 6 months post injury have been reported as well.[26] Abnormalities of WM microstructure in mTBI patients with persistent cognitive impairment have been found[27] in the anterior corona radiata, the uncinate fasciculus, corpus callosum, inferior longitudinal fasciculus, and the cingulum bundle. Furthermore, significant correlations between attentional control and FA were found within the left anterior corona radiata as well as memory performance and FA within the uncinate fasciculus.[28] A DTI study on patients with mTBI[29] demonstrated increased FA and decreased RD in the subacute phase after injury and subsequent partial normalization of FA values in left corona radiata and splenium. These studies provide evidence that anisotropy measurements cannot only be used to assess alterations in the microstructure of the WM but also provide a biomarker of cognitive function and dysfunction. Such markers may prove critical in refining the diagnosis, prognosis and management of mTBI. It should, however, be emphasized that the DTI studies using measurements of anisotropy discussed so far include diverse

individuals with mild TBI and a GCS ranging between 13-15; athletes were not targeted for investigation. Although the mechanism of injury in SRC is believed to be comparable to non sports related mTBI, SRC represents the mildest form of mTBI. Most individuals with SRC will not score below 15 on the GCS, but will present with rapid onset of short-lived neurological impairment; they typically show no structural changes in traditional MRI and CT scans. It therefore seems prudent to exploit DTI technology to separately examine the case of SRC, especially given the prevalence of this condition (see above).

Only a few studies have assessed structural changes in adult athletes with SRC who do not score below 15 on the GCS. Increased RD and axial diffusivity after repetitive concussive head impacts in adult ice hockey players over the course of a single season were observed in the right precentral region, corona radiata and the anterior, posterior limb of the internal capsule[11]. Decreased FA (in temporo-occipital WM) and lower cognitive function (CogState[30]) were found to be associated with high frequency heading rate (> 885–1800 headings per year) in amateur soccer players.[31] In college athletes exhibiting prolonged symptoms after SRC, increased MD has been reported in parts of the left inferior/superior longitudinal and fronto-occipital fasciculi, the retrolenticular part of the internal capsule, posterior thalamic and acoustic radiations.[12] Persistent microstructural alterations in deep WM have been shown in female contact sports athletes at 7 months post injury;[6] all participants were symptom free at this point of their recovery, suggesting that in female athletes, structural recovery may lag behind

behaviorally assessed recovery by up to 7 months post injury. Finally, changes in WM microstructure were observed in a cohort of contact sports athletes with subconcussive blows to the head (26-399 hits), whereas no such changes were identified in six control participants.[32]

There is thus growing evidence suggesting that even in the absence of clinically symptomatic concussions, i.e. subconcussive hits [31–33] or at a stage of recovery when athletes are symptom free[6] they are likely to exhibit WM alterations when advanced neuroimaging techniques are used to examine their brains. These findings suggest, that DTI may be a useful imaging tool to assess the severity of a concussion and may provide a biomarker for structural injury. DTI examination of the brain may thus serve to monitor the reorganization and reversal of WM injury, and to predict recovery. The aim of the present study was to track changes of WM fiber tract integrity during the two months following SRC using advanced DTI.

Materials and Methods

Participants

All concussed participants were varsity level college students enrolled in the Princeton University concussion program for high-risk sports. The program ensures systematic documentation of athletic history, physical exam, and baseline NP testing including SCAT2[13] and Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT).[34] Princeton's concussion program also provides acute care and long term monitoring. All athletes involved in this study were diagnosed with a concussion by team physicians using criteria outlined by the 4th International Consensus Conference on Concussion in Sport.[5] Post-injury testing included SCAT2, traditional "paper and pencil" NP tests, ImPACT, the Patient Health Questionnaire (PHQ-9)[35] and the Generalized Anxiety Disorder (GAD-7) questionnaire.[36] The PHQ-9 and the GAD-7 are assessments for depression and generalized anxiety respectively. The baseline and post-injury testing protocols were identical to those described in our earlier publication.[10]

Following their most recent concussion, a certified athletic trainer and team physician at the University Health Services evaluated athletes within 48 hours post injury. None of the athletes experienced a loss of consciousness and their overall symptomatology did not warrant further assessment by the Glasgow

Coma Scale or the use of a clinical radiological exam. All concussed athletes underwent NP testing within 24-48 hours after injury. Abnormal NP performance was determined through comparison of post injury NP scores to the athlete's baseline scores. Specifically, abnormality of ImPACT clinical composites was based on Reliable Change Indices at the 0.8 Confidence Interval.[34,37] Similarly, scores on the traditional NP test performance were examined using Princeton specific normative data. Data from both ImPACT and the NP test were integrated and interpreted by an experienced clinical neuropsychologist.

Athletes were kept out of activity until they were symptom free and their clinical exam including balance and NP evaluations were considered to have returned to baseline levels. Return to activity decisions were made by the team physician, who supervised a personalized return to play progression that exposed athletes to gradual increases in physical exertion as per the 1st International Consensus Conference on Concussion guidelines.[38] Athletes were cleared to return to full contact play once they were symptom free at rest, had successfully completed the exertional program and were neurocognitively functioning at baseline levels.

A total of 21 right-handed, male, varsity level contact sport athletes (mean age 20.19, S.D. 1.03, age range 18-22 years) who suffered a SRC were enrolled in the study. In addition to having no contraindications to MR imaging, participants had no self reported history of medical, genetic, or psychiatric disorder. History of concussion was obtained through self-report. It should be noted that under-

reporting of concussion by athletes has been suggested in previous studies.[39] An objective evaluation of the number of previous concussions in contact sport athletes is therefore difficult. Among the pool of 21 concussed athletes, 12 reported no prior history of concussion, five-reported one prior concussion; three reported two prior concussions and one reported three prior concussions. The mean time since the last self reported concussion for the latter nine concussed athletes was 2.75 years, SD 3.02 years; see Table 1.

< Please insert Table 1 here>

Healthy control participants included 16 age matched, right-handed, male varsity non-contact athletes (mean age 19.9, S.D. 1.67, age range 18-22 years), with no contraindications to MR imaging and no self reported history of prior head trauma, psychiatric, neurological or developmental disorders. All athletes (concussed and controls) gave written consent to participate in the study, which was approved by the Princeton University's Institutional Review Panel for Human Subjects Research. The concussed athletes were scanned at ~2 days, ~2 weeks and ~2 months post injury. The controls were scanned once. All athletes repeated SCAT2, PHQ-9, GAD-7 and NP testing assessments synchronized with the three imaging sessions of the concussed athletes. Concussed athletes

participated in additional NP testing in between imaging sessions, as clinically indicated and requested by the team physician. There were 8 instances (during a single contiguous time period identified by 'X' in Table 1) when data collection was not possible on concussed athletes in the time interval required by the experimental design of the present study due to hardware maintenance issues. There was also one instance of a concussed athlete deciding to discontinue participation in the study (identified by 'D' in Table 1). A strict data quality assurance protocol (described in the data pre-processing section) resulted in the exclusion of scans for 10 concussed and 2 controls (identified by 'M' in Table 1). In total, 14 controls, 16 concussed athletes at ~2 days, 17 concussed athletes at 2 weeks and 13 concussed athletes at 2 months were included in the analyses (identified by 'Y' in Table 1). From this pool of concussed athletes (see Table 1) only 12 concussed were imaged at both the 2 day time point and at 2 weeks, 11 concussed athletes were imaged at both 2 weeks and 2 months post injury.

Imaging protocol

Diffusion weighted images (single-shot spin echo pulse sequence with parameters adapted from our earlier publication[12]) were acquired with a 16 channel, phase array coil (Siemens, Erlangen, Germany) on a whole body 3T Siemens 'Skyra' scanner; TR = 12100 ms, TE = 96 ms, 70 axial slices, voxel size 1.88 × 1.88 mm^2 in plane, slice thickness = 1.9 mm, field of view = 256 mm, 64 gradient directions, b-value 1000 s/mm^2, 8 volumes with no diffusion weighting (b

= 0), 2 runs, yielding a total scan time of 26 minutes 52 seconds. In order to facilitate image volume registration to the Montreal Neurological Institute (MNI) space, a high resolution T1 weighted MPRAGE image was additionally acquired at the start of each imaging session; TR = 1900 ms, TE = 2.13 ms, 192 sagittal slices, 0.90 × 0.94 × 0.94 mm^3 voxel resolution, flip angle = 9 degrees, field of view = 240 mm and a total anatomical scan time of 4 minutes 26 seconds. Care was taken to minimize subject motion with pre-scan instructions and comfortable neck padding. Participants watched a pre-selected, movie of their choice from an online streaming service during the entire scanning session.

Data preprocessing and quality assurance

All data processing was done within the FSL suite (version 4.1.9).[40] The two averages of the acquired diffusion weighted images of each subject were concatenated in the order of image acquisition and visually inspected for signal drop offs and other imaging artifacts. All acquired data passed visual inspection. Eddy current correction was done for each subject's concatenated dataset, employing the first B0 volume for reference. Each volume's registration parameters from the eddy correction step was then used to implement a strict, quantitative, quality assurance protocol based on recent findings.[41] Mean motion estimates (translation, rotation in three dimensions) were calculated for each group separately. All individual subject-scans with motion estimates greater than three standard deviations from the mean or scans with a net translational motion

estimate exceeding two voxels were excluded in their entirety (10 scans of concussed athletes and 2 controls). The concatenated B vectors corresponding to the applied diffusion gradients were then corrected for motion (rotation component[42]) before FSL function 'dtifit'[43] was applied to fit a diffusion tensor model,[44] generating the three principal eigenvalues $\boldsymbol{\lambda_1}, \boldsymbol{\lambda_2}, \boldsymbol{\lambda_3}$ at each voxel. This step additionally provides scalar diffusion measures of WM microstructure, FA = $\sqrt{((\boldsymbol{\lambda_1} - \boldsymbol{\lambda_2})^2 + (\boldsymbol{\lambda_2} - \boldsymbol{\lambda_3})^2 + (\boldsymbol{\lambda_3} - \boldsymbol{\lambda_1})^2)/(2(\boldsymbol{\lambda_1^2} + \boldsymbol{\lambda_2^2} + \boldsymbol{\lambda_3^2}))}$ and AD = $\boldsymbol{\lambda_1}$. RD = $[\boldsymbol{\lambda_2} + \boldsymbol{\lambda_3}]/\mathbf{2}$ and MD = $[\boldsymbol{\lambda_1} + \boldsymbol{\lambda_2} + \boldsymbol{\lambda_3}]/\mathbf{3}$ volumes were generated using the radial eigenvalues $\boldsymbol{\lambda_2}, \boldsymbol{\lambda_3}$.

Statistical analyses

Between group T-tests, using the function 'randomise', were performed via Tract Based Spatial Statistics[43] (TBSS, FSL version 4.1.9) on the skeletonized WM fiber tracts for all derived scalar diffusion measures of WM microstructure FA, AD, RD and MD. All TBSS processing steps followed recommended guidelines[43]. The 'FMRIB58_FA_1mm' image volume in MNI space, included in FSL version 4.1.9, served as the target for initial non-linear registration[45] of subject FA volumes. The mean WM skeleton based on the included participants FA volumes was thresholded to only include voxels with FA > 0.25 in order to restrict the analyses to the core WM tracts. The brain stem and the cerebellum were removed (mask included in the FSL suite 4.1.9) from all analyses since individual subject variability in brain volumes resulted in omission of inferior parts of these

structures in a few cases. The number of 'randomise' permutations was set at 10000 with Threshold Free Cluster Enhancement (TFCE) option enabled.[46] Between sessions comparisons of the concussed athletes were made via paired T-tests (TBSS, TFCE, all permutations, variance smoothing of 2 voxel sizes) for all four diffusion measures of WM microstructure (FA, AD, RD and MD).

Two Post hoc tests, a traditional univariate Mixed Effects approach[47] and a multivariate bootstrap method[48] were selected to test if regions identified via a whole brain TBSS analysis differed (in terms of diffusion metrics of WM microstructure) between groups over time. The Mixed Effects model incorporates both 'fixed effects' and 'random effects' and is particularly useful in longitudinal studies because of it's ability to deal with repeated measures and missing values (See Appendix A1 for details). The multivariate Bootstrap has the added advantage of accounting for combined responses of identified diffusion measures WM microstructure and is preferred in situations of moderate sample sizes such as this study. (See Appendix A2 for the algorithm). All post-hoc tests were run using the open source statistical software R (http://www.r-project.org).

Results

No between group differences were found in TBSS analyses via pooled T-tests at $p < 0.05$ (two sided), Family Wise Error (FWE) corrected with TFCE option enabled. Significant differences (pointing to structural alterations) were observed in the paired, between concussed sessions T-test (2 days vs. 2 weeks, $p < 0.025$, FWE corrected, TFCE) of the RD measure, with the cluster indicating greater RD values at 2 days as compared to 2 weeks. The significant RD cluster consisted of 469 contiguous voxels in standard space (MNI, FMRIB58_FA_1mm). The regions implicated are all in the right hemisphere; posterior limb of the internal capsule (IC), retrolenticular part of the IC, sagittal stratum (inferior longitudinal fasciculus & inferior fronto-occipital fasciculus) and anterior thalamic radiation. The John Hopkins University (JHU) ICBM-DTI-81 WM and JHU WM tractography atlases[49–51] included in FSL version 4.1.9 were used to determine the anatomical regions referenced. Interestingly, these regions are almost identical to those reported earlier[12] in the contralateral hemisphere. In addition, a trend ($p<0.05$, one sided, FWE, TFCE, two clusters for a total of 348 voxels in MNI space; FMRIB58_FA_1mm) was observed in the FA measure with both clusters overlaying about 58 % of the aforementioned significant RD cluster but with the result trending in the opposite direction. FA values were greater at 2 weeks as compared to the values at 2 days post injury. No paired, significant differences in FA, RD and AD measures were observed between sessions two and three or session one and three.

<Please insert Fig. 1 here>

<Please insert Fig. 2 here>

The RD voxel (paired TBSS, two days vs. two weeks, $p<0.025$, FWE corrected, TFCE) mask was used to download individual mean RD and FA values from all eligible subject's volumes in order to conduct post-hoc between group statistical tests. Fig. 2 illustrates the individual trajectories of the downloaded mean RD values.

The results of the between group, Mixed Effects analyses for RD and FA are presented in Table 2. The results of the Mixed Effects model suggest that RD values are on average significantly higher 2 days post injury (two sided p-value = 0.002) as compared to the controls, but the difference at 2 months represents more of a trend (two sided p-value = 0.11). At two weeks post injury there is no statistical difference between the groups with regard to the RD measure. The FA results show a similar but inverted pattern. FA values are on average lower for the injured athletes at all three time points as compared to the controls. At two days post injury, FA values are significantly lower in the concussed as compared

to the controls (two sided p-value = 0.0008). At two months the differences persist (two sided p-value = 0.044), but at two weeks the average difference from controls is not statistically significant. The results of the multivariate (FA and RD) Bootstrap analysis are presented in Table 3. These results point to significant differences between groups at two days and a trend at two months.

<Please insert Table 2 here>

<Please insert Table 3 here>

Mean RD values from the significant RD cluster and its local vicinity within the WM tract i.e. the inflated RD cluster (see Fig. 1) were correlated with mean RD measures of the remaining deep WM tracts to assess if the same trend existed 'globally'. The deep WM tracts for each individual volume in MNI space were masked by JHU ICBM-DTI-81 WM atlas. They were further constrained to include only WM by thresholding the corresponding FA volumes at 0.25. Two tailed p values (testing the null hypothesis of no correlation) of the control group was 0.14 and less than 0.05 for the concussed across all three imaging sessions. Fig. 3 illustrates the individual trajectories of the mean RD values from the aforementioned deep WM region.

<Please insert Fig. 3 here>

Discussion

Results of the current study reveal structural alterations in the deep WM of the brain over the course of the two months following injury. Our primary finding is the significant difference observed between sessions one and two (two days vs. two weeks), within the concussed group in the paired TBSS t-test of the RD measure with greater values at two days as compared to two weeks. In addition, the same TBSS comparison revealed a reverse trend for the FA measure (within concussed session, paired TBSS t-test) with greater values at two weeks as compared to two days post injury with significant overlap of the FA with the RD cluster. The significant RD cluster, spans across parts of the posterior limb, the retrolenticular part of the IC, the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus (sagittal stratum) and extends into the anterior thalamic radiation. Of specific note are two recent TBSS studies; a pilot study of veterans with combat related TBI[52] and a comparable study in athletes with prolonged symptoms after SRC,[12] both of which reported nearly the same anatomic region in the contralateral hemisphere as compared to the significant RD cluster identified in this study. Other research, involving patients with a GCS 13-15, using a broad range of analyses, including TBSS, have reported abnormal diffusion measures in a subset of regions covered by the significant RD cluster reported in the current study. Specifically, such regions were observed in the internal capsule,[24,26,29,32,53–58] in either the inferior fronto-occipital and/or inferior long fasciculus[27,28,59–61] and the anterior thalamic radiation.[60] The current study

lends further support to the an earlier hypothesis,[12] which suggested the prevalence of crossing and merging WM fiber tracts in the anatomic region of the RD cluster might make this particular area more vulnerable to the type of forces acting on the brain during the course of a concussion. This hypothesis posits that certain anatomic regions are more vulnerable to trauma than others, independent of the biomechanical load dynamics of the injury. Finite Element Method (FEM) based reconstructions[62] of head impacts from the National Football League found early strain 'hot spots' along the temporal lobe. These strain 'hot spots' then migrated to the fornix, midbrain and corpus callosum and were manifest in 9 out of 22 concussion reconstructions. A later FEM study[63] correlating FA and MD values in a different ROI (corpus callosum) with computer simulations of the impact appear to show strain resulting in 'hot spots' in the temporal lobe, as well, although secondary in intensity to the corpus callosum. These findings provide additional support for the increased vulnerability of the anatomical regions of the significant RD cluster identified in the current study. Future studies might further elucidate the effect of impact forces by correlating injury mechanism and load dynamics to brain pathology (via post injury in-vivo imaging) with retrospective video analyses coupled to a head impact telemetry system.[64]

Given the variability of patient characteristics and concussive injury mechanisms, one may question the validity of searching for common regions of pathology, which is inherent to any between group, voxel wise analyses of mTBI.[57,65,66] Instead, mTBI may have a unique spatial pattern of injury in each individual

patient's brain. Researchers taking this perspective compare the voxels of individual patients (diffusion measures) in standard space with the corresponding voxel set of a control group. Extreme voxels, deviating either positively or negatively from the control group, are then labeled and clustered (with multiple comparisons correction). The summary statistics of such abnormal loci reported in recent mTBI literature[57,67] reveal significant positive and negative clusters with significant between group differences.[65] Future approaches to tracking recovery of individual concussions should compare the efficacy of the latter techniques against monitoring of diffusion measures over time, obtained from predetermined regions of vulnerability, such as the mask of the significant clusters arising in the current study.

No previous study has assessed the type of SRC examined here (with no LOC) at three time points (2 days, 2 weeks and 2 months). Although our permutation tests on the whole brain WM skeleton did not reveal any significant between group differences, the comparisons of the voxels within the RD cluster showed significant between group difference at 2 days and a trend at 2 months. Closely related studies have demonstrated RD as a useful measure to assess the continuum of the mild end of TBI. RD values have been shown to increase (paired TBSS t-tests) over the course of a season in individual contact sport athletes[11] demonstrating significant increases only in Trace, AD and RD measures when comparing preseason with postseason images. The posterior limb of the IC was reported as a region (among others) with significant

differences in structural measures between pre and post season, which incidentally is a region implicated in the current study. Furthermore a significant increase in RD was found in 3 athletes as compared to the rest of the players in the study, who sustained a medically diagnosed concussion during the course of the season. No significant difference was found in Trace, FA or AD. Another study[33] compared the WM integrity of swimmers to professional soccer players, with exposure to 'headings' (without a symptomatic concussion), and found increased RD in several areas including inferior fronto-occipital fasciculus (a region implicated in the current study). No significant differences were found in the FA and MD measures. These studies[11,33] suggest that RD might be a potentially sensitive measure to sub-concussive hits. A recent DTI study[68] on cerebral WM in 74 boxers and 81 mixed martial arts fighters found that a history of prior knockouts (the 'knockout' measure includes 'technical knockouts' with no subsequent LOC) could predict increased RD in the corpus callosum, isthmus of the cingulate gyrus, pericalcarine sulcus, the precuneus and the amygdala in the group of boxers. The same regions had increased MD and decreased FA values. The 'knockout' measure additionally predicted significantly increased RD in the posterior cingulate in the group of mixed martial arts fighters. In addition they found that the number of prior fights did not predict differences in diffusion measures, suggesting that diffusion measures were sensitive to potential sub-concussive hits or concussions, as opposed to time of exposure to the sport. In a longitudinal mTBI study (GCS 13-15) with imaging sessions at 24 hours, 1 week and 1 month post injury, statistical trends were reported[69] in the paired between

concussed session, based on TBSS t-tests of RD (greater at 24 hours vs. 1 month post injury) and FA (lower at 24 hours vs. 1 week post injury). It should, however, be noted, that the lack of significant differences might have been due to random assignment of participants to two different scanners. A region of interest study[70] reported increased RD in a sample of mild and moderate TBI patients; imaging occurred an average of 8.9 days post injury. Despite the fact that these findings appear to lend support to the sensitivity of RD with regard to mTBI, future DTI studies should additionally assess the validity of RD as a diffusion measure for the assessment of mTBI.

The major finding of the current study is the occurrence of significant temporal changes in radial diffusivity between ~2 days and 2 weeks post injury in a sample of concussed athletes. Multiple cross-sectional mTBI studies with one or more time points[67,69,71] have broadly discussed the coupled, inverse expression of RD/MD and FA measures in the acute and sub-acute phases post injury i.e. increased RD/MD and/or decreased FA or decreased RD/MD and/or increased FA with respect to matched controls. The results of the current study support an earlier hypothesis[24] on the role of focal neurofilament misalignment, as an initializing mechanism leading to decreased FA, increased RD and reduced AD in human mTBI patients (GCS 13-15) imaged around 24 hours post injury.[24,71] Such misalignment had been observed to be manifest within 6 hours of axonal injury in animal models.[72–75] While the increased RD/MD and/or decreased FA mode is frequently reported in mTBI as well as moderate/severe TBI literature[76]

and in studies of sub-concussive hits,[32] there is a lack of consensus on the broad directionality of the diffusion measures after a concussive injury.[29,53,77] Earlier findings of increased FA and reduced RD following mTBI,[29] have been replicated.[67] The authors reported a significant reduction in both the count and the volume of positive clusters representing regions of high FA over a 4-month period with the corresponding reduction in self-reported symptomatology suggesting recovery. Cytotoxic edema[53] was suggested as a potential explanation for the increased FA findings during the recovery interval. A recent longitudinal study[57] assessed individual FA abnormalities in mTBI patients at ~ 2 weeks, 3 months and 6 months post injury. They found that the count of low FA voxels decreases at both 3 and 6 months, but the count of high FA voxels increased at 3 months followed by a decrease at 6 months as compared to their initial assessments at 2 weeks post injury. The authors note that the continued expression of the positive clusters is inconsistent with cytotoxic edema, which drives ionic edema and signals a premorbid cellular process leading to necrotic cell death.[78] In discussing these findings, other researchers[68] suggest the possibility that contact sport athletes represent a distinct population due to their continued exposure to sub-concussive hits leading to constant WM injury and recovery cycles and therefore might present a different recovery profile from the civilian, non-contact sport population suffering a single mTBI episode. It must be noted that at least one study on SRC[79] showed significantly higher FA, AD and lower MD (as compared to non contact controls) values at two time points; ~ 81 hours (on average) and 6 months post injury, suggesting no significant recovery

in diffusion measures during that time interval. Future work is needed to address these observed differences of diffusion metrics during recovery after SRC.

Animal models of TBI additionally support the findings of the present study. For example, a recent controlled cortical impact study on rats[80] showed significantly increased RD and decreased FA in WM. RD may also be selectively sensitive to alterations of the myelin sheath[81] as shown in the mouse model[22,82] and more recently in optic neuritis.[23] Recovery as observed by histology after controlled cortical impact induced TBI in a rat model have correlated with increases in FA;[83,84] this has been attributed to axonal recovery and increased oligodendrocyte generation. A recent histology study scaling biomechanical loads to approximate mTBI in swine found axonal swellings and an accumulation of neurofilament protein.[85] These observations could be expected to increase RD and lower FA according to the focal neurofilament misalignment hypothesis discussed earlier.[24] Further evidence is needed to confirm these findings in humans.

A traditional interpretation of FA increases from 2 days to 2 weeks post injury and corresponding decreases in RD would indicate that patients are recovering from mTBI. This interpretation has been proposed in more severe TBI.[86] The fact that no differences (in all four diffusion metrics considered) were identified between two weeks and two months in the current study, might in part be due to inter subject variability of these measures. The finding of significant between group differences of the cluster at two days provides support for the view that diffusion measures may offer the required sensitivity to assess injuries as mild as the ones

examined in this study. Diffusion measures at the identified anatomic location might have future diagnostic potential as a signature of concussion. Individual subject baselines or a database of normative values in the early phase of concussion might allow for identification of athletes at greater risk of prolonged recovery. There were no significant between group differences at two weeks. While this could be interpreted to be indicative of recovery, it should be noted that inter subject variability could potentially mask an ongoing or unresolved recovery process at two weeks. A future study should include a baseline MRI scan and a time point at one month to further clarify the course of the recovery process, exhibited through diffusion abnormalities.

A majority (80 - 90%) of concussions resolve between 7-10 days post injury as measured by behavioral assessments.[13] However the results of the present study provide evidence of neural recovery extending to at least 2 weeks from a structural perspective. Although these data do not inform us about the absolute maxima and minima of the diffusion metric trajectories due to the absence of measurements between two weeks and two months post injury, the statistical trend detected via the between group analyses at two months, suggests a minor relapse in the recovery of the structural measures of WM integrity. This finding, taken together with the observed variability in the trajectories of RD and FA between two weeks and two months, might be reflective of the athletes' exposure to sub-concussive hits following return to play (see earlier discussion[11,33,68]). A more recent study[87] and the first to relate diffusion measures to biomarkers in athletes with sub-concussive hits, reported a positive correlation between the

percentage change in football post minus preseason levels of serum auto-antibodies of the astrocytic protein S100B (considered a peripheral marker of blood brain barrier dysfunction) and the percentage of voxels with changes in MD during the corresponding time period. The same study reported a significant positive trend between the Head Hit Index (a derived measure accounting for both the number and severity of sub-concussive hits during a single game) and the increased post game (as compared to baseline) S100B levels of individual athletes. In addition, these, significant post game increases in S100B levels were detected only in athletes with sub-concussive hits (confirmed via game video analyses). These findings suggest that subject specific exposure to sub-concussive hits after return to play may be a potential factor affecting recovery of diffusion measures.[32,87]

Furthermore, variability in the trajectories of the diffusion measures might be affected by differences in number of prior concussions,[68] timing of each athlete's return to play (see Table 1) and individual genetic predisposition.[88,89] Experimental designs of future studies should include the assessment of subconcussive hits, extending at least to the end of season.

Conclusions

This is the first longitudinal study that tracks diffusion measures of contact sport athletes following a single episode of SRC with no LOC at ~2 days, 2 weeks and 2 months. This study provides support for the hypothesis of increased RD and reduced FA within 72 hours post injury followed by patterns of recovery. It further suggests that neural recovery may extend beyond 2 weeks as described in other similar imaging studies.[8,10] RD was found to be sensitive marker of SRC with potential for personalized imaging based diagnosis.

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Author Disclosure Statement

No competing financial interests exist.

Appendix

A1. Mixed Effect Model

The Mixed Effect model was selected based on the following observations unique to this experimental design:

1. 'Participant' is a random effect and there are repeated measures over the same individuals. The repeated measures are unbalanced due to the missing values. This model also accounts for any correlations between the scalar diffusion measures of WM microstructure.
2. Concussed participants cannot be matched (paired) to controls as controls are imaged at only one time point.
3. This model treats time as a fixed effect, with time = 0, denoting the controls. It then allows for comparison of the concussion effect at time = 2 days, 2 weeks and 2 months in relation to the controls.

The model was implemented using the lme4 library in R, specifically, using the 'lmer' function.

A2. Multivariate analysis using Bootstrap method for hypothesis testing

The following procedure describes the bootstrap method employed for testing the mean effect at 2 days, 2 weeks and 2 months with respect to the controls. This test preserves repeated measures, the missing value structure and any correlation structure between the scalar diffusion measures of WM

microstructure.

Step 1. Use the data to construct the null distribution of the observed variables, by centering the empirical distributions around zero so that the means of the scalar diffusion measures of WM microstructure for the controls and the 3 time points of the concussed are all zero. Then the effect at 2 days, 2 weeks and 2 months with respect to the control group are exactly zero.

Step 2. Generate a dataset with the same variables, groups and dimensions as the original data but sampled with replacement from the null distribution defined in step 1, which is also called bootstrap resampling.

Step 3. Calculate the Hoteling T^2 statistic from the data set generated in Step 2 and save the value.

Step 4. Repeat steps 2 and 3, 10000 times and store the 10000 values of the T^2 statistic. These 10000 values form the bootstrap distribution of the statistic T^2 under the null hypothesis.

Step 5. Calculate the bootstrap p-values by comparing the observed T^2 from the real data to their corresponding bootstrap distribution under the null.

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Controls				Concussed									
											MRI Inclusion		
Subject	Age (years)	Sport	MRI Inclusion	Subject	Age (years)	Sport	# Prior concussions	NP Normal post injury (days)	Symptom free (days)	return to play (days)	2 days scan	2 wks. scan	2 mon. scan
1	18	Crew	Y	1	20	Football	1	24	17 (1st), 77(2nd)	31[a]	Y	Y	Y
2	18	Squash	Y	2	19	Water polo	0	6	6	24	Y	Y	Y
3	21	Crew	Y	3	18	Lacrosse	0	14	11	20	Y	Y	Y
4	18	Crew	Y	4	21	Ice hockey	3	15	162	no return to play	M	Y	Y
5	20	Track + Cross country	Y	5	22	Lacrosse	0	17	10	23	Y	Y	Y
6	18	Crew	Y	6	20	Wrestling	0	11	3	18	Y	Y	M
7	21	Track	Y	7	19	Ice hockey	1	2	4	23	Y	M	M
8	21	Volleyball	Y	8	20	Basketball	0	10	8	15	Y	Y	M
9	22	Track + Cross country	Y	9	20	Rugby	0	23	10	31	Y	Y	Y
10	22	Cross country	Y	10	21	Rugby	0	11	2	12	Y	Y	Y
11	19	Crew	Y	11	21	Rugby	0	6	10	16	Y	Y	M
12	22	Track	Y	12	20	Rugby	1	17	12	no return to play[e]	Y	Y	Y
13	19	Track	Y	13	22	Rugby	2	na[b]	7	na[b]	Y	Y	M
14	18	Swimming	Y	14	20	Basketball	1	na[b]	31	na[b]	M	Y	Y
15	19	Volleyball	M	15	19	Football	0	60[c]	14	no return to play	Y	X	Y
16	22	Cross country	M	16	21	soccer	1	na[d]	11	32	Y	Y	D
				17	21	Ice hockey	0	3	5	12	X	Y	Y
				18	20	Football	0	6	5	15	Y	M	X
				19	20	Basketball	1	9	6	12	Y	X	Y
				20	19	Football	0	18	10	22	X	Y	Y
				21	21	Sprint Football	1	13	7	16	X	Y	M

[a] Returned to play after 1st injury at 31 days, sustained a 2nd concussion and decided not to return to play

[b] NP testing never reached normal range before athlete graduated/season ended

[c] Not normal at 2 weeks, not repeated until 2 months since season over

[d] Subjected discontinued from study and decided not to return to play (although cleared to do so for next, season)

[e] no return to play, graduated

Y: Scan included in analyses, M: Scan excluded due to motion exceeding threshold, X: Period of study during which scanner amplifier failed, D: Subject discontinued

Table 1: Demographics of controls and concussed athletes

FA	Estimate	Std. Error	t value	one-tailed p value	two-tailed p value
(Intercept)	0.619967	0.004693	32.09		
2 days	-0.030114	0.008278	-3.64	0.0004	0.0008*
2 wks.	-0.007985	0.007536	-1.06	0.14	0.28
2 mon.	-0.017821	0.008550	-2.08	0.022	0.044*
RD	Estimate	Std. Error	t value	one-tailed p value	two-tailed p value
(Intercept)	4.48E-04	4.56E-06	98.15		
2 days	3.06E-05	9.21E-06	3.32	0.001	0.002*
2 wks.	3.90E-06	8.07E-06	0.48	0.32	0.64
2 mon.	1.53E-05	9.54E-06	1.6	0.059	0.11

Table 2: Between group Mixed effects analysis of FA and RD

Hotelling T^2 statistic for 3 time points			
	2 days	2 wks.	2 mon.
T^2	8.253	3.154	4.315
Boot p value	0.0273*	0.189	0.092
Percentiles of Null Bootstrap distribution of T^2			
95%	6.463	6.076	5.558
97.5%	8.469	7.825	7.038
99%	10.988	9.930	9.569

Table 3: Results of the between group, multivariate analysis of FA, RD measures using the Bootstrap method for hypothesis testing.

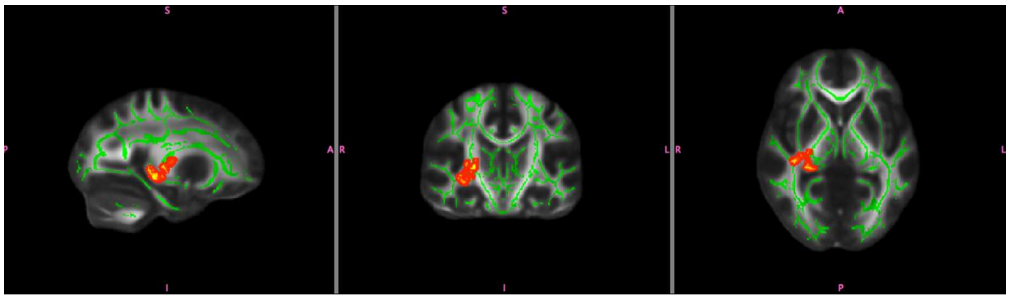
Figure Legend

Figure 1: Results of the paired, between concussed session (two days vs. two weeks, corrected $p < 0.025$), TBSS T- test of the RD values on the WM skeleton. The voxels (inflated into adjoining local tracts for visualization) showing significantly higher RD values at two days as compared to two weeks have been highlighted by color mapping (red-yellow). These voxels have been overlaid onto their corresponding WM skeleton (green). The underlay is the 'FMRIB58_FA_1mm' image volume (grayscale).

Figure 2: Individual trajectories of the mean RD values downloaded from the paired TBSS T-test RD mask (two days vs. two weeks, $p < 0.025$ corrected) for all concussed athletes across all three sessions. The red circles indicate those concussed athletes who participated at all three sessions. The red stars indicate those athletes who had imaging data at a maximum of two time points. Solid red lines connect athletes with consecutive measurements. Dashed red lines connect non-consecutive measurements (i.e. session 1 to 3). The blue line marks the mean value of the controls while the solid black line connects the mean of the concussed across the three time points.

Figure 3: Individual trajectories of the mean RD values from whole deep WM region (with the significant RD cluster from the between session TBSS analysis masked out) for

individual concussed athletes at all three sessions. The red circles indicate those concussed athletes who participated at all three sessions. The red stars indicate those athletes who had imaging data at a maximum of two time points. Solid red lines connect athletes with consecutive measurements. Dashed red lines connect non-consecutive measurements (i.e. session 1 to 3). The blue line marks the mean value of the controls (with the significant RD cluster from the between session TBSS analysis masked out) while the solid black line connects the mean of the concussed across the three time points.



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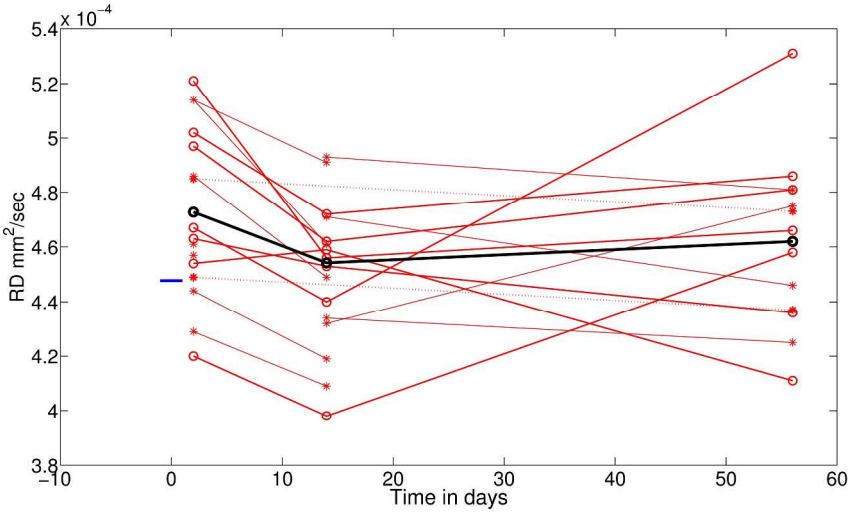

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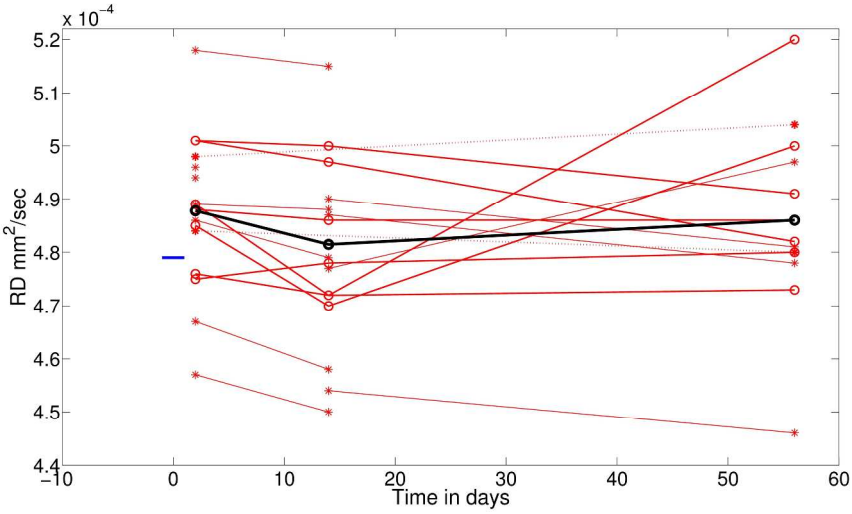
272x146mm (300 x 300 DPI)



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Persistent differences in patterns of brain activation after sports-related concussion: a longitudinal fMRI study

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Introduction:

Avoiding recurrent injury in sports-related concussion (SRC) requires understanding the neural mechanisms involved in recovery. The purpose of this study was to define neural correlates of SRC during the 2 months following injury, using a working memory task and fMRI.

Methods:

15 right-handed, varsity level contact sport athletes who suffered a SRC were scanned doing an N-back memory task (N = 1 to 3) at 72 hrs, 2 and 8 weeks post injury. 15 age and sex matched control subjects (non-contact varsity level athletes) were scanned at baseline and 2 weeks. All athletes were evaluated prospectively, using baseline clinical evaluation (SCAT2) and neuropsychological (NP) testing (ImPACT). All athletes who subsequently sustained a SRC were evaluated again within 24-48 hrs on SCAT2 and a hybrid NP battery (ImPACT, paper & pencil tests), which was repeated at 2 and 8 weeks post injury. Images were acquired on a 3T Siemens Skyra scanner (EPI PACE, FoV read/phase 192 mm/100 %, slice thickness 3 mm, base/phase resolution 64/100 %, TR/TE 2020/30 msec, flip angle 76 deg, 36 slices, 284 volumes, 0.9 mm isotropic whole brain MPRAGE for registration). Each imaging session consisted of 30 randomized blocks of N-back tasks with 15 sec rest intervals. Behavioral responses were collected using a Psychtoolbox/MATLAB program. Neuroimaging data were processed using standard block design processing routines in FSL - FEAT. In this abstract we report results based on paired, post-hoc, whole brain T-tests of the 2-1back contrast across the 3 sessions (Mixed effects modeling, $z > 2.3$, corrected cluster thresholding 0.05).

Results:
Across all 3 sessions, concussed athletes demonstrated significantly increased activation in the right dorsolateral prefrontal cortex, compared to controls. In session 1, concussed subjects also demonstrated increased activation in the left dorsolateral prefrontal cortex, bilateral inferior parietal and supplementary motor cortex. No significant between-group differences were observed in subjects' performance on any N-back task during any of the three scanning sessions. Clinical interpretation of the initial hybrid NP testing within 24-48 hrs demonstrated deficits in neurocognitive function, which normalized by 8 weeks.

Conclusions:
These data suggest that functional brain activation differences persist at 8 weeks after injury in concussed athletes, despite the fact that their performance on a standard working memory task is comparable to normal controls and normalization of clinical, /hybrid NP tests. These results might indicate a delay between neural and behaviorally-assessed recovery.

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A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports related concussion

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Abstract:

Introduction

The presence of structural injury after Sports Related Concussions (SRC) continues to be a question of great concern, in particular with regard to the long term effects of this particular type of injury, the individual course of recovery and the decision to return to play. The purpose of this study was to *longitudinally* assess, white matter fiber tract integrity in individuals with SRC.

Methods

A total of 21 contact athletes (males, 20.2 ± 1.0 years (y), right-handed (rh), varsity) with a medically diagnosed SRC (Glasgow coma scale > 15, no loss of consciousness) were enrolled in the study along with 16 male controls (19.9 ± 1.7 y, rh, varsity, non contact athletes).

All athletes were evaluated prospectively using baseline clinical evaluation (SCAT2, *ImPACT*). All concussed athletes were tested post injury (SCAT2, *Im*PACT, paper/pencil testing battery) concomitantly with the DTI protocol at 72 hours (h), 2 weeks (w) and 2 months (m).

DTI parameters: single-shot spin echo, axial, TR/TE = 12100/96 ms, 1.88×1.88x1.9 mm^3, 64 gradient directions, b-value 1000 s/mm^2, 8 B0 volumes, 2 runs, scan time 26.88 mins, 3T Skyra.

After accounting for scan/subject loss and exclusion of scans with motion (greater than 2 voxels and 3 S.D. > cohort mean); 14 controls (single baseline), 16, 17 and 13 concussed subjects (at 72 h, 2 w and 2 m respectively) were included in the Tract Based Spatial Statistics (TBSS, FSL 4.1.9, TFCE, 10k iterations) between group t-tests on derived measures of WM integrity, fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD). From the pool of subjects eligible for statistical analyses, only 12 concussed athletes were imaged at both the 72 h time point and at 2 w, 11 concussed athletes were imaged at both 2 w and 2 m post injury. Between sessions comparisons of the concussed athletes were made via paired t-tests (TBSS, TFCE, all permutations).

Results

Results of the TBSS analysis (*paired, between session* comparison, 72 h vs. 2 w, $p < 0.05$, multiple comparisons corrected) yielded one cluster in the right hemisphere comprising voxels in which both FA ($p<0.05$) and RD ($p<0.025$) were significant (posterior limb of the internal capsule (IC) sagittal stratum (inferior longitudinal fasciculus & inferior fronto-occipital fasciculus), retrolenticular part of the IC). No paired differences were observed in the *other between session* analyses and *the between group* comparisons.

Conclusions

These data provide evidence of structural changes in the acute phase after a single SRC. Mean FA trajectories of the implicated white matter fiber tracts appear to support a loss of structural integrity within 72 h extending to a minimum of 2 w.

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