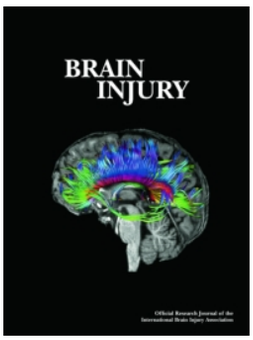

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Changes in functional connectivity of the brain associated with a history of sport concussion: A preliminary investigation

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ABSTRACT

Objective: There is evidence of long-term clinical consequences associated with a history of sport concussion. However, there remains limited information about the underlying changes in brain function. The goal of this study was to identify brain regions where abnormal resting-state function is associated with chronic concussion, for athletes without persistent symptoms.

Methods: Functional Magnetic Resonance Imaging (fMRI) was performed on a group of athletes with prior concussion (n = 22) and a group without documented injury (n = 21). Multivariate predictive modelling was used to localize reliable changes in brain connectivity that are associated with a history of concussion and with clinical factors, including number of prior concussions and recovery time from last injury.

Results: No significant differences were found between athletes with and without a history of concussion, but functional connectivity was significantly associated with clinical history. The number of prior concussions was associated with most extensive connectivity changes, particularly for elements of the visual attention network and cerebellum.

Conclusion: The findings of this preliminary study indicate that functional brain abnormalities associated with chronic concussion may be significantly dependent on clinical history. In addition, elements of the visual and cerebellar systems may be most sensitive to the long-term effects of sport concussion.

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Concussion; functional MRI; neuroimaging; MRI scan

Introduction

Sport concussion is defined as biomechanical injury leading to altered brain function, and is associated with a variety of debilitating outcomes, including physical symptoms (e.g. dizziness, headache), emotional disturbances (e.g. anxiety, depression) and impaired cognition (e.g. confusion, feeling 'slowed down') [1]. Symptom recovery usually occurs within 7–10 days, with medical determination of return to play (RTP) typically within a month post-injury [2]. However, there is growing evidence of long-term consequences of sport concussion that persist beyond RTP. Athletes with prior injury are prone to repeated injury and longer recovery times [3] and retired athletes with a history of concussion are at elevated risk for depression and mild cognitive impairment [4,5]. Post-concussion brain changes may play a key role in these long-term risk factors and the identification of reliable brain biomarkers of concussion history may lead to improvements in risk assessment and patient management.

Concussion significantly alters brain function, as impulsive force leads to cellular injury and disturbances in metabolism, cerebral blood flow and neural activity [6,7]. Blood-Oxygenation Level Dependent functional Magnetic Resonance Imaging (BOLD fMRI) provides a sensitive neuroimaging tool for identifying such alterations in brain function, based on fluctuations in cerebral blood oxygenation. To date, the majority of fMRI studies of concussion have focused on the 'sub-acute' time window between 1 week and 1 month post-injury, during which athletes are asymptomatic at rest and beginning a graded return to full activity. In this phase, athletes show increased BOLD activity when performing cognitive tasks, which may reflect decreased efficiency of neural processing [8]. Resting-state fMRI studies have also shown disruptions in functional connectivity between brain regions within the first month post-injury [9,10], although, in some cases, no significant differences were observed [11].

Despite these important early findings, there is currently a limited understanding of the long-term alterations in brain function associated with sport concussion. Only a few studies have used fMRI to evaluate long-term effects of concussion, focusing on brain activity during vision and motor tasks [12], and tests of working memory [13]. These studies have reported that athletes with a history of concussion show no significant differences in brain activity relative to those without prior injury, and they have generally comparable performance on neurological tests [12,13]. Nonetheless, further study is required, particularly in examining alternative biomarkers and analysis techniques, which may have greater sensitivity to the functional changes associated with a history of concussion.

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To date, there have been no rigorous examinations of the long-term effects of concussion on BOLD functional connectivity. Functional connectivity is typically studied in the resting brain, and is most commonly measured by the pairwise correlation between BOLD time series of different brain regions. It is believed to reflect the degree of shared information (i.e., functional integration) between brain regions and abnormalities in functional connectivity have been identified for a variety of neurological conditions [14–16]. This analysis approach is sensitive to changes in the functional organization of the brain in response to injury, which may not be detected in standard task-based fMRI studies. Functional connectivity may, therefore, be an important tool for studying athletes with a history of concussion that have no significant symptoms or functional impairments. The detection of abnormal brain function in this cohort potentially indicates neurobiological alterations that are not captured by standard clinical assessments, and may help to identify brain regions that play a key role in successful long-term recovery.

This study provides preliminary evidence addressing this knowledge gap, using resting-state fMRI data acquired from two cohorts of varsity athletes, with and without a prior history of concussion. Both cohorts included a balanced sample of male/female athletes and contact/non-contact sports, making these findings relevant to the overall sporting community. Analyses were conducted to identify brain regions where the seed-based functional connectivity map significantly distinguished athletes with prior injury from those without. For athletes with a prior history of concussion, subsequent testing examined whether seed-based functional connectivity patterns correlated with clinical factors, including the number of previous concussions and length of recovery from their last concussion. For this study, a multivariate predictive modelling approach was used, in order to characterize the relative sensitivity to chronic concussion throughout the entire brain. This approach was used to identify brain regions of interest motivating future research of brain function and sport concussion.

Methods

Participants

Athletes were recruited from seven varsity sport teams (volleyball, hockey, soccer, football, rugby, basketball and lacrosse), through the University of Toronto Sport Medicine Clinic. At the time of recruitment, demographic data were collected, along with clinical variables including the number of previous concussions, time since their last concussion (in months) and recovery time for their last concussion, based on the number of days from reported injury to medical clearance. In addition, the SCAT3 (sport concussion assessment tool) was administered to assess for potential post-concussion symptoms [17], and the BESS (balance error scoring system) was used to test for postural deficits [18]. The resulting cohorts included a set of 22 athletes with prior concussion, which were matched on age, sex and contact sport participation to a set of 21 athletes with no documented concussions (see Table I for demographics). The study procedures were approved by the University of Toronto and St Michael's Hospital research ethics boards and all participants provided written informed consent before the study began.

MRI acquisition and pre-processing

For each participant, anatomical MRI was performed using a 3D T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence (field-of-view (FOV) = 22 × 16.5 cm, 256 × 196 × 192 acquisition matrix, 1.1 × 0.9 × 1.4 mm voxels, bandwidth = 250 Hz/Pixel, TI/TE/TR = 850/2.63/2000 ms, flip angle = 15°). In order to screen for structural abnormalities, including lesions and micro-haemorrhage, participants were also imaged with fluid attenuated inversion recovery imaging (FLAIR; FOV = 22 × 18.6 cm, 256 × 196 acquisition matrix, 1.1 × 0.9 × 3.0 mm voxels, bandwidth = 315 Hz/Pixel, TI/TE/TR = 2200/96/9000 ms) and susceptibility-weighted imaging (SWI; 220 × 192 FOV, 0.6 × 0.6 × 1.2 mm

Table I. Clinical and demographic measures for participants, including test scores for the Sport Concussion Assessment Tool (SCAT) and sub-scales, including the Balance Error Scoring System (BESS).

	No prior injury	Previous concussion
Age, mean ± SD	19.5 ± 1.5	21.0 ± 1.7
Contact sport	9/22	8/21
Female	11/22	11/21
Time since injury, median (range)	—	26 months (9–120)
Number of concussions, median (range)	—	2 (1–5)
Recovery time, median (range)	—	18 days (2–120)
SCAT, mean ± SD		
Symptoms	3.45 ± 3.98	2.59 ± 3.50
Symptom Severity	5.27 ± 7.05	3.77 ± 5.01
Orientation	4.90 ± 0.29	4.86 ± 0.35
Immediate Memory	14.27 ± 1.49	14.55 ± 0.60
Concentration	3.27 ± 1.28	3.86 ± 1.23
Delayed Memory	3.48 ± 1.29	4.06 ± 1.05
BESS Total Errors	3.86 ± 3.37	1.81 ± 2.23
Sports	Volleyball (4 M, 7 F) Basketball (2 F) Ice Hockey (1 M) Soccer (3 M) Football (1 M) Lacrosse (2 M) Rugby (2 F)	Volleyball (5 M, 3 F) Ice Hockey (3 M, 5 F) Football (1 M) Lacrosse (1 M) Soccer (3 F)

The 'sports' row lists the sports represented in each group, with numbers of male (M) and female (F) athletes.

voxels. TR/TE 28/20 ms, FA = 15°, 384 × 307 with encoding gap of 0.2 mm, 120 Hz/px bandwidth).

Resting-state fMRI data were then acquired via multi-slice T2*-weighted echo planar imaging (FOV = 20 × 20 cm, 64 × 64 matrix, 32 slices, 3.125 × 3.125 × 4.5 mm voxels, bandwidth = 2232 Hz/Pixel, TE/TR = 30/2000, flip angle = 70°, oblique axial interleaved) to produce a time-series of 193 samples images. During fMRI acquisition, participants were instructed to lie still with their eyes closed and not focus on anything in particular. Subsequent data processing and analysis were performed using software from the Analysis of Functional Neuroimages (AFNI) package (afni.nimh.nih.gov) and customized algorithms developed in the laboratory. This included rigid-body motion correction (AFNI *3dvolreg*), removal of outlier scan volumes (using nitrc.org/projects/spike-cor), slice-timing correction (AFNI *3dTshift*), spatial smoothing with a 6 mm isotropic 3D Gaussian kernel (AFNI *3dmerge*), regression of motion parameters and linear-quadratic trends as nuisance covariates. To control for physiological noise, the data-driven physiological model PHYCAA+ was used to down-weight non-neuronal tissue contributions (nitrc.org/projects/phycaa_-plus) and white matter signal was regressed out, by segmenting the brain with FSL's *fast* algorithm (fsl.fmrib.ox.ac.uk/fsl/fslwiki/FAST) and regressing out the mean signal in voxels with p(white matter) > 0.95. Co-registration of fMRI images was obtained by computing the rigid-body transform of the time averaged fMRI data for each participant to their T1-weighted anatomical image, and the 12-parameter affine transformation of the anatomical image for each participant to the MNI152 template. The transformation matrices were concatenated and the net transform was applied to all fMRI data, re-sampled at 2 × 2 × 2 mm^3 resolution.

Data analysis

The primary goal of this paper was to determine which brain regions showed greatest functional connectivity disturbances associated with chronic sport concussion, and secondarily to characterize the specific connectivity changes for these regions. Brain parcellation was performed by using FSL's *fast* algorithm to exclude white matter voxels where p(white matter) > 0.95, and applying the Automated Anatomical Labelling (AAL) atlas on remaining grey matter voxels, which segments the brain into 116 cortical domains (58 per hemisphere) [19]. For each parcel, the grey matter centre-of-mass voxel was identified and the average seed time-course computed for a Gaussian kernel centred on this voxel, with a standard deviation of 4 mm. Whole-brain functional connectivity maps were obtained by measuring the correlation between the seed fMRI time-series and all other brain voxels. This was done for each parcel, producing a set of 116 functional connectivity maps per participant.

Subsequent analyses tested the relative sensitivity of each brain region in distinguishing between athletes with and without prior concussion. For each of the 116 AAL seeds, the set of participant connectivity maps was analyzed using principal component regression (PCR). This predictive multivariate model was used to determine whether athletes had a prior concussion based on their functional connectivity maps, by generating regression scores for each participant (see Appendix 1 for details). PCR was performed in a cross-validation framework, where 90% of participants were randomly selected to fit the PCR model, and model prediction accuracy was evaluated on the remaining 10% of participants, using the predictive squared correlation coefficient Q^2 (averaged over 1000 re-sampling iterations) [20]. The Q^2 metric provides an unbiased estimate of how strongly the fitted regression model predicts the dependent variable (i.e. concussion history) for held-out test data. A value of $Q^2 = 1$ indicates perfect prediction accuracy, and $Q^2 \leq 0$ indicates no association. Significance was assessed via bootstrap re-sampling on the set of 1000 cross-validated Q^2 estimates and computing a distribution over mean Q^2 values (1000 iterations). This was used to estimate 95% confidence bounds on Q^2 and an empirical p-value, based on the fraction of bootstrap samples with mean $Q^2 > 0$. This analysis was performed on the set of connectivity patterns generated by each seed region, giving an estimate of mean Q^2 and significance, for each of the 116 AAL seeds.

For athletes with a history of concussion, additional PCR analyses were conducted to identify brain regions where the connectivity pattern is associated with clinical outcome, for number of concussions and recovery time from their last concussion. This was done using the same approach as above, based on cross-validated PCR. To improve model robustness and reduce potential model biases of participants with a high number of concussions or recovery days, stepwise regression was performed. For number of concussions, athletes were binned as one concussion, two concussions, and more than two concussions. For recovery time, athletes were binned as within 10 days, from 10–30 days and greater than 30 days. The PCR analysis was used to estimate mean Q^2 values and bootstrapped significance for all 116 AAL seeds.

For all analyses (prior history of concussion, number of concussions, recovery time), a map was produced of the seed regions of interest (ROIs) with significant Q^2 values. Significant regions were identified at both $p \leq 0.05$ (uncorrected) and after correcting for multiple comparisons at a False Discovery Rate (FDR) threshold of 0.05. In addition, for seed ROIs showing greatest mean Q^2 scores, bootstrap ratios were calculated on the regression coefficient weights for each brain voxel (the mean/SE of coefficient weights, for 1000 bootstrap re-samples). This produced brain maps of z-scored statistics [21], indicating the relative importance of individual brain regions in predicting clinical factors. Positive bootstrap ratios indicate that the clinical factor is associated with increased connectivity between the seed ROI and this brain region, whereas negative bootstrap ratios indicate decreased connectivity with the seed ROI. These brain maps were also corrected for multiple comparisons at FDR = 0.05.

Results

Clinical data

For all participants in the present study, the inspection of anatomical neuroimaging (SWI, FLAIR, T1) revealed no significant structural brain abnormalities. The clinical and demographic data of the study cohort are summarized in Table I. Both groups included balanced samples of male and female athletes, along with contact sports (men's hockey and lacrosse, rugby, soccer, football) and non-contact/collision sports (women's hockey, basketball, volleyball). The

studied cohort had an average SCAT total score of 3.02 (SD = 3.7), with no significant differences identified between athletes with and without prior concussion ($p = 0.42$, Mann-Whitney test). The groups showed no significant differences for any symptom severity or cognitive scores.

Significant differences were observed for BESS Total Score ($p = 0.037$, Mann-Whitney test), although fewer errors were seen for athletes with prior concussion. This is consistent with a greater proportion of ice hockey players among athletes with prior concussion, as this sport requires highly refined balance [22]. However, among athletes with prior concussion, no significant association was seen between participation in hockey and total number of concussions (Spearman correlation = 0.23; $p = 0.30$), or recovery time from their last concussion (Spearman correlation = 0.30; $p = 0.16$), indicating that this group is unlikely to be a confound in subsequent fMRI analyses of these clinical factors.

fMRI analyses

Figure 1 depicts seed ROIs where a change in functional connectivity is significantly associated with history of concussion or clinical factors. Table II summarizes the ROIs, with Q^2 confidence bounds and empirical p-values. Comparing athletes with and without prior history of concussion (Figure 1 (*a*)), no significant differences were seen in functional connectivity after correcting for multiple comparisons. Thus, a history of concussion is not associated with consistent changes in seed-based functional connectivity for this cohort. For athletes with a prior history of concussion, however (Figures 1(*b*) and (*c*)), multiple ROIs were associated with the number of prior concussions and recovery from the last concussion, after correcting for multiple comparisons.

Of the two clinical factors analysed, the number of concussions shows the greatest number of significant ROIs (six regions at FDR = 0.05 threshold) and tends to have the highest Q^2 values among all analyses. Significant ROIs are mainly posterior and show highest Q^2 for elements of the visual attention network, including the precuneus and superior occipital lobe. However, the functional connectivity of cerebellar seed regions is also significantly associated with number of concussions. In comparison, recovery time shows fewer significant brain ROIs (two regions at an FDR of 0.05), both of which are also located in the cerebellum. These results highlight the importance of both the visual system and cerebellum as regions of altered functional connectivity post-concussion. Notably, trends indicate that the inferior frontal lobes are also weakly associated with history of concussion and number of prior concussions, but not after correcting for multiple comparisons.

Figure 2 shows statistical maps of brain regions where changes in functional connectivity are significantly associated with clinical factors, for the seed ROIs showing highest Q^2 statistics in Table II, including precuneus (R) and cerebellum 7B(R). In these plots, positive bootstrap ratios indicate that the clinical measure (more concussions, longer recovery time) is associated with higher connectivity in this region, while negative bootstrap ratios indicate the opposite trend. As shown in Figure 2(*a*), athletes with a higher number of concussions tend to have greater connectivity between the precuneus (R) and insula ($z = -4$), orbitofrontal lobe ($z = -4$), motor cortex ($z = 46, 66$) and superior frontal lobe ($z = 66$), but decreased connectivity with the cerebellum ($z = -38$), amygdala ($z = -18$), anterior cingulate ($z = -4, 6$) and caudate ($z = 6$). As shown in Figure 2(*b*), athletes with a longer recovery time tend to have greater connectivity between the cerebellum 7B (R) and insula ($z = -4$), orbitofrontal lobe ($z = -4$) and motor cortex ($z = 34, 46$), but decreased connectivity with anterior cingulate ($z = -4, 6$) and precentral gyri ($z = 46, 66$). See Appendix 2 for the mean connectivity patterns of the clinical sub-groups, which can be compared to the bootstrapped regression maps of Figure 2.

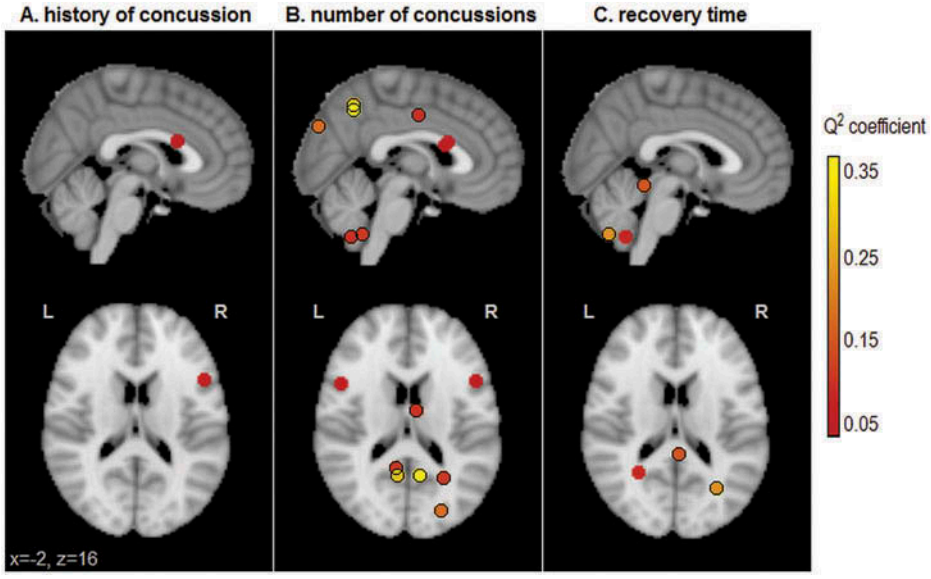

Figure 1. Seed regions of interest (ROIs) where a change in functional connectivity is significantly predictive of (*a*) prior concussion or (*b, c*) clinical factors including number of concussions and recovery time. All displayed ROIs are significant at $p < 0.05$ (uncorrected), with significant ROIs after correcting for multiple comparisons (at a False Discovery Rate of 0.05) circled in black.

Table II. Regions where functional connectivity significantly predicts prior concussion or clinical factors.

Analysis	Seed region	Q^2 coeff, mean (95% CI)	*p*-value
History of concussion	inferior frontal gyrus, oper. (R)	0.045 (0.001–0.063)	0.017
Number of concussions	inferior frontal gyrus, oper. (L)	0.055 (0.001–0.118)	0.047
	inferior frontal gyrus, oper. (R)	0.056 (0.001–0.110)	0.027
	middle cingulum (R)	0.108 (0.048–0.166)	< 0.001*
	superior occipital lobe (R)	0.181 (0.122–0.237)	< 0.001*
	precuneus (L)	0.279 (0.230–0.327)	< 0.001*
	precuneus (R)	0.388 (0.341–0.433)	< 0.001*
	cerebellum 8 (R)	0.108 (0.055–0.159)	< 0.001*
	cerebellum 9 (L)	0.116 (0.053–0.178)	< 0.001*
Recovery time	cerebellum 7B (R)	0.226 (0.184–0.267)	< 0.001*
	cerebellum 8 (L)	0.091 (0.026–0.156)	0.003
	vermis 3	0.152 (0.109–0.195)	< 0.001*

* Significant regions with a significant predictive correlation coefficient (Q^2 coeff.) after correcting for multiple comparisons at FDR = 0.05. The mean and 95% confidence intervals (95% CI) of Q^2 values are also reported.

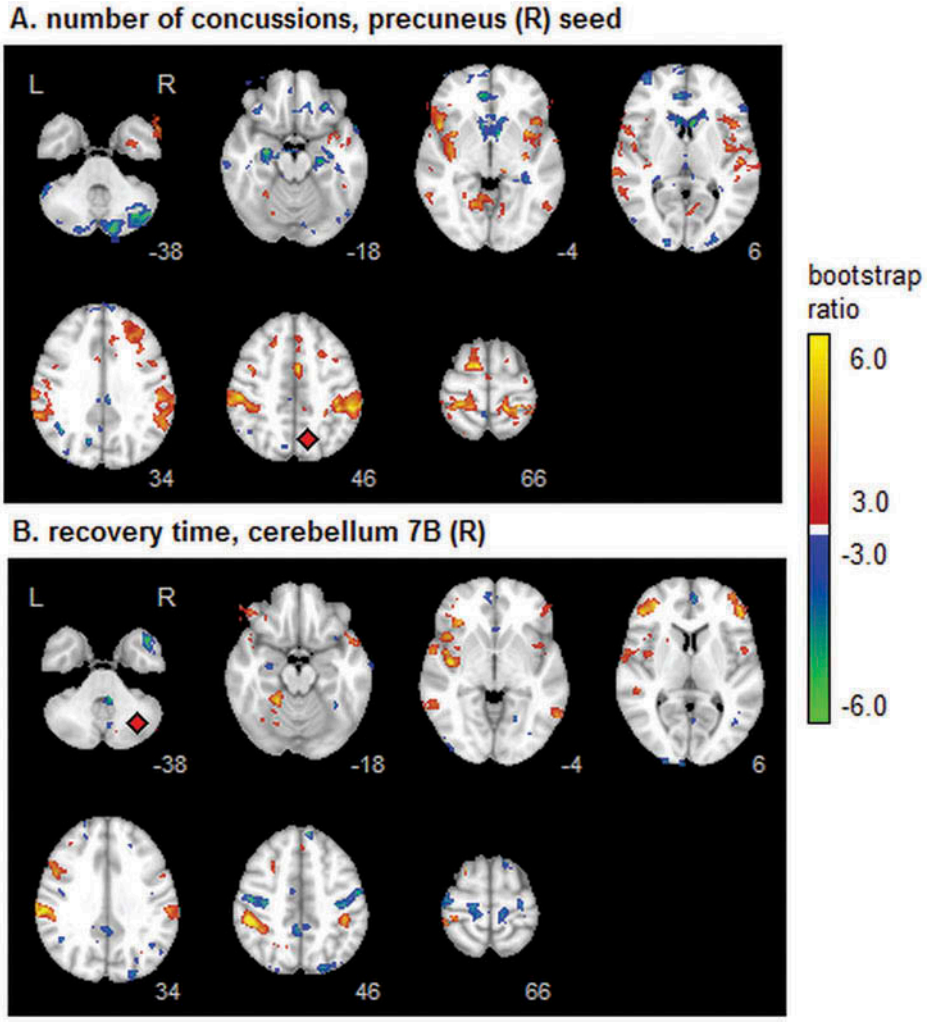

Figure 2. Bootstrap ratio maps of regression coefficients for ROIs showing highest prediction of patient outcome (mean Q^2 statistic) in Table I, for (*a*) number of concussions, and (*b*) recovery time. Positive bootstrap ratios indicate that higher functional connectivity with the seed (red diamond) predicts greater number of concussions/recovery time; negative bootstrap values indicate the opposite. Maps are thresholded at a False Discovery Rate of 0.05 to correct for multiple comparisons.

Figure 3 shows the distribution of participant regression scores generated by PCR analysis, for the ROIs showing highest Q^2 statistic in Table II, including precuneus (R) and cerebellum 7B(R). There is relatively large within-group spread in scores, reflecting heterogeneity in the long-term effects of concussion. Nonetheless, Figure 3(*a*) shows consistent separation between groups with 1, 2, and > 2 concussions. Conversely, Figure 3(*b*) shows athletes with < 10 days of recovery time and 10–30 days

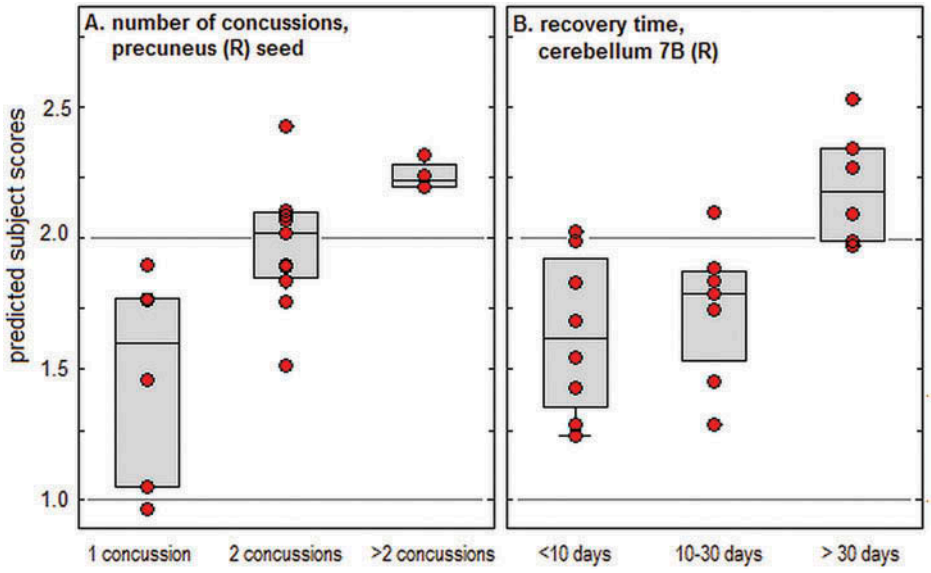

Figure 3. Predicted subject scores for ROIs showing highest prediction of patient outcome (mean Q^2 statistic) in Table I, for (*a*) number of concussions and (*b*) recovery time. Red circles indicate individual subject scores, whereas grey boxes indicate quartile bounds (25^{th}, 50^{th} and 75^{th}).

have highly similar scores, whereas athletes with > 30 days recovery time show the greatest difference between groups.

Discussion

Outcome after sport concussion is a major public health concern. Currently, there is insufficient scientific knowledge of how the brain recovers from concussion over the long-term. This paper provides a preliminary examination of resting-state brain function for athletes with a history of concussion, but no persistent symptoms or postural deficits, using BOLD fMRI to measure functional connectivity. In addition, the studied cohort consists of a mixture of male and female athletes, along with contact and non-contact sports, making these findings relevant to the overall sporting community. The focus of this paper is on identifying brain regions where abnormalities in resting-state fMRI are associated with a history of concussion, potentially providing information to future studies about which regions are most important to investigate when mapping long-term recovery from brain injury.

An important finding was that athletes with and without a history of concussion showed no significant differences in functional connectivity, after correcting for multiple comparisons. This is supported by task-based fMRI studies, which also showed no significant long-term abnormalities for athletes with no persistent functional deficits [12,13]. However, this study also identified significant effects of clinical history among athletes with concussion, which suggests that the lack of significant differences between athletes with and without history of concussion is partly due to the heterogeneity of long-term functional changes among athletes with prior concussion. Hence, the present findings emphasize the importance of examining clinical covariates when studying the effects of concussion on brain function.

Functional connectivity changes were significantly associated with the number of prior concussions and recovery time from last injury. Critically, more significant ROIs identified when analysing the number of prior concussions, compared to recovery time. This indicates that the cumulative effects of repeated injury may have a more consistent overall effect on brain function across participants, compared to the severity of their last concussion. The effects of multiple injuries on brain function is also consistent with prior resting-state fMRI studies of sub-acute concussion [9].

Examining significant brain regions in Figure 1, the prominence of effects in superior dorsal brain regions suggests that the visual attention system might be most sensitive to concussion. This is a major concern, as visual dysfunction is a frequent symptom of concussion [1], and there is evidence that visual screening may be effective for early concussion detection [23]. Moreover, visual function is essential for sport performance and avoiding future injuries in contact sports. The cerebellum also exhibited altered functional connectivity in athletes with prior concussion, for both a greater number of injuries and longer recovery time. This is of interest, as cerebellar function is not widely studied in functional imaging of concussion, despite the area being potentially vulnerable to compression during head impact [24]. The cerebellum plays a critical role in numerous domains, including visual-motor integration, gait and balance [25], all of which are critical for sport performance and avoiding re-injury [26]. These findings suggest that it may be important to further investigate neural correlates of different vision and motor processes in athletes with prior injuries, to better understand the potential risks of re-injury during sport.

The regression maps of Figure 2 show brain regions where connectivity with the seed is most affected by clinical factors. The maps highlight the complexity of long-term changes in response to injury, as they do not show a uniform increase or decrease in connectivity throughout the brain. This is consistent with prior research, which has reported relatively complex sub-acute functional connectivity changes for athletes with concussion [9–11]. Two key findings were seen in the regression maps. First, both a

greater number of concussions and longer recovery were associated with hyper-connectivity of the prefrontal and motor cortices (with precuneus and cerebellar seeds). This is supported by a recent meta-analysis, which found that traumatic brain injury is associated with hyper-connectivity in dorsal brain regions [27]. These results indicate greater recruitment of visual-motor and sensory domains in athletes with more severe history of injury, potentially as a compensatory mechanism. Importantly, athletes with prior concussion did not have any significant deficits in posture and balance, based on their BESS scores. Second, hypo-connectivity was seen in elements of the limbic system (e.g. the amygdala and anterior cingulate), which is also consistent with the meta-analysis of brain injury [27]. This is an important area of future study, as these regions are implicated in emotional response [28] and athletes with a history of concussion are at higher risk for emotional disorders, such as depression [5].

Although this study identified significant resting-state fMRI markers of concussion, there were also a few limitations which should be addressed in future work. This study was cross-sectional and, for athletes with concussion, the time since injury was variable, ranging from 9–120 months. Hence, future research using a longitudinal design and fixed imaging time points post-injury will be crucial, in order to establish how brain abnormalities evolve from acute injury and whether they reflect long-term brain dysfunction. In addition, the clinical data on concussion history was self-reported and may be, therefore, subject to reporting errors. Nonetheless, the identification of predictive fMRI markers of altered connectivity in the present study suggest that these effects are highly robust. In terms of methodology, the current findings were based on ROIs derived from the AAL atlas. While this is highly relevant due to the widespread use of this template in fMRI literature, anatomical parcellations may have sub-optimal correspondence to functional boundaries in the brain [29]. Future research should, therefore, examine data-driven functional parcellations (i.e. clustering models) as an alternative approach to generating seed ROIs.

An additional area of future investigation is how the effects of concussion vary between different sports. While this study focused on changes in resting-state fMRI that are consistent across a variety of sports, there may also be significant differences between sport sub-groups. In this study, higher BESS scores were reported for athletes with prior concussion. As noted in the Results section, this may be attributed to a greater proportion of hockey players in the concussed cohort, as it has been previously shown that BESS scores show systematic differences across sports [30] and hockey requires highly refined balance [22]. Although this is unlikely to be a confound in the present study, given the lack of significant correlations with number of concussions and recovery time, there may be connectivity differences for regions implicated in balance, e.g. the cerebellum and motor cortex. Similarly, there is evidence that athletes in contact sports show systematic alterations in functional connectivity, putatively due to sub-concussive impacts [31]. In order to determine whether this has a significant effect on long-term brain changes associated with concussion, large-scale studies will be required, with greater representation across different sports.

The results of this paper demonstrate significant functional brain changes associated with concussion history, despite all athletes being imaged a minimum of 6 months post-injury and showing no persistent signs or symptoms of injury based on standardized assessment tools of SCAT3 and BESS. Therefore, the identified brain abnormalities likely reflect adaptation in response to injury, rather than indicating persistent, gross dysfunction. The present findings indicate that functional connectivity of resting-state fMRI is an important potential biomarker to consider when assessing long-term brain changes in response to sport concussion.

Declaration of interest

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Appendix 1: Principal Component Regression

All analyses were based on Principal Component Regression (PCR), which represents brain maps in a low-dimensional basis that explains the most data variance, in order to reduce noise and ensure a well-posed solution. This approach is chosen due to the diffuse nature of concussion effects, as PCR is able to identify distributed patterns of brain change. In brief, the process is defined as follows:

1. For a set of S subjects with MRI images consisting of V voxels, data are represented as a matrix $\boldsymbol{X}$, of dimensions $(V \times N)$, where column n=1…N corresponds to the brain map of the n^{th} subject. We subtract row means of $\boldsymbol{X}$ and a perform principal component analysis (PCA) $\boldsymbol{XX^T} = \boldsymbol{U\Lambda^2U^T}$, where $\boldsymbol{U}$ is a matrix of orthonormal brain patterns (eigenimages) that explain greatest covariance in $\boldsymbol{XX^T}$, and diagonal elements of $\boldsymbol{\Lambda^2}$ indicate variance expressed per component.
2. After subtracting row means, the data matrix data has rank (N-1), and thus we retain components $\boldsymbol{U^*} = [\boldsymbol{u1}, \boldsymbol{u2}, \ldots, \boldsymbol{u_{N-1}}]$. Data are transformed into PCA space via $\boldsymbol{Q} = \boldsymbol{U^{*T}X}$, where the nth column of $\boldsymbol{Q}$ gives PCA-space coordinates for the n^{th} subject.
3. The PCA scores are now regressed onto a vector of clinical factors $\boldsymbol{y}$. When analyzing athletes with/without prior injury, this is a binary vector (0=no injury /1=prior concussion). For other analyses, this is simply a vector of clinical measures (e.g. number of previous concussions). We obtain a map of regression weights on brain voxels by solving the expression:
$$\boldsymbol{w} = \boldsymbol{U^*}(\boldsymbol{QQ^T})^{-1}\boldsymbol{Qy}.$$
4. This model is fit on a "training" dataset, then used to predict clinical factors $\mathbf{y}^*$ for an independent "test" dataset $\boldsymbol{X^*}$, by computing regression weights $\hat{\boldsymbol{y}} = \boldsymbol{X^{*T}w}$. We then measure how well predicted values $\hat{\boldsymbol{y}}$ agree with the true values $\boldsymbol{y^*}$ using the Q^2 statistic [18]. For the current paper, 90% of subjects were randomly selected to train the model, and the remaining 10% used as test data. This was repeated for 1000 iterations, and the average Q^2 computed
5. To measure the importance of brain regions in predicting clinical factors, we employed non-parametric bootstrap resampling. This was done by randomly sampling subjects with replacement and estimating w, repeated for 1000 iterations. We then computed per-voxel Bootstrap ratios, defined as mean/Standard-Error, which were used to obtain empirical p-values, adjusted for multiple comparisons using at an FDR threshold of 0.05.

Appendix 2: mean connectivity patterns for clinical sub-groups

This section directly plots the mean functional connectivity patterns produced by the seeds with highest regression Q^2 scores, for each of the discretized clinical subgroups. These maps are also compared to the regression map of significant brain regions (originally depicted in Fig. 2). Supplementary Figure S1 shows connectivity maps for precuneus (R), as a function of number of prior concussions. Supplementary Figure S2 depicts connectivity maps for cerebellum 7B (R), as a function of recovery time from last concussion.

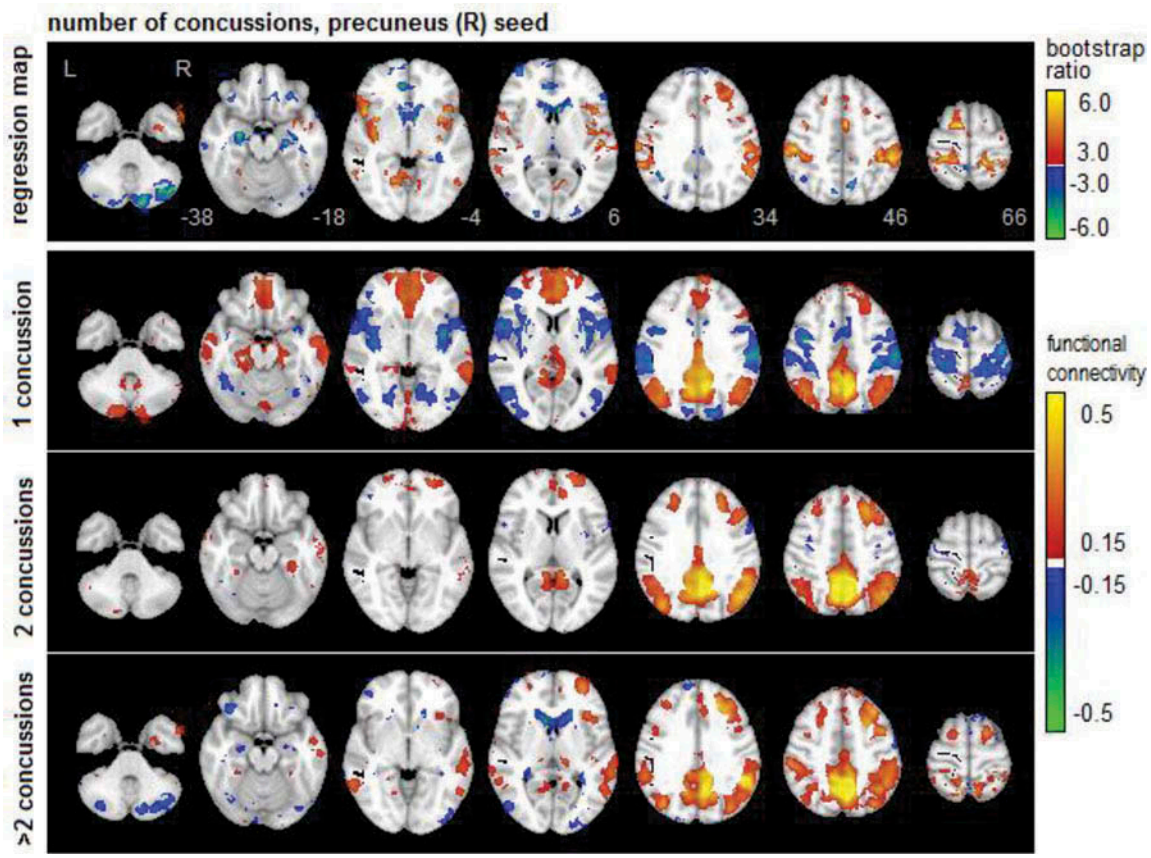

Figure S1: (top) bootstrap ratio map of regression coefficients for precuneus (R), which shows highest prediction of number of concussions, as originally reported in Fig. 2A. Image is thresholded at a False-Discovery Rate of 0.05. (bottom) mean functional connectivity maps in this seed region, for athletes with 1 concussion, 2 concussions and >2 prior concussions.

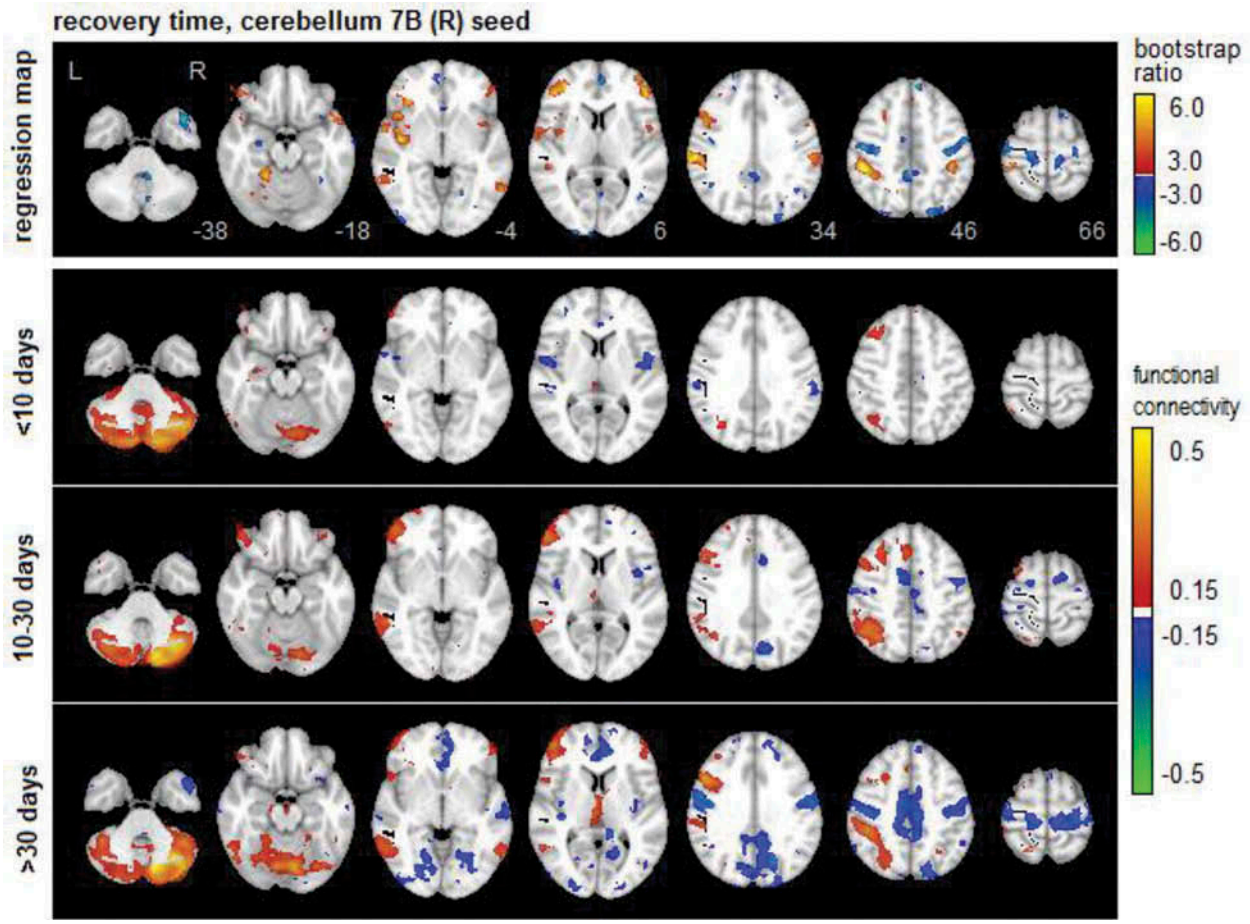

Figure S2: (top) bootstrap ratio map of regression coefficients for cerebellum 7B (R), which shows highest prediction of recovery time, as originally reported in Fig. 2B. Image is thresholded at a False-Discovery Rate of 0.05. (bottom) mean functional connectivity maps in this seed region, for athletes with <10 days recovery, 10-30 days recovery and >30 days recovery.