Supplemental Data

Appendix e-1

Study Details: The regular season was approximately 25 weeks, starting the first of October and lasting until mid-March. Players on teams that made the playoffs may have played into April. On average players practiced twice a week and had a minimum of one game per week. An independent group of healthy, age-matched, non-concussed male hockey players were assessed and acted as our controls (n = 26, age 13.0 ± 1 years). Most of the players had not experienced a diagnosed concussion, and those that had were not concussed within the 6-months leading up to the study. Players suspected of a potential concussion were assessed at the Fowler Kennedy Sports Medicine Clinic within 24-72 hours of their injury where an experienced Sports Medicine physician confirmed a concussion diagnosis. Concussed players (age 13.3 ± 0.6 years) were assessed within 24-72 hours of injury (n = 17) and again 3-months later (n = 14). Controls were assessed in September, and those who suffered a concussion after enrollment into the study were permitted to enroll in the concussion aspect of the study (n = 2). Players were screened for participation in the MRI portion of the study and the total numbers of included datasets for each MRI protocol are detailed in table e-1.

Clinical Protocol: Clinical assessment of both controls (n = 26) and concussed players (24-72 hours n = 17, 3-month follow-up n = 14) occurred at the Fowler-Kennedy Sports Medicine Clinic. Portions of these clinical results have been reported previously[e1]. The assessment for both groups included a short medical history obtained by a Sports Medicine physician including past concussion history, cognitive assessments including Sports Concussion Assessment Tool (SCAT 3) and Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT), balance testing, and an MRI scan. SCAT 3 is composed of various sub-tests including cognitive orientation, immediate memory, concentration ability, number of errors during balance attempt, coordination, delayed recall, number and severity of symptoms[e2]. ImPACT is comprised of visual and verbal memory composites, reaction time, impulse control, cognitive efficiency index and total symptom score[e3]. Players who suffered a concussion underwent an examination by a physician and were subjected to all the same testing as the non-concussed players. However, it should be noted that for concussed players the initial ImPACT testing post-concussion was performed at the discretion of the physician and was often delayed due to the potential for the test to exacerbate symptoms. Players were cleared to return to play by the Sports Medicine physician and the number of days to recover was recorded.

Balance Testing: Balance data were successfully recorded for the control group (n = 26), for concussed players at 24-72 hours post-concussion (n = 14) and 3-months follow-up (n = 13). Two concussed players dropped out of the study by the 3-month time-point, one opted not to participate in the balance testing, two initial post-concussion visits were not collected due to technical difficulties with the testing platform or the participant was too unbalanced to remain on the board. Standing balance was assessed using a pressure sensitive balance platform interfaced with a laptop computer using custom-written software (Labview 8.5 National Instruments, Austin, TX, USA) and calibrated as per the outlined protocol[e4]. The platform has a useable surface of 45 cm x 26.5 cm, and has been previously demonstrated to be a valid tool to objectively evaluate balance within healthy[e4] and neurologically impaired[e5] populations.

The standing balance of each player was evaluated as they stood on the pressure sensitive balance platform and completed the following three increasingly complex balance tasks that comprise the balance assessment within the SCAT 3 testing protocol: i) Double limb stance (standing with feet together side by side); ii) Single limb stance (standing on non-dominant foot with contralateral hip flexed to 30° and knee flexed to 45°), and iii) Tandem stance (standing heel-to-toe with non-dominant limb at the back). Each trial lasted 20 seconds and was completed once per testing session. Immediately prior to the start of each trial players were instructed to keep their hands on their hips, head facing forward with eyes closed, and to stand as still as possible for the duration of the trial. All trials were completed with the player in bare feet and 30 seconds of rest were provided between successive trials. Balance testing was completed within a quiet room housed within the Fowler-Kennedy Sports Medicine Clinic.

The outcome measures used to quantify balance were the total centre of pressure length (COPL), the COPL amplitude, and the standard deviation of the COPL in both the anterior and posterior directions. These measures have been shown to be a valid and reliable indication of postural stability[e6]. The COPL was calculated from data acquired from sensors within the platform via custom-written software (Labview 8.5 National Instruments, Austin, TX, U.S.A.). Data for each individual sensor were streamed to the software, with interpolation of the data and the time point of data acquisition ensuring a stable 100 Hz sampling rate. To remove signal noise, the data were processed as follows: filtered using a 12.5 Hz low-pass filter utilizing a two-level undecimated Symlet-8 wavelet with the detail levels removed; converted to center-of-pressure coordinates using the equation outlined previously; and then low-pass filtered at 6.25 Hz using a three-level undecimated Symlet-8 wavelet with the detail levels removed.

Table e-1: Total number of datasets included in each portion of the study.

Data	Control Group	24-72 hours Post-Concussion	3-Months Post-Concussion	Reasons for missing data
SCAT/ImPACT	26	17	14	Three players dropped out by the 3-month timepoint.
Balance	26	14	13	One concussed player opted to not participate in balance testing; two 24-72 hour datasets were not collected due to technical issues with the balance board.
MRI	18	15	13	Two control players were claustrophobic, two controls were uncomfortable with the MRI, and four control players had braces; one concussed player declined to participate and one had braces; 2 scanned concussed players dropped out by the 3-month timepoint.
RS-fMRI	16	14	13	2 healthy controls and one 24-72 hour post-concussion participant moved excessively (> 1 mm, relative mean displacement > 0.5 mm) during the RS-fMRI portion.
DTI	18	12	11	A small portion of post-concussion data was acquired on the Tim Trio scanner and only the RS-fMRI data were included in the overall analysis. The DTI and MRS data were excluded for these participants because these quantitative techniques are more sensitive to scanner-related differences.
MRS	18	12	11	

Appendix e-2

MRI Acquisition: All MRI data were acquired using a 64-channel human head coil in the 3T MRI Tim Trio or Prisma Fit scanners (Siemens, Erlangen, Germany) at the Robarts Research Institute. Players were screened for participation in the MRI session. The scan was approximately 50 minutes long and involved an MPRAGE (TE/TR = 2.94/2300 ms, TI = 900 ms, flip angle = 9˚, matrix size = 256x256, FOV = 256 mm x 240 mm, Number of slices = 160, slice thickness = 1.2 mm) for registration purposes. Turbo spin echo (TSE) sequence (TE/TR = 95/5690 ms, flip angle = 120˚, matrix size = 320x256, FOV = 220 mm x 178 mm, Number of slices = 32, slice thickness = 4 mm) and FLAIR (TE/TR = 139/15000 ms, TI = 2850 ms, flip angle = 90˚, matrix size = 256x256, FOV = 256 mm x 256 mm, Number of slices = 50, slice thickness = 3 mm) were acquired to rule out cerebral edema indicative of a more serious TBI. Water suppressed (number of acquisitions = 192) and unsuppressed (number of acquisitions = 8) spectroscopy data were acquired from the prefrontal white matter ROI using a single voxel point-resolved spectroscopy (PRESS) pulse sequences (TE/TR=135/2000 ms, dwell time = 833 µs, number of points = 1024, voxel=2x2x1.5 cm^3). A diffusion weighted spin echo sequence (TE/TR = 79/7200 ms, flip angle = 2˚, matrix size = 98x98, FOV = 200 mm x 200 mm, Number of slices = 64, slice thickness = 2 mm, b1 = 0 s/mm^2, b2 = 1000 s/mm^2, gradient directions = 64), and a ten-minute resting state functional MRI EPI sequence (TE/TR = 30/2500 ms, flip angle = 90˚, matrix size = 80x80, FOV = 240 mm x 240 mm, Number of interleaved slices = 45, slice thickness = 3 mm) were also acquired.

Appendix e-3

Clinical Results: The SCAT and ImPACT scores generally reflected symptoms that were present and more severe within 24-72 hours post-concussion, and returned to control levels by 3-months. On average, players required 23.6 ± 10 days to recover after their injury and be cleared by a physician to return to play, though notably this ranged from 10 to 46 days. The SCAT symptom score (number of symptoms) and symptom severity scores (rated on a scale from 0-6) were significantly elevated within 24-72 hours post-concussion (figure e-1) and 3-month data were significantly decreased compared to the acute post-concussion data indicating a return to control levels ($F > 28.3$, $p < 0.0001$). SCAT cognitive orientation (score based on number of correct answers to five questions) was significantly decreased at 3-months post-concussion, however this was not a clinically relevant change (4/14 players answered 1/5 basic questions incorrectly). The balance composite (the number of errors while attempting to balance) was significantly increased acutely post-concussion and returned to control levels at 3-months. ImPACT data was gathered about a week after injury once the physician determined that symptoms would not be aggravated by completing the clinical test, however the total symptom score remained significantly different from controls ($F = 4.7$, $p < 0.05$). ImPACT visual motor processing speed improved significantly by 3-months post-concussion, most likely due to practice and a learning effect.

Balance Results: Detailed balance scores revealed deficits in balance performance 24-72 hours post-concussion compared to controls while the 3-month follow-up data demonstrated a significant recovery to control levels. In particular, there were non-significant increases in COPL during double limb stance at 24-72 hours post-concussion ($F = 2.84$, $p = 0.068$). The amplitude and standard deviation of the player’s sway in the anterior-posterior and mediolateral directions were significantly increased 24-72 hours post-concussion ($F > 8.0$, $p < 0.005$) and in the mediolateral direction these metrics decreased significantly by 3-months ($F > 8.3$, $p < 0.001$) and were not significantly different from the controls (figure e-1).

Figure e-1: (a) The SCAT 3 symptom score relaying the number of reported symptoms out of a possible 22 and (b) their self-reported severity rated based on 0 = none, 1-2 = mild, 3-4 = moderate and 5-6 = severe, for a maximum of 132. Balance measures include the amplitude and SD of sway in the mediolateral (c-d) and anterior-posterior directions (e-f). Significance is indicated using the star symbol ($p < 0.05$).

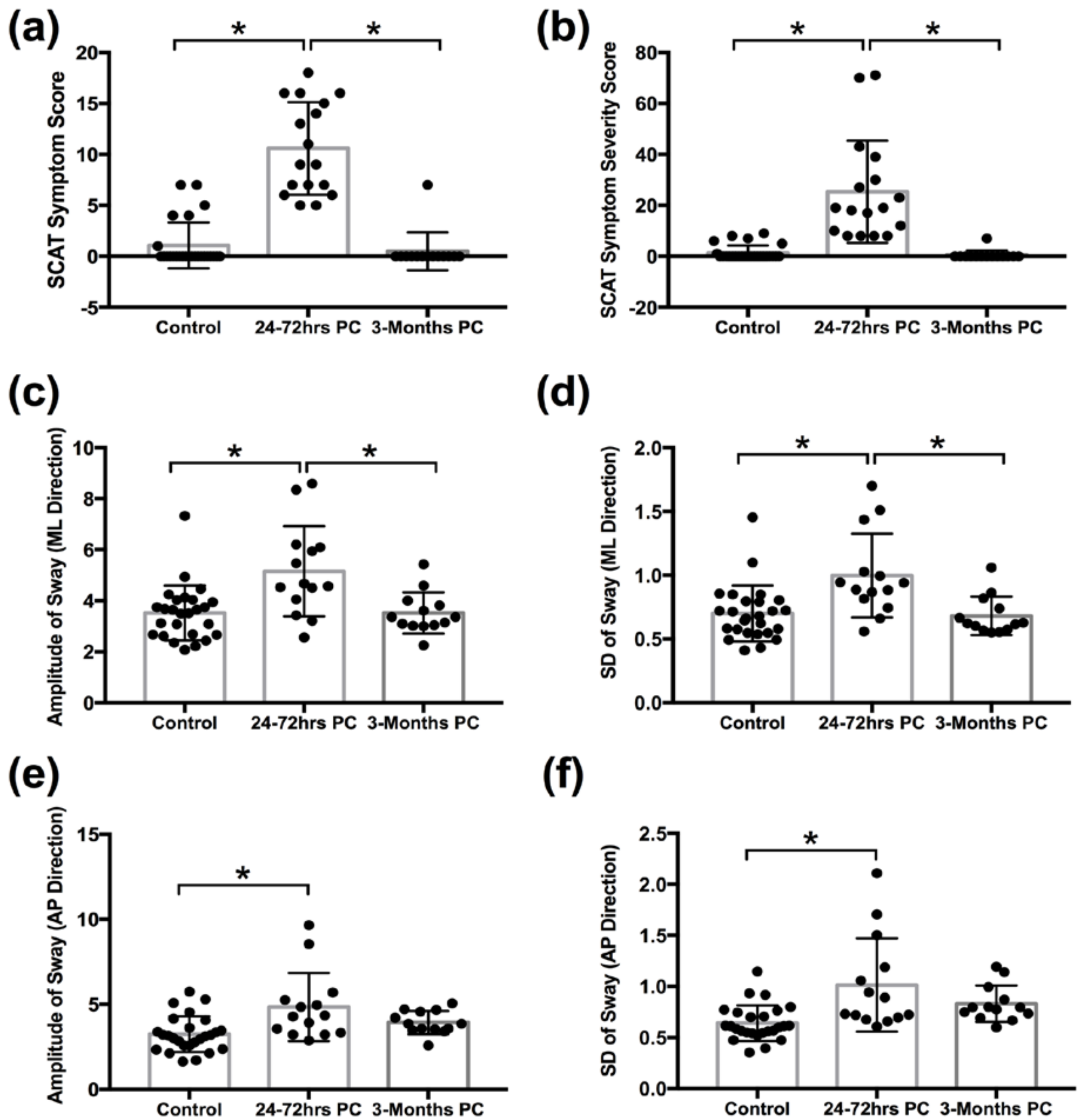

Table e-2: Correlation analysis details relating different MRI measures and clinical deficits. Right and left tracts or regions are indicated with brackets. Uncorrected p-values are shown and the * symbol indicates correlation values that survived FDR-correction.

Data from 24-72 hours post-concussion		**Pearson correlation coefficient, r**	***p*-value**
Connectivity between cerebellum 2(r) and posterior parahippocampal gyrus(l)	COPL (double limb)	0.660	0.014*
	Mediolateral amplitude of sway	0.585	0.036
	Anterior-posterior amplitude of sway	0.600	0.030
	Anterior-posterior SD of sway	0.584	0.036
Number of days to recover	SCAT symptom score	0.615	0.011*
AD in DTI_{max}	ImPACT total symptom score	-0.747	0.008*
	SCAT Balance Errors	-0.732	0.007*
AD in right CST	COPL (double limb)	0.548	0.081
Data from 3-months post-concussion		**Pearson correlation coefficient, r**	***p*-value**
Connectivity within the cerebellum 8(r) and 2(l)	Mediolateral amplitude of sway	-0.867	0.000*
	Mediolateral SD of sway	-0.880	0.000*
AD in right SLF	Connectivity between temporal pole(l) and parietal occipital pole(r)	0.812	0.002*
RD in DTI_{max}	Connectivity between occipital pole(r) and post central gyrus(l)	-0.680	0.021
	Connectivity between occipital pole(l) and post central gyrus(l)	-0.741	0.014*
AD in DTI_{max}	Connectivity between anterior supramarginal gyrus(r) and medial prefrontal cortex	-0.768	0.006*
MRS Choline	Connectivity between anterior supramarginal gyrus(l) and paracingulate gyrus(l)	-0.620	0.042
	Connectivity between anterior supramarginal gyrus(l) and paracingulate gyrus(r)	-0.715	0.013*
Data from 24-72 hours post-concussion	**Data from 3-months post-concussion**	**Pearson correlation coefficient, r**	***p*-value**
	AD in the right CST	0.735	0.015*

Connectivity between anterior supramarginal gyrus(r) and frontal pole(l)	SCAT Balance Errors	0.751	0.005*
MRS Choline	ImPACT total symptom score	-0.665	0.026
	Connectivity between temporal pole(l) and parietal operculum(r)	0.818	0.002*
	Connectivity between cerebellum 2(r) and posterior parahippocampal gyrus(l)	-0.659	0.027
	Connectivity between post central gyrus(r) and occipital pole(r)	0.690	0.019
	Connectivity between post central gyrus(r) and occipital pole(l)	0.620	0.039
Number of days to recover	Connectivity between occipital pole(r) and superior lateral occipital cortex(l)	-0.668	0.013*
	Connectivity between occipital pole(l) and inferior temporal gyrus(r)	-0.610	0.027
	Connectivity between occipital pole(r) and inferior temporal gyrus(r)	-0.595	0.032
	Connectivity between superior parietal lobule(l) and occipital fusiform gyrus(l)	-0.574	0.040
AD in DTI_{max}	Connectivity between anterior supramarginal gyrus(r) and frontal pole(l)	-0.568	0.087
SCAT symptom severity score	Connectivity between superior parietal lobule(l) and temporal pole(r)	-0.711	0.006*
	Connectivity between superior parietal lobule(r) and temporal pole(r)	-0.692	0.009*
	AD in DTI_{max}	-0.654	0.029
SCAT symptom score	Connectivity between cerebellum 2(l) and brainstem	-0.894	0.032
ImPACT total symptom score	Connectivity between anterior supramarginal gyrus(r) and paracingulate gyrus(l)	0.609	0.027
	Connectivity between anterior supramarginal gyrus(r) and medial prefrontal cortex	0.627	0.022
	Connectivity between cerebellum 8(r) and 7(l)	-0.767	0.002*
	Connectivity between cerebellum 8(r) and 2(l)	-0.941	0.000*

e-References

e1. Daley M, Dekaban G, Bartha R, et al. Metabolomics profiling of concussion in adolescent male hockey players: a novel diagnostic method. Metabolomics. Springer US; 2016;12:185.

e2. SCAT3. Br J Sport Med. BMJ Publishing Group Ltd and British Association of Sport and Exercise Medicine; 2013;47:259.

e3. Schatz P, Pardini JE, Lovell MR, Collins MW, Podell K. Sensitivity and specificity of the ImPACT Test Battery for concussion in athletes. Arch Clin Neuropsychol. 2006;21:91–99.

e4. Clark RA, Bryant AL, Pua Y, McCrory P, Bennell K, Hunt M. Validity and reliability of the Nintendo Wii Balance Board for assessment of standing balance. Gait Posture. 2010;31:307–310.

e5. Holmes JD, Jenkins ME, Johnson AM, Hunt MA, Clark RA. Validity of the Nintendo Wii ® balance board for the assessment of standing balance in Parkinson’s disease. Clin Rehabil. 2013;27:361–366.

e6. Salavati M, Hadian MR, Mazaheri M, et al. Test-retest reliabty of center of pressure measures of postural stability during quiet standing in a group with musculoskeletal disorders consisting of low back pain, anterior cruciate ligament injury and functional ankle instability. Gait Posture. 2009;29:460–464.