Supplementary Online Content


eAppendix 1. Concussion Diagnosis and Exclusion Criteria, Behavioral Information, Imaging Parameters, Processing and Analysis, and Additional Linear-Mixed Models and Correlations

eAppendix 2. ANAM Results and Effects of Prior Concussions

eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix 1.

Concussion Diagnosis and Exclusion Criteria

Exclusion criteria included a past or current diagnosis of mood disorders, anxiety disorders, alcohol abuse, or substance abuse. Concussion diagnosis was made independently of the study at the time of injury by clinicians trained in sports medicine based on a clinical exam assessing symptoms, a cranial nerve check, manual muscle testing for strength deficits, the Rhomberg’s test for balance deficits, on-field cognitive testing developed by the UPMC Center for Sports Medicine, and the King-Devick test.

Behavioral Information

Primary and secondary behavioral measures were collected in a confidential research setting. For the structured interviews (primary measures; described in main text), trained professionals assessed symptom scores since the day of injury at T1, since the last visit at T2, and over the previous week at T3. Identical structured interviews were conducted on healthy athletes, assessing symptoms over the previous week.

The ANAM4 was used for secondary measures of interest. The ANAM4 includes a 21-item overall symptom inventory, a 12-item concussion symptom inventory, and a mood scale that measures self-reported levels of anger, anxiety, depression, fatigue, happiness, restlessness, and vigor. The cognitive battery from the ANAM4 also includes measures of simple processing speed (two simple reaction time tasks), information processing speed (code substitution task, procedural reaction time task), visual-spatial processing (spatial processing task), working memory (matching-to-sample task, memory search task, mathematical processing), memory (delayed code-substitution task), and response inhibition (go-no-go task) tasks. Throughput, the number of correct responses per minute, was used to measure performance on each ANAM4 cognitive task, except for the go-no-go task, for which d’ was used. With the exception of the go-no-go and mathematical processing tasks, all scores were standardized relative to a normative sample of male collegiate athletes available as part of the ANAM4 software package. The ANAM4 Validity Indicator Report was used to flag cognitive subtests on which participants demonstrated questionable effort or a possible misunderstanding of subset instructions. These data were excluded from analyses (see Table 2 in main text for final N in all analyses).

Imaging Parameters, Processing, and Analysis

Neuroimaging data were collected using a General Electric (GE) Discovery MR750 whole body 3-Tesla MRI scanner and a brain-optimized receive-only 32-element coil array head coil. T1-weighted magnetization-prepared rapid gradient-echo sequence with sensitivity encoding was used for anatomical reference with the following parameters: FOV=240 mm, 130 axial slices, slice thickness=1.1 mm, voxel size 0.938x0.938x1.1 mm, image matrix=256 x 256, TR/TE=5/1.948 ms, acceleration factor R=2 in the phase encoding direction, flip angle=8 degrees, delay time, Ti=725 ms, sampling bandwidth=31.25 kHz, scan time=5:05 minutes. CBF data were collected using the GE 3dASL pulsed-continuous arterial spin labeling sequence with the following default parameters: TR/TE=5161/12.08 ms, FOV=240 mm, spiral readout of 8 arms and 512 samples, number of excitations=3, post-label delay=1.525 s, label duration 1.45 s, sampling bandwidth=62.5 Hz, slice thickness=2 mm, voxel size 1.875x1.875x2.0 mm. The number of slices was tailored to each participant to maximize brain coverage and ranged from 62 to 72. This resulted in slightly varied TR varied across participants, ranging from 5.088 to 5.233 seconds, but should not affect quantitative CBF measurements.

CBF was quantified to ml/100gm/min using the GE FuncTool algorithm provided for the GE 3dASL sequence using the following equation:

\[ CBF = 6000 \cdot \frac{\lambda}{2T_{1b}} \left( 1 - \exp \left( - \frac{ST}{T_{1b}} \right) \right) \exp \left( \frac{PLD}{T_{1b}} \right) \cdot \left( \frac{PW * NEX}{SF * PD} \right) \]

Following GE specifications, the T1 of blood (T_{1b}) was assumed to be 1.6s, the T1 of gray matter (T_{1g}) was assumed to be 1.2s, the saturation time (ST) was set to 2s, the partial coefficient (\lambda) was set to 0.9, the overall efficiency (\epsilon), which is a combination of inversion efficiency and background suppression efficiency, was set to 0.6, and the scaling factor (SF) was set to 32. PLD, LT, and NEX are the post-label delay, label duration, and number of excitations used in data collection, respectively. PW represents the perfusion-weighted image and PD is the proton density image generated by the 3dASL sequence.
To facilitate future multimodal analyses, quantified CBF images were first aligned with anatomical images using an affine registration. Both images were then transformed to EPI data, followed by a non-linear registration to the Colin N27 brain template using the Advanced Normalization Tools (ANTS) program. The final resolution of the resampled data was 1.75 mm isotropic voxels. The quantified CBF image was then spatially smoothed with a 6 mm full-width at half-maximum Gaussian kernel. An intersection mask of the final CBF image and the anatomical image for each participant was created, and the average CBF within this mask was calculated for each participant. A relative CBF image was then calculated by dividing the smoothed quantified CBF image by the average CBF value. The AFNI program suite was used for all voxel-wise calculations and analyses.

Group level voxel-wise analysis was limited to gray matter by applying a binary gray matter mask created from the segmentation of the standard template. After estimating the smoothness of the CBF data, Monte Carlo simulations (10,000 iterations) were used to determine the necessary correction for family-wise error at \( p < 0.05 \) within the gray matter mask (\( p = 0.005; \) minimum cluster volume = 3,189 µl).

**Additional Linear-Mixed Models and Correlations**

Additional linear-mixed models were performed to determine whether the number of prior concussions had an effect on CBF recovery following concussion. Each model included the fixed factor of time, number of previous concussions, and the interaction between previous concussions and time. A random intercept of considered for each participant and compound symmetric correlation was used as the covariance structure. Four analyses were performed: one for CBF in the dorsal-mid insular cortex (dmIC), one for CBF in the superior temporal sulcus (STS), and one each for primary behavioral measure (Hamilton Anxiety Rating Scale [HAM-A]; Hamilton Depression Rating Scale (HAM-D)).

Spearman correlations were conducted separately for athletes in the control group to confirm there was no relationship between our primary behavioral measures and prior concussions, or between prior concussions and CBF in dmIC and STS.

**eAppendix 2.**

**ANAM Results**

There was a significant main effect of time for secondary ANAM4 measures included overall symptom severity (\( F(2,26)=16.4, p<0.001 \)), overall symptom frequency (\( F(2,26)=23.5, p<0.001 \)), concussion symptom inventory frequency (\( F(2,26)=28.5, p<0.001 \)), concussion symptom inventory severity (\( F(2,26)=19.3, p<0.001 \)), self-reported fatigue (\( F(2,26)=9.4, p<0.001 \)), and both simple reaction time subtests (\( F(2,26)=10.3, p<0.001; F(2,26)=10.1, p<0.001 \)). Concussed athletes showed a significant improvement in scores at T2 and T3 relative to T1 for overall symptom frequency (all \( p's<0.001 \)), overall symptom severity (all \( p's<0.001 \)), concussion symptom inventory frequency (all \( p's<0.001 \)), concussion symptom inventory severity (all \( p's<0.001 \)), self-reported fatigue (all \( p's<0.005 \)), and both simple reaction time subtests (all \( p's<0.005 \)). In contrast to our primary measures, no difference between scores at T2 and T3 were observed for any ANAM4 measures (all \( p's>0.30 \)), suggestive of faster recovery of these symptoms.

Furthermore, one-sample independent samples t-tests confirmed that all ANAM4 measures exhibiting a significant effect of time were also significantly worse (i.e., slower reaction time and more reported symptoms) at T1 for the post-concussion athletes relative to healthy athletes. This includes self-reported fatigue (\( t(35)=2.55, p=0.008 \)), overall symptom frequency (\( t(35)=4.45, p<0.001 \)), overall symptom severity (\( t(35)=3.92, p<0.001 \)), concussion symptom inventory frequency (\( t(35)=5.04, p<0.001 \)), concussion symptom inventory severity (\( t(35)=4.29, p<0.001 \)), and the first (\( t(34)=-3.63, p<0.001 \)) and second (\( t(34)=-4.16, p<0.001 \)) simple reaction time. In contrast, symptom scores and reaction times were not significantly worse at T2 or T3 compared to healthy control scores (all \( p's>0.10 \), with the exception of a trend for worse performance at T2 in the second simple reaction time subtest (\( t(32)=-1.62, p=0.057 \)).

**Effects of Prior Concussions**

The main effect of prior concussions and the interaction between prior concussions and time were not significant for any of the supplementary mixed-models. For dmIC CBF, neither the main effect of prior concussions \( F(1,24) = 0.19, p = 0.67 \), nor the interaction of prior concussion or time point \( F(2,24) = 0.70, p = 0.50 \), was significant. The main effect of time was still significant \( F(1,24) = 6.28, p < 0.01 \). Similarly, for STS CBF, the main effect of prior concussions \( F(1,24) = 1.32, p = 0.26 \), the interaction of prior concussion or time point \( F(2,24) = 2.30 \),
p = 0.12, and the main effect of time F(2,24) = 2.25, p = 0.13) were not significant. The main effect of prior concussion was not significant for HAM-A or HAM-D measures F(1,24) = 0.40, p = 0.53 and F(1,24) = 0.01, p = 0.93, respectively. The interaction between time and prior concussion was also not significant for HAM-A or HAM-D measures F(2,24) = 0.15, p = 0.86 and F(2,24) = 0.39, p = 0.68, respectively. The main effect of time was still significant for both HAM-A and HAM-D measures F(1,24) = 11.6, p < 0.001 and F(1,24) = 8.71, p = 0.001, respectively.

Spearman correlations in the healthy athlete group found no significant relationship between the number of previous concussions and CBF in the STS (Rs = 0.06, p = 0.75) or dmiC (Rs = -0.08, p = 0.68), or in the HAM-A (Rs = 0.18, p = 0.37) or HAM-D (Rs = 0.13, p = 0.51) behavioral measures.

eReferences