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Association of acute depressive symptoms and functional connectivity of emotional processing regions following sport-related concussion

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ABSTRACT

Acute mood disturbance following sport-related concussion is common and is known to adversely affect post-concussion symptoms and recovery. The physiological underpinnings of depressive symptoms following concussion, however, are relatively understudied. We hypothesized that functional connectivity of the emotional processing network would be altered in concussed athletes and associated with the severity of depressive symptoms following concussion. Forty-three concussed collegiate athletes were assessed at approximately one day ($N = 34$), one week ($N = 34$), and one month post-concussion ($N = 30$). Fifty-one healthy contact-sport athletes served as controls and completed a single visit. The Hamilton Rating Scale for Depression (HAM-D) was used to measure depressive symptoms. Resting state fMRI data was collected on a 3 T scanner (TR = 2 s) and functional connectivity was calculated in a meta-analytically derived network of regions associated with emotional processing. Concussed athletes had elevated depressive symptoms across the first month post-concussion relative to control athletes, but showed partial recovery by one month relative to more acute visits ($ps < 0.05$). Concussed athletes had significantly different connectivity in regions associated with emotional processing at one month post-concussion relative to one day post-concussion ($p = 0.002$) and relative to controls ($p = 0.003$), with higher connectivity between default mode and attention regions being common across analyses. Additionally, depressive symptoms in concussed athletes at one day ($p = 0.003$) and one week post-concussion ($p = 7 \times 10^{-8}$) were inversely correlated with connectivity between attention (e.g., right anterior insula) and default mode regions (e.g., medial prefrontal cortex). Finally, the relationships with HAM-D scores were not driven by a general increase in somatic complaints captured by the HAM-D, but were strongly associated with mood-specific HAM-D items. These results suggest that connectivity of emotional processing regions is associated with acute mood disturbance following sport-related concussion. Increased connectivity between attention and default mode regions may reflect compensatory mechanisms.

1. Introduction

Sport-related concussions (SRCs) are a major public health issue, with an estimated 1.6–3.8 million occurring in the United States every year (Langlois et al., 2006). Mood disturbance, a common consequence of brain injury, occurs in up to 50% of athletes following SRC (Kontos et al., 2012; Ellis et al., 2015). While previous work suggests that persistent depressive symptoms following SRC are associated with neural abnormalities consistent with the limbic-frontal model of depression (Chen et al., 2008), the precise physiological underpinnings of mood disturbance following *acute* brain injury remain unknown.

A better understanding of the physiological underpinnings of acute depressive symptoms following SRC is particularly important given evidence that depression adversely affects post-concussive symptoms

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and recovery. For example, emergency room patients with mild traumatic brain injury (mTBI) who developed major depressive disorder (MDD) acutely following injury experienced higher levels of post-concussive symptoms and worse behavioral outcomes (Rapoport et al., 2003). In similar samples, MDD following mild to moderate TBI was associated with increased disability and cognitive impairment (Levin et al., 2001) and significantly reduced functional outcome and health-related quality of life (Haagsma et al., 2015). Thus, isolating the relationships between neurophysiology and depressive symptoms following mTBI may provide novel prognostic information.

Resting state functional magnetic resonance imaging (rs-fMRI) has emerged as a popular method to assess neurophysiological changes brought on by mTBI. This method allows for the indirect study of large scale neural networks by measuring correlated fluctuations in the blood-oxygen-level dependent (BOLD) signal in the absence of external task demands. Although rs-fMRI may develop into a viable biomarker candidate, previous investigations regarding functional connectivity following SRC have yielded mixed results (McCrea et al., 2017). Such discrepant findings are likely the product of methodological differences, limited sample sizes, and variations in assessment timelines. Additionally, the natural heterogeneity of concussion sequelae, particularly as it pertains to mood disturbance, may account for the observed inconsistencies across studies (McCrory et al., 2017). Efforts to categorize this heterogeneity have led to attempts at identifying specific symptom clusters describing and predicting unique clinical trajectories (Collins et al., 2014). Identifying the physiological signatures of specific SRC indicators may eventually lead to the development of objective diagnostic or prognostic biomarkers for symptoms that largely depend on subjective self-report (e.g., depressive mood symptoms).

The present study sought to determine the extent to which depressive symptoms following SRC are associated with physiological abnormalities in brain regions associated with emotional processing. Specifically, we investigated patterns of functional connectivity between regions of interest (ROI) comprising a meta-analytically derived emotion network in collegiate athletes with SRC across the acute and sub-acute phase of injury. We hypothesized that: 1) SRC would be associated with disrupted resting state connectivity in regions associated with emotional processing relative to healthy contact sport athletes; and 2) greater post-concussion depressive symptoms would be associated with greater disruption of functional connectivity in regions associated with emotional processing.

2. Method

2.1. Participants and behavioral data

Data from this sample have been previously reported (Meier et al., 2017). This study was approved by an institutional review board. A total of 94 NCAA Division I student-athletes were referred by sports medicine professionals and provided written informed consent. From this sample, 43 concussed athletes completed at least one visit following SRC that occurred at one day (T1: 1.74 ± 0.93 days; $N = 34$), one week (T2: 8.44 ± 2.15 days; $N = 34$), and one month (T3: 32.47 ± 4.68 days $N = 30$) post-concussion. Of the 43 concussed athletes, 19 participated in all three visits and 36 participated in at least two visits. Concussions were diagnosed independent of the study at the time of injury by physicians trained in sports medicine following recommended guidelines (McCrory et al., 2013). Diagnosis was based on a clinical exam assessing symptoms, manual muscle testing for strength deficits, a cranial nerve check, on-field cognitive testing, the King-Devick test, and the Rhomberg's test for balance deficits. Fifty-one non-injured collegiate contact sport athletes served as healthy controls (HA). No participants reported past or current mood disorders, anxiety disorders, or alcohol/substance abuse.

For the purposes of this study, depressive symptoms were quantified using a structured interview for the Hamilton Depression Rating Scale (HAM-D) collected at each study visit. Sample characteristics are presented in Table 1.

Table 1
Sample characteristics.

	Healthy athletes ($N = 51$) Mean (*SD*)	Concussed athletes ($N = 43$) Mean (*SD*)
Demographics		
Gender (male/female)	35/16	34/9
Age	20.26 (1.44)	20.29 (1.31)
Education	13.31 (1.29)	13.12 (0.98)
Previous concussions	0.59 (1.10)	0.93 (1.14)
Concussion information		
Post-traumatic amnesia (# of athletes)	NA	1 of 37
Retrograde amnesia (# of athletes)	NA	6 of 37
Loss of consciousness (# of athletes)	NA	4 of 37
Sport		
Basketball	0	6
Football	31	31
Volleyball	0	1
Rowing	0	1
Soccer	20	4
Final n of usable data	HA	T1/T2/T3
Enrolled	51	34/34/30
HAM-D	50	34/34/30
Rs-fMRI	50	28/29/26

HAM-D = Hamilton Depression Rating Scale, HA = healthy athletes, T1 = one day post-concussion, T2 = one week post-concussion, T3 = one month post-concussion, NA = not applicable.

2.2. MRI parameters

Imaging data were collected on a General Electric Healthcare Discovery MR750 3-Tesla whole body scanner and a brain-dedicated receive-only 32-element coil array (Nova Medical, Inc.). T_1-weighted structural images were obtained using a parallelized magnetization-prepared rapid gradient-echo sequence with sensitivity encoding, FOV = 240 mm, 130 1.1 mm axial slices, image matrix 256 × 256, TR = 5 ms, TE = 1.948 ms, TI = 725 ms, acceleration factor R = 2, flip angle = 8°, sampling bandwidth 32.25 kHz, and voxel size = 0.9375 × 0.9375 × 1.1 mm. 180 volumes of rs-fMRI data were collected in a six-minute gradient-echo echo-planar image (EPI) during which participants were instructed to fixate on a cross. Rs-fMRI data had the following parameters: TR = 2 s, TE = 30 ms, flip angle = 90°, sampling bandwidth = 250 kHz, acceleration factor R = 2, FOV = 240 mm, acquisition matrix = 96 × 96, 37 axial slices, slice thickness = 3 mm, inter-slice spacing = 0.2 mm acquired voxel size 2.5 × 2.5 × 3.2 mm interpolated to 1.875 × 1.875 × 3.2 mm.

2.3. MRI processing

Image processing for these data has been previously described (Meier et al., 2017). T_1-weighted images were skull stripped and transformed to the TT_N27 template available in the Analysis of Functional NeuroImages software package (AFNI) (Cox, 1996) by applying a 12-parameter affine transformation followed by a non-linear warp as implemented in the Advanced Normalization Tools (ANTS; Avants et al., 2011). An eroded white matter mask was created following tissue segmentation. FreeSurfer segmentation v5 (Fischl et al., 2002) was used to create a bilateral lateral ventricle mask in native space, which was transformed to standard space and eroded to exclude non-CSF voxels.

The first four EPI volumes were removed, anomalous time-series data were replaced using AFNI's despike program, and images were slice-time corrected. EPI volumes were registered to the first volume to account for head motion using a 6-degree of freedom transformation. Motion-corrected volumes were then aligned to the TT_N27 template

using an affine transformation and non-linear warp and resampled to 1.75 mm isotropic voxel size using ANTS. EPI images were then spatially smoothed using a Gaussian kernel with full-width at half-maximum of 4 mm. The six rigid-body motion parameters and their derivatives, the average lateral ventricle signal, the average local white matter signal, and bandpass filter frequencies (0.01–0.10 Hz), were regressed from the spatially smoothed data. Head motion was calculated as the Euclidian norm of the six motion parameters. Time points with volume-to-volume head motion > 0.30 mm were censored from analyses along with the preceding time point. Athletes with < 128 usable volumes following motion censoring or image artifacts were excluded from analyses. Rs-fMRI data from six concussed athletes at T1, five concussed athletes at T2, four concussed athletes at T3, and one control athlete were excluded due to poor scan quality or excessive head motion. The final number of subjects with usable rs-fMRI data is found in Table 1. All analyses were limited to gray matter voxels based on a binary mask from a tissue segmentation of the standard template (gray matter probability > 0.25) and further restricted to voxels in which all participants had EPI coverage.

2.4. Region of interest selection

A multivariate modeling method was used to assess connectivity at the network-level, where regions of interest (ROI) across a defined network are analyzed as simultaneous response variables (Chen et al., 2014; Taylor et al., 2016). A meta-analytic approach was used to define the ROI comprising an emotional processing network. Specifically, an automated meta-analysis of 427 studies that were selected based on the term “emotion” was performed using forward inference via the Neurosynth program, with false discovery rate (FDR) criterion of 0.01 (Yarkoni et al., 2011).

Results from the meta-analysis were warped to the TT_N27 template and resampled to 1.75 mm isotropic voxels. To limit the number of ROI, results were masked to include clusters larger than 200 voxels in resampled space (1072 μl), resulting in twelve distinct clusters. Several large clusters covered multiple anatomically distinct regions and were therefore split into multiple ROI. A total of 23 ROI were identified and created as 5 mm radius spheres at their respective centers of mass (Supplementary Fig. 1; Supplementary Table 1). The AFNI program 3dNetCorr was used to create a connectivity matrix of Fisher-Z transformed correlation coefficients between all ROI for each participant and time point (Taylor and Saad, 2013). Anatomical localization of ROI was based on online Neurosynth meta-analytic maps linked through AFNI (Yarkoni et al., 2011) and the macro-labels of the Eickhoff-Ziles brain atlases distributed with AFNI (Eickhoff et al., 2005). ROI were additionally characterized by their location relative to common resting state networks (RSN) based on their overlap with the Yeo cortical parcellation provided in FreeSurfer (Yeo et al., 2011). The Yeo volume parcellation was warped to TT_N27 template and the mode of Yeo mask values was calculated for each spherical ROI and used to assign each ROI a single cortical RSN. Subcortical ROI were considered separately as a unique network.

2.5. Statistics

Statistics were completed in IBM SPSS version 21 (IBM, Armonk, NY) and AFNI (Cox, 1996). Outliers for HAM-D scores were defined as being outside 3 times the interquartile range. The number of concussions and censored time points were square root transformed (with addition of a constant) to better approximate a normal distribution. Linear mixed-effects models with time as a fixed factor and a random intercept were used to determine changes in HAM-D scores over time. Independent samples *t*-tests were used to compare HAM-D scores between concussed and healthy athletes. For rs-fMRI analyses, group differences (i.e., healthy athletes versus concussed athletes at each visit) in connectivity across all ROI at the emotional processing network level were tested using AFNI's fat_mvm python programs, where pairs of ROI were treated as simultaneous response variables (Chen et al., 2014; Taylor et al., 2016). Similarly, longitudinal differences in connectivity across all ROI at the network level were assessed in concussed athletes with pairs of ROI treated as simultaneous response variables using a pairwise approach (i.e., T1 vs. T2, T1 vs. T3, and T2 vs. T3). For each individual visit post-concussion, pairs of ROI were treated as simultaneous response variables to assess the relationship between HAM-D scores and connectivity across the defined emotional processing network in concussed athletes only. For each analysis, effects of individual pairs of ROI were further assessed for descriptive purposes following significant effects across the entire emotional processing network (Taylor et al., 2016). A Bonferroni corrected alpha of 0.0055 was used to determine significance at the network level (i.e., emotional processing network), correcting for the number of a priori comparisons. An alpha of 0.05 was used to determine significance for post-hoc analyses of ROI within significant networks without correction for multiple comparisons. Finally, Spearman correlations were conducted between ROI that were correlated with the HAM-D total and each individual HAM-D item score (see Results) to determine which items were driving the observed relationship, corrected at FDR alpha = 0.05 for each visit.

3. Results

3.1. Demographic and behavioral comparisons

There were no significant differences in age, education, or the number of self-reported prior concussions between concussed and healthy athletes (*ps* > 0.05). The HAM-D score of one healthy athlete was an extreme outlier and was excluded from analyses. There was a significant effect of time point on depressive symptoms post-injury in concussed athletes ($F(2,61.23) = 37.69$, $p < 0.001$; Fig. 1), with higher HAM-D scores in concussed athletes at T1 relative to T2 ($p = 0.024$) and T3 ($p < 0.001$), and at T2 relative to T3 ($p < 0.001$). Concussed athletes also had higher HAM-D scores relative to healthy athletes at T1 ($t(82) = 10.34$, $p < 0.001$), T2 ($t(82) = 9.26$, $p < 0.001$), and T3 ($t(78) = 3.49$, $p = 0.001$).

3.2. rs-fMRI quality assurance and differences relative to controls

As previously reported, there were no differences in head motion or the number of censored time points between concussed or healthy athletes, or across time in concussed athletes (*ps* > 0.05) (Meier et al., 2017).

The effect of group (i.e., healthy athletes versus concussed athletes) on rs-fMRI across emotion processing ROI was not significant at T1

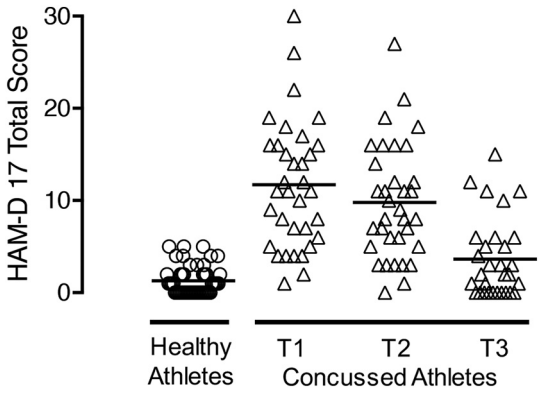

Fig. 1. HAM-D scores.
Shown are the total scores for Hamilton Depression Rating Scale (HAM-D) for healthy athletes as well as concussed athletes at one day (T1), one week (T2), and one month (T3) post-concussion. Horizontal bars represent the group mean.

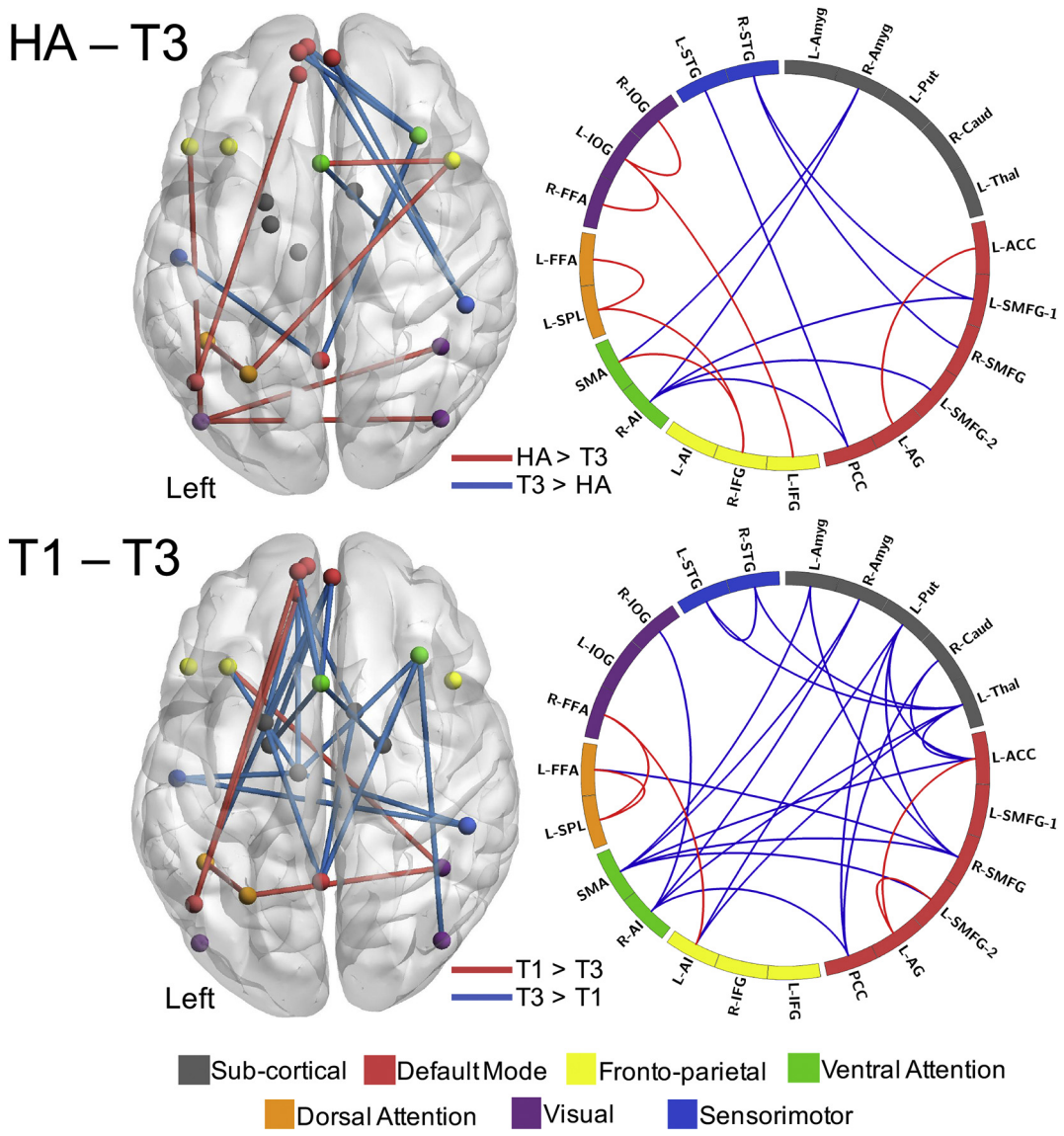

Fig. 2. Cross-sectional and longitudinal differences in functional connectivity at one month post-concussion.
Shown are pairs of regions of interest in which functional connectivity was significantly different at one month post-concussion (T3) relative to controls (top) and at one month post-concussion relative to one day post-concussion (T1; bottom). On the left, centers of mass for each region of interest are represented on the standard template. Images on the right show circle plots of significant region of interest connections. Colors of nodes and individual region of interest labels represent resting state network affiliations. Red connections represent connections stronger in controls (top) or at T1 (bottom) relative to T3. Blue connections represent connections stronger in concussed athletes at T3. L = Left, R = Right, Amyg = amygdala, Put = putamen, Caud = caudate, Thal = thalamus, ACC = anterior cingulate cortex, SMFG = superior medial frontal gyrus, AG = angular gyrus, PCC = posterior cingulate cortex, IFG = inferior frontal gyrus, AI = anterior insula, SMA = supplementary motor area, SPL = superior parietal lobe, FFA = fusiform face area, IOG = inferior occipital gyrus, STG = superior temporal gyrus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

($\chi^2 = 1.27$, $p = 0.26$) or T2 ($\chi^2 = 0.04$, $p = 0.85$). However, there was a significant difference in connectivity of the meta-analytically derived emotional processing network at T3 relative to controls ($\chi^2 = 8.95$, $p = 0.0028$). Follow-up analyses showed that this effect was driven by fifteen ROI-pairs ($p < 0.05$, uncorrected; Fig. 2, Supplementary Table 2). The right anterior insula and left inferior occipital gyrus were the ROI that had the greatest number of connections that were different between groups (Table 2). Additional characterization of ROI-pairs was performed based on the RSN of each ROI as previously defined (Yeo et al., 2011). The most common finding was increased connectivity between regions of the default mode and ventral attention networks at one month post-concussion, as well as increased connectivity between regions of the default mode and sensorimotor networks (Table 3).

3.3. Longitudinal rs-fMRI differences in concussed athletes

Pairwise longitudinal comparisons in concussed athletes demonstrated that connectivity in the emotional processing network was significantly different at T3 relative to T1 ($\chi^2 = 9.66$, $p = 0.0019$). Follow-up analyses showed that this effect was driven by twenty-seven ROI-pairs ($p < 0.05$, uncorrected; Fig. 2, Supplementary Table 3), with the supplementary motor area, left thalamus, and the left anterior cingulate cortex being the most common ROI with significantly different connectivity between T1 and T3 (Table 2). The most frequent findings included increased connectivity at T3 between the regions of the default mode network and sub-cortical regions, as well as increased connectivity at T3 between regions of the default mode and ventral attention networks (Table 3).

There were no differences in connectivity between T1 and T2

Table 2
Most common ROI significantly different at one month post-concussion.

HA vs. T3		T1 vs. T3	
ROI	Frequency	ROI	Frequency
R AI	4	L Thal	5
L IOG	3	L ACC	5
L SMFG-1	2	SMA	5
L SPL	2	L Put	4
PCC	2	R AI	4
R Amyg	2	R SMFG	4
SMA	2	L AI	3
R STG	2	PCC	3
R IFG	2	L Amyg	2
L IFG	1	L SPL	2
L SMFG-2	1	L STG	2
L ACC	1	L SMFG-2	2
R FFA	1	R Amyg	2
R SMFG	1	R Caud	2
R IOG	1	R FFA	2
L FFA	1	L FFA	2
L AG	1	R STG	2
L STG	1	L AG	2
		R IOG	1

Frequency = the total number of instances in which an ROI was significantly different at one month post-concussion. HA = healthy athletes, T1 = one day post-concussion, T3 = one month post-concussion, R = right, L = left, AI = anterior insula, SPL = superior parietal lobule, Amyg = amygdala, Caud = caudate, ACC = anterior cingulate cortex, SMFG = superior medial frontal gyrus, AG = angular gyrus, PCC = posterior cingulate cortex, IFG = inferior frontal gyrus, IOG = inferior occipital gyrus, Put = putamen, Thal = thalamus, FFA = fusiform face area, STG = superior temporal gyrus, SMA = supplementary motor area.

Table 3
Resting state networks significantly different at one month post-concussion.

HA vs. T3			T1 vs. T3		
Resting state network	Frequency	Direction	Resting state network	Frequency	Direction
DMN-VAN	3	T3 > HA	Sub BG-DMN	6	T3 > T1
DMN-SM	3	T3 > HA	DMN-VAN	4	T3 > T1
Sub Amyg-VAN	2	T3 > HA	Sub Amyg-VAN	3	T3 > T1
Visual-Visual	2	HA > T3	DMN-DMN	2	T1 > T3
DAN-DAN	1	HA > T3	Sub BG-FP	2	T3 > T1
DAN-FP	1	HA > T3	Sub BG-SM	2	T3 > T1
DMN-DMN	1	HA > T3	DAN-Visual	1	T1 > T3
FP-Visual	1	HA > T3	DAN-DAN	1	T1 > T3
VAN-FP	1	HA > T3	DMN-DAN	1	T3 > T1
			FP-Visual	1	T3 > T1
			SM-SM	1	T3 > T1
			Sub Amyg-DMN	1	T3 > T1
			Sub BG-VAN	1	T3 > T1
			VAN-Visual	1	T3 > T1

Frequency = the total number of instances in which resting state network pairs were significantly different at one month post-concussion. HA = healthy athletes, T1 = one day post-concussion, T3 = one month post-concussion, DMN = default mode network, VAN = ventral attention network, FP = fronto-parietal, Sub = sub-cortical, Amyg = amygdala, BG = basal ganglia, DAN = dorsal attention network, SM = sensorimotor.

($\chi^2 = 0.83$, $p = 0.36$) or between T2 and T3 ($\chi^2 = 0.75$, $p = 0.39$).

3.4. Association between rs-fMRI and total HAM-D following SRC

Associations between rs-fMRI and post-concussion HAM-D scores were assessed in the concussed group only. There was a significant relationship between HAM-D scores and rs-fMRI of the emotional processing network at T1 ($\chi^2 = 9.04$, $p = 0.0026$). Follow-up analyses showed that the effect was driven by nineteen ROI-pairs ($p < 0.05$ uncorrected; Fig. 3, Supplementary Table 4). As seen in Table 4, the right anterior insula and left superior parietal lobule ROI were most commonly associated with HAM-D scores. The most frequent finding was an inverse association of HAM-D scores and connectivity between regions in the default mode network to regions in attention and frontal networks, including the ventral attention network, dorsal attention network, and fronto-parietal network (Table 5). In contrast, one of the strongest observed effects was a positive relationship between depressive symptoms and connectivity of the right amygdala to the right caudate and left thalamus (Supplementary Table 4).

There was also a significant relationship between HAM-D scores and rs-fMRI at T2 ($\chi^2 = 29.07$, $p = 7 \times 10^{-8}$). Follow-up analyses showed that the network effect was driven by twenty-five ROI-pairs ($p < 0.05$ uncorrected, Fig. 3, Supplementary Table 5). ROI that were associated with HAM-D scores at T2 were like results at T1 and included right superior medial frontal gyrus, the left superior parietal lobule, and the right anterior insula (Table 4). As at the first visit, the most frequent finding at the second visit was an inverse relationship between HAM-D scores and connectivity of the default mode network to dorsal and ventral attention networks (Table 5).

There was no relationship between HAM-D scores and rs-fMRI at T3 ($\chi^2 = 0.47$, $p = 0.49$).

3.5. rs-fMRI differences relative to controls based on median split of HAM-D scores

Exploratory group analyses were conducted to compare emotional network connectivity in concussed athletes with high (SRC-high) or low HAM-D scores (SRC-low), based on median split, to healthy athletes to further characterize the nature of connectivity associated with HAM-D scores in concussed athletes. At T1, neither the SRC-high ($\chi^2 = 1.67$, $p = 0.20$) nor the SRC-low ($\chi^2 = 1.56$, $p = 0.21$) groups differed from controls. Similarly, at T2, neither the SRC-high ($\chi^2 = 0.10$, $p = 0.75$) nor the SRC-low ($\chi^2 = 0.36$, $p = 0.55$) groups differed from controls.

3.6. Association between rs-fMRI and specific HAM-D item scores

At T1, there were seven significant relationships following FDR correction (Fig. 4). The HAM-D item 'Depressed Mood' was inversely correlated with connectivity between the right anterior insula to three default mode regions ($r(s) = -0.58$ to -0.66; see Supplement for specific regions and item prompts). The items 'Genital Symptoms' and 'Hypochondriasis' were both inversely correlated with connectivity of the right anterior insula to separate default mode regions ($r(s) = -0.58$ for both). 'Anxiety Somatic' responses were positively correlated with connectivity of the right amygdala to the right caudate ($r(s) = 0.58$). 'Retardation' was inversely correlated with connectivity of the posterior cingulate cortex to the right inferior frontal gyrus ($r(s) = -0.59$).

At T2 (Fig. 4), an inverse correlation between 'Depressed Mood' and connectivity of the left angular gyrus to supplementary motor area was the only significant finding with FDR correction ($r(s) = -0.69$).

4. Discussion

Few studies have examined the physiological correlates of depressive symptoms following SRC, despite the prevalence of these symptoms following brain injury. This work investigated alterations in functional connectivity in regions associated with emotional processing across the acute and sub-acute phases of concussion. Overall, concussed athletes showed significantly different connectivity in regions associated with emotional processing at one month post-concussion relative to connectivity at one day post-injury and healthy controls. These differences were prominently characterized by increases in connectivity between the DMN and attention regions at one month post-concussion.

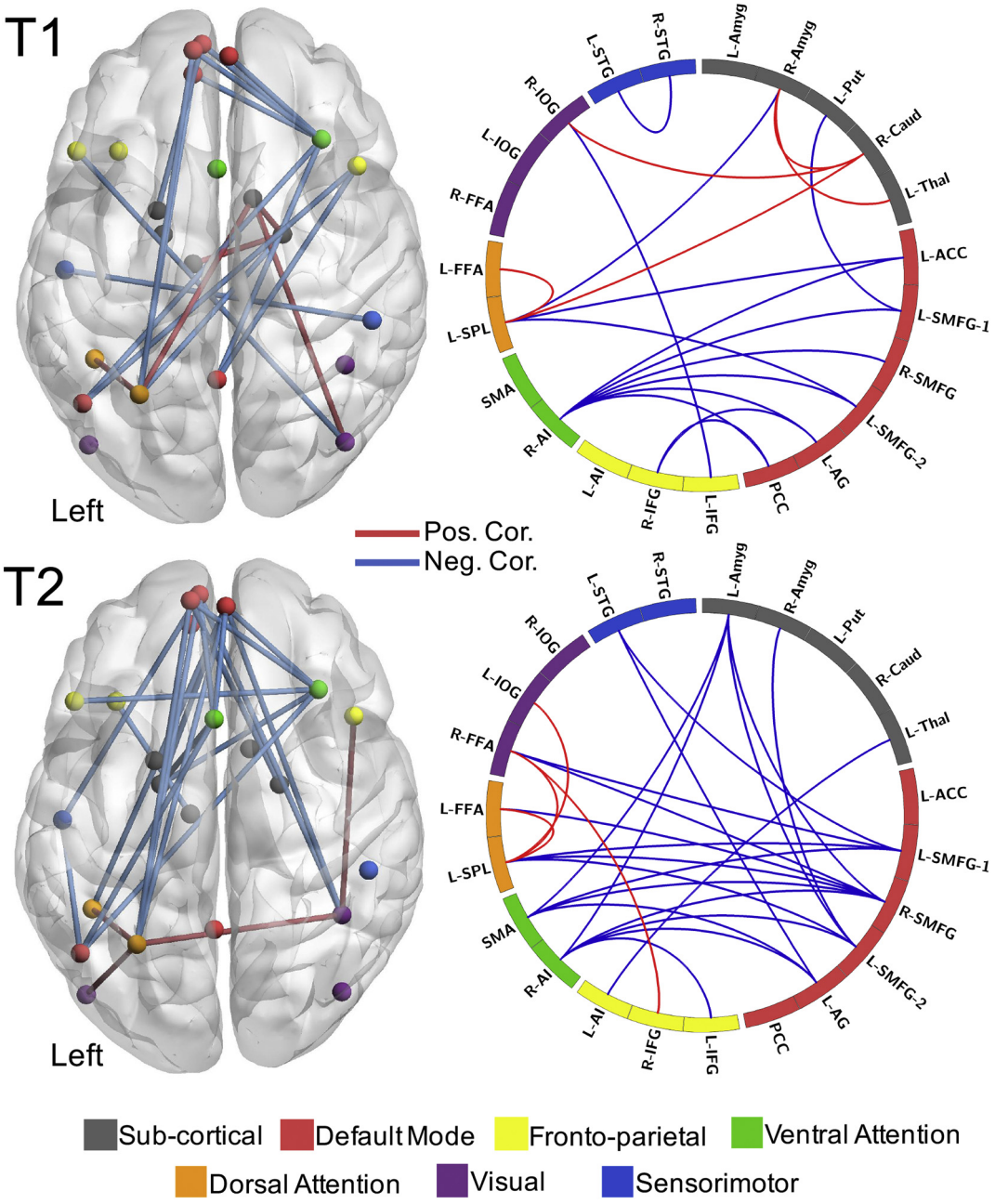

Fig. 3. Association between functional connectivity and HAM-D scores following SRC.
Shown are pairs of regions of interest in which functional connectivity was significantly associated with Hamilton Depression Rating Scale (HAM-D) scores at one day (T1; top) and one week post-concussion (T2; bottom). On the left, centers of mass for each region of interest are represented on the standard template. Images on the right show circle plots of significant region of interest connections. Colors of nodes and individual region of interest labels represent resting state network affiliations. Red connections represent positive correlations between connectivity and HAM-D scores, while blue connections represent negative correlations. L = Left, R = Right, Amyg = amygdala, Put = putamen, Caud = caudate, Thal = thalamus, ACC = anterior cingulate cortex, SMFG = superior medial frontal gyrus, AG = angular gyrus, PCC = posterior cingulate cortex, IFG = inferior frontal gyrus, AI = anterior insula, SMA = supplementary motor area, SPL = superior parietal lobe, FFA = fusiform face area, IOG = inferior occipital gyrus, STG = superior temporal gyrus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Furthermore, as hypothesized, post-concussion depressive symptoms were also associated with functional connectivity of emotional processing regions at one day and one week post-concussion. Primarily, concussed athletes with greater HAM-D scores had lower connectivity between attention regions and regions of the DMN. Finally, correlations with individual HAM-D item scores showed that this relationship was not driven by a general increase in somatic complaints commonly observed after SRC (e.g., headaches). These results suggest that connectivity between emotional processing regions is associated with post-concussion depressive symptoms.

As previously reported, concussed athletes had elevated HAM-D scores across the acute and sub-acute phase relative to control athletes, with partial recovery toward control levels by one month post-injury (Meier et al., 2017). While our results of increased depressive symptoms during the acute phase are consistent with other reports (Kontos et al., 2012), the observed delayed recovery contrasts with the more rapid recovery of self-report symptoms, neurocognitive testing deficits, or balance deficits observed in large-scale clinical studies (i.e., within

Table 4
Most common ROI with HAM-D association.

One day post-concussion		One week post-concussion	
ROI	Frequency	ROI	Frequency
R AI	6	L SPL	6
L SPL	5	R SMFG	6
R Amyg	3	R AI	5
R Caud	3	L SMFG-1	4
L ACC	2	L SMFG-2	4
L SMFG-1	2	L Amyg	4
L SMFG-2	2	SMA	4
L AG	2	R FFA	4
PCC	2	L AG	3
R IFG	2	L FFA	2
R IOG	2	L STG	2
L Put	1	R Amyg	1
L Thal	1	R IFG	1
R SMFG	1	L Thal	1
L IFG	1	L IFG	1
L FFA	1	L AI	1
L STG	1	L IOG	1
R STG	1	R Caud	0
L Amyg	0	L ACC	0
L AI	0	PCC	0
SMA	0	R IOG	0
R FFA	0	L Put	0
L IOG	0	R STG	0

Frequency = the total number of instances in which an ROI was significantly associated with HAM-D scores. R = right, L = left, AI = anterior insula, SPL = superior parietal lobule, Amyg = amygdala, Caud = caudate, ACC = anterior cingulate cortex, SMFG = superior medial frontal gyrus, AG = angular gyrus, PCC = posterior cingulate cortex, IFG = inferior frontal gyrus, IOG = inferior occipital gyrus, Put = putamen, Thal = thalamus, FFA = fusiform face area, STG = superior temporal gyrus, SMA = supplementary motor area.

Table 5
Most common resting state networks with HAM-D association.

One day post-concussion			One week post-concussion		
Resting state network	Frequency	Direction	Resting state network	Frequency	Direction
DMN-VAN	6	Negative	DMN-VAN	6	Negative
DMN-FP	2	Negative	DMN-DAN	4	Negative
Sub Amyg-Sub BG	2	Positive	Sub Amyg-DMN	3	Negative
DMN-DAN	2	Negative	DAN-Visual	2	Positive
SM-SM	1	Negative	DMN-SM	2	Negative
Sub Amyg-DAN	1	Negative	DMN-Visual	2	Negative
Sub BG-DAN	1	Positive	Sub Amyg-VAN	2	Negative
Sub BG-DMN	1	Negative	DAN-DAN	1	Positive
Sub BG-Visual	1	Positive	FP-Visual	1	Positive
DAN-DAN	1	Positive	Sub BG-FP	1	Negative
FP-Visual	1	Negative	VAN-FP	1	Negative

Frequency = the total number of instances in which resting state network pairs were significantly associated with HAM-D scores. DMN = default mode network, VAN = ventral attention network, FP = fronto-parietal, Sub = sub-cortical, Amyg = amygdala, BG = basal ganglia, DAN = dorsal attention network, SM = sensorimotor.

7 days in collegiate athletes) (Williams et al., 2015). It is possible that post-concussion depressive symptoms persist relative to other post-concussion symptoms. Factors including the relatively small sample size, variations in baseline symptoms, or variations in assessment methodology (i.e., self-report measures versus structured interviews) could also account for the extended recovery timeline for depressive symptoms in the current study.

Contrary to our hypothesis, no acute connectivity differences in

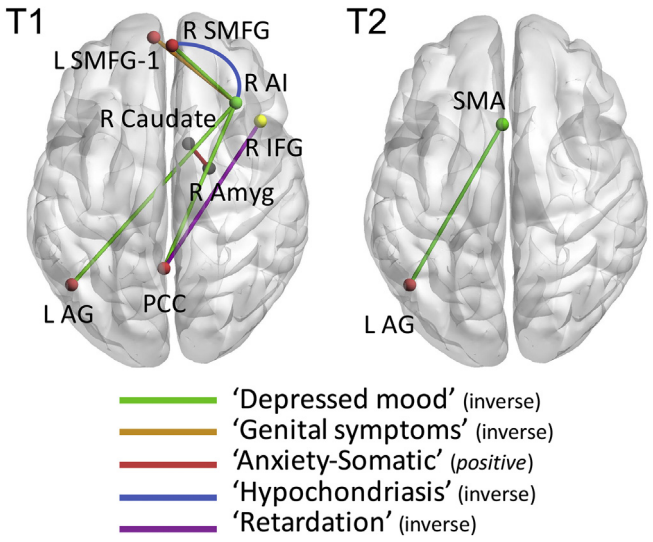

Fig. 4. Association between specific HAM-D item scores and functional connectivity.
Shown are connections between regions of interest that significantly correlated with an individual Hamilton Depression Rating Scale (HAM-D) item at one day (T1) and one week post-concussion (T2). The color of connections indicates the HAM-D item; the direction of the relationship is indicated for each item (i.e., a positive or inverse relationship between connectivity and HAM-D item scores). Question prompts for relative items can be found in the Supplement. L = left, R = right, SMFG = superior medial frontal gyrus, AG = angular gyrus, PCC = posterior cingulate cortex, AI = anterior insula, IFG = inferior frontal gyrus, Caud = caudate, Amyg = amygdala, SMA = supplementary motor area. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

emotional processing regions were observed in the current study. Several limiting factors may have contributed to this null finding at the acute phases, including the innate heterogeneity of SRC and a lack of pre-injury imaging. Null functional connectivity results following mTBI are not unprecedented (Mayer et al., 2015; van der Horn et al., 2016; Zhang et al., 2012). Differences, however, were observed at one month post-concussion relative to the acute visit (i.e., T1) and controls, with the majority of connections being stronger at one month post-injury in concussed athletes. Significant patterns of connectivity that were consistent across both analyses (i.e., T1–T3 and HA–T3) included stronger connections at one month post-SRC between regions of the DMN and ventral attention network. As previously reported, one explanation for the delayed onset of connectivity differences is that these effects are driven by secondary injury factors that are common following mTBI, such as inflammation (Meier et al., 2017; Giza and Hovda, 2014). It is also possible that the increase in connectivity between the DMN and ventral attention network at one-month post-concussion beyond levels observed in control athletes reflects a compensatory response to injury, which is discussed in more detail below.

Although there were no group differences relative to controls at the first two visits, higher post-concussion HAM-D scores in concussed athletes were associated with lower connectivity of the meta-analytically derived emotional processing network. Specifically, concussed athletes with more depressive symptoms had reduced connectivity between attention and DMN regions at one day and one week post-concussion. Alterations in the functional connectivity of DMN are among the most commonly reported findings following SRC (McCrea et al., 2017), with several previous studies observing altered connectivity of the DMN across the first month following SRC (Meier et al., 2017; Johnson et al., 2012; Zhu et al., 2015). This is consistent with the known sensitivity of midline structures (e.g., medial prefrontal cortex) to shearing associated with rotational forces of head injury (Zhang et al., 2004). Disruption of attention networks including insular

connectivity is also commonly reported following mTBI (van der Horn et al., 2016; Bharath et al., 2015), which may be indicative of concussion-related sensitivity in highly interconnected networks and structures.

The relationship between post-concussive functional connectivity and depressive symptoms observed in the current study is also consistent with prior work that suggests hyperconnectivity following mTBI may be compensatory in nature, though the timing of the observed hyperconnectivity has varied across studies (Iraji et al., 2015; Sours et al., 2013). With regard to emotional processing, higher connectivity of the medial prefrontal cortex has been associated with fewer mood symptoms at two months post-concussion (Zhou et al., 2012). Similarly, reduced functional connectivity between the salience network (i.e., ventral attention network) and a bilateral frontal network was linked to increased self-reported depression symptoms while reduced connectivity between the medial prefrontal cortex and bilateral frontal regions was linked to increased anxiety symptoms one month post-mTBI (van der Horn et al., 2016). This evidence of post-injury hyperconnectivity as being compensatory is not limited to indicators of emotional well-being. For example, increased functional connectivity of attention and default networks was associated with decreased cognitive symptoms approximately one month post-concussion as well (Sours et al., 2013).

One hypothesis relating to the hyperconnectivity observed in the present results is that they reflect compensatory top-down regulation of negative self-referential thoughts following SRC. The DMN is associated with self-referential processing (Buckner et al., 2008) whereas the attention networks play a critical role in assessing stimuli relevance and are more active during goal-directed cognitive processes (Fox and Raichle, 2007). Attention networks have also been associated with top-down modulation of emotional processing, including the suppression and reappraisal of negative emotions (Sliz and Hayley, 2012). Accordingly, MDD has been associated with negative self-referential processing, poor stimuli appraisal, and impairments in cognitive control (Beck, 2008). The results of the present study, however, depart from the literature on MDD in that DMN alterations associated with clinically significant levels of depression typically report increased connectivity within and between the DMN (Hamilton et al., 2015). This distinction is not surprising given markedly different duration of depressive symptoms between MDD and typical SRC patients (i.e., chronic symptoms in MDD versus transient symptoms observed in SRC). Additional research is needed to delineate specific processes underlying mood disturbance following SRC and to determine if the physiological mechanisms of post-concussion depressive symptoms are similar to, or distinct from, those of MDD (e.g., enrollment of a non-concussed, depressed control group).

An alternative hypothesis for the relationship between HAM-D scores and connectivity in concussed athletes observed in the current study is that concussed athletes with greater HAM-D symptoms have lower than normal connectivity (i.e., hypoconnectivity), in contrast to the hypothesis that concussed athletes with low HAM-D symptoms have hyperconnectivity. The observed group differences at one month post-concussion, however, are consistent with the hyperconnectivity hypothesis. Importantly, regions that showed increased connectivity across both analyses at one month post-SRC (i.e., the time point in which post-SRC depressive symptoms were lowest) largely included connections between DMN and attention networks. This is consistent with the observed inverse relationship between internetwork connectivity of DMN to attention areas and depressive symptoms at the earlier time points. Nevertheless, exploratory analyses at one day and one week post-concussion comparing concussed groups with high or low HAM-D scores to controls were not significant. Thus, we cannot rule out the hypoconnectivity hypothesis.

The exact neurophysiological underpinnings of the observed functional connectivity effects are undetermined. The acute neurometabolic cascade of concussion includes increases in excitatory neurotransmission, altered glucose metabolism, reductions in cerebral blood flow, and altered white matter integrity via shear-strain forces (Giza and Hovda, 2014). Each of these factors could affect the BOLD signal. Thus, we cannot definitively state that differences in post-concussion functional connectivity reflect neuronal connectivity, per se. It is possible, for example, that the increased connectivity observed in concussed athletes with lower depressive symptoms is secondary to these metabolic and/or axonal effects, rather than active compensatory recruitment processes. The inability to rule out these alternative explanations demonstrates a need for large-scale studies implementing multi-modal imaging techniques capable of assessing these other factors.

The current results should be interpreted within the study limitations. First, although this study included multiple post-concussion visits in injured athletes, no pre-injury data were available (e.g., imaging or depressive symptoms). Future work assessing changes in connectivity and depressive symptoms from pre- to post-injury is needed. Second, the analytical approach used provides only ‘weak’ family-wise error correction for the post-hoc comparisons that were used to describe connections most responsible for the significant effects observed at the network level. Thus, we cannot rule out Type I error at the level of individual ROI connections. Finally, previous work has demonstrated that the test-retest reliability of resting state metrics is improved with scan durations longer than the 6 min used in the current study (Birn et al., 2013), though earlier work has demonstrated that scans of this duration can be used to calculate stable resting state metrics (Van Dijk et al., 2009). Nevertheless, it is possible that there are dynamics at lower resting state frequencies that were not captured in the current study.

5. Conclusion

Post-concussion depressive symptoms correlate with resting state functional connectivity of brain regions associated with emotional processing. Increased connectivity between nodes of attention and default mode networks is associated with lower depressive symptoms and may represent compensatory connectivity. In addition, across all SRC patients, connectivity between DMN and attention regions showed a delayed elevation at one month post-concussion, corresponding to the visit at which concussed athletes had the fewest depressive symptoms. Additional research aimed at identifying pathophysiological signatures of specific post-concussion symptoms is needed. In the SRC literature, the objective quantification of depressive symptoms has the potential to unearth unique prognostic trajectories and ultimately inform clinical treatment.

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Disclosures

The authors have no competing interests to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2018.05.011.

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