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Positive association between serum quinolinic acid and functional connectivity following concussion

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Abstract

The molecular mechanisms underlying the diverse psychiatric and neuropathological sequalae documented in subsets of athletes with concussion have not been identified. We have previously reported elevated quinolinic acid (QuinA), a neurotoxic kynurenine pathway metabolite, acutely following concussion in football players with prior concussion. Similarly, work from our group and others has shown that increased functional connectivity strength, assessed using resting state fMRI, occurs following concussion and is associated with worse concussion-related symptoms and outcome. Moreover, other work has shown that repetitive concussion may have cumulative effects on functional connectivity and is a risk factor for adverse outcomes. Understanding the molecular mechanisms underlying these cumulative effects may ultimately be important for therapeutic interventions or the development of prognostic biomarkers. Thus, in this work, we tested the hypothesis that the relationship between QuinA in serum and functional connectivity following concussion would depend on the presence of a prior concussion. Concussed football players with prior concussion (N=21) and without prior concussion (N=16) completed a MRI session and provided a blood sample at approximately 1 days, 8 days, 15 days, and 45 days post-injury. Matched, uninjured football players with (N=18) and without prior concussion (N=24) completed similar visits. The association between QuinA and global connectivity strength differed based on

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group (F(3, 127)=3.46, p=0.019); post-hoc analyses showed a positive association between QuinA and connectivity strength in concussed athletes with prior concussion (B=16.05, SE = 5.06, *p*=0.002, 95%CI[6.06, 26.03]), but no relationship in concussed athletes without prior concussion or controls. Region-specific analyses showed that this association was strongest in bilateral orbitofrontal cortices, insulae, and basal ganglia. Finally, exploratory analyses found elevated global connectivity strength in concussed athletes with prior concussion who reported depressive symptoms at the 1-day visit compared to those who did not report depressive symptoms (*t*(15)=2.37, mean difference=13.50, SE=5.69, *p*=0.032, 95%CI[1.36, 25.63], Cohen's d =1.15.). The results highlight a potential role of kynurenine pathway (KP) metabolites in altered functional connectivity following concussion and raise the possibility that repeated concussion has a "priming" effect on KP metabolism.

Keywords

kynurenine pathway; resting state; mild traumatic brain injury

1. Introduction

Reports of increased psychiatric (e.g., major depressive disorder [MDD]) and neurodegenerative disease (e.g., chronic traumatic encephalopathy) in retired and deceased athletes have led to an increase in public concern regarding the potential adverse, long-term effects of sport-related concussion (SRC) on athletes of all ages (Guskiewicz et al., 2007; Manley et al., 2017; McKee et al., 2013). However, the molecular mechanisms underlying the diverse psychiatric and neuropathological sequalae documented in subsets of athletes with concussion have yet to be definitively determined. Here, we focus on the kynurenine pathway (KP; Supplementary Figure 1), a key energy-regulating pathway that has been hypothesized to link inflammation and glutamatergic signaling in numerous psychiatric (e.g., MDD) and neurodegenerative diseases (e.g., Alzheimer's disease) (Savitz, 2020).

Activated immune cells require more energy than naïve cells and the KP is the major endogenous source of nicotinamide adenine dinucleotide (NAD+). Inflammatory mediators increase the catabolism of tryptophan (TRP) into kynurenine (KYN) via the enzyme indoleamine 2,3-dioxygenase (*IDO*). Kynurenine-3-monooxygenase (*KMO*), which is also activated by inflammatory cytokines, converts KYN into 3-hydroxykynurenine (3HK), ultimately leading to the formation of quinolinic acid (QuinA) and NAD+. 3HK is a potent free-radical generator while QuinA is a N-methyl-D-aspartate (NMDA) receptor agonist that potentiates the release and impairs the reuptake of glutamate (Guillemin, 2012). Alternatively, KYN is metabolized by kynurenine aminotransferase (*KAT*) enzymes into kynurenic acid (KynA), a competitive antagonist of ionotropic excitatory amino acid receptors, including NMDA, that is generally considered to be neuroprotective (Foster et al., 1984; Kessler et al., 1989). As an illustrative example, we have previously shown reductions in KynA relative to QuinA (KynA/QuinA; a putative neuroprotective index) in MDD patients compared to controls (Savitz et al., 2015c, 2015b), and decreases in KynA and/or elevations in QuinA have been reported in several neurodegenerative disorders (Heilman et al., 2020; Sorgdrager et al., 2019).

Evidence suggests that after concussion, and traumatic brain injury (TBI) of all severities, the KP is activated and metabolism down the QuinA/NAD+ branch of the KP is favored - although the increase in QuinA could also arise from decreased breakdown of QuinA into NAD+, as has been shown in a number of *in vitro* and non-TBI preclinical models (Jones et al., 2015; Minhas et al., 2019; Poyan Mehr et al., 2018; Sahm et al., 2013). Irrespective, elevated KYN relative to TRP (KYN/TRP; a proxy for *IDO* activity) has been reported in chronic TBI patients (Mackay et al., 2006) and acutely injured TBI patients have elevated QuinA in cerebral spinal fluid compared to controls (Bell et al., 1999; Sinz et al., 1998; Yan et al., 2015). Regarding concussion, our group has previously shown decreased KynA/QuinA and elevated QuinA in plasma in concussed football players at approximately 1 day, 1 week and 1 month post-concussion relative to uninjured football players (Singh et al., 2016). Likewise, we have reported elevated plasma QuinA in football players with a remote history of concussion (i.e., latest concussion on average 10 months prior to visit) versus football players without prior concussion (Meier et al., 2016b). Finally, we have recently shown in an independent cohort of high school and collegiate athletes that recently concussed football players with a prior concussion had reduced serum KynA/QuinA and elevated QuinA across multiple time points up to 45 days post-injury compared to controls and acutely concussed athletes without a prior concussion (Meier et al., 2020b). Taken together, these findings suggest that prior concussion may result in a predisposition towards metabolism down the QuinA pathway or its preferential accumulation.

Parallel findings demonstrate that acute concussion is associated with differences in intrinsic brain connectivity as measured by resting state functional magnetic resonance imaging (rs-fMRI). Functional connectivity abnormalities have been repeatedly demonstrated acutely and sub-acutely following SRC, though specific findings have varied across studies likely due to differences in methodology (e.g., selection of seed-regions or networks versus global metrics), differences in cohorts, and differences in study design (e.g., time since injury, comparison groups) (for review; [Mayer et al., 2015; McCrea et al., 2017]). In an overlapping sample with the aforementioned study investigating KP metabolites in football players up to 45 days post-injury, we recently found elevated global connectivity strength at the sub-acute phase in concussed football players relative to controls, with sub-analyses demonstrating that this effect was driven by symptomatic athletes after concussion, rather than those that no longer reported symptoms (Kaushal et al., 2019). Additional work further supports the hypothesis that hyperconnectivity (i.e., stronger connectivity relative to controls) may be pathological following concussion (i.e., is associated with poorer recovery or more symptoms) (N. W. Churchill et al., 2017; Meier et al., 2020a; van der Horn et al., 2017), though opposite patterns have also been reported (for review; (Puig et al., 2020)).

There is also evidence of altered functional connectivity due to chronic or repetitive SRC. Prior work has shown both positive and negative associations between connectivity and prior concussion. For example, retired football players with multiple prior concussions had increased connectivity between the anterior temporal lobe and orbitofrontal cortex relative to healthy controls (Goswami et al., 2016). Asymptomatic hockey players with prior concussion had region specific increases and decreases in connectivity of the default mode network relative to players with no prior concussion (Orr et al., 2016). Finally, the number of

prior concussions was inversely or positively associated with connectivity across several seed regions in men and women collegiate athletes (N. Churchill et al., 2017).

We have previously reported increased connectivity between the motor cortex and supplementary cortex in football players with prior concussion relative to those without (Meier et al., 2017). Moreover, in that same work we identified associations between rs-fMRI and neuroactive KP metabolites in collegiate athletes with varying concussion history, consistent with the indirect relationship between glutamatergic neurotransmitter flux (i.e., cycling of glutamate and glutamine) and the blood-oxygen-level-dependent (BOLD) signal (Hyder et al., 2002; Smith et al., 2002). Specifically, across all participants, lower plasma KynA/QuinA was associated with greater functional connectivity between the anterior cingulate cortex, orbitofrontal cortex, hippocampus, and motor cortex to several regions including the insula, superior temporal gyrus, and visual cortex. Most relevant to the current work, prior concussion status moderated the association between connectivity and KP metabolites. That is, football players with prior concussion predominantly showed an inverse association between KynA/QuinA and connectivity of the ACC (to frontal cortex and anterior insula) as well as connectivity of the hippocampus (to visual cortex) (Meier et al., 2017).

The current study expands upon the aforementioned findings of increased functional connectivity and elevated QuinA (and reduced KynA/QuinA) following concussion in overlapping samples of high school and collegiate athletes (Kaushal et al., 2019; Meier et al., 2020b). Our goal was to determine the extent to which associations between functional connectivity and neuroactive KP metabolites differ based on acute concussion status (i.e., recent concussion versus. no recent concussion) and prior concussion history (i.e., prior concussion versus no prior concussion). Based on our prior work (Kaushal et al., 2019; Meier et al., 2020b, 2017, 2016b; Singh et al., 2016), we hypothesized that elevated QuinA and lower KynA/QuinA would be associated with increased global functional connectivity in acutely injured athletes with prior concussion.

2. Materials and Methods

2.1 Participants

High school and collegiate football players were enrolled as part of a prospective study of concussion, which has been detailed previously (Kaushal et al., 2019; Meier et al., 2020b). Exclusion criteria for the current study included: injury precluding participation in the study or other contraindications to study procedures, current narcotic use, conditions known to cause cognitive dysfunction (e.g., moderate to severe TBI, epilepsy), psychopathology (e.g., mood disorders), migraines or recurrent headaches, attention deficit/hyperactivity disorder, memory difficulties, structural MRI findings that required clinical follow-up (Klein et al., 2019), and a history of a potentially confounding illness/disease (e.g., meningitis; full list of exclusionary diseases can be found in Supplementary Table 1). The study was approved by the institutional review board at the Medical College of Wisconsin. Adult participants and parents of minors provided written informed consent; minors provided written assent.

Football players completed preseason baseline clinical assessments. Players that sustained a concussion during the study period completed up to four follow-up visits that included an MRI session and blood collection: approximately 24–48 hours (1d), 8 days (8d), 15 days (15d), and 45 days (45d) post-injury. Certified athletic trainers or team physicians trained in sports medicine initially identified and diagnosed concussions. Study investigators screened all injuries to ensure they met the study definition of concussion, which was based on the Centers for Disease Control and Prevention HEADS UP educational initiative: “An injury resulting from a forceful bump, blow, or jolt to the head that results in rapid movement of the head and causes a change in the athlete’s behavior, thinking, physical functioning, or the following symptoms: headache, nausea, vomiting, dizziness/balance problems, fatigue, difficulty sleeping, drowsiness, sensitivity to light/noise, blurred vision, memory difficulty, and difficulty concentrating”.

Uninjured football players without concussion in the last 6 months were selected from enrolled athletes to match injured athletes based on the following criteria: level of competition, institution, team, estimated intellectual functioning (word reading performance at baseline), race, handedness, concussion history, and position. Control participants completed the same study protocol as concussed participants at similar intervals.

A total of 37 football players with concussion and 42 uninjured football players met the study criteria and had MRI and blood data from at least one follow-up visit. For the purposes of the current study, concussed and uninjured athletes were further characterized based on concussion history, as in our prior work (Meier et al., 2020b). Final groups included concussed athletes without prior concussion (SRC-; n=16), concussed athletes with prior concussion (SRC+; n=21), contact controls without prior concussion (CC-; n=24), and contact controls with prior concussion (CC+; n=18). The demographic details for each group are presented in Table 1.

2.2 Clinical battery

The clinical battery has been described in detail previously (Kaushal et al., 2019; Meier et al., 2020b). Data collected at baseline included demographic and health information and the Wechsler Test of Adult Reading to estimate intellectual functioning, (WTAR). In addition, athletes were asked about their concussion history at baseline after being provided with a standard definition based on the United States Department of Defense (Carney et al., 2014). The clinical battery included measures of psychological distress (Brief Symptom Inventory–18; BSI-18), concussion symptom severity (The Sport Concussion Assessment Tool–3rd Edition symptom; SCAT), balance deficits (Balance Error Scoring System; BESS), and neurocognitive performance (Standardized Assessment of Concussion; SAC). Information regarding acute injury characteristics and length of recovery was collected at follow-up visits.

2.3 Blood biomarker data

Venous blood was collected using Red Top BD Vacutainer tubes, left to clot at room temperature for 30 min, centrifuged at 1,500 RCF for 15 min and stored at -80 °C. Quinolinic acid (QuinA), kynurenic acid (KynA), 3-hydroxykynurenine (3HK), tryptophan

(TRP), and kynurenine (KYN) concentrations were determined from serum blind to diagnosis using high-performance liquid chromatography with tandem mass spectrometry detection by Charles River Laboratories, Inc. according to their standard protocol.

2.4 Imaging parameters and processing

Imaging data were obtained on a 3 Tesla General Electric MR750 whole-body MR scanner using 32-channel receiver coil array. Rs-fMRI data were collected using a gradient-echo echo-planar image (EPI) with the following parameters: 501 volumes, FOV=210 mm, acquisition matrix=104 × 104, slice thickness=2 mm, 72 sagittal slices, TR/TE=720/30 ms, flip angle=50°, hyperband acceleration factor=8. During the rs-fMRI scan, participants were instructed to keep their eyes open and think of nothing in particular. A reverse phase-encoded scan was collected to allow susceptibility-induced distortion correction. High-resolution T1-weighted structural images were obtained for anatomical reference using a magnetization-prepared rapid gradient-echo sequence with the following parameters: FOV=256 mm, acquisition matrix=256, slice thickness=1 mm, 160 slices, TR/TE/TI=7.592/3.008/900 ms, flip angle=8°.

Unless otherwise noted, preprocessing was performed using Analysis of Functional NeuroImages programs (AFNI) (Cox, 1996) as previously described (Kaushal et al., 2019). Anatomical images were skull-stripped in native space using a union mask of segmented gray matter and white matter from SPM 12. The skull-stripped brain was registered to the MNI-152 template using an affine registration with correlation ratio cost function and trilinear interpolation followed by a nonlinear warp, implemented in FSL (Jenkinson et al., 2002). The first 29 volumes of the resting-state scan were removed to account for auto-calibration data and allow for stabilization of longitudinal magnetization, and the AFNI program 3dDespike was used to remove signal spike artifacts. Susceptibility-induced distortion correction was performed using FSL's topup (Andersson et al., 2003). Volumes were registered to the first volume to account for head motion. A single transformation matrix was created for spatial normalization by concatenating the anatomy-to-MNI-152 matrix and the matrix resulting from a 6°-of-freedom registration of the rs-fMRI volume to the anatomical scan calculated using FSL's FLIRT with the boundary-based registration cost-function (Greve and Fischl, 2009; Jenkinson et al., 2002). The resulting matrix and the nonlinear warp from the anatomy-to-MNI-152 brain were applied to the motion corrected image to bring the image in standard space with 2 mm isotropic resolution.

Signals of no-interest, including the average CSF signal, average white matter signal, the six motion parameters and their derivatives, and the zero- through third-order polynomial trends were then regressed from the rs-fMRI data. Volumes with excessive head motion (i.e., Euclidian norm of the six motion parameters >0.30) were removed along with the preceding volume and replaced using interpolation. Denoised images were then bandpass filtered (0.01 to 0.10 Hz). Resting-state scans with visually-identified artifacts and scans in which the average Euclidian norm of motion parameters was greater than 0.2 were excluded from analyses to minimize potential effects of motion on group analyses. Table 1 shows the sample size with usable data at each time point.

The AFNI program 3dNetCorr was used to calculate a connectivity matrix for each participant at each time point using regions-of-interest (ROI; or nodes) derived from the automated anatomical labeling atlas (AAL2) (Rolls et al., 2015; Taylor and Saad, 2013). The connectivity strength (i.e., nodal strength) of each individual ROI was calculated from the weighted connectivity matrices as the sum of weights of all connections to that ROI, implemented in the BRain analysis using GraPH theory (BRAPH) software (Mijalkov et al., 2017; Rubinov and Sporns, 2010). The average strength across all ROIs was calculated as a measure of global connectivity strength (i.e., average nodal strength). Negative correlations were not included in calculations of connectivity strength. To ensure that the observed results were not sensitive to the selected brain atlas, identical procedures were performed using the Craddock whole-brain functional atlas (200 ROI atlas based on r_t 2-level parcellation) (Craddock et al., 2012).

2.5 Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics version 24 (Armonk, NY) unless otherwise indicated. Analyses of variance, Kruskal-Wallis tests, chi-square tests, or Fisher's exact tests compared demographic variables, clinical measures, and head motion during scanning across groups. For descriptive purposes, clinical variables (i.e., BSI-18 global severity index, SCAT, BESS, SAC) were compared between groups at baseline and at the 1d visit. Individual KP markers (e.g., QuinA) and the relevant ratios (e.g., KynA/QuinA) were natural log-transformed to normalize their distribution. Linear mixed-effects (LME) models were fit to determine the interaction between group (SRC+, SRC-, CC+, CC-) and log-transformed KP metabolite (each metabolite and ratio run separately) on the global connectivity strength measure; the main effects of group and KP metabolite were included. To account for repeat scans and KP measurements, visit was modeled and participant was included as a random factor. Primary analyses focused on the global connectivity strength measure derived using the AAL2 parcellation. To confirm that the results were not biased by the parcellation scheme, identical LME were fit using the Craddock parcellation as sensitivity analyses. An alpha of 0.05 was used for primary KP metabolites (QuinA, KynA/Quin); while an alpha of 0.0083 (Bonferroni corrected 0.05/6) was considered significant for secondary KP outcomes (KynA/3HK, KynA, 3HK, KYN/TRP, KYN, TRP).

Based on significant global connectivity results of the above analyses, LME were fit in the SRC+ group to characterize the association between QuinA and connectivity strength of each individual node from the AAL2 parcellation. Visit was modeled and participant treated as a random factor as above. For these analyses, Benjamini-Hochberg False-Discovery Rate (FDR) of $q<0.05$ determined significance to account for multiple testing across 120 nodes (i.e., regions).

Given the known associations between QuinA and depression (Savitz, 2017), additional exploratory analyses were conducted in SRC+ participants to determine if either global connectivity strength or connectivity strength of individual ROI (limited to those showing associations with QuinA, see Results) were associated with BSI-18 depression sub-scale scores. SRC+ were differentiated into two subgroups based on the presence (n=8) or absence (n=9) of depressive symptoms from the BSI depression sub-scale at the 1d visit (i.e., the

visit with the most severe post-concussion depressive symptoms). Exploratory independent samples t-tests compared connectivity strength at the 1d visit in SRC+ athletes with or without depressive symptoms. For individual ROI, an FDR of q<0.05 determined significance to account for multiple testing.

3. Results

3.1 Demographic and clinical data

Groups did not differ in demographic information or clinical data at baseline (Table 1), with the exception of number of prior concussions (p<0.001). By design, SRC+ and CC+ had more prior concussions than SRC- and CC- (all p<0.001). There were no group differences in head motion during the rs-fMRI scan at any visit (all p>0.10). Acutely following injury (i.e., 1d visit), SRC+ and SRC- reported more severe SCAT and BSI-GSI symptoms than CC + and CC- (all p<0.05); SRC- reported less severe BSI-GSI symptoms than SRC+ (p<0.05). One SRC+ reported post-traumatic amnesia and one SRC- reported retrograde amnesia following their current injury; no injured athletes reported loss of consciousness due to their current injury.

3.2 Association of KP markers and global connectivity strength

There was a significant interaction between group and QuinA on connectivity strength, F(3, 127)=3.46, p=0.019. Follow-up analyses showed that this effect was driven by a significant association between QuinA and connectivity strength in SRC+, B=16.05, SE = 5.06, p=0.002, 95%CI[6.06, 26.03] (Figure 1). The association between QuinA and connectivity was not significant in SRC-, B=-2.76, SE=4.11, p=0.50, 95%CI[-10.91, 5.38], CC-, B=4.22, SE=4.41, p=0.34, 95%CI[-4.54, 12.97], or CC+, B=-4.28, SE=5.64, p=0.45, 95%CI[-15.42, 6.86]. There were no significant main effects or interactions for KynA/QuinA or any secondary KP marker (all p>0.10; Table 2).

Sensitivity analyses were performed as above using an alternative atlas to define ROI. As in the primary analysis, there was a significant interaction between group and QuinA on connectivity strength, F(3, 123.34)=3.27, p=0.024. As above, SRC+ had a significant association between QuinA and connectivity strength, B=27.92, SE=9.22, p=0.003, 95%CI[9.71, 46.12] (Figure 1). There were no significant associations for SRC-, B=-3.39, SE=7.44, p=0.65, 95%CI[-18.15, 11.38], CC-, B=7.72, SE=7.99, p=0.34, 95%CI[-8.14, 23.57], or CC+, B=-10.44, SE=10.29, p=0.31, 95%CI[-30.76, 9.88]. As in the primary analysis, there were no significant main effects or interactions for KynA/QuinA or any secondary KP marker (all p>0.10; Table 2).

3.3 Association of QuinA and connectivity strength in individual regions

Analyses were performed to determine the association between QuinA and connectivity strength in individual regions-of-interest in SRC+ given the observed association between global QuinA and global connectivity strength. There was a significant association in 31 of the 120 regions following FDR correction (Table 3). As seen in Figure 2, several of the strongest associations were observed bilaterally in the orbitofrontal cortices, insulae, and basal ganglia.

3.4 Association between connectivity strength and depressive symptoms

Exploratory analyses were conducted to determine if connectivity strength was association with post-concussion depressive symptom status in SRC+ participants, limited to the global connectivity measure and individual ROIs that showed associations with QuinA (see above). Global connectivity strength was significantly higher in SRC+ athletes with depressive symptoms compared to those without, $t(15)=2.37$, mean difference=13.50, SE=5.69, $p=0.032$, 95%CI[1.36, 25.63], Cohen's d =1.15. Several individual ROI showed similar associations with depressive symptom status at 1d post-concussion (Table 4), though they did not survive FDR correction at $q<0.05$ despite several having large effect sizes (i.e., Cohen's $d > 1.0$).

4. Discussion

The current study in high school and collegiate football players tested the hypothesis that the association between neuroactive KP metabolites and a global metric of functional connectivity would depend on both acute injury status and prior concussion. Consistent with our hypothesis, QuinA was significantly associated with functional connectivity strength in acutely injured athletes with prior concussion across the acute to sub-acute phase post-concussion, but not in injured athletes without prior concussion or in uninjured controls. Follow-up analyses identified several regions where this association was the strongest, including multiple regions in medial prefrontal cortex, insulae, and basal ganglia. Finally, exploratory analyses found that greater global connectivity strength was associated with the presence of acute depressive symptoms in concussed athletes with prior concussion. These results are discussed in detail in the following sections.

The positive association between QuinA and functional connectivity strength is mostly consistent with our work in an independent sample of collegiate athletes. In the prior work, the association between KynA/QuinA and connectivity of seed-regions in the anterior cingulate cortex and hippocampus to multiple brain regions differed based on concussion history as well as football exposure, with football players with prior concussion typically showing inverse associations between KynA/QuinA and connectivity (Meier et al., 2017). Although we did not observe any statistically significant effects for KynA/QuinA in the current study, the patterns of lower KynA/QuinA and higher QuinA are consistent (KynA/QuinA is negatively correlated with QuinA). Moreover, the current results extend our prior work by focusing on a global metric of functional connectivity strength that did not require the *a priori* selection of a ROI, the inclusion of high school athletes in addition to collegiate athletes, and the availability of MRI and blood draws collected across multiple, longitudinal visits.

The current study critically extends upon our prior work in that, here, the association between connectivity and QuinA was only observed in recently concussed athletes with prior concussion, whereas prior work focused on prior concussion in isolation in an independent cohort (Meier et al., 2017). It is important to note that the same association was not observed in acutely injured athletes without prior concussion, suggesting that this effect was not solely due to acute injury. Similarly, the same association was also not observed in uninjured football players with prior concussion, highlighting the fact that the effect is not

solely due to prior injury. We hypothesize that this finding reflects a priming effect of prior concussion and acute/sub-acute concussion on the relationship between connectivity and QuinA. In our previous work in an overlapping sample, QuinA was elevated (and KynA/QuinA reduced) at all visits in the recently concussed group with prior concussion (Meier et al., 2020b). Based on that finding, and the fact that QuinA is produced by microglia and macrophages (Espey et al., 1997; Guillemin et al., 2005), we hypothesized that the observed elevation in QuinA reflects the long-term priming of monocyte lineage cells by prior concussion. That is, chronic inflammation (e.g., due to prior concussion) may sensitize the immune system to subsequent triggers (e.g., recent concussion), resulting in a greater inflammatory response (Dilger and Johnson, 2008; Perry and Holmes, 2014; Witcher et al., 2015). Nevertheless, we cannot rule out the possibility that association between QuinA and hyperconnectivity reflects the residual effect of multiple concussions.

Given the proposed association between glutamatergic neurotransmission and the BOLD signal (Hyder et al., 2002; Smith et al., 2002), one possible explanation for the observed association between QuinA and functional connectivity is that the effects of QuinA on the NMDA receptor (e.g., promotion of glutamate release and inhibition of reuptake) alters functional connectivity. In addition to its role in altering glutamatergic activity, QuinA has other deleterious effects that could impact intrinsic connectivity, including blood brain barrier disruption, the generation of reactive oxygen species, and the destabilization of cellular cytoskeletons (Guillemin, 2012). Regardless of the potential mechanisms, we cannot prove a causal relationship. Nevertheless, current results are consistent with abnormalities in QuinA (elevated QuinA, reduced KynA/QuinA) reported in a variety of psychiatric and neurodegenerative diseases that are associated with brain injury (Amaral et al., 2013; Guskiewicz et al., 2007; Savitz, 2020).

Hyperconnectivity has been posited to be a common response of the brain to neurological injuries with a known inflammatory component (i.e., multiple sclerosis and TBI) (Hillary et al., 2015). Although there are reports of hypoconnectivity following concussion (for review, see (Puig et al., 2020)), hyperconnectivity has been documented in several studies at the acute, sub-acute, and chronic phase following concussion and is associated with worse symptoms or prolonged recovery time (N. W. Churchill et al., 2017; Goswami et al., 2016; Kaushal et al., 2019; Meier et al., 2020a, 2017; van der Horn et al., 2017). The current work provides further support that hyperconnectivity is pathological based on our finding in concussed athletes with prior concussion that greater connectivity strength was associated with greater global functional connectivity strength. Therefore, in the context of the primed immune system hypothesis outlined above, the increased release of QuinA in concussed athletes with prior concussion leads to greater glutamatergic dysregulation that is ultimately reflected in the rs-fMRI BOLD signal connectivity and potentially mood dysregulation. Additional work is needed to directly test this proposed pathway. However, it should be noted that we cannot rule out that the observed hyperconnectivity is not pathological, per se, but rather reflects a compensatory increase in connectivity strength in response to elevated symptoms.

Finally, although we focused on a global connectivity metric, follow-up analyses demonstrated that the strongest associations with QuinA were observed in the medial and

orbital frontal cortex, insula, as well as the basal ganglia. We have previously shown that various KP measures are associated with striatal and medial PFC structure in patients with major depressive disorder (MDD) (Meier et al., 2016a; Savitz et al., 2015a). Specifically, neuroactive KP metabolites (KynA/3HK, KynA/QuinA) mediated group differences (controls versus MDD) in mPFC thickness, while KYN and KYN/TRP were inversely associated with striatal volume in MDD patients. Similarly, multiple papers have shown that inflammatory challenges (e.g., endotoxin, IFN-alpha) impact glucose metabolism, BOLD signal response, and glutamate levels in the medial frontal cortex, insula, and/or striatum (Capuron et al., 2007, 2005; Eisenberger et al., 2010; Hannestad et al., 2012; Haroon et al., 2014; Harrison et al., 2009). Therefore, the current results add to the literature documenting associations between KP activity, as well as inflammation in general, with these brain regions. This is further supported by the finding that increased functional connectivity in several of these regions was also associated with the presence of depressive symptoms with large effect sizes, though none survived multiple comparison correction.

Current results were limited to male football players; therefore, we are unable to test for potential sex-differences in the associations between concussion, KP metabolites, and functional connectivity. Sex differences have been documented in previous concussion research, with indications that women report more symptoms following concussion, take longer to recover, and have higher risk of sustaining a concussion (Bretzin et al., 2018; Covassin et al., 2003; Iverson et al., 2017; Merritt et al., 2019; Zemek et al., 2016). Moreover, we have previously documented sex differences in serum KP metabolites, with women having lower KynA/QuinA and KynA/3HK, driven by lower KynA, compared to men (Meier et al., 2018). Given these differences, as well as the well-established sex differences in immune function, in general, future work is required to determine the extent to which sex moderates the associations observed in the current study (Klein et al., 2010; Klein and Flanagan, 2016; vom Steeg and Klein, 2016).

Finally, the current study investigated associations of KP metabolites and concussion with intrinsic brain connectivity as measured by rs-fMRI. Rs-fMRI, compared to task-based fMRI, allows for the mapping of intrinsic functional connectivity without confounds associated with tasks, such as performance or effort issues (for a review of fMRI use in TBI research, see (Mayer et al., 2015). There are alternative methods to assess intrinsic brain connectivity, such as electroencephalogram (EEG), magnetoencephalography (MEG), or functional near-infrared spectroscopy (fNIRS). While some of these methods provide more direct assessment of neural activity (i.e., EEG, MEG), the spatial resolution throughout the entirety of brain (including critical subcortical regions) is typically superior for fMRI. Finally, there are neuroimaging measures of brain microstructure that are sensitive to the effects of concussion, such as diffusion MRI (Gardner et al., 2012). The possible associations of concussion and KP metabolites with these alternative neuroimaging metrics merits future research.

4.1 Limitations

Current results were limited to high school and collegiate American football players and may not generalize to other sports, women, or athletes of different ages. The presence and

number of prior concussions was based on participant self-report, which may be biased. Serum samples were non-fasting and collection time of blood samples was non-standardized across the study due to confines of the parent study. Although the repeated sampling over multiple visits could help mitigate these factors, they could impact KP measurements in blood. The extent to which serum KP metabolites reflect levels in the brain is uncertain, though blood and brain/CSF levels of QuinA have been shown to be significantly correlated (Haroon et al., 2020; Heyes et al., 1992; Heyes and Morrison, 1997; Raison et al., 2010). Finally, the sample size of participants with both blood and available scan data is relatively small in terms of individual participants. Because of the longitudinal design, however, KP metabolites and connectivity strength were measured in up to four visits per participants (e.g., 262 visits with blood and imaging). Nevertheless, it is likely that certain analyses (e.g., exploratory analyses in SRC+ group) were underpowered.

5. Conclusions

Elevated serum concentrations of QuinA are associated with increased functional brain connectivity in recently concussed athletes with a history of prior concussion. Future studies are needed to directly test the hypothesis that negative sequalae of multiple concussions may be, at least in part, mediated by a priming of the KP that triggers greater production of QuinA upon subsequent injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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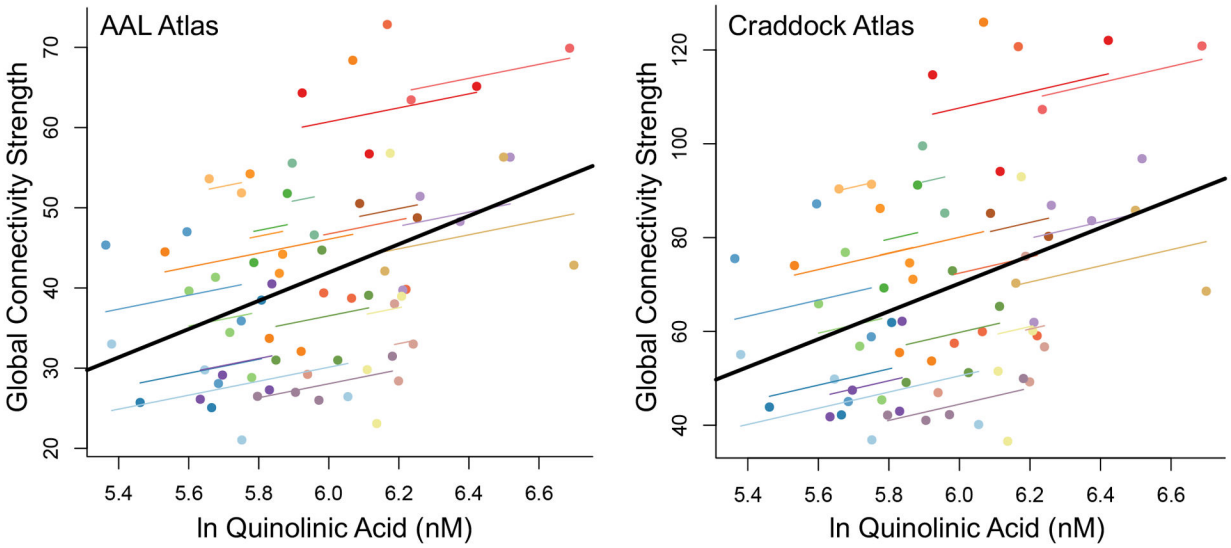

Figure 1:

Shown is the association between natural log transformed (ln) quinolinic acid and global connectivity strength for both the Automated Anatomical Labeling atlas, version 2 (AAL2) and the Craddock atlas for concussed participants with prior concussion. For illustrative purposes, individual participants are indicated using a single color, with smaller lines demonstrating the relationship between connectivity and quinolinic acid in each participant over the repeated sessions. The solid black line represents the association between connectivity and quinolinic acid across all participants and time points. nM = nanomolar

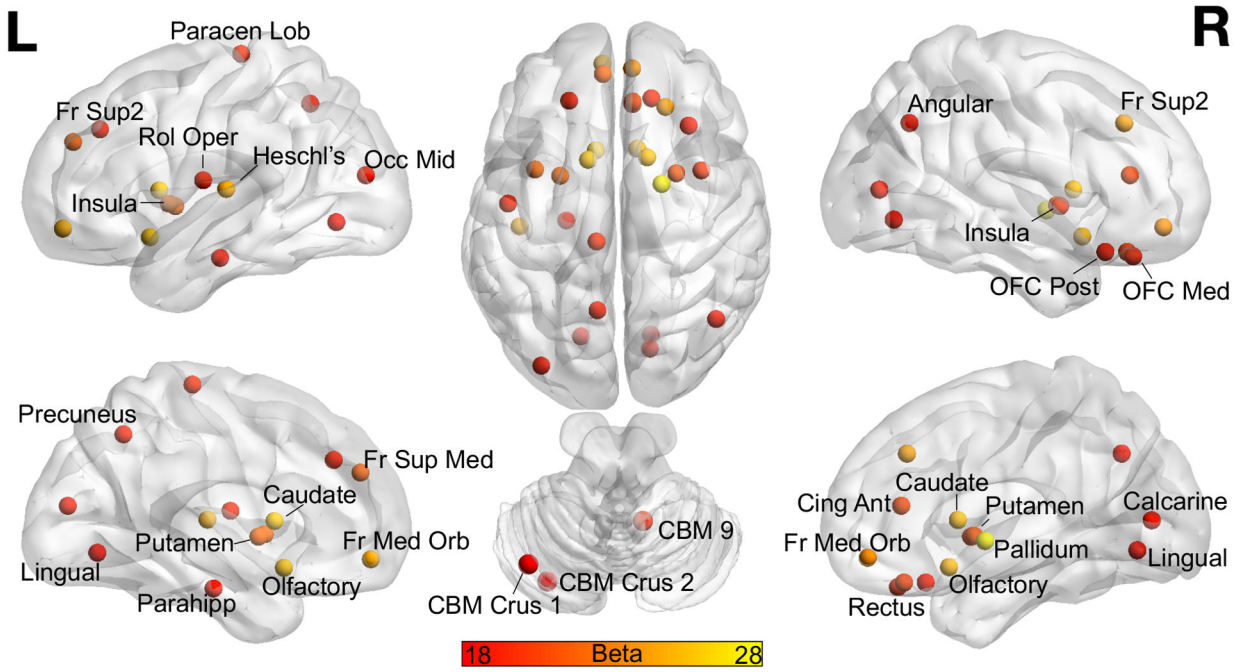

Figure 2:

Displayed are the regions of interest from the Automatic Anatomical Labeling atlas, version 2 that showed a significant association between natural log transformed quinolinic acid and connectivity strength in concussed athletes with prior concussion following False Discovery Rate correction. Regions are labeled once. Color bar corresponds to the unstandardized Beta. L = left, R = Right, Paracen Lob = paracentral lobule, Fr = frontal, Sup = superior, Rol Oper = rolandic operculum, Occ = occipital, Mid = middle, Med = medial, Orb = orbital, OFC = orbitofrontal cortex, Cing = cingulate, Ant = anterior, Parahipp = parahippocampal, CBM = cerebellum, Post = posterior.

Table 1:

Sample Characteristics and Demographics

	SRC−	SRC+	CC−	CC+	Statistic
Total No. Participants	16	21	24	18	
No. by Visit (1d/8d/15d/45d)	13/12/12/12	17/19/15/15	21/21/20/19	16/18/17/15	$X^2(9)=0.47$, $p=1.00$
Age	17.81(1.83)	18.05(1.75)	18.08(1.47)	18.39(1.91)	F(3, 75)=0.32, $p=0.81$
Race, No. White/Non-White, NR, or Unknown	10/6	15/6	14/10	15/3	$X^2(3)=3.33$, $p=0.34$
Ethnicity, No. Not Hispanic/Hispanic, NR or Unknown	16/0	16/5	19/5	17/1	FET=6.06, $p=0.08$
WTAR Standard Score	99.94(18.88)	99.10(11.97)	98.17(12.40)	105.22(14.41)	F(3, 75)=0.94, $p=0.42$
No. Participants in College	12	15	19	12	FET=0.99, $p=0.86$
Body Mass Index	27.68(4.83)	28.12(4.56)	30.31(5.74)	28.20(5.22)	F(3, 75)=1.14, $p=0.34$
Years of Participation in Sport	7.13(2.50)	7.60(2.80)	7.83(2.33)	8.39(2.75)	F(3, 74)=0.71, $p=0.55$
Median [IQR] No. of Prior Concussions	0 [0,0]	1 [1,2]	0 [0,0]	1 [1,2]	H=69.59, $p<0.001$
Clinical Measures at Baseline					
BSI-GSI Raw Score	1.88(2.68)	4.76(8.83)	2.79(3.22)	1.94(2.59)	Welch's F(3, 40)=0.94, $p=0.43$
SCAT-3 Symptom Severity	1.88(2.66)	3.24(6.46)	3.04(6.23)	1.83(2.62)	F(3, 74)=0.41, $p=0.74$
BESS Total Score	12.87(2.80)	11.71(4.44)	12.04(3.11)	9.72(3.80)	F(3, 74)=2.34, $p=0.08$
SAC Total Score	26.13(2.31)	26.19(2.02)	25.83(2.08)	25.78(2.96)	F(3, 75)=0.15, $p=0.93$
Clinical Measures at 1d					
BSI-GSI Raw Score	7.31(6.93)	4.24(5.07)	0.81(1.33)	1.00(1.63)	Welch's F(3, 28)=5.73, $p=0.003$
SCAT-3 Symptom Severity	25.08(23.31)	18.12(16.57)	2.24(3.42)	0.69(1.40)	Welch's F(3, 28)=11.25, $p<0.001$
BESS Total Score	13.00(6.81)	9.75(5.26)	9.50(4.21)	8.31(3.77)	F(3, 60)=2.16, $p=0.10$
SAC Total Score	25.62 (2.22)	26.41(2.00)	25.71(2.63)	25.81(3.31)	F(3, 63)=0.31, $p=0.82$
Median [IQR] Duration of Symptoms in Days	6.5 [4.75, 9.5]	7 [5, 12]	-	-	H=0.14, $p=0.71$

Note: Values are expressed as mean (standard deviation) unless otherwise noted. SRC- = concussed participants without prior concussion, SRC+ = concussed participants with prior concussion, CC- = contact controls without prior concussion, CC+ = contact controls with prior concussion, No. = Number, 1d = 1 day visit, 8d = 8 day visit, 15d = 15 day visit, 45d = 45 day visit, NR = not reported, WTAR = Wechsler Test of Adult Reading, IQR = interquartile range, BSI-GSI = Brief Symptom Inventory Global Severity Index, SCAT = The Sport Concussion Assessment Tool, BESS = Balance Error Scoring System, SAC = Standardized Assessment of Concussion, FET = Fisher's Exact Test, H = Kruskal-Wallis chi-square.

Table 2:

Associated statistics for kynurenine pathway marker effects on global connectivity strength

	AAL2	AAL2	Craddock	Craddock
Measure	**Main Effect, KP marker**	**KP marker × Group Interaction**	**Main Effect, KP marker**	**KP marker × Group Interaction**
Primary				
QuinA	F(1, 134)=1.86, *p*=0.18	F(3, 127)=3.46, *p*=0.019	F(1, 130)=1.53, *p*=0.22	F(3, 123)=3.27, *p*=0.024
KynA/QuinA	F(1, 149)=0.00, *p*=0.99	F(3, 147)=0.32, *p*=0.81	F(1, 143)=0.00, *p*=0.96	F(3, 141)=0.26, *p*=0.86
Secondary				
KynA/3HK	F(1, 185)=0.01, *p*=0.94	F(3, 190)=1.00, *p*=0.39	F(1, 177)=0.00, *p*=0.98	F(3, 181)=0.92, *p*=0.43
KynA	F(1, 243)=1.11, *p*=0.29	F(3, 241)=0.77, *p*=0.51	F(1, 240)=1.28, *p*=0.26	F(3, 238)=1.00, *p*=0.39
3HK	F(1, 245)=1.60, *p*=0.21	F(3, 239)=0.44, *p*=0.73	F(1, 243)=2.16, *p*=0.14	F(3, 237)=0.63, *p*=0.60
KYN/TRP	F(1, 212)=0.03, *p*=0.86	F(3, 179)=0.49, *p*=0.69	F(1, 207)=0.04, *p*=0.84	F(3, 173)=0.38, *p*=0.76
KYN	F(1, 170)=0.86, *p*=0.35	F(3, 158)=0.15, *p*=0.93	F(1, 164)=0.79, *p*=0.38	F(3, 153)=0.24, *p*=0.87
TRP	F(1, 228)=0.91, *p*=0.34	F(3, 223)=0.92, *p*=0.43	F(1, 221)=0.84, *p*=0.36	F(3, 216)=1.10, *p*=0.35

Note: AAL2 = Automated Anatomical Labeling atlas, Craddock = Craddock atlas, KP = kynurenine pathway, QuinA = quinolinic acid, KynA = kynurenic acid, 3HK = 3-hydroxykynurenine, KYN = kynurenine, TRP = tryptophan.

Table 3:

Association of quinolinic acid and functional connectivity strength in SRC+

Region-of-Interest	Beta	SE	*t*	df	*p*-value	FDR-corrected *p*
Pallidum_R	27.66	7.18	3.85	43.12	< 0.001	< 0.001
Caudate_L	25.80	6.77	3.81	31.45	0.001	0.017
Olfactory_L	25.55	7.37	3.47	45.39	0.001	0.017
Caudate_R	25.30	7.22	3.51	46.09	0.001	0.017
Olfactory_R	25.04	7.38	3.39	39.94	0.002	0.018
Heschl_L	24.69	7.64	3.23	42.42	0.002	0.018
Frontal_Med_Orb_L	24.67	6.76	3.65	55.46	0.001	0.017
Frontal_Sup_2_R	24.16	7.08	3.41	56.49	0.001	0.017
Frontal_Med_Orb_R	23.62	7.01	3.37	47.39	0.001	0.017
Insula_L	22.00	7.22	3.05	40.95	0.004	0.030
Putamen_L	21.98	6.79	3.24	44.81	0.002	0.018
Frontal_Sup_Medial_L	21.68	7.33	2.96	51.57	0.005	0.034
Putamen_R	20.95	6.38	3.28	44.35	0.002	0.018
Cingulate_Ant_R	20.53	7.16	2.87	47.68	0.006	0.034
Rectus_R	20.46	6.74	3.04	47.94	0.004	0.030
Insula_R	19.68	7.14	2.76	33.43	0.009	0.043
Paracentral_Lobule_L	19.54	7.47	2.62	48.54	0.012	0.046
OFCmed_R	19.50	5.87	3.32	34.07	0.002	0.018
Cerebellum_9_R	19.42	6.06	3.21	60.94	0.002	0.018
Rolandic_Oper_L	19.33	6.85	2.82	32.92	0.008	0.042
Precuneus_L	19.10	7.06	2.70	48.82	0.009	0.043
Frontal_Sup_2_L	19.05	6.66	2.86	47.02	0.006	0.034
ParaHippocampal_L	18.99	6.55	2.90	44.99	0.006	0.034
OFCpost_R	18.93	6.98	2.71	36.45	0.010	0.044
Occipital_Mid_L	18.90	6.67	2.83	52.81	0.006	0.034
Calcarine_R	18.88	6.75	2.80	45.16	0.008	0.042
Angular_R	18.72	7.22	2.59	59.77	0.012	0.046
Lingual_L	18.64	7.12	2.62	44.09	0.012	0.046
Lingual_R	18.63	7.00	2.66	51.31	0.010	0.044
Cerebellum_Crus2_L	17.52	5.81	3.02	44.48	0.004	0.030
Cerebellum_Crus1_L	17.10	6.58	2.60	52.82	0.012	0.046

Note: Shown are the parameter estimates for the significant associations between natural log transformed quinolinic acid and connectivity strength for specific regions-of-interest from the Automated Anatomical Labeling (version 2) atlas following False Discovery Rate (FDR) correction. SE = standard error, df = degrees of freedom, t = t statistic, _R = right hemisphere, _L = left hemisphere, Med = medial, Orb = orbital, Sup = superior, Ant = anterior, Oper = operculum, Post = posterior, Mid = middle, OFC = orbitofrontal cortex.

Table 4:

Differences in individual ROI connectivity strength based on depression symptom status in SRC+

Region-of-Interest	MD*	SE	*t*	df	*p*-value	FDR-corrected *p*	Cohen's d
Lingual_L	−19.205	6.580	−2.919	15	0.011	0.075	−1.418
Cingulate_Ant_R	−21.310	7.573	−2.814	15	0.013	0.075	−1.367
Heschl_L	−20.173	7.575	−2.663	15	0.018	0.075	−1.294
Frontal_Sup_2_L	−17.606	6.869	−2.563	15	0.022	0.075	−1.245
Occipital_Mid_L	−15.896	6.243	−2.546	15	0.022	0.075	−1.237
Rolandic_Oper_L	−17.910	7.033	−2.547	15	0.022	0.075	−1.237
Frontal_Med_Orb_R	−18.290	7.194	−2.542	15	0.023	0.075	−1.235
Lingual_R	−17.840	7.068	−2.524	15	0.023	0.075	−1.227
Pallidum_R	−14.385	5.403	−2.662	9.540*	0.025	0.075	−1.348
Putamen_R	−14.375	5.836	−2.463	15	0.026	0.075	−1.197
Putamen_L	−13.683	5.659	−2.418	15	0.029	0.075	−1.175
Calcarine_R	−15.678	6.629	−2.365	15	0.032	0.075	−1.149
ParaHippocampal_L	−17.036	7.271	−2.343	15	0.033	0.075	−1.138
Frontal_Sup_Medial_L	−17.932	7.665	−2.339	15	0.034	0.075	−1.137
Insula_L	−14.768	6.474	−2.281	15	0.038	0.076	−1.108
Caudate_R	−18.285	7.761	−2.356	9.827*	0.041	0.076	−1.191
Insula_R	−15.070	6.760	−2.229	15	0.042	0.076	−1.083
Frontal_Med_Orb_L	−14.993	6.830	−2.195	15	0.044	0.076	−1.067
Frontal_Sup_2_R	−17.028	8.077	−2.108	15	0.052	0.085	−1.024
Paracentral_Lobule_L	−16.507	8.003	−2.063	15	0.057	0.087	−1.002
Precuneus_L	−16.238	7.941	−2.045	15	0.059	0.087	−0.994
Angular_R	−15.685	8.577	−1.829	15	0.087	0.122	−0.889
Cerebellum_9_R	−11.894	6.946	−1.712	15	0.107	0.142	−0.832
OFCpost_R	−13.794	8.112	−1.700	15	0.110	0.142	−0.826
Olfactory_L	−14.420	8.879	−1.624	15	0.125	0.150	−0.789
Cerebellum_Crus1_L	−13.025	8.033	−1.621	15	0.126	0.150	−0.788
Olfactory_R	−14.340	8.904	−1.610	9.495*	0.140	0.161	−0.816
Rectus_R	−9.302	6.977	−1.333	15	0.202	0.223	−0.648
Caudate_L	−11.613	8.566	−1.356	8.708*	0.209	0.223	−0.691
OFCmed_R	−8.561	7.329	−1.168	15	0.261	0.270	−0.568
Cerebellum_Crus2_L	−1.434	6.034	−0.238	15	0.815	0.815	−0.115

Note: Shown are the associated statistics for comparison of connectivity strength for specific regions-of-interest in concussed athletes with prior concussion based on depression symptom status at 1day post-injury. Asterisk indicates t-test with unequal variance, MD = mean difference, SE = standard error, DF = degrees of freedom, T = T-statistic, FDR = False Discovery Rate, _R = right hemisphere, _L = left hemisphere, Med = medial, Orb = orbital, Sup = superior, Ant = anterior, Oper = operculum, Post = posterior, Mid = middle, OFC = orbitofrontal cortex.

*Comparison is athletes without versus those with depression symptoms.