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Default Mode Network in Concussed Individuals in Response to the YMCA Physical Stress Test

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Abstract

We hypothesize that the evolution of mild traumatic brain injury (mTBI) may be related to differential effects of a concussive blow on the functional integrity of the brain default mode network (DMN) at rest and/or in response to physical stress. Accordingly, in this resting-state functional magnetic resonance imaging (fMRI) study, we examined 14 subjects 10 ± 2 days post-sports-related mTBI and 15 age-matched normal volunteers (NVs) to investigate the possibility that the integrity of the DMN is disrupted at the resting state and/or following the physical stress test. First, all mTBI subjects were asymptomatic based upon clinical evaluation and neuropsychological (NP) assessments prior to the MRI session. Second, the functional integrity within the DMN, a main resting-state network, remained resilient to a single concussive blow. Specifically, the major regions of interest (ROIs) constituting the DMN (e.g., the posterior cingulate cortex [PCC]/precuneus area, the medial prefrontal cortex [MPFC], and left and right lateral parietal cortices [LLP and RLP]) and the connectivity within these four ROIs was similar between NVs and mTBI subjects prior to the YMCA physical stress test. However, the YMCA physical stress test disrupted the DMN, significantly reducing the magnitude of the connection between the PCC and left lateral parietal ROI, and PCC and right lateral parietal ROI, as well as between the PCC and MPFC in mTBI subjects. Thus while the DMN remained resilient to a single mTBI without exertion at 10 days post-injury, it was altered in response to limited physical stress. This may explain some clinical features of mTBI and provide some insight into its mechanism. This important finding should be considered by clinical practitioners when making decisions regarding return-to-play and clearing mTBI athletes for sports participation.

Key words: default mode network; resting state functional magnetic resonance imaging; subacute phase of mild traumatic brain injury

Introduction

Most commonly known as concussion, mild traumatic brain injury (mTBI), with an annual reported incidence of 1.4 million cases in the United States alone (Bazarian et al., 2005), accounts for 80% of all traumatic brain injuries (Risdall and Menon, 2011; Ruff, 2011). There are several immediate physical, cognitive, and emotional symptoms arising from mTBI that include: headache, dizziness, unsteady gait, nausea, slurred speech, poor concentration, and short-term memory loss (McCrory et al., 2009). For the most part, sports-related mTBI recovery is rapid, with spontaneous symptoms resolving within 10 days post-injury (Webbe and Barth, 2003). However, more than 15% of individuals suffering from mTBI have symptoms persisting beyond 3 months post-injury (Kiraly and Kiraly, 2007; Sedney et al., 2011; Witt et al., 2010). Prolonged recovery time is referred to as atypical, but its physiology is unknown. The debate on the time it takes for mTBI symptoms to resolve, the time frame for the susceptibility to a recurrent brain injury, the cumulative effects of multiple brain injuries, and return-to-play criteria are still ongoing.

Currently there is no concrete definition for mTBI, and diagnosis largely depends upon subjective measures that include patient self-reports of symptoms (Ruff, 2011). Clinical imaging methods that include computed tomography (CT) and traditional magnetic resonance imaging (MRI) are neither sensitive nor sufficiently specific to detect subtle functional

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and/or structural alterations in concussed individuals (Cantu, 2006; Provenzale, 2010). Neuropsychological tests such as automated neuropsychological assessment metrics (ANAM) and Immediate Post-Concussion Assessment and Cognitive Testing (ImPact) are widely used in athletics as assessment tools for mTBI, although these current clinical measures show no correlation in either detecting neurocognitive deficits beyond 10 days post-injury, or in predicting the development of post-concussive syndrome (PCS; Williams et al., 2010). Overall, current clinical tools cannot detect the subtle underlying causes of persistent neurocognitive and/or motor deficits in mTBI (Belanger et al., 2005; Signoretti et al., 2010).

The concept of a default mode network (DMN), originally introduced in 2001 by Raichle and associates, has rapidly become a central theme in contemporary cognitive and clinical neuroscience. DMN emerges with regional task-nonspecific deactivations during goal-oriented activity (Raichle et al., 2001), and is characterized by coherent low-frequency blood oxygen level dependent (BOLD) signals (<0.1 Hz; Greicius et al., 2003). Several brain regions including the posterior cingulate cortex (PCC), medial prefrontal cortex (MPFC), and the medial, lateral, and inferior parietal cortex, are associated with the DMN (Buckner et al., 2008; Greicius et al., 2003). In contrast to the DMN, the task-positive networks (TPN) correspond to specific domains, including the sensorimotor, episodic memory, visual-attention, and emotion-attention networks (Broyd et al., 2009). The high degree of temporal anti-correlation between the DMN and TPN is thought to reflect a low-frequency toggling between their associated psychological functions of introspective and self-referential thought and extrospective attentional orienting, thereby allowing an individual to remain alert during unexpected environmental events (Broyd et al., 2009; Buckner et al., 2008). Advances in technology and methodology that include resting-state functional connectivity magnetic resonance imaging (rs-fcMRI) and independent component analysis (ICA) have shown promise in investigating brain network architecture. The most common network studied is the DMN (Laird et al., 2009), and there is promise that evaluating the DMN may have potential clinical applications, as it has been shown to be altered in numerous neurological and neuropsychological populations, including Alzheimer's disease (AD), schizophrenia, depression, and attention-deficit hyperactivity disorder (ADHD; Broyd et al., 2009,2011; Greicius et al., 2004; Swanson et al., 2011; Wu et al., 2011). Overall, altered connectivity is a conspicuous characteristic of the altered integrity of the DMN and affiliated functions in pathological populations and during aging in the resting state (Damoiseaux et al., 2008). The DMN is also altered during changes in levels of consciousness, both during physiological events such as deep sleep (Horovitz et al., 2009), and pathological disorders of consciousness (Noirhomme et al., 2010).

Both electroencephalography (EEG) and magneto-electroencephalography (MEG) studies of brain connectivity in mTBI revealed decreased long-distance functional connectivity (Cao and Slobounov, 2010; Sponheim et al., 2011), as well as reduced interhemispheric connectivity (Kumar et al., 2009; Sponheim et al., 2011). Similarly to EEG and MEG studies, mTBI rs-fcMRI studies also revealed reduced interhemispheric connectivity (Marquez de la Plata et al., 2011; Slobounov et al., 2011). Recent studies by Mayer and colleagues (2001) and Johnson and associates (2011) showed hypo-connectivity in the posterior regions and hyper-connectivity in the anterior areas within the DMN architecture. These alterations may be important in explaining residual physical, cognitive, and emotional symptoms associated with mTBI, as well as clinically inaccurately assessing the injury evolution that may lead to improper management (e.g., premature return-to-play).

Current return-to-play guidelines following mTBI include: (1) waiting until the player is symptom-free according to self-reported symptoms scales, and neurological and neuropsychological evaluations; (2) an athlete's response to aerobic exercises including the YMCA physical stress test; (3) an athlete's response to sports specific training and drills; (4) and finally full participation or game play (Lovell et al., 2004). The YMCA physical stress test and similar bike tests are widely used and accepted measures to help determine an athlete's ability to return to play, but the question remains whether or not it is sufficiently sensitive for residual cognitive, behavioral, and underlying brain network deficits resulting from mTBI. Accordingly, in this study we examined the effect of the YMCA physical stress test on the functional integrity of the brain DMN in mTBI subjects just cleared for sports participation based on clinical symptom resolution.

In our previous work of a subgroup of these subjects, we documented: (1) reduced interhemispheric connectivity between the right and left visual cortex, dorsolateral prefrontal cortex (DLPFC), and hippocampus in mTBI subjects within 30 days post-injury (Slobounov et al., 2011); and (2) reduced magnitude and number of significant connections between the primary regions of interest (ROIs) that make up the DMN (i.e., PCC, MPFC, medial, and lateral and inferior parietal cortices; Raichle et al., 2001), and other ROIs under study, including the DLPFC and bilateral parietal cortex. Moreover, the connections between the parahippocampal gyrus with the left and right parietal lobes were absent in mTBI during the subacute phase of an injury (Johnson et al., 2011). The functional integrity of the DMN in general, and the strength of brain functional connections that make up the DMN in subacutely concussed individuals in particular, has not been examined yet. We hypothesized that the functional integrity of the DMN may be resilient toward a single concussive blow, but could be jeopardized in response to the YMCA physical stress test. Specifically, we anticipated reduction in the strength of the brain functional connections that constitute the DMN in response to the YMCA physical stress test.

Methods

Participants

Seventeen neurologically normal student athletes with no history of mTBI and 17 student athletes who had recently suffered a sports-related mTBI (e.g., collegiate rugby, ice hockey, or lacrosse) were recruited for this study. Five subjects were excluded from the final analysis because of imaging artifacts and failure to maintain resting state. Data from 15 normal volunteers (mean age 20.9 ± 1.1 years) and 14 mTBI patients (mean age 20.8 ± 1.5 years) were included in the final analysis and are reported in this paper. The sample was 58% male and 42% female. Academic grade average score for all subjects was 3.2 (± 0.5). All injured subjects suffered from grade 1 mTBI (Data Driven Revised Concussion Grading Guideline; Cantu, 2006). The initial diagnosis of mTBI was

made on the field by certified athletic trainers (ATs), and as a part of the routine protocol of the Sport Concussion Program at the Pennsylvania State University. All athletes were evaluated by a physician at Penn State Athletic Medicine, which has expertise in evaluating and treating concussed athletes. Each athlete completed the clinical Sports Concussion Assessment Tool-2 (SCAT-2) and Balance Error Scoring System (BESS) examination (McCrory et al., 2009) administered by the physician, and had returned to their baseline taken prior to the season. In addition, each athlete had reported at least a 24-h self-reported symptom-free period, at which point they had been cleared for aerobic activity (<70% maximal heart rate [MHR]; Third International Consensus Statement on Concussion). Scanning took place on day 10 (±2 days) post-injury, and within 24 h of clinical symptom resolution and medical clearance for the first stage of aerobic activity by their supervising physician (International Cerebellar Ataxia Rating Scale [ICARS], World Federation of Neurology; Trouillas et al., 1997). Inclusion criteria for this study were the commonly accepted clinical symptoms of mTBI, such as: complaints of loss of concentration, dizziness, fatigue, headache, irritability, visual disturbances, and light sensitivity (Bryant and Harvey, 1999). All subjects were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971), with a handedness score above 90. All subjects signed an informed consent form and the Institutional Review Board of the Pennsylvania State University approved this protocol.

Experimental procedure

Subjects reported to the scanning facility and were interviewed about their symptoms. Subjects signed an informed consent form and MRI safety checklist prior to being scanned. All were clinically asymptomatic before scanning took place, and were excluded if they reported any symptoms before or during scanning.

The YMCA physical stress test bike protocol begins with a 2- to 3-min warm-up to acquaint the subjects with the cycle ergometer and prepare them for the exercise intensity during the first stage of the test. The specific protocol consists of four stages of increasing resistance lasting 3 min per stage. Subject heart rates were monitored to determine progression to the next stage of the bike protocol, and also to ensure that the athletes' heart rates stayed within the prescribed range of <70% of age-adjusted MHR, as recommended by the most recent consensus on return to sporting activities achieved at the Third International Conference on Concussion in Sports (McCrory et al., 2009).

During the bike stress test, physiological monitoring of the heart rate was recorded using the Polar F4 Fitness Heart Rate Monitor (Polar Electro Oy, Kempele, Finland), which consists of a transmitter that is secured to the subject across the chest and relays physiological information to a receiver worn on the subject's wrist. While acquiring fcMRI data, heart rate was monitored and recorded with a physiological monitoring unit pulse oximeter that is integrated into the Siemens (Erlangen, Germany) 3T Trio system.

After completing the bike test, the subjects were placed back in the scanner for another round of fcMRI scanning. Transition times from the bike to the initiation of the post-bike scanning sequences were all under 2 min. Overall, fMRI data were acquired before the YMCA physical stress test (rest), right after its completion (physical stress), and during recovery, which was on average within 15–20 min after completing the bike test. Heart rate was monitored throughout the entire experimental session, including rest, prior to bike testing, during the bike test, and while in the scanner for all three sessions.

MRI data acquisition

Functional and anatomical images were acquired on a 3.0 Tesla Siemens Trio whole-body scanner using a 12-channel head coil. T1 anatomical images and fMRI were acquired in the axial plane parallel to the anterior and posterior commissure axis covering the entire brain. Anatomical images were collected using a three-dimensional isotropic T1-weighted magnetization prepared rapid gradient echo (MP-RAGE: 0.9 mm × 0.9 mm × 0.9 mm resolution, TE = 3.46 msec, TR = 2300 msec, TI = 900 msec, flip angle = 9°, 160 slices, iPAT = none, NSA = 1). Two-dimensional BOLD echo planar fMRI (3.1 mm × 3.1 mm × 5 mm resolution, TE = 25 msec, TR = 2000 msec, EPI factor = 64, flip angle = 79°, 30 slices, iPAT = none, NSA = 1, acquisition time = 6:04) resting-state images were obtained. During resting-state fMRI acquisition, subjects were asked to lie motionless with their eyes closed. No radiological findings such as lesions or hyperintense signals were present in both the mTBI and NV subjects participating in this study.

Resting-state fMRI data analysis

Voxel-based and ROI-based correlation analyses were performed using the Functional Connectivity (CONN) toolbox (http://web.mit.edu/swg/software.htm) of SPM version 8 (http://www.fil.ion.ucl.ac.uk/spm/). Typical pre-processing of fMRI images was carried out with SPM 8, including realignment, slice timing, co-registration, segmentation, and normalization. After pre-processing, the images were then band-pass filtered to 0.01 ~ 0.10 Hz, and motion parameters were regressed to reduce the influence of noise and artifact. The CONN toolbox performed seed-based analysis by computing the temporal correlation between the BOLD signals from a given seed region to all other voxels in the brain. White matter, cerebrospinal fluid (CSF), and physiological noise source reduction were taken as confounders, following the implemented CompCor strategy (Behzadi et al., 2007). Whole-brain BOLD signal was excluded as a regressor to eliminate erroneous anti-correlations (Murphy et al., 2009). CONN also allowed ROI-based analysis by grouping voxels into ROIs based on anatomical partitions, such as Brodmann areas and Talairach Daemon labels. As for the time series standing for ROIs, a principal component analysis (PCA) was done for each ROI to extract the most representative voxels that could explain most of the variance of the BOLD signal from that ROI. Bivariate correlations were calculated between each pair of ROIs as reflections of connections. All Brodmann areas (BA) were imported as possible connections for our selected seed ROIs. Z-score standardizing was introduced to validate the multiple comparisons, and the significance tests were based on the Z-scores.

Specifically, we selected the ROIs that make up the default mode network (Raichle et al., 2001) as seed points for ROI-based analysis, including the MPFC, PCC, LLP, and RLP. Correlations were computed between each voxel and the seed

signals. CONN illustrated brain maps showing areas correlated with the seed ROIs. These processes were performed with all the subjects' data, and statistical parametric mapping tests were done to determine significant resting-state DMN connections at the individual level (first level). Based on the first-level results, the correlation coefficients were converted into Z scores and an unpaired *t*-test was carried out on each connection in the resting-state DMN to determine whether there was a significant difference between the NV and mTBI groups. Significance threshold was set at $p < 0.05$ false discovery rate (FDR) corrected.

Furthermore, we extracted the correlation coefficients among the 4 ROIs and converted them to Z scores. Two-way analysis of variance (ANOVA) was performed on each connection to test the effects of group and stress test. Average connection strengths were calculated to form DMN graphs for each group and condition.

Results

ROI-based analysis

PCC and MPFC were chosen as seeds for the ROI and voxel-based correlation analyses, similarly to other DMN studies (Uddin et al., 2009), to test the existence and reproducibility of DMN in this subject population. All the Brodmann areas (separated laterally), LLL, RLP, MPFC, and PCC were imported as ROIs. PCA analyses were done to all the ROIs to extract the representative voxels for the coherence calculation. Color maps identifying the DNW architecture in both groups at rest and in response to the YMCA physical stress test are shown in Figure 1 (see Table 1 for details).

PCC has significant connections with the MPFC and bilateral parietal cortex. The pattern of connectivity remains almost the same across all three testing conditions regardless of group. Specifically, compared to NVs, the mTBI group showed similar connections from the PCC, which is supported by the statistical tests. Also, ROI-based analyses of other DMN ROIs did not show significant differences between groups. It should be noted that in the NV group, right after the stress test, the number of connections increased compared to the rest condition and returned to the same level in the recovery phase. In contrast, in the mTBI group, both the number and magnitude of connections from the PCC were less and lower in the recovery phase.

DMN connectivity statistics

Two-way ANOVAs were performed for all six connections among the four DMN ROIs. Neither main effect of group (2) nor main effect of condition (3) was significant ($p > 0.05$). However, there was a significant main effect of group regarding the magnitude of the PCC connection to the LLP ($F = 5.90$, $p = 0.018$).

t-Tests between different conditions or groups were conducted for the major DMN ROIs under study. There was a significantly lower magnitude of connection between the PPC and MPFC in the mTBI group during the recovery phase ($T = 2.85$, $p = 0.010$; Fig. 2).

There was also a significantly lower magnitude of connection between the PCC and LLP in the mTBI group during the recovery phase, $T = 2.12$, $p = 0.047$; Fig. 3).

Finally, there was a significant reduction in the magnitude of PPC-RLP connections in the mTBI group during transition from the stress to recovery phase ($T = 2.62$, $p = 0.017$; Fig. 4)

Discussion

The functional role of the DMN and its interaction with other resting-state networks is still a debatable topic, but is

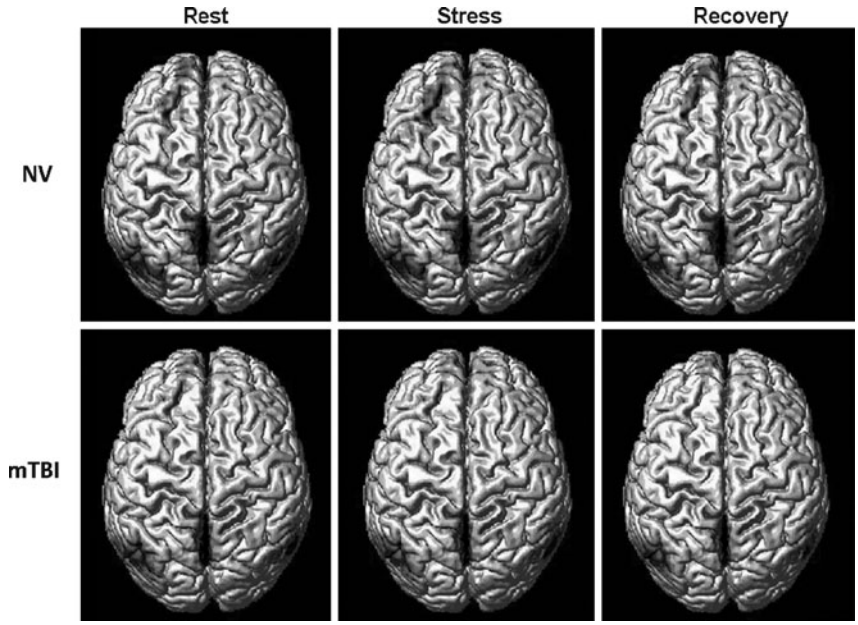

FIG. 1. Maps of the default mode network (DMN) at rest and in response to the YMCA physical stress test. Maps were generated by voxel-based correlation from the posterior cingulate cortex; cluster thresholds were set at $p < 0.05$ family-wise error (FWE), $k > 5000$. Clearly, similar DMNs were present in both groups in the resting state, but were reduced in mTBI in response to exercise (NV, normal volunteer; mTBI, mild traumatic brain injury).

Table 1. Evidence of Similar Default Mode Network (DMN) Architecture in Both the Normal Volunteer (NV) and Mild Traumatic Brain Injury (mTBI) Groups

		NV			*mTBI*			*Between group*
		r	*T*	p *(FDR)*	*r*	*T*	p *(FDR)*	p
Rest	PCC-MPFC	0.42	4.34	0.003	0.44	5.82	0.000	0.773
	PCC-LLP	0.77	14.09	0.000	0.58	5.48	0.001	0.090
	PCC-RLP	0.49	6.88	0.000	0.36	4.35	0.003	0.224
	LLP-RLP	0.71	10.13	0.000	0.61	11.11	0.000	0.326
Stress	PCC-MPFC	0.48	5.01	0.001	0.37	5.87	0.000	0.432
	PCC-LLP	0.70	8.79	0.000	0.63	6.74	0.000	0.452
	PCC-RLP	0.45	5.11	0.001	0.52	6.21	0.001	0.784
	LLP-RLP	0.74	10.47	0.000	0.68	9.37	0.000	0.551
Recovery	PCC-MPFC	0.50	6.57	0.000	0.32	4.34	0.003	0.010
	PCC-LLP	0.75	10.17	0.000	0.59	6.60	0.000	0.047
	PCC-RLP	0.43	5.83	0.000	0.32	4.38	0.002	0.305
	LLP-RLP	0.67	7.57	0.000	0.64	8.58	0.000	0.861

Correlation coefficients and corresponding T and *p* values are shown for each connection. PCC is connected to all the other DMN ROIs with significant *t*-tests as confirmation.

ROI, region of interest; FDR, false discovery rate; LLP, left lateral parietal cortex; RLP, right lateral parietal cortex; MPFC, medial prefrontal cortex; PCC, posterior cingulate cortex.

believed to correspond to task-independent introspection or self-referential thought (Broyd et al., 2009; Greicius et al., 2003). It has also been suggested that the DMN may include temporal binding of information (Engel et al., 2001), coordination, and neuronal organization of brain activity between regions that frequently work in combination (Fox and Raichle, 2007), and act as a toggling mechanism between introspective and extrospective attention (Fransson, 2006). A more recent elaboration is that the DMN may exhibit ongoing intrinsic activity that initiates maintenance of information for interpreting, responding to, and even predicting environmental demands (Raichle and Snyder, 2007). Considering that mTBI induces deterioration of cognitive functions including the speed of information processing, attention, and ability to predict forthcoming events, at least during the acute phase of injury, DMN examination may be a promising tool to explore the alterations in brain network architecture seen in concussed individuals.

There are several studies examining DMN integrity with specific focus on its interaction with other networks or ROIs in pathological populations. Specifically, Greicius and associates (Greicius et al., 2004) documented decreased resting-state activity in the PCC area and reduced connectivity between the PCC and hippocampus in AD patients. In a more recent study, the overall segregation of the DMN from other brain regions including the PCC has been shown in AD patients (Ciftci, 2011). Our major finding from the current study suggests that the functional integrity within the DMN, a main resting-state network (Raichle and Snyder, 2007), remained resilient to a single concussive blow without premature

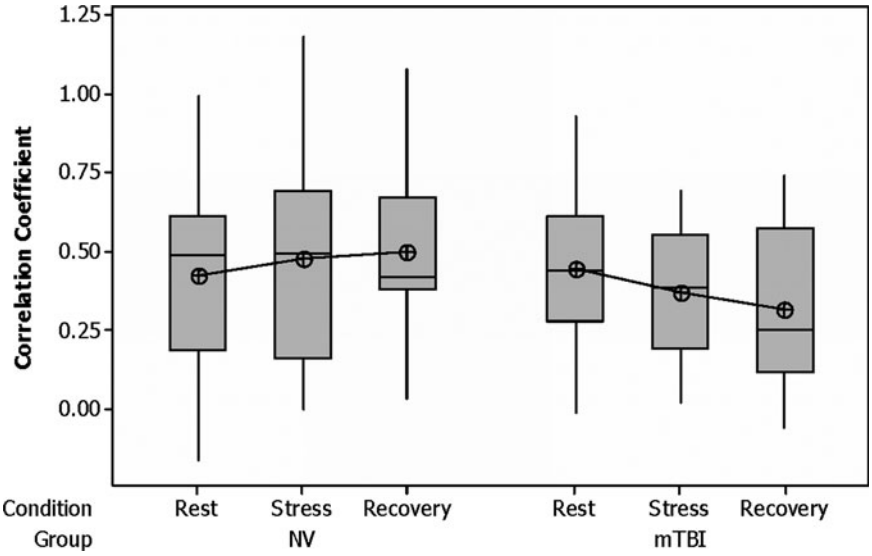

FIG. 2. Correlation between the posterior cingulate cortex (PCC) and medial prefrontal cortex (MPFC) versus group boxplot. The trend line shows the reduced average correlation coefficients from NV to mTBI (NV, normal volunteer; mTBI, mild traumatic brain injury).

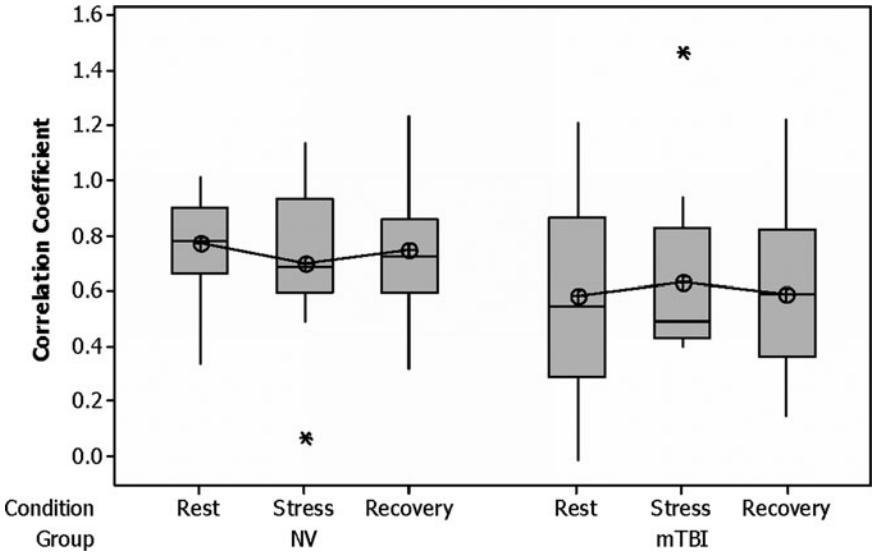

FIG. 3. Correlation between the posterior cingulate cortex (PCC) and left lateral parietal cortex (LLP) versus group boxplot. The trend line shows the reduced average correlation coefficients from NV to MTBI. Asterisks denote the outliers (NV, normal volunteer; mTBI, mild traumatic brain injury).

physical stress. Specifically, the major ROIs constituting the DMN (e.g., PCC/precuneus, MPFC, LLP, and RLP), and the connectivity within these four ROIs appeared to be similar between NVs and mTBI subjects (Fig. 5).

There are a few possible explanations for this major finding, including : (1) these four ROIs are connected through major white matter tract bundles (Greicius et al., 2009; Supekar et al., 2010); therefore, their physical size and densely abundant neuropaths make their major functions resilient to the randomness of a mTBI attack; (2) these four ROIs are relatively large regions that may have their own small-scale networks within each ROI; thus, the neural plasticity based on redundancy and compensation phenomena (Hillary, 2008) may take place following a single concussive blow, and allow the large-scale DMN to recover quickly after partial disruptions. In support of the latter, it should be noted that low-frequency oscillations are likely associated with connectivity within the larger-scale neuronal networks, while higher frequencies are constrained in smaller networks, and may be modulated by activity in the slower-oscillating larger networks (Buzsaki and Draguhn, 2004; Fox and Raichle, 2007). In fact, low-frequency resting-state fluctuations were identified in the DMN, sensory-motor networks (Biswal et al., 1995), visual and auditory, and other networks (Hampson et al., 2002; Lowe et al., 1998) involved in memory and executive functions in the adult brain (Damoiseaux et al., 2006).

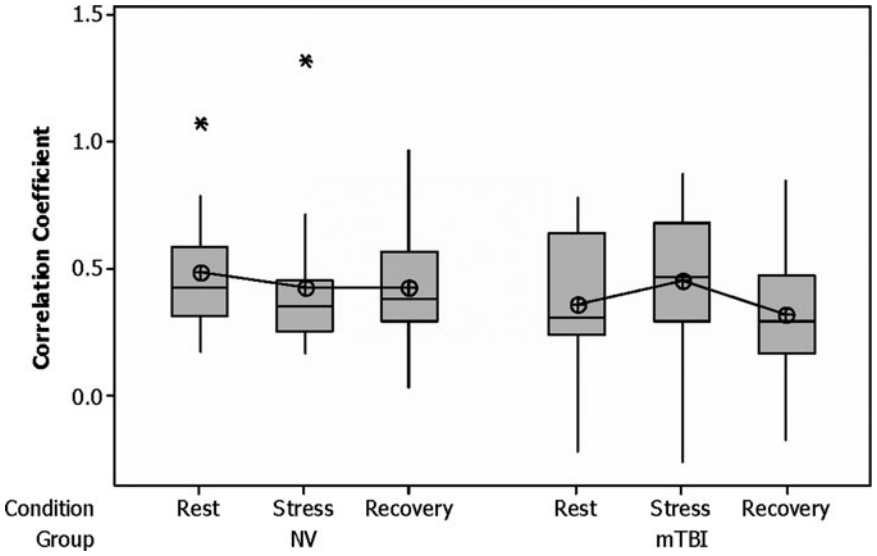

FIG. 4. Correlation between the posterior cingulate cortex (PCC) and right lateral parietal cortex (RLP) versus group boxplot. The trend line shows the reduced average correlation coefficients from NV to MTBI. Asterisks denote the outliers (NV, normal volunteer; mTBI, mild traumatic brain injury).

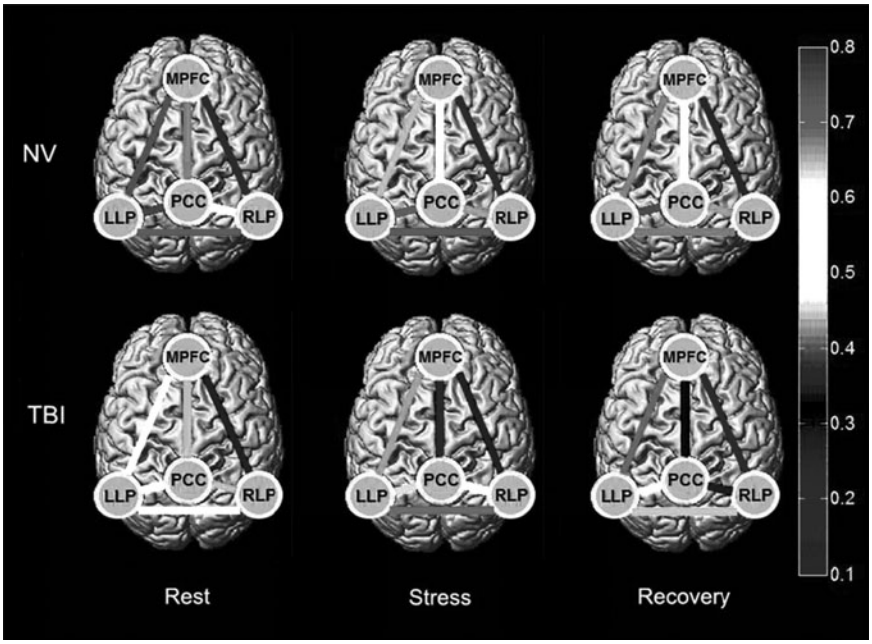

FIG. 5. Graphic representation of the default mode network (DMN) network. The strength of connectivity is reflected in terms of correlation coefficients (r). Usually $r > 0.3$ is used as a threshold of valid connection (LLP, left lateral parietal cortex; RLP, right lateral parietal cortex; MPFC, medial prefrontal cortex; PCC, posterior cingulate cortex; NV, normal volunteer; TBI, traumatic brain injury).

Even though a concussive blow may lead to diffuse axonal injury (Browne et al., 2011; Topal et al., 2008), and could alter white matter integrity in the acute and subacute phases of mTBI (Miles et al., 2008; Zhang et al., 2010), major connections within the DMN remained unaffected and may not be compromised. It should be noted, however, that severe TBI may cause major white matter injuries that disrupt the basic function of the DMN (Sharp et al., 2010). This is a novel finding that sheds additional light on the brain's functional integrity as an injury evolves over time.

As documented in this study, the major connections within the DMN remained intact during the subacute phase of typical sport-related mTBI, but resting-state activity in the PCC, visual cortex, hippocampus, and DLPFC became compromised, particularly with regard to inter-hemispheric connectivity (Mayer et al., 2011; Slobounov et al., 2010). Compared to DMN integrity, the interhemispheric connectivity appears to be a more sensitive measure of the effects of concussive blows. More recently, we documented disrupted connectivity between the DMN and the rest of the brain (Johnson et al., 2011). These recent findings are consistent with clinical studies of brain resting-state functional connectivity in other pathological populations (see Broyd et al., 2009 for review). For example, it has been shown that there is a raised motivational threshold at which task-relevant stimuli become sufficiently salient to deactivate the DMN in children with ADHD while performing an inhibitory control task (Liddle et al., 2011). Alterations in DMN connectivity in the prefrontal cortex have also been implicated in the psychopathology of schizophrenia (Jang et al., 2011). The integrity of resting-state DMN in atypical mTBI subjects with persistent post-concussive symptoms requires additional exploration.

The resting-state DMN retained normal functional connectivity following a single concussive blow in the subacute phase of mTBI, but appeared to be altered in response to the light aerobic activity (<70% MHR) induced by the YMCA physical stress test (Fig. 5). Light physical exertion is the first step of incremental loads of exercise, which may assist the clinician in determining whether an athlete recovering from mTBI may return to play (McCrory et al., 2009). Our findings suggest that: (1) the YMCA physical stress test significantly reduced the magnitude of the connection between the PCC and the left lateral parietal ROI; (2) the YMCA physical stress test significantly reduced the magnitude of the connection between the PCC and the right lateral parietal ROI in mTBI subjects during the transition from physical stress to recovery; and (3) the magnitude of the connections between the PCC and MPFC during the recovery phase was reduced in mTBI subjects. It should be noted that mTBI subjects experienced and reported no clinical symptoms (PCS), including abnormal hart rate readings, during any phase of the study. Furthermore, on follow-up, each athlete studied in this cohort resumed normal levels of activity within the prescribed progressive return-to-play protocol. We anticipate that more rigorous physical stress, such as the full range of athletic activities, may induce the reappearance of clinical symptoms due to altering brain functional connectivity during the subacute phase of mTBI, and may increase the risk for recurrent concussions. There are several reasons to support this hypothesis, considering the functional role of primary ROIs that make up the DMN.

LLP-PCC connections

The LLP is believed to be involved in multiple brain functions, including memory retrieval and encoding and language and mathematics ability (Grabner et al., 2007; Nelson et al., 2010). The connections from the precuneus to the lateral

parietal areas and the premotor cortex are believed to play a pivotal role in the visual guidance of hand movements (Johnson et al., 1993,1996; Wise et al., 1997). The reason why the strength of LLP-PCC connections is reduced in response to physical stress testing, but the RLP-PCC connections remained intact is unclear. It is possible that the coincidental similarity of position and strength or some kinematic characteristics of the concussive blow may cause this effect. Note that mTBI subjects reports indicated that the injury occurred while attempting to avoid a head-to-head collision, and that the left side of the head was the site of contact inducing the concussive blow. Indeed, further research is needed to fully explore this issue.

PCC-MPFC connections

The reduced connectivity between the MPFC and PCC regions of the DMN is associated with aging, deep sleep, and disorders of consciousness. Also, the anterior region of the DMN is correlated with cognitive decline (Broyd et al., 2009). Alteration of functional connectivity within the DMN in adolescents with ADHD has been reported by Tian and colleagues (Tian et al., 2006). This finding is consistent with other reports (Castellanos et al., 2008; Uddin et al., 2008). Castellanos and associates (2008) speculate that this atypical pattern of connectivity may reflect neural underpinnings of an altered relationship between working memory and attentional control. Moreover, the reduced connectivity within the DMN, particularly between the ACC and PCC, reflecting disturbance of self-referential and emotional processing, was observed in autistic subjects (Cherkassky et al., 2006; Kennedy and Courchesne, 2008; Kennedy et al., 2006). Alteration of MPFC-PCC connections in mTBI subjects in response to physical stress test may reflect the residual effect of symptoms (i.e., working memory, attention, and emotional processing) present during the acute phase of injury, but that had resolved at the time of fMRI scanning.

In conclusion, there is no solid evidence to argue whether physical exercise is good or bad for recovery of brain function during the subacute and chronic phases of mTBI. In this study we used the YMCA physical stress test, and documented alteration of intra-DMN connectivity in immediate response to this test and during recovery. The duration of this effect is unknown, since we had only one recovery resting-state fMRI session. As noted in the previous discussion, it would also be of clinical interest to explore the relationship between the amount of physical load and the magnitude of DMN alterations. Additional studies are needed to examine the dynamics of DMN connectivity during the acute to chronic phases of mTBI, including those in response to differential physical and cognitive loads, both of which provoke clinical symptoms in acute and sub-acute stages of recovery from concussions. Clearly, the DMN remained resilient to a single mTBI attack, but was altered in response to light-intensity physical stress. This important finding should be considered by clinical practitioners when making decisions regarding return to play and clearing mTBI athletes for sports participation.

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