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Alteration of Brain Functional Network at Rest and in Response to YMCA Physical Stress Test in Concussed Athletes: rsFMRI study

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Abstract

There is still controversy in the literature whether a single episode of mild traumatic brain injury (mTBI) results in short- and/or long-term functional and structural deficits in the concussed brain. With the inability of traditional brain imaging techniques to properly assess the severity of brain damage induced by a concussive blow, there is hope that more advanced applications such as resting state functional magnetic resonance imaging (rsFMRI) will be more specific in accurately diagnosing mTBI. In this rsFMRI study, we examined 17 subjects 10 +/− 2 days post-sports-related mTBI and 17 age-matched normal volunteers (NVs) to investigate the possibility that the integrity of the of resting state brain network is disrupted following a single concussive blow. We hypothesized that advanced brain imaging techniques may reveal subtle alterations of functional brain connections in asymptomatic mTBI subjects. There are several findings of interest. All mTBI subjects were asymptomatic based upon clinical evaluation and neuropsychological (NP) assessments prior to the MRI session. The mTBI subjects revealed a disrupted functional network both at rest and in response to the YMCA physical stress test. Specifically, interhemispheric connectivity was significantly reduced in the primary visual cortex, hippocampal and dorsolateral prefrontal cortex networks ($p<0.05$). The YMCA physical stress induced nonspecific and similar changes in brain network connectivity patterns in both the mTBI and NV groups. These major findings are discussed in relation to underlying mechanisms, clinical assessment of mTBI, and current debate regarding functional brain connectivity in a clinical population. Overall, our major findings clearly indicate that functional brain alterations in the acute phase of injury are overlooked when conventional clinical and neuropsychological examinations are used.

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Keywords

Mild traumatic brain injury (mTBI); Resting state fMRI (rsFMRI); YMCA physical stress test

1. Introduction

Over the last two decades, it has been challenging to establish clear links between the observable neuropsychological and behavioral symptoms of mild traumatic brain injury (mTBI) and the underlying structural/functional deficits using clinically available brain imaging techniques (see Schrader et al., 2009 for review). However, advances in brain imaging methodologies have revealed important information regarding both structural (Bazarian et al., 2007; Wozniak et al., 2007; Wilde et al., 2008) and functional (McAllister et al., 2001, 2006; Slobounov et al., 2010) alteration in subjects suffering from mTBI. It should be noted that "traditional" analysis of the brain correlating with behavior is mostly implemented by focusing on alteration of the brain signal from local regions of interests (ROIs). For example there is evidence that suggests a variety of functional deficits in concussed individuals that correlate with brain imaging (functional magnetic resonance imaging: fMRI) data (Ptito et al 2007). McAllister et al. (2001) have shown enhanced and diffuse activation primarily in the prefrontal cortex (PFC) of concussed subjects who have successfully performed the required cognitive tasks. Similarly, Jantzen et al. (2004) observed increased activation in the parietal frontal and cerebellar regions in concussed individuals when compared with pre-injury fMRI data although there were no changes in the subjects' cognitive performance. In our recent fMRI study, we observed larger cortical networks and additional increases in activity outside common ROIs in mTBI subjects when compared to normal volunteers (NVs), during spatial navigation working memory tasks (Slobounov et al., 2010). Both mTBI and NVs displayed nearly identical performance and success rates for each task. In contrast, Chen et al. (2004) reported the opposite fMRI findings suggesting a reduced blood oxygen level dependent (BOLD) signal in the mid-dorsolateral prefrontal cortex (DL-PFC) in symptomatic concussed athletes, who also performed poorly on the working memory tasks. Although the nature of these discrepancies is beyond the scope of this report, it should be noted that one of the possible reasons is variation in subjects' inclusion criteria and performance level.

Clearly the human brain has two contradictory properties: (1) "segregation", which means localization of specific functions; and (2) "integration", which means combining all the information and functions at a global level within the conceptual framework of a "global integrated network" (Varera et al., 2001; Reijneveld et al., 2007). Consistent with this conceptual framework, Biswal et al. (1995) were the first to document the spontaneous fluctuations within the somatosensory system and high potential for functional connectivity in resting state functional MRI (rsFMRI) using intrinsic activity correlations. Since this discovery of coherent spontaneous fluctuations, a many studies have shown that several brain regions engaged during various cognitive tasks also form coherent large-scale brain networks that can be identified using rsFMRI (Smith et al., 2009). More recently, neurophysiological correlates underlying rsFMRI were documented specifically emphasizing the links between rsFMRI connectivity and inter-areal synchronization observed with intracranial EEG (see Jerbi et al., 2010 for review).

The existence of a "default network" in the brain during the resting state was reported by Greicius et al. (2003). Both functional and structural connectivity between brain regions were examined to detect whether there are orderly sets of regions that have particularly high local connections (forming families of clusters) as well as a limited number of regions that serve as relay stations or hubs (Sporns et al 2007). It was suggested that the neural network

of the brain has a small-world structure, namely, high-cluster coefficients and low average path length allowing optimization of information processing (Reijneveld et al 2007). Overall, network analysis is necessary to explore the integration phenomena observed in both resting states and in response to high-level information processing in the brain induced by cognitive and/or motor tasks.

Recent advances in brain imaging technologies offer tremendous promise for improving clinical applicability of fMRI with specific focus on spontaneous modulations in the BOLD signal that occur during resting state conditions (see Fox & Raichle, 2007 for review). In contrast to the traditional task-related approach, resting state studies observe the brain in the absence of overt task performance and/or stimulation. In this approach, subjects are generally asked to lie quietly with eyes closed or while fixating on a crosshair. In fact, no consensus exists as to whether there is a significant impact of the precise experimental setting (e.g., with eyes open, eyes closed, or fixation) on the stability of correlation matrix (see Cole et al. 2010, for details). Spontaneous modulation in the BOLD signal in the absence of any explicit input or output is then recorded and analyzed. One of the reasons to use resting state functional connectivity (fcFMRI) for clinical applications is that the task-related increases in neuronal metabolism are usually small (< 5%) when compared to the large resting energy consumption (20% of the body's energy, most of which supports ongoing neuronal signaling) (Raichle & Mintun, 2006). Overall, ongoing spontaneous activity provides a window into the neural processing that appears to consume the vast majority of the brain resources, which might provide a more accurate and richer source of disease-related BOLD signal change (Fox & Greicius, 2010).

Functional abnormalities of the brain are associated with pathological changes in connectivity and network structures. There are varying methodologies [e.g., graph analysis, Granger causality, independent component analysis (ICA), seed-based correlation, and more recently, extended unified structural equation modeling (SEM) approach, Gates et al., 2010] to examine spontaneous BOLD oscillations which reflect enduring and intrinsic properties of the brain and can allow us to obtain a more general characterization of brain dysfunction in psychiatric populations, including ADHD, autism, depression, PTSD, and schizophrenia (see Fortino & Bullmore, 2010; Fox & Creisius, 2010 for review). A few recent fMRI reports also indicated a predominant loss of long-distance functional connections in the resting state in patients suffering from Alzheimer's disease (AD) (Zhow et al 2008; Rosenbaum et al., 2008). Departures from a "small world" network (Watts and Strogatz, 1998) configuration in neurological populations including stroke, tumors, multiple sclerosis, epilepsy have been reported, and have also brought new insight into better understanding the pathophysiology of these diseases affecting specific local and/or global brain networks (see Guye et al., 2010 for review). More recently Nakamura et al. (2010), using the graph theory, examined neural network properties at separate time points during recovery from severe traumatic brain injury. They reported that the strength but not the number of network connections diminished during the acute phase of TBI indicating disruption of the neural system. Marguez de la Plata (2010) also reported a deficit in the functional connectivity of the hippocampus and frontal lobe circuits six months after traumatic diffuse axonal injury (DAI).

The majority of approaches to analyze rsFMRI data have been driven using spatial models with a strong *a priori hypothesis* regarding the functional connectivity of a small number of brain ROIs or individual voxel locations of interest (Cole et al., 2010). With regard to traumatic brain injury (TBI), hippocampal and DL-PFC networks were recently identified and studied (Marquez de la Plata et al., 2010). Few other TBI-related fMRI studies have focused on these ROIs due to their functional alteration, in isolation or in conjunction with other brain regions (McAllister et al., 2001; Cheng et al., 2004; Nakamura et al., 2009;

Slobounov et al., 2010). In the present study, we utilized an *a priori hypothesis* approach focusing on alterations of several interhemispheric brain functional networks at rest and in response to the YMCA physical stress test in subjects suffering from sports-related mTBI. We hypothesized that interhemispheric connectivity within hippocampal, visual, DL-PFC and precuneus networks may be altered in mTBI subjects with a more pronounced effect induced by the physical stress test.

2. Methods

2.1 Participants

Seventeen neurologically normal student-athletes with no history of mTBI (mean age 21.3 +/− 1.5 years) and 17 student-athletes (mean age 20.8 +/− 1.7 years) who had recently suffered a sports-related mTBI (collegiate rugby, ice hockey and soccer players) were recruited for this study. The sample was 65% male and 35 % female. Academic grade average score for all subjects under study was 3.2 +/− 0.5. All injured subjects suffered from grade 1 mTBI (Cantu Data Driven Revised Concussion Grading Guideline, 2006) and were scanned on day 10 (+/−2 days) post-injury right after clearance to perform the YMCA stress test (see below). Inclusion criteria for this rsFMRI 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). The initial diagnosis of mTBI was made on the field by certified athletic trainers (AT) and as a part of the routine protocol of the Sport Concussion Program at Pennsylvania State University. The research team administered a Post-Concussive Symptoms Checklist prior to the scanning. Several conventional neuropsychological measures were also employed, including the: Hopkins Verbal Learning Test –Revised (HVLT), Stroop tests, Trailmaking tests A and B, and the Symbol Digit Modalities Test (SDMT). Both traditional pen-and-pencil procedures and computerized testing (ImPact) were used. All mTBI subjects were asymptomatic based upon the aforementioned neuropsychological (NP) measures and resolution of clinical symptoms. Prior to the MRI scanning, the athletes were cleared both for the first stage of aerobic activity by their supervising physician as well and for sports participation by the medical practitioners at the Penn State Center for Sport Medicine based upon neurological assessments (Co-operative Ataxia Rating Scale, World Federation of Neurology, Trouillas et al., 1997). 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 protocol was approved by the Institutional Review Board of the Pennsylvania State University. Neither lesions nor hyperintense signals were present in either the mTBI or NVs in our study.

2.2. Procedure

Subjects reported to the scanning facility and were interviewed about their symptoms. Subjects were then given an informed consent and MRI safety guidelines prior to being scanned. They were given a post-concussion symptom checklist and asked to rate their current symptom intensities. Subjects were excluded if they reported any symptoms before being scanned. Once they signed the consent form, they were placed in the 3Tesla MRI scanner and were scanned using the rsFMRI protocol. At any time during testing or scanning, procedures were stopped if the subjects reported any presence of post-concussive symptoms. Subjects were then asked to perform the modified YMCA bike protocol. The motivation and rational for examining the brain functional responses to physical stress test came from the clinical practice dealing with mild TBI. Specifically, the YMCA physical stress test is a common clinical procedure used for detecting residual physiological and behavioral abnormalities prior to clearance of concussed athletes for sport participation. We initially hypothesized that rsfMRI data, specifically functional connectivity, may shed

additional light and be capable of detecting subtle brain abnormal functions otherwise overlooked by clinicians while administering the YMCA stress test. The YMCA bike protocol begins with a 2 – 3 minute warm-up in order to acquaint the subjects with the cycle ergometer and prepare them for the exercise intensity in the first stage of the test. The specific protocol consists of 4 stages of increasing resistance lasting 3minutes per stage. Subjects' 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% age adjusted maximum heart rate (MPHR), as recommended by the most recent consensus on return to sporting activities achieved at the 3rd International Conference on Concussion in Sport (see also McGrory 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 rsFMRI 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 series of rsFMRI scanning. Transition times from bike to the initiation of the post-bike scanning sequences were all under 2 minutes. Overall, fMRI data were acquired before the YMCA bike test (rest), right after its completion (physical stress), and during recovery, which was on average within 15–20 minutes after completing the bike test. Heart rate was monitored throughout the entire experimental session including rest, prior to bike test, during the bike test, and while in scanner for all 3 scan sessions.

2.3. MRI Data acquisition

Functional and anatomical images were acquired on a 3.0 Tesla Siemens Trio whole-body scanner (Siemens, Erlangen, Germany) using a 12-channel head coil. fMRI and T1 anatomical images were acquired in the axial plane parallel with 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: 0.9mm × 0.9mm × 0.9mm resolution, TE= 3.46ms, TR= 2300ms, TI= 900ms, flip angle= 9°, 160 slices, iPAT= none, NSA= 1). Identical two-dimensional BOLD echo planar fMRI (3.1mm × 3.1mm × 5mm resolution, TE= 25ms, TR= 2000ms, EPI factor=64, flip angle= 79°, 30 slices, iPAT= none, NSA= 1, acquisition time= 6:04) resting-state images were obtained at each rest, stress, and recovery stage. A ten-minute relaxation time was given to subjects in between the stress and recovery stages to allow for the subjects' heart rates to return to baseline.

2.4. Data analysis

Data analysis was performed using Statistical Parametric Mapping (SPM) version 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), in addition to the Functional Connectivity (CONN) toolbox (http://web.mit.edu/swg/software.htm). Both are brain imaging data analysis toolboxes written in the MATLAB language (http://www.mathworks.com/). The first step carried out was preprocessing which included: realignment, co-registration, segmentation, normalization and band filtering. During preprocessing, images were motion-corrected, registered with structural images, and normalized to the standard brain template from the Montreal Neurological Institute (MNI). The resulting preprocessed images were then band-pass filtered to 0.01Hz~0.1Hz to reduce the influence of noise.

The CONN toolbox performs seed-based analysis by computing the temporal correlation between the BOLD signals from a given voxel to all other voxels in the brain. White matter, cerebrospinal fluid (CSF) and physiological (and other) noise source reduction were taken as confounders, following the implemented CompCor strategy (Behzadi et al., 2007). Specifically, the heart rate (assumed associated with blood flow intensity) and motion artifact in the scanner were taken as confounders. CONN also allows for ROI-based analysis by grouping voxels into ROIs based upon Brodmann's Areas. Bi-variate correlations were calculated between each pair of ROIs as reflections of connections. All Brodmann areas were imported as possible connections for our selected four ROI sources. Z-score standardizing was introduced to validate the multiple comparisons, and the significance tests were based on the Z-scores.

Specifically, we selected the following areas: right dorsal-lateral prefrontal cortex (DL-PFC), bilateral precuneus, bilateral primary visual cortex, and bilateral hippocampus, as our 4 ROIs. The selection of the 4 ROIs is based on our recent fMRI results (Slobounov et al., 2010), and agrees with other studies indicating the relevance and/or alterations of these ROIs in concussed subjects. Several studies showed that the right DL-PFC and hippocampus are linked to alterations of spatial memory tasks performance (Chen et al., 2004; Slobounov et al., 2010). Visual cortex and precuneus are not only limited to vision and motor coordination, but are areas most vulnerable to concussive blows.

Based on the 1st level results, an ANOVA factorial analysis was conducted aimed at exploring the effects of different conditions (rest, stress, and recovery) and the two groups (NVs and mTBI). We used the F-test implemented in SPM8 to illustrate the main effects of each group together with the main and cross effects of the conditions. Post-hoc t-tests were then performed on those areas that had a significant F-test. Therefore, it was possible to determine exactly which condition caused the effect of group and which group showed significantly differential connectivity.

A further comparison was performed with respect to the interhemispheric connectivity, since some DTI studies revealed alteration in the white matter integrity of the corpus callosum, which is the main bundle of axons in interhemispheric connections (Wilde et al., 2008; Zhang et al., 2010). We chose the DL-PFC and hippocampus as our focus ROIs and extracted the correlation coefficients between the bilateral ROIs for each subject. The ROIs were defined anatomically by Brodmann areas (BA) in the MNI template. A two-sample t-test was then carried out to determine if there was a significant difference in the strength of interhemispheric connectivity between NVs and mTBI patients. This connectivity was illustrated in a graph views using the CONN functional connectivity toolbox. For each target ROI, CONN determined the inter-ROI connectivity according to the correlation analysis. A t-test was used to test the significant connections. The threshold was set at $p<0.05$ FDR. ROI-based connectivity maps were generated by CONN and the connections of interest were highlighted by thick lines (see Figures 4 and 6).

3. Results

3.1. Neuropsychological (NP) Test Performance Data

Table 1 shows the neuropsychological data for the concussion (mTBI) and normal volunteers (NV) groups. As can be seen from the data presented in Table 1, there were no significant differences between mTBI subjects and NVs for all the variables under NP testing ($p >0.05$).

3.2. Heart Rate Dynamics Data

As noted before (see Methods section), the subjects' heart rates were constantly monitored to accommodate the requirements for the YMCA bike protocol as well as to control for the rate of recovery while subjects were in the scanner. As can be seen from Fig. 1, there was a similar trend of heart rate dynamics in both NV and mTBI groups. Specifically, there was an initial increased heart rate at the beginning of the stress test, with ultimate recovery within 10 minutes after completion. Paired t-tests revealed no significant differences between groups within any of these phases during the entire testing protocol ($p < 0.05$). mTBI subjects reported no subjective symptoms of concussion, an accepted indication that all of them were clinically asymptomatic at the time of scanning.

3.3. rsFMRI Data

3.3.1 Primary Visual Cortex Network—The connectivity maps of the primary visual cortex (V1) network pooled across all subjects for both groups (mTBI and NV) as well as for all three testing conditions (rest, stress and recovery) are shown in Figure 2. Visually, V1 connectivity revealed similar patterns between mTBI and NVs. However, ANOVA showed that there was a significant main effect of group (NV versus TBI), $F = 30.95$, $p < 0.005$, Family-Wise Error rate (FWE) corrected, as well as significant main effect of conditions (rest, stress and recovery), $F=17.65$, $p < 0.05$ (FWE) on connectivity maps. There was no effect of interaction ($p > 0.05$), indicating that stress induced non-specific and similar changes in connectivity patterns in both mTBI and NV groups. Table 2 shows, for each of the four ROIs, the connected areas common to both groups. Functional V1 interhemispheric connectivity was significantly reduced in mTBI patients compared to NVs at rest ($p = 0.01$), as well as following stress and recovery ($p < 0.05$). This was true regardless of whether the left or right V1 was chosen as seeds.

Post-hoc analysis within the groups between conditions revealed that stress induced increased magnitude of interhemispheric connections between left and right V1 ($p < 0.05$) in both groups of subjects. This pattern of increased magnitude of significant functional connections induced by stress remained during the recovery stage in both groups of subjects. Finally, there were additional significant connections between primary visual cortex (R) with secondary visual cortex bilaterally (BA 18) and dorsal posterior cingulate cortex (R, BA 31) during recovery in both groups ($p < 0.05$). Moreover, we observed additional significant connection between primary visual cortex (L) with secondary visual cortex bilaterally ($p<0.05$) in both groups during recovery. The magnitude of V1 interhemispheric connections in mTBI subjects following stress was comparable with those of NVs at rest, $t = 15.3 +/- 1.2$).

3.3.2. Hippocampal Network—The connectivity maps of the hippocampal network pooled across all subjects for both groups of subjects (mTBI and NV) as well as for the three testing conditions (rest, stress and recovery) are shown in Figure 3a. Time series of the BOLD signal fluctuation for right and left hippocampi plotted over time are shown in Figure 3b. Visually, there was reduced interhemispheric hippocampal connectivity at rest in mTBI patients and tended to increase following stress and recovery. ANOVA revealed that there was a significant main effect of group (NV versus mTBI), $F = 31.07$, $p < 0.005$ (FWE) as well as significant main effect of conditions (rest, stress and recovery), $F=18.32$, $p < 0.05$ (FWE). Similar to the visual cortex network, the interaction was not significant ($p > 0.05$), indicating that stress induced nonspecific and similar changes in connectivity maps within the hippocampal network in both mTBI and NV groups.

T-tests revealed that the degree of interhemispheric hippocampal connections was significantly lower in mTBI compared to NV at rest as well as following stress and recovery

($p < 0.05$, see Table 1 for details). There was a similar pattern of connections between the right and left hippocampus at rest with additional connections with the parahippocampal cortex and perirhinal cortex contralaterally. The magnitude of contralateral connections with the perirhinal cortex was significant ($p < 0.05$), indicating reduced connectivity in mTBI patients at rest with this ROI. The degree of connectivity within the parahippocampal cortex was not significantly different between mTBI and NV ($p < 0.05$).

Post-hoc analysis revealed increased magnitude of connections within the interhemispheric hippocampal network following stress ($p < 0.05$) in both groups. This trend of increased degree of connectivity was maintained during recovery (the differences between rest and recovery were significant, $p < 0.05$). Moreover, stress induced an additional significant connection within the right hippocampal network to the ipsilateral perirhinal cortex (BA 35) in both groups. Again, the degree of this connection was significantly reduced in mTBI than in NV subjects ($p < 0.05$).

Finally, for both groups, physical stress induced a similar increase in the number of connections of the left hippocampal network extending to the left perirhinal cortex (BA 35), middle temporal gyrus (R, BA 21), and dorsal posterior cingulate cortex (R, BA 31). Similar to the visual network, the degree of hippocampal interhemispheric connections in mTBI subjects following stress was comparable those of NV at rest, $t\text{-}v = 8.15 +/- 1.3$).

To further illustrate the alteration of interhemispheric hippocampal connectivity in mTBI we obtained the connectivity diagram using the CONN toolbox (see Fig. 4). Clearly, the magnitude of interhemispheric hippocampal connectivity was reduced in mTBI subjects as indicated by the thickness of lines connecting the left and right hippocampi. This was true for all three study conditions.

3.3.3 Precuneus Network—Visually, there were similar patterns of connectivity at rest, stress, and recovery between groups. The ANOVA confirmed this observation indicating that the main effect of group was not significant, $p > 0.05$ (see Fig. 5). The degree of precuneus interhemispheric connectivity did not differ significantly in both groups of subjects. However, there was a significant main effect of conditions, $F = 17.61$, $p < 0.05$ (FWE), indicating that the degree of resting state functional connectivity increased in response to stress in both groups of subjects. Post-hoc analysis revealed significant differences between stress and recovery, $p < 0.05$ in both groups. The main effect of interaction was not significant, indicating that stress induced a nonspecific (group independent) effect on functional connectivity within the precuneus network during recovery.

3.3.4 Dorsolateral Prefrontal Cortex DL-PFC (R) Network—The samples of the pooled average connectivity maps of DL-PFC (R) network for both groups of subjects (mTBI and NV) as well as for the three testing conditions (rest, stress and recovery) are shown in Figure 6. Visually, connectivity maps appeared to be similar between mTBI and NV. However, ANOVA showed that there was a significant main effect of group (NV versus mTBI), $F=30.87$, $p < 0.05$ (FWE). Neither main effect of conditions nor effect of interaction was significant ($P > 0.05$) indicating that unlike other ROIs under study, physical stress did not induce significant changes in connectivity patterns in both groups of subjects. As can be seen from Table 2 and Fig 5b, functional DL-PFC (R) interhemispheric connectivity was significantly reduced in mTBI patients compared to NV at rest ($p = 0.01$), as well as following stress and recovery ($p < 0.05$). In addition, the degree of functional connectivity in mTBI patients was significantly lower between the DL-PFC (R) and the supramarginal gyrus, dorsal frontal cortex ipsilaterally ($p < 0.05$), and dorsal frontal cortex contralaterally ($p < 0.05$). Also, the magnitude of DL-PFC interhemispheric connections in

mTBI subjects following physical stress was comparable to those of NV at rest, $t = 6.12 +/- 1.5$.

To further illustrate alteration of the DL-PFC interhemispheric connectivity in mTBI, we have elaborated the connectivity diagram using the CONN toolbox (see Fig. 7). Clearly, the magnitude of DL-PFC interhemispheric connectivity is reduced in mTBI subjects as indicated by the thickness of lines connecting these brain regions. This was true for all three study conditions.

4. Discussion

It is important to better delineate alterations in the brain's functional network possibly linked to both short- and long-term subtle cognitive and/or behavioral impairment in mild TBI. Indeed, there is a growing evidence to support the notion that "asymptomatic" mTBI subjects may have residual brain abnormalities, which may be assessed by advanced brain imaging techniques, putting these individual at high risk for recurrent brain injuries (Slobounov et al., 2009, 2010). The resting state functional connectivity, as assessed by fMRI measures, have a neurophysiological basis (Jerbi et al., 2010), is sensitive to various neurological and psychiatric abnormalities (Fox and Greicius, 2010), and therefore is a good candidate to understand the behavior in normal and pathological populations. In this rsFMRI study we focused primarily on interhemispheric functional brain connectivity that appeared to be present in both groups but altered in terms of their magnitude in mTBI subjects. It should be noted that all mTBI subjects reported in this study were clinically asymptomatic by the time of scanning, symptom-free based on neuropsychological examination, and, therefore cleared by medical professionals to participate in the fMRI and YMCA physical stress protocol.

We used an *a priori hypothesis* approach based on our own previous fMRI findings (Slobounov et al., 2010) and other mTBI studies (McAllister, 2001; Nakamura et al., 2009, Chen et al., 2004) and focused on the primary visual, hippocampal, DL-PFC and precuneus networks due to their susceptibility toward mild TBI including those induced by a concussive blow. It has been shown that synchronized activation between prefrontal, frontal, and central sites correlates with the efficiency of working memory and speed of information processing (Silberstein et al., 2004). In our previous EEG study, altered functional interhemispheric connectivity in concussed subjects was consistently observed in ROIs centered at frontal, occipital and parietal sites (Cheng & Slobounov, 2009). Several other studies have also indicated that the cognitive impairment in concussed individuals is due to dysfunction of the frontal and parietal lobes (Bakay et al., 1997; Abdel-Dayem et al., 1998; Chen et al., 2003).

It was documented in numerous previous studies that the general pattern emerging from neurological patients is that the abnormalities are mostly relegated to frontal areas (Elbert et al., 1992; Jeong, et al., 1998; Foster et al., 1994; Vavera et al., 2007). Specifically, the frontal, temporal and parietal lobes are shown to be associated with efficacy of retrieving declarative memories (Purves et al, 2007) and/or intact sustained and directed attention (Foster et al., 1994). Accordingly, damage to the frontal, temporal and parietal areas is believed to be associated with memory loss and attention problems, which are typical symptoms of mTBI (Trimble, 1990). It is important to note that most of our subjects had suffered from memory and attention deficits within 3–7 days post-injury as revealed by subjective reports.

There are three major findings in this study. *First*, all our mTBI subjects were clinically asymptomatic at the time of MRI scanning based on physician examination, NP testing and

the self-reported symptom scale as being zero. This finding is consistent with numerous NP studies demonstrating the high sensitivity and specificity of the NP testing procedure within the acute stage of mTBI (e.g., 7–10 days post-injury). In fact, subjective symptoms reported by mTBI subjects within 7–10 days post-injury are highly correlated with NP measures (Lovell et al., 2003). Interestingly, the YMCA physical stress test protocol, which is a common clinical procedure to rule out residual abnormalities (McGrory, 2009), also demonstrated that all mTBI subjects under study were asymptomatic at the time of scanning. Specifically, heart rate dynamics over the course of experiment were similar in both subject groups characterized by an initial increase during physical stress and then saturation during recovery.

Second, interhemispheric connectivity was obviously reduced in mTBI for the primary visual cortex, hippocampus and DL-PFC networks. In fMRI activation studies in the clinical working memory literature, a nearly universal finding is that neurological insult, including a concussive blow, results in recruitment of additional neural resources during the period of cognitive challenge (Fitzpatric et al., 2008; McAllister et al., 2001, 2006; Jantzen et al., 2004; Slobounov et al., 2010). In the current resting state fMRI study, we observed a significantly reduced hippocampal interhemispheric connectivity in mTBI subjects recovering from mTBI. This major finding is consistent with an earlier report by MacDonald et al (2008), suggesting compromised hippocampal connectivity in a single patient who had suffered a TBI. More recently, Marcuez de la Plata et al (2010) also reported that patients with traumatic DAI showed significantly lower hippocampal interhemispheric functional connectivity up to 6 months post-injury. The reduced interhemispheric functional connectivity within the anterior cingulate (AC) and DL-PFC in their study correlated with the functional and neurocognitive outcomes.

The question may be raised why there was a difference in connectivity between two groups in the visual cortex. There at least two possible explanations we can offer to address this question. First, impairment of vision including blurred vision, light sensitivity, double vision, impaired ocular-vestibular reflexes, impaired eye movement (saccades, nystagmus, etc.) is a common symptom of mild TBI observed both in acute and sub-acute phases of injury. All our subjects were tested within 10 days post-injury and may have residual subtle visual abnormalities that were overlooked using routine neurological examination. Second, in our previous EEG research we have documented the departure from the small-world like network in mild TBI (Cao & Slobounov, 2010) as well as alterations of EEG signal non-stationarity (Cao & Slobounov, in press). The occipital ROI (visual cortex) was the prominent brain area subjected to these brain functional alterations.

Reduced interhemispheric functional connectivity observed in our study agrees with other clinical reports. Specifically, patients with Alzheimer's disease have shown disrupted hippocampal and frontal lobe connectivity throughout the whole brain as compared to healthy volunteers (Alen et al., 2007; Wang et al 2006). Also, interhemispheric functional connectivity is significantly reduced in patients with a compromised corpus callosum (CC) (Quigley et al., 2003), indicating the relationship between CC integrity and functional connectivity. Likewise, Johnston et al. (2008) demonstrated significant reductions in interhemispheric connectivity of various functional systems after a complete callosotomy, while intrahemispheric connectivity was preserved. Considering that the CC is the most commonly injured white matter structure following TBI (Afrikanis et al., 2002; Inglese et al., 2005; Rutgers et al., 2008; Wilde et al., 2008), it is reasonable to hypothesize that compromised integrity of the CC may result in reduced interhemispheric functional connectivity in mTBI, as we observed in this report. Our current research is focused on directly examining the relationship between CC integrity and resting state interhemispheric

functional connectivity (rsFMRI) in acute, subacute, and chronic phases of mTBI to further support this hypothesis.

Third, the YMCA physical stress test induced nonspecific and similar changes in connectivity patterns in both mTBI and NV groups. The rsFMRI data in Nakamura et al. (2009) study indicated such a change in the context of "sparsity", meaning that the magnitude of the cortical connections between nodes within the whole brain is reduced in TBI patients. They hypothesized that alterations in the neural connections observed during recovery may not signify formal brain reorganization (e.g., creation of novel connections). Instead these changes represent utilization of existing support resources early after neural disruption and this demand on auxiliary resources diminishes later, thereby resulting in a less costly network with greater neural efficiency. In fact, in this study we observed increased interhemispheric connectivity in mTBI subjects following physical stress. Specifically, the degree of interhemispheric connectivity in all mTBI subjects under study following physical stress significantly increased for the primary visual, hippocampal, and DL-PFC networks and was comparable to those observed in NV at rest prior to the physical stress test. This finding may indicate the efficacy of moderate aerobic exercise for enhancing the neural efficiency during the acute and subacute phases of recovery from mTBI. Whether this effect is short-term or may last longer and therefore be considered as a potential rehabilitation procedure aimed to facilitate the recovery from mTBI, has yet to be examined.

In conclusion, our initial hypothesis that interhemispheric connectivity within hippocampal, visual, DL-PFC, and precuneus networks may be altered in mTBI subjects with more pronounced effect induced by physical stress was partly supported by experimental findings. Indeed, we found reduced interhemispheric functional connectivity during rest and in response to physical stress within the visual, hippocampal and DL-PFC (but not precuneus) networks in "asymptomatic" athletes who had suffered mTBI. We speculate that this reduced interhemispheric connectivity may result from compromised CC integrity; direct examination of this hypothesis will be conducted in future research. Such research should consider various MRI modalities (e.g., DTI, MRS, rsFMRI) as well as several factors including severity of trauma, differential rate of recovery from brain injury, age of participants, time since injury, and present and previous health history. The precuneus is known to show deactivation during sleep, coma, and other pathophysiological altered states of consciousness (Trimble and Cavanna 2008). After performing the YMCA bike protocol subjects may have become more aroused after exercise which might explain why the precuneus network was enhanced during recovery compared to both rest and stress. *Second*, the YMCA physical stress test, although sensitive toward modulating brain functional connectivity, appeared to be nonspecific as evidenced by inducing similar effects (e.g., increased interhemispheric connectivity) in both mTBI and NV. *Third*, considering the fact that a physical stress test indeed results in strengthening the degree of interhemispheric connectivity, it is logical to suggest that moderate aerobic exercise shortly after clinical symptom resolution may be considered within a rehabilitation protocol for mTBI.

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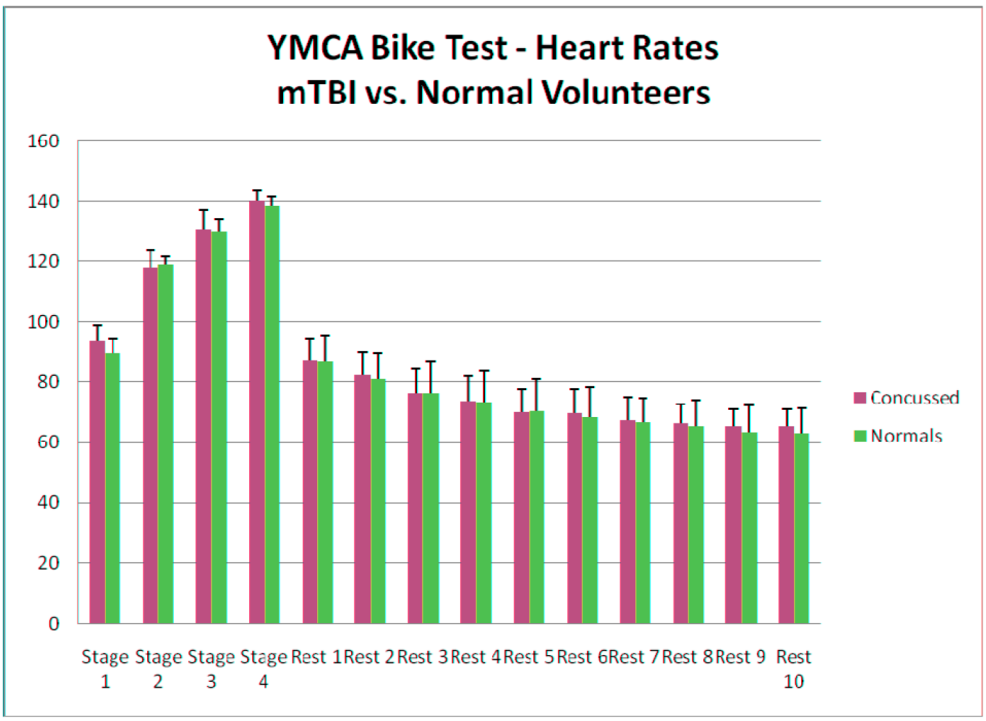

Fig. 1.

Mean values and SD of heart rate dynamics prior to participating in the YMCA bike protocol (rest while subjects in scanner), during the stress test (subjects actually performed the bike protocol) and during recovery (rest while subject in scanner). Note that no group differences were observed throughout the entire experimental session.

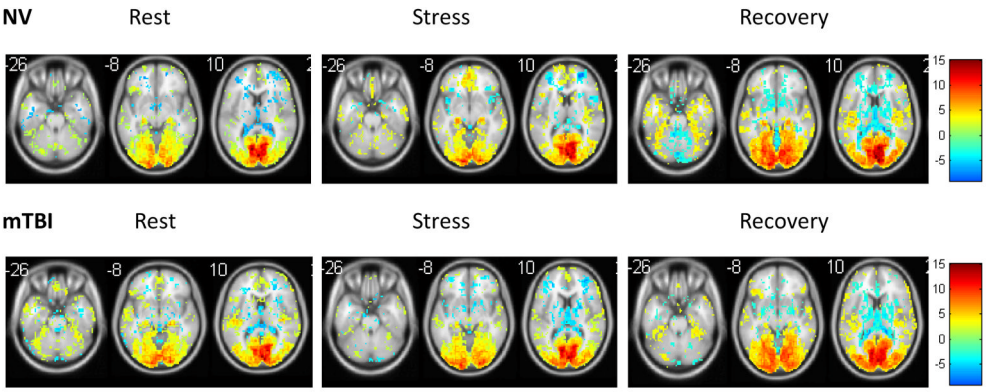

Fig. 2.

Functional connectivity maps of the primary visual cortex network pooled across all subjects for both groups (mTBI and NV) as well as for three testing conditions (rest, stress and recovery) under study. Statistical thresholds were set at k=100, $p < 0.05$, False Discovery Rate (FDR) corrected. Positive correlations are depicted in warm colors and their overlap is depicted in red in the conjunction analysis. Negative correlations are depicted in cool colors and their overlap is depicted in blue in the conjunction analysis.

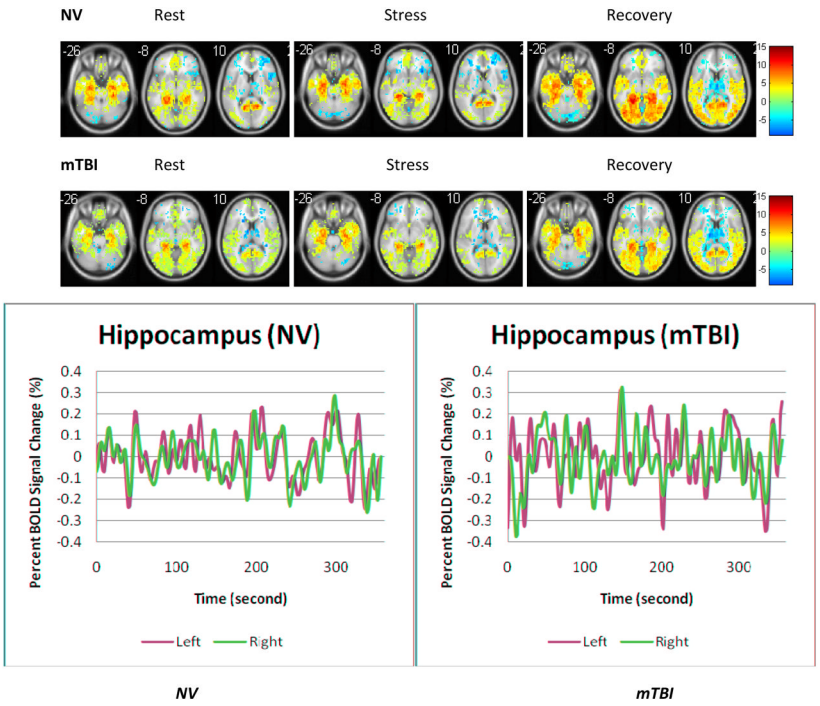

Fig. 3.

(**A**) Functional connectivity maps of the hippocampal network pooled across all subjects indicating correlations between right and left hippocampi as well as with other voxels in the brain (k=100, p< 0.05, FDR corrected). Positive correlations are depicted in warm colors and their overlap is depicted in red in the conjunction analysis. Negative correlations are depicted in cool colors and their overlap is depicted in blue in the conjunction analysis. Resting state functional connectivity revealed correlations within the hippocampal network (specifically interhemispheric hippocampal connectivity), which were observed in normal volunteers (NVs) and subjects suffering from mild traumatic brain injury (mTBI). This pattern of hippocampal network qualitatively was preserved in response to physical stress and during recovery. (**B**) BOLD signal percent change for the right and left hippocampi is plotted over time. Visually, the BOLD signal fluctuates more synchronously in NVs as opposed to mTBI subjects. Time course of a single run shown for the seed region (right hippocampus is highly correlated with the left hippocampus (r= 0.78 p< 0.05) for NVs and mTBI subjects (r=0.56, p < 0.05).

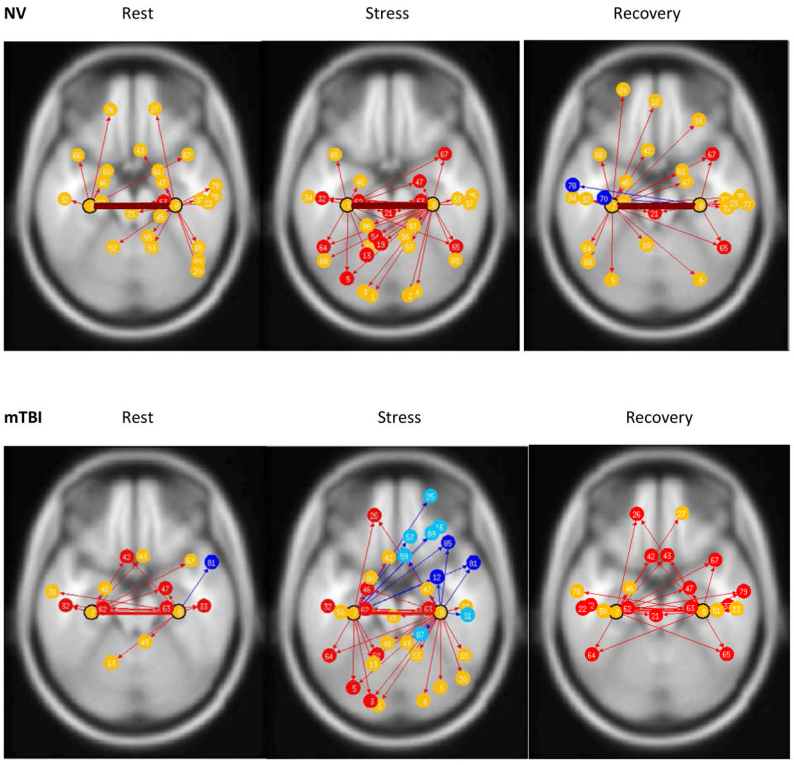

Fig. 4.

Functional connectivity diagrams developed by CONN toolbox for (A) NV and (B) mTBI subjects, indicating reduction of hippocampal interhemispheric correlations in mTBI subjects as reflected in the thickness of the line between the right and left hippocampi (k=100, p< 0.05, FDR corrected). The thicknesses depends on the average correlation coefficients from the 2 groups. Positive correlations are depicted in warm colors and their overlap is depicted in red in the conjunction analysis. Negative correlations are depicted in cool colors and their overlap is depicted in blue in the conjunction analysis. The number in the circle indicates the index of the BA-based ROIs. See CONN toolbox manual for details.

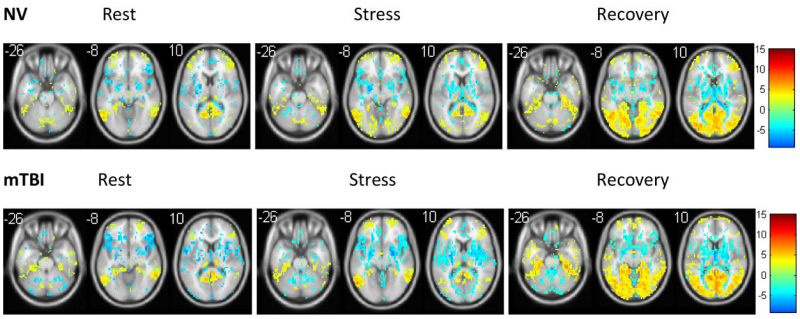

Fig 5.

Functional connectivity maps of precuneus network pooled across all subjects for both groups (mTBI and NV) as well as for three testing conditions (rest, stress and recovery) under study (k=100, $p < 0.05$, FDR corrected). Positive correlations are depicted in warm colors and their overlap is depicted in red in the conjunction analysis. Negative correlations are depicted in cool colors and their overlap is depicted in blue in the conjunction analysis. Visually, similar patterns were observed in both groups of subjects during the resting state, in response to the YMCA bike stress test, and during recovery.

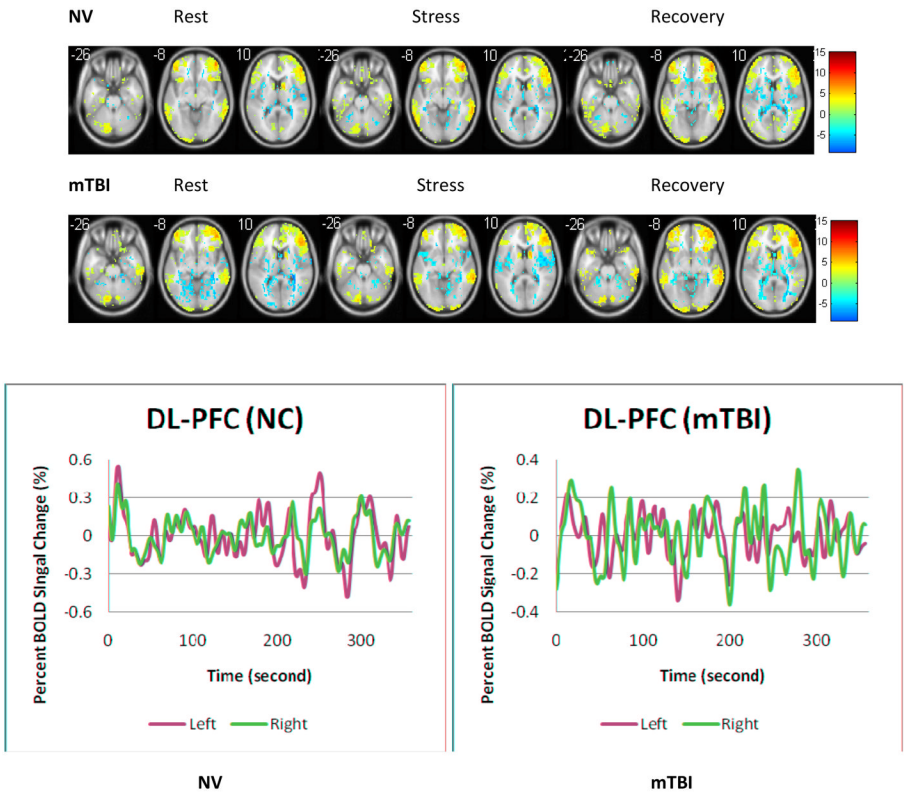

Fig. 6.

(**A**) Functional connectivity maps of the right DL-PFC network pooled across all subjects indicating significant correlations with left DL-PFC as well as with other voxels in the brain (k=100, p< 0.05, FDR corrected). Positive correlations are depicted in warm colors and their overlap is depicted in red in the conjunction analysis. Negative correlations are depicted in cool colors and their overlap is depicted in blue in the conjunction analysis. Similar to the hippocampal network, resting state functional connectivity revealed interhemispheric DLPFC correlations which were observed in normal volunteers (NVs) and subjects suffering from mild traumatic brain injury (mTBI). This pattern of DL-PFCl network qualitatively was preserved in response to physical stress and during recovery. (**B**) BOLD signal percent change for the right and left DL-PFC is plotted over time. Visually, the BOLD signal fluctuates more synchronously in NVs as opposed to mTBI subjects. Time course of a single run shown for the seed region (right DL-PFC is highly correlated with the left DL-PFC (r= 0.69 p< 0.05) for a NV and mTBI patient (r=0.51, p < 0.05).

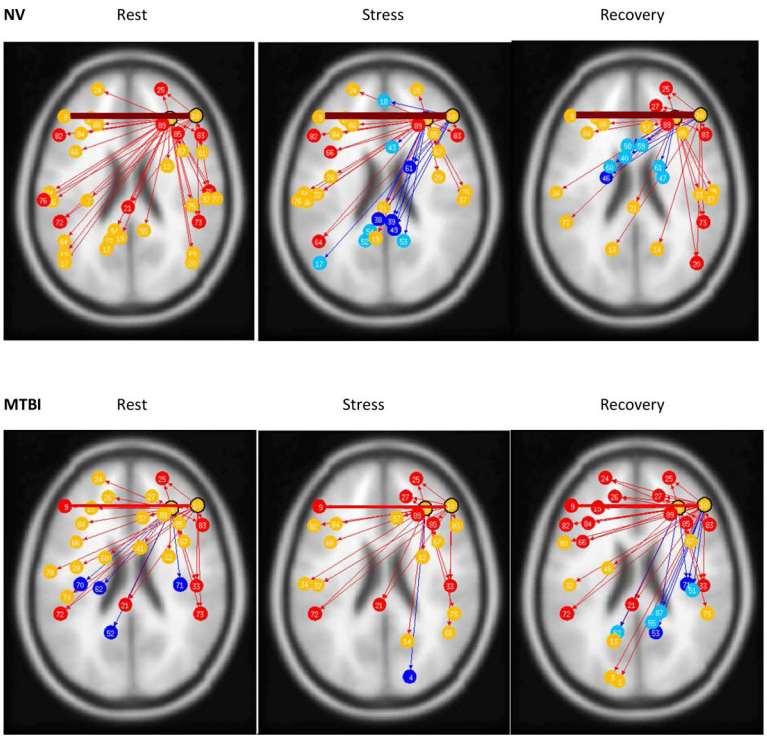

Fig. 7.

Functional connectivity diagrams developed by CONN toolbox for (A) NV and (B) mTBI subjects, indicating reduction of DL-PFC interhemispheric correlations in mTBI subjects as reflected in the thickness of the line between the right and left DL-PFC (k=100, $p < 0.05$, FDR corrected). The thicknesses depends on the average correlation coefficients from the 2 groups. Positive correlations are depicted in warm colors and their overlap is depicted in red in the conjunction analysis. Negative correlations are depicted in cool colors and their overlap is depicted in blue in the conjunction analysis. The number in the circle indicates the index of the BA-based ROIs. See CONN toolbox manual for details.

Table 1

Neuropsychological Test Performance Variables including Reported Fatigue Scores prior to MRI scanning for normal volunteers (NVs) and mTBI subjects.

Subject Groups	**NC**	**MTBI**		
	M (SD)	**M (SD)**	**t-test**	**p-value**
Reported fatigue:				
Beatty Test Fatigue Rating				
Cognitive fatigue	9.3 (3.3)	10.4 (3.7)	2.05	.071
Physical fatigue	9.9 (3.1)	10.7 (4.2)	2.05	.086
Total fatigue	21.6 (5.1)	22.3 (11.3)	2.074	.081
Neuropsychological (NS) test performance:				
Trailmaking A	31(4)	30(6)	1.93	.071
Trailmaking B	88 (5)	87(1)	1.28	.133
Stroop Color-Word (CS) & Color-Word Interference (CW-I)				
CW total time	51.9 (7.8)	50.3 (6.1)	3.25	.077
CW-I total time	101.5 (25.5)	101.4 (21.6)	3.12	.077
CW total errors	0.0 (0.0)	0.3 (0.5)	1.41	>.10
CW-I total errors	1.5 (0.7)	1.6(1.4)	1.46	.088

Note, no differences were observed between subject groups, indicating that mTBI subjects were clinically asymptomatic on the day testing.

Table 2

Correlation matrix during resting state prior to participating in the bike protocol (1), right after the bike protocol - stress (2) and during recovery (3). Only common connectivity regions observed in both normal volunteers (NVs) and mTBI subjects are included. Only statistically significant connections ($p<0.05$, FDR) within both NV and mTBI groups are included.

(1) Rest		NV		mTBI	
ROI	**BA**	***t*-v**	***p*-v FDR**	***t*-v**	***p*-v FDR**
Dorsolateral Prefrontal Cortex(R)					
Dorsal frontal cortex (R)	8	15.97	0.0079	**8.85****	0.0277
Supramarginal gyrus (R)	40	9.61	0.0208	**5.49***	0.0447
Dorsal frontal cortex (L)	8	9.41	0.0208	**5.13****	0.0446
Dorsolateral prefrontal cortex (L)	9	6.93	0.0319	**4.65***	0.0447
Anterior prefrontal cortex (R)	10	6.18	0.0341	5.03	0.0448
Hippocampus (L)					
Hippocampus (R)	37	8.43	0.0254	**5.48***	0.0454
Perirhinal cortex (R)	35	7.81	0.0459	**5.91***	0.0425
Parahippocampal cortex(R)	36	7.35	0.0459	8.41	0.0290
Hippocampus(R)					
Hippocampus (L)	37	8.43	0.0254	**5.48***	0.0454
Perirhinal cortex (R)	35	9.82	0.0259	**6.33***	0.0349
Parahippocampal cortex (L)	36	7.35	0.0459	8.41	0.0290
Precuneus (L)					
Dorsolateral prefrontal cortex (R)	46	17.21	0.0017	16.42	0.0070
Precuneus (R)	7	8.58	0.0489	9.60	0.0237
Dorsal posterior cingulate cortex (L)	31	8.02	0.0223	9.42	0.0270
Angular gyrus (R)	39	7.71	0.0223	8.81	0.0284
Anterior prefrontal cortex (L)	10	5.92	0.0325	6.89	0.0337
Precuneus (R)					
Precuneus (L)	7	8.58	0.0248	10.60	0.0237
Angular gyrus (R)	39	7.55	0.0223	6.81	0.0389
Retrosplenial cingulate cortex (R)	29	5.95	0.0325	4.93	0.0468
Primary Visual Cortex (R)					
Primary visual cortex (L)	17	15.46	0.0075	**11.51****	0.0033

(1) Rest		**NV**		**mTBI**	
ROI	**BA**	***t*-v**	***p*-v FDR**	***t*-v**	***p*-v FDR**
Primary Visual Cortex (L)					
Primary visual cortex (R)	17	15.46	0.0075	**11.51****	0.0033
(2) Stress		**NC**		**mTBI**	
ROI	BA	*t*-v	*p*-v FDR	*t*-v	*p*-v FDR
Dorsolateral Prefrontal Cortex(R)					
Dorsal frontal cortex (R)	8	8.34	0.0281	**6.85***	0.0347
Dorsolateral prefrontal cortex (L)	46	7.97	0.0482	**5.08***	0.0481
Anterior prefrontal cortex (R)	10	5.40	0.0481	5.83	0.0446
Dorsal frontal cortex (L)	8	5.01	0.0481	6.13	0.0446
Hippocampus (R)					
Hippocampus (L)	37	10.43	0.0343	**7.18***	0.0454
Perirhinal cortex (R)	35	9.52	0.0487	**8.53** *	0.0412
Hippocampus (L)					
Hippocampus (R)	37	10.43	0.0343	**8.43***	0.0343
Parahippocampal cortex(R)	36	6.91	0.0487	5.91	0.0487
Perirhinal cortex (L)	35	4.90	0.0487	4.67	0.0487
Middle temporal gyrus (R)	21	4.79	0.0487	4.09	0.0481
Dorsal posterior cingulate cortex (R)	31	4.24	0.0487	3.24	0.0487
Precuneus (R)					
Precuneus (L)	7	9.95	0.0478	**7.42***	0.0395
Precuneus (L)					
Precuneus (R)	7	9.95	0.0478	**7.82***	0.0395
Primary Visual Cortex(R)					
Primary visual cortex (L)	17	17.77	0.0003	**15.52***	0.0035
Primary Visual Cortex (L)					
Primary visual cortex (R)	17	17.77	0.0003	**15.52***	0.0035
(3) Recovery		**NC**		**mTBI**	
ROI	BA	*t*-v	*p*-v FDR	*t*-v	*p*-v FDR
Dorsolateral Prefrontal Cortex(R)					

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(1) Rest		NV		mTBI	
ROI	**BA**	***t*-v**	***p*-v FDR**	***t*-v**	***p*-v FDR**
Dorsolateral prefrontal cortex (L)	46	16.97	0.0278	**6.08****	0.0331
Dorsal frontal cortex (R)	8	6.58	0.0482	**4.21***	0.0405
Hippocampus(R)					
Hippocampus (L)	37	11.43	0.0313	**8.18***	0.0421
Perirhinal cortex (R)	35	13.33	0.0161	**9.20****	0.0136
Hippocampus(L)					
Hippocampus (R)	37	8.18	0.0421	**6.24***	0.0365
Perirhinal cortex (L)	35	12.96	0.0094	**10.96***	0.0094
Parahippocampal cortex(R)	36	12.79	0.0094	**9.79***	0.0096
Precuneus (R)					
Precuneus (L)	7	15.83	0.0316	**13.18***	0.0105
Dorsal posterior cingulate cortex (L)	31	7.04	0.0366	**9.31***	0.0097
Angular gyrus (R)	39	6.46	0.0366	7.48	0.0105
Associative visual cortex (L)	19	5.73	0.0444	7.55	0.0110
Precuneus (L)					
Precuneus (R)	7	15.83	0.0316	14.75	0.0163
Associative visual cortex (L)	19	5.58	0.0444	**11.40***	0.0097
Primary Visual Cortex(R)					
Primary visual cortex (L)	17	18.39	0.0374	**14.51****	0.0064
Secondary visual cortex (R)	18	12.27	0.0474	**7.39****	0.0085
Dorsal posterior cingulate cortex (R)	31	7.03	0.0474	8.01	0.0136
Secondary visual cortex (L)	18	9.60	0.0479	7.39	0.0083
Primary Visual Cortex (L)					
Primary visual cortex (R)	17	18.39	0.0374	**14.51****	0.0064
Secondary visual cortex (L)	18	12.93	0.0085	**10.93***	0.0854
Secondary visual cortex(R)	18	11.46	0.0096	10.76	0.0196

Statistically significant differences (**p < 0.05*** & **p<0.01*****) in magnitude of connectivity between NV and mTBI subjects are identified in **BOLD.** t-v and p-v stand for t-value and p-value.