Title

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Journal

Journal of neurotrauma, 34(8)

ISSN

0897-7151

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Publication Date

2017-04-01

DOI

10.1089/neu.2016.4752

Peer reviewed

JOURNAL OF NEUROTRAUMA 34:1546–1557 (April 15, 2017)

DOI: 10.1089/neu.2016.4752

Resting-State Functional Connectivity Alterations Associated with Six-Month Outcomes in Mild Traumatic Brain Injury

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Abstract

Brain lesions are subtle or absent in most patients with mild traumatic brain injury (mTBI) and the standard clinical criteria are not reliable for predicting long-term outcome. This study investigates resting-state functional MRI (rsfMRI) to assess semiacute alterations in brain connectivity and its relationship with outcome measures assessed 6 months after injury. Seventy-five mTBI patients were recruited as part of the prospective multicenter Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) pilot study and compared with matched 47 healthy subjects. Patients were classified following radiological criteria: CT/MRI positive, evidence of lesions; CT/MRI negative, without evidence of brain lesions. rsfMRI data were acquired and then processed using probabilistic independent component analysis. We compared the functional connectivity of the resting-state networks (RSNs) between patients and controls, as well as group differences in the interactions between RSNs, and related both to cognitive and behavioral performance at 6 months post-injury. Alterations were found in the spatial maps of the RSNs between mTBI patients and healthy controls in networks involved in behavioral and cognition processes. These alterations were predictive of mTBI patients' outcomes at 6 months post-injury. Moreover, different patterns of reduced network interactions were found between the CT/MRI positive and CT/MRI negative patients and the control group. These rsfMRI results demonstrate that even mTBI patients not showing brain lesions on conventional CT/MRI scans can have alterations of functional connectivity at the semiacute stage that help explain their outcomes. These results suggest rsfMRI as a sensitive biomarker both for early diagnosis and for prediction of the cognitive and behavioral performance of these patients.

Keywords: cognitive and behavioral outcome; rsfMRI; TBI

Introduction

Symptoms after mild traumatic brain injury (mTBI) may be somatic, cognitive, or psychiatric, and although it is often assumed that there will be total recovery within the first 3 months after an episode of mTBI, in some patients symptoms may be persistent and may result in lifelong disability.[1] The diagnosis and prognosis of mTBI continue to be a challenge, and misdiagnosis is common.[2,3] Symptomatology and clinical neuroimaging are not sufficiently sensitive to allow the detection of subtle brain changes that occur after mTBI. These changes may be the cause of persistent postconcussive symptoms and cognitive/behavioral impairments. Therefore, it is extremely important to find biomarkers capable of diagnosing changes in the brain that occur after mTBI, permitting the identification of patients who will require specific short- and long-term therapeutic interventions.

Through the analysis of temporal correlations of the blood-oxygenation-level- dependent (BOLD) signal in different gray matter regions, resting-state functional MRI (rsfMRI) allows the noninvasive study of brain networks and their interactions. The

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main resting-state networks (RSNs) are well characterized,[4] and maps of functional connectivity within RSNs have been shown to match patterns of task-related increases in brain activity associated with a variety of cognitive and behavioral domains.[5] Abnormal functional connectivity has been reported in mTBI patients in the default mode network (DMN),[6–10] which consists of a set of the brain regions that remain active while the brain is at rest, and that deactivate when there are external behavioral demands.[11] Although the default mode network is the most widely studied RSN, other networks and regions, such as the thalamic network,[12,13] or the frontoparietal and motor-striatal networks,[14] have also been reported as being disrupted after mTBI. Stevens and coworkers[15] also found abnormal increases and decreases in the connectivity of numerous networks in addition to the DMN. Although most studies have focused on the status of specific networks in isolation, it is important also to address how RSNs interact with one another to give efficient responses to environmental stimuli.

To date, rsfMRI studies of mTBI have been limited by factors such as small sample size, wide spectrum of injury severity, large variation in time point after injury ranging from acute to chronic, and variability in the clinical criteria used for evaluating patients.[16] There have also been relatively few data published on the correlation of early rsfMRI changes with long-term outcome in mTBI. In this study, we hypothesize that altered functional connectivity within and between RSNs at the semi-acute stage, $\sim$1–2 weeks after mTBI, will be related to postconcussive symptoms and to cognitive deficits 6 months after injury.

Methods

Participants

A sample of 75 mTBI patients recruited at San Francisco General Hospital (SFGH) as part of the prospective multi-center Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) pilot study[17] was included in this investigation. The detailed characteristics of this sample have been described in detail elsewhere.[16] The inclusion criteria included CT scan to assess for evidence of acute TBI within 24 h of injury, Glasgow Coma Scale (GCS) score 13–15 (upon emergency department [ED] arrival), loss of consciousness (LOC) <30 min, post-traumatic amnesia (PTA) duration <24 h, and age 18–55 years (inclusive). Exclusion criteria were: lack of fluency in English, contraindication for MRI, and a reported history of previous TBI resulting in LOC >5 min. Four of the subjects were excluded because of artifacts in the rsfMRI data.

CT was performed within 2–3 h of TBI. MRI was performed within 11.2 ± 3.3 days (range, 5–18) post-injury. A 7 min. rsfMRI single shot gradient-echo echo planar imaging (EPI) sequence was acquired (repetition time [TR] = 2000 ms, echo time [TE] = 28 ms; flip angle = 90 grad; field of view [FOV] = 220 mm; voxel size = 3.4 × 3.4 × 4.0 mm). The subjects were asked to close their eyes, relax, not focus their attention on anything specific, and not fall asleep. All CT examinations were performed on a GE Lightspeed 64-row-detector CT scanner, and all MRIs were performed on the same 3T GE Signa EXCITE scanner equipped with an eight channel phased array head radiofrequency coil (GE Healthcare, Waukesha, WI), using the same scanner software version. The following conventional 3T MRI sequences were performed: 1) axial three-dimensional (3D) inversion recovery fast spoiled gradient recalled echo T1-weighted images (TE = 1.5 ms; TR = 6.3 ms; inversion time [TI] = 400 ms; flip angle, 15 degrees) with 230 mm FOV, 156 contiguous partitions (1.0 mm) at 256 × 256 matrix; 2) axial T2-weighted fluid-attenuated inversion recovery images (TE = 126 ms; TR = 10 sec; TI = 2200 ms) with 220 mm FOV, 47–48 contiguous slices (3.0 mm) at 256 × 256 matrix; and 3) axial magnetization-prepared gradient echo T2*- weighted images (TE = 15 ms; TR = 500 ms; flip angle 20 degrees) with 220 × 170 mm FOV and 47–48 contiguous slices (3.0 mm) at 256 × 192 matrix. The MRI scanner and the scanning protocol used were the same for the group of patients and for the healthy control group.

Each patient's head CT upon ED presentation and semiacute brain MRI were characterized using the TBI common data elements (TBI-CDE).[17] Each CT and MRI was anonymized and reviewed by a board certified neuroradiologist blinded to the data. The mTBI patients were divided into two subgroups: 1) CT/MRI positive ($n = 31$; age: $\bar{x} = 34 \pm 12.2$ years), defined as patients with any acute traumatic intracranial lesion (epidural hematoma [EDH], subdural

Table 1. MRI Radiological Findings of the CT/MRI Positive TBI Group

1	2 microhemorrhages (R fr opercular)
2	5–7 microhemorrhages (L sup fr gyr, L sup parietal, L splenium, L genu, R PLIC); small R/L SDH
3	2 microhemorrhages
4	1 microhemorrhage (L temp)
5	5–7 microhemorrhages (L/Rmid-inf temp gyr, R inf fr gyr, genu); contusions (R middle fr gyr, R parietal) R/L fr EDH. SDH; R fr-parietal skull fractures
6	Contusions (L sup fr gyr, R sup fr gyr, R/L ant orbit, R inf temp gyr, L med temp; SDHs
7	2 microhemorrhages (L sup fr gyr, L cing)
8	2 microhemorrhages (R rostrum/genu, L sup parietal); L fr-temp EDH; contusions (R fusiform gyr, R mid temp gyr, R inf temp gyr); SDH.
9	1 microhemorrhage (L cingulum single focus); L med orb gyr encephalomalacia
10	3 microhemorrhages (R genu, R frontal horn, R subinsular WM)
11	1 microhemorrhage (R fr hem shear); contusions (R med orb, L inf fr gyr, R inf temp gyr, L ant temp gyr)
12	Contusions (R mid and inf temp); SDH
13	3 microhemorrhages (R mid fr gyr, L postcentral gyr, L parietal); contusion (R sup temp gyr); SDH
14	1 microhemorrhage (R frontal shear)
15	2 microhemorrhages L fr subcortical white matter
16	2 microhemorrhages (R post limb of internal capsule)
17	1 microhemorrhage (post L temp WM)
18	2 microhemorrhages (R periventricular); contusion (R medial orbital)
19	2 microhemorrhages (L & R CGH); contusions (L sup, mid, inf temp gyr, L fr opercular)
20	2 microhemorrhages (L CGH, L sup fr gyr)
21	3 microhemorrhages (R genu, L sup fr gyr)
22	2 microhemorrhages (L post temp, R postcentral gyr); contusions (L mid- inf fr gyr, L sup- mid temp gyr)
23	3 microhemorrhages (B ant temp and R occ WM); contusions (R frontal, B occ contusions)
24	2 microhemorrhages (R CGH, L post temp WM)
25	2 microhemorrhages (L precentral gyr, L sup fr gyr)
26	4 microhemorrhages (L sup fr gyr, R fr operculum)
27	2 microhemorrhages (L ant and post temp WM)
28	1 microhemorrhage (R ant temp)
29	2 microhemorrhages (L sup parietal lobule)
30	2 microhemorrhages (L and R ant temp WM)
31	Small L SDH

TBI, traumatic brain injury; PLIC, posterior limb of the internal capsule; CGH, cingulum hippocampal gyrus; SDH, subdural hematoma; L, left; R, right; B, bilateral, EDH, epidural hematoma; WM, white matter; sup, superior; mid, middle; inf, inferior; ant, anterior; post, posterior; fr, frontal; temp, temporal; occ, occipital; gyr, gyrus; orb, orbital.

hematoma [SDH], subarachnoid hemorrhage [SAH], contusion, or evidence of traumatic axonal injury[TAI]) and/or depressed skull fracture on either CT or MRI, and 2) CT/MRI negative ($n=44$; age: $\bar{x}=31\pm9.5$ years), defined as patients without any such abnormality on either CT or MRI.[14] The radiological MRI findings of the CT/MRI positive TBI group are displayed in Table 1. The age group comparisons between mTBI groups were not statistically significant ($n=75$; $\bar{x}=32.36\pm10.7$ years, $p=0.28$). The shapes of the age distributions of the two groups were also not statistically significant, as measured by Kolmogórov–Smirnov test (K-S) ($p=0.60$). The patients' GCS scores ranged from 13 to 15 (mTBI positive GCS [15/14/13]=19/11/1; mTBI negative GCS [15/14/13]=36/7/1).

The outcome measures included the Extended Glasgow Outcome Scale (GOS-E) at 6 months post-injury performed through structured interviews with each participant by research assistants trained to uniformly assess the GOS-E. A trained neuropsychologist administered the following behavioral and cognitive tests to the mTBI patients 6 months after injury: the Rivermead Postconcussion Symptoms Questionnaire (RPQ) consisting of 16 physical and psychosocial symptoms frequently reported after mTBI, the California Verbal Learning Test–Second Edition (CVLT-II) to evaluate learning, short and long-term memory, and the Trail Making Tests (TMT) A and B to evaluate attention, processing speed, and cognitive flexibility to switch tasks as well as executive function. No cognitive testing data were available for the control group.

The control group consisted of 47 healthy subjects matched with the patients group by age ($\bar{x}=28.8\pm9$ years; ANOVA: $F=2.5$ $p=0.08$) and education (ANOVA: $F=2.5$ $p=0.08$) without previous diagnosis of TBI, or neurological or psychiatric disorders. The shape of the age distributions measured by K-S was not significant between the control group and either the mTBI CT/MRI positive or the mTBI CT/MRI negative group ($p=0.30$ and $p=0.40$, respectively).

Statistical analysis

The summary of the imaging data preprocessing and analysis is shown in Figure 1.

Resting-state fMRI data were first preprocessed and then analyzed using probabilistic independent component analysis (ICA), implemented in MELODIC, followed by a network-based approach with FSLNets, a toolbox for performing basic network modelling from fMRI time series data. All procedures are part of FSL (http://fsl.fmrib.ox.ac.uk/fsl).

First we performed standard preprocessing of resting-state fMRI data, which included brain extraction,[18] motion correction,[19] and spatial smoothing using a Gaussian kernel with a full-width at half maximum (FWHM) of 6 mm and high-pass temporal filtering with a 100 sec cutoff. Functional scans were then registered to each subject's high-resolution MPRAGE scan using affine linear registration (FMRIB's Linear Image Registration Tool [FLIRT]) and further registered to the common Montreal Neurological Institute (MNI) standard space using linear affine registration with 12 degrees of freedom.

We then used ICA-based Xnoiseifier artifact removal (FIX) to de-noise single subject data.[20] For this, we performed a single-session ICA and the resulting components were introduced into FIX, which identified the ''bad'' components and removed them from the individual preprocessed fMRI timeseries. Fifteen subjects were selected to create a training data set to classify the ICA components into ''good'' or ''bad.'' Then, in order to obtain the study group maps, a group-level ICA was performed in the new ''clean'' data using temporal concatenation of fMRI data from all the subjects, and restricted to 25 independent components (ICs).

A dual regression approach[21] was then used to find between-group differences in the connectivity maps for each component. The group-ICA maps were first regressed against each individual

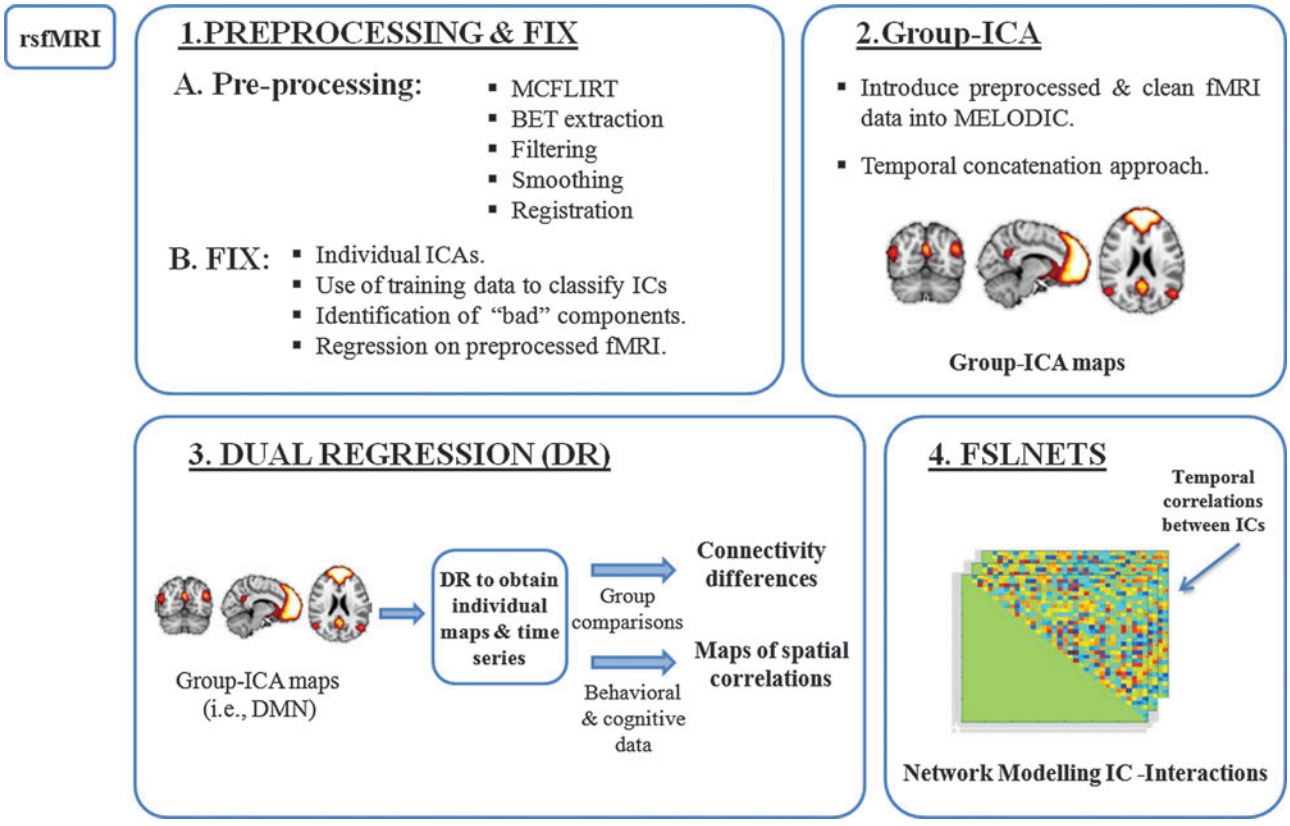

FIG. 1. Imaging data preprocessing and analysis summary. Color image is available online at www.liebertpub.com/neu

preprocessed fMRI series (spatial regression) to obtain sets of time series that are specific to each subject and each IC. At a second stage, these time series were regressed again to the individual fMRI data (temporal regression) to obtain IC Z-maps specific for each subject and each component. Finally, these individual maps were compared between subjects using a voxelwise general linear model (GLM) analysis with permutation testing to correct for multiple comparisons[22] using threshold-free cluster enhancement (TFCE) family-wise error corrected (FWE) corrected at $p \leq 0.05$. Further, to assess resting-state network interactions, whole brain

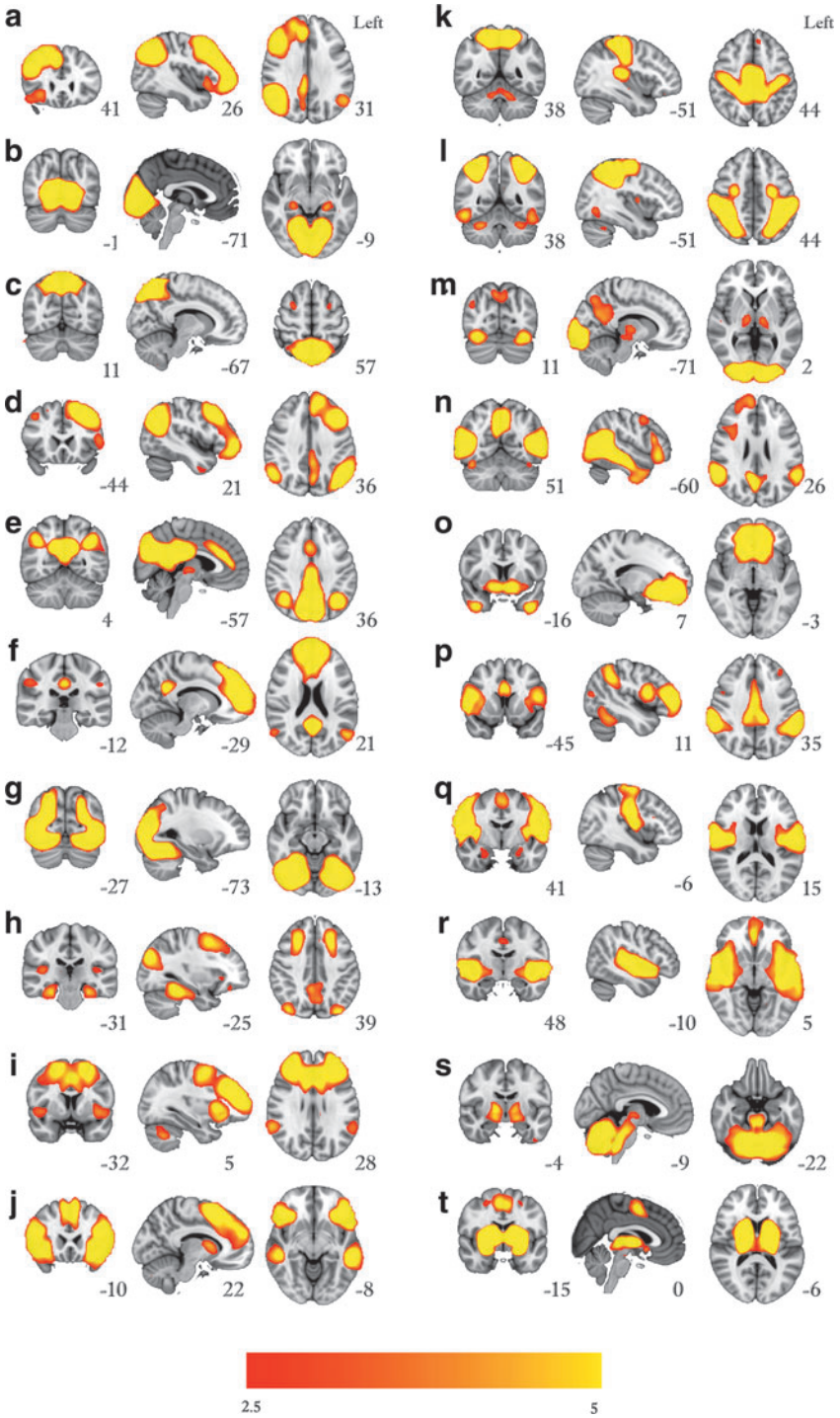

FIG. 2. Resting-state networks (RSNs) from the independent component analysis (ICA) group decomposition. **(a)** Frontoparietal right network; **(b)** primary visual network; **(c)** superior parietal network; **(d)** frontoparietal left; **(e)** default mode network (DMN) posterior part; **(f)** DMN; **(g)** occipito-cerebellar network; **(h)** ventral attentional network; **(i)** executive control network; **(j)** salience network; **(k)** upper somatomotor network; **(l)** dorsal attentional network; **(m)** visual network; **(n)** dorsal and ventral visual stream; **(o)** orbitofrontal network; **(p)** cingular opercular network; **(q)** lower somatomotor network; **(r)** auditory network**; (s)** brainstem and cerebellum network; **(t)** basal ganglia. Color image is available online at www.liebertpub.com/neu

connectivity matrices were created from the individual network time series with FSLNETs[23] (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). In these matrices, nodes were defined from the group ICA maps. Time series were then obtained by the spatial regression of these maps to the preprocessed 4D data sets (i.e., first stage of the dual regression procedure). Because of the nature of ICA maps, each node would be a map covering the whole brain with the strongest weight of the regions showing higher Z-scores in that specific map. After defining the nodes and their associated time series, each edge represents the connectivity between pairs of nodes, computed using full correlation. These matrices were then analyzed in a group-level approach, and GLM was used to find group differences and correlations with cognitive and clinical outcomes. Results of the RSNs interactions were corrected for multiple comparisons using the false discovery rate (FDR).

Results

Cognitive and behavioral data

No significant differences were found in any of the cognitive and behavioral data between the CT/MRI mTBI positive and CT/MRI mTBI negative groups.[16]

RSN spatial maps

We identified 20 RSNs from the group ICA decomposition (Fig. 2) by visual inspection and using templates available in the literature.[4] The detailed description of the networks is described in Table S1 (see online supplementary material at http://www.liebertpub.com).

We performed four group RSN spatial maps comparisons analysis: 1) mTBI CT/MRI positive and negative ($n=75$) versus healthy control group; 2) CT/MRI mTBI positive ($n=31$) versus healthy control group; 3) CT/MRI mTBI negative ($n=44$) versus healthy controls; and 4) mTBI CT/MRI positive group ($n=31$) versus CT/MRI mTBI negative group ($n=44$).

We found significant differences in connectivity within the spatial patterns of the main RSNs for the mTBI patient group as a whole when compared with the control group (Fig. 3). mTBI patients showed reduced connectivity in the frontal nodes of the DMN, executive control network, frontal nodes of the frontoparietal network (FP-right), parietal areas of the dorsal attentional network, and the frontal node of the orbitofrontal network, together with an increase in the connectivity of the visual network.

We also found significant differences for each of the two subgroups of mTBI patients compared with the healthy controls (Fig. 4). mTBI patients with CT/MRI positive scans showed reductions in connectivity in frontal brain areas in the same abovementioned RSNs, whereas the mTBI patients with CT/MRI negative scans showed reduced connectivity in the orbitofrontal network and the DMN and, additionally, demonstrated reductions in the salience network and an increase in the connectivity of the visual network. No significant differences in connectivity were found when comparing the RSN spatial maps between the CT/MRI mTBI positive versus the CT/MRI mTBI negative subgroups.

All results were corrected for multiple comparisons by using FWE correction at $p<0.05$.

Correlations of RSNs with 6months postconcussive symptoms

Negative correlations between semiacute connectivity and RPQ score at 6 months were found within the posterior regions of several networks only in the CT/MRI negative group (Fig. 5).

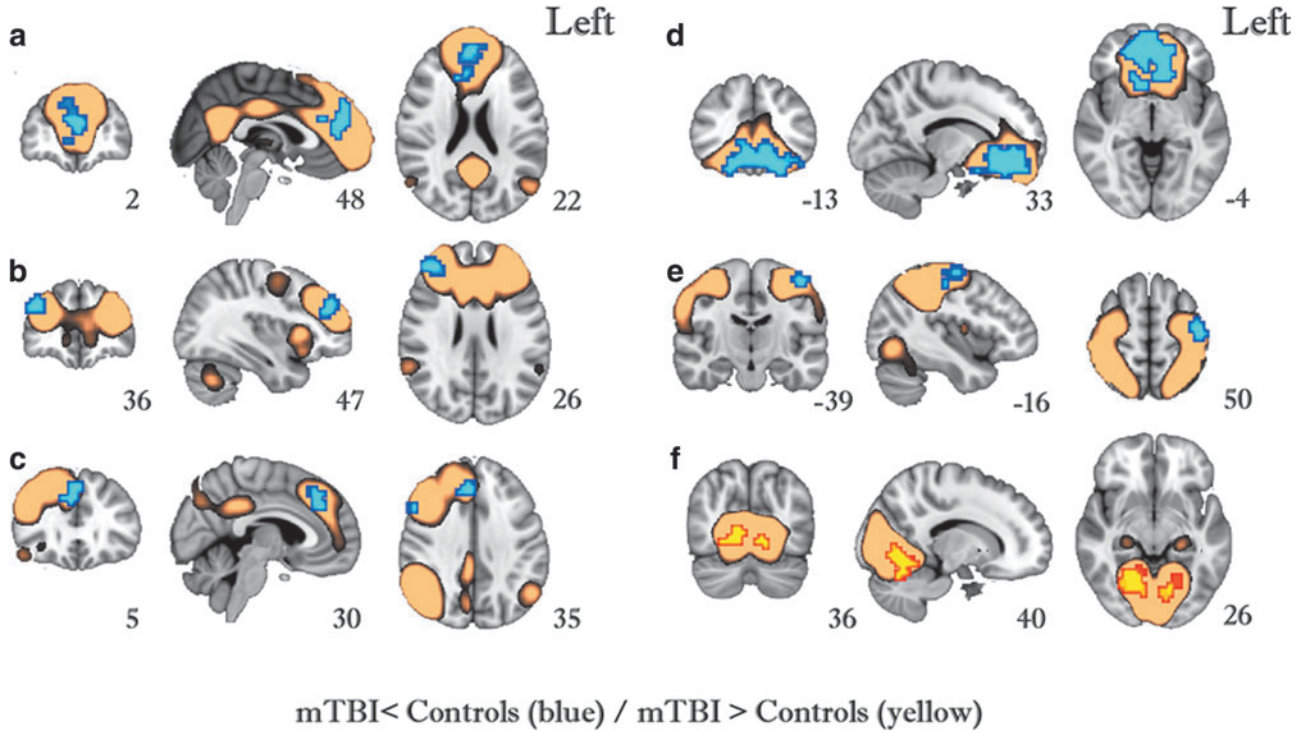

FIG. 3. Resting-state networks' (RSNs') significant differences between the whole sample of mTBI patients and healthy controls. **(a)** Default mode network (DMN); **(b)** executive control network; **(c)** frontoparietal network; **(d)** orbitofrontal network; **(e)** dorsal attentional network; **(f)** visual network. In blue: reductions in connectivity. In red-yellow: increases in connectivity. Color image is available online at www.liebertpub.com/neu

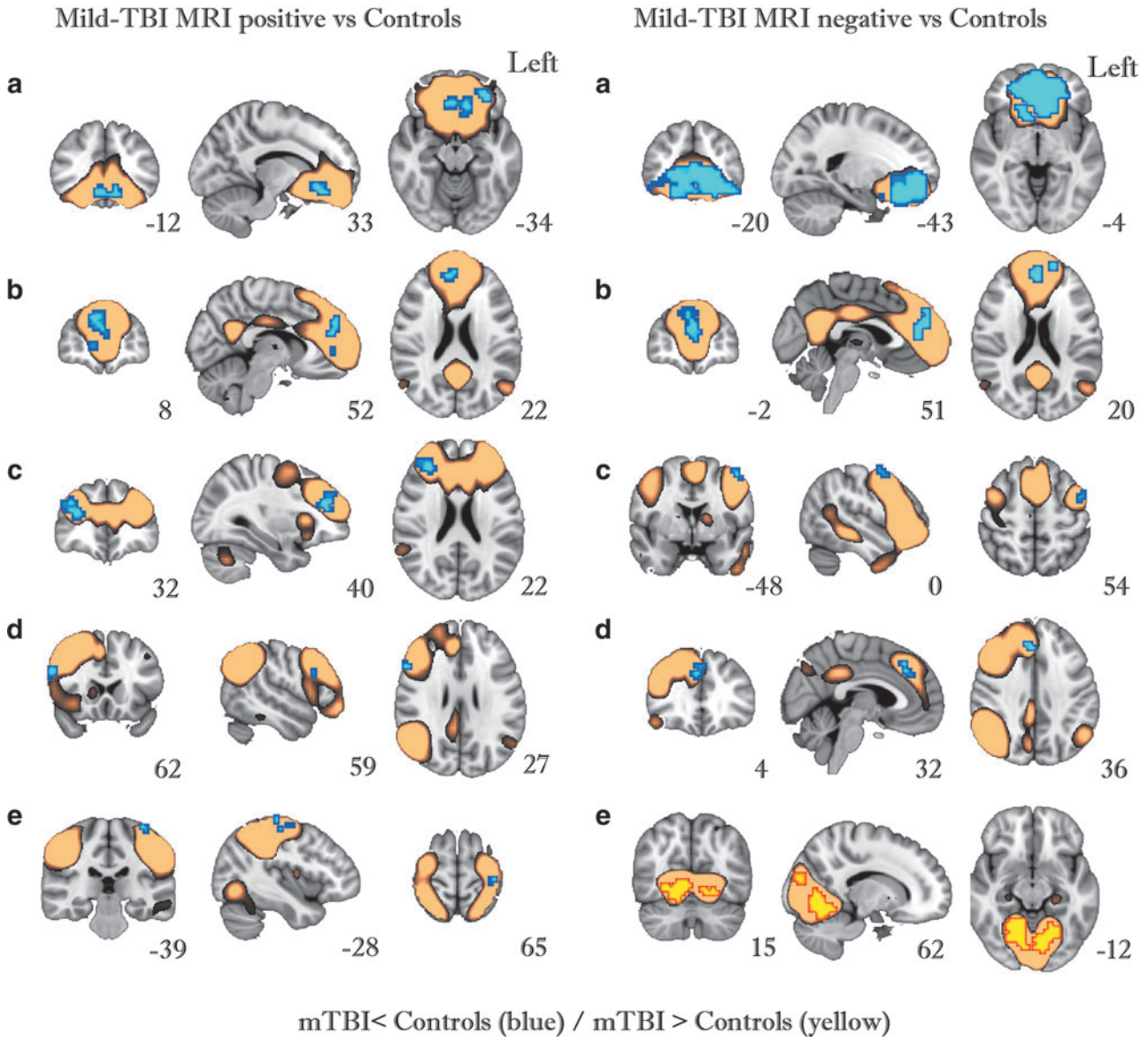

FIG. 4. Group comparisons of CT/MRI positive or negative patients versus controls. Left side: **(a)** orbitofrontal network; **(b)** default mode network (DMN); **(c)** executive control network; **(d)** frontoparietal network; **(e)** dorsal attentional network. Right side: **(a)** orbitofrontal network; **(b)** DMN; **(c)** salience network; **(d)** fronto-parietal network**; (e)** visual network. In blue: reductions in connectivity. In red-yellow: increases in connectivity. Color image is available online at www.liebertpub.com/neu

Patients with decreased connectivity presented more post-concussive symptoms.

Correlations of RSNs with 6 month cognitive performance

TMT. We found positive correlations of semiacute functional connectivity in brain regions corresponding to the DMN, salience network, and dorsal attentional network with TMT A scores at 6 months after injury in mTBI patients with CT/MRI positive scans, but not in those with CT/MRI negative scans (Fig. 6). On the other hand, in the mTBI patient group with CT/MR negative scans, we found that the measure of executive function, TMT B-A (obtained by substracting the time taken to complete the TMT-A and TMT-B), correlated positively with the connectivity corresponding to the orbitofrontal RSN.

California Verbal Learning Test. Increased semiacute connectivity within the occipito-cerebellar RSN was correlated positively with the learning memory scores at 6 months after injury in the CT/MRI negative subgroup.

All results were corrected for multiple comparisons by using FWE correction at $p < 0.05$.

Temporal interactions between RSNs

Group comparisons. After multiple comparisons correction, reduced inter-network functional connectivity in mTBI patients versus controls was found between different pairs of RSNs (Fig. 7).

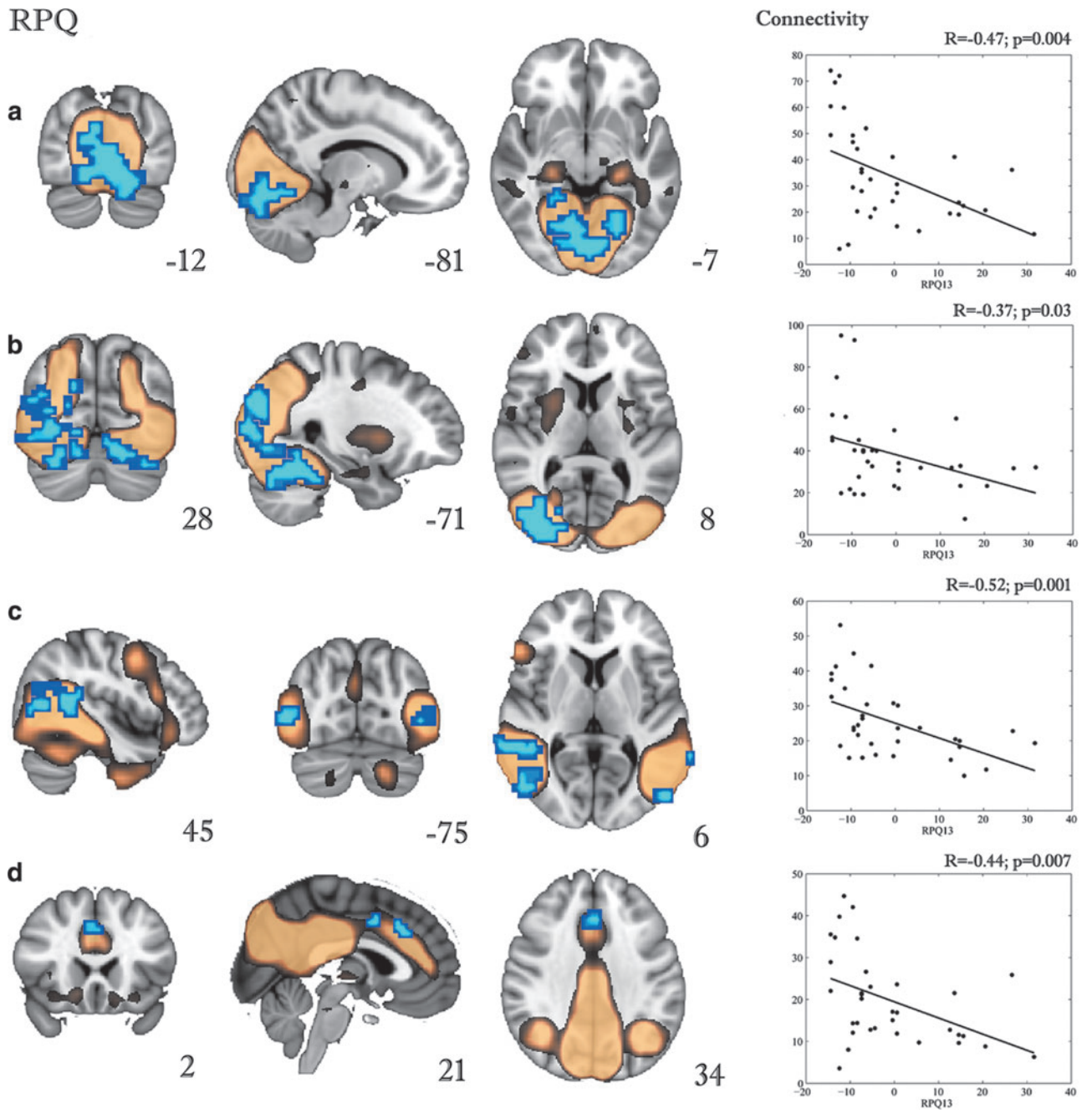

FIG. 5. Rivermead Post Concussion Questionnaire versus functional connectivity in the CT/MRI negative mTBI subgroup. **(a)** Visual network; **(b)** occipito-cerebellar network; **(c)** dorsal visual stream; **(d)** posterior default mode network. In blue: negative correlations with the behavioral test. Scatter plots show individual mean values for connectivity within the significant areas, in relation to the demeaned results of the behavioral test. Color image is available online at www.liebertpub.com/neu

Reduced network connectivity interactions were found in the CT/MRI positive subgroup of mTBI patients versus controls between two pairs of networks: 1) the auditory network with the ventral attentional network ($p = 0.04$), and 2) basal ganglia network with dorsal attentional network ($p = 0.016$).

We also found reduced network connectivity interactions in the CT/MRI negative subgroup of mTBI patients versus controls in one pair of networks: the visual network with the dorsal and ventral visual stream network ($p = 0.04$).

No differences were found when comparison was made between mTBI subgroups.

Correlations with cognitive measures. Correlations were found only in the CT/MRI negative subgroup of mTBI patients with the TMTB-A. This measure of executive function was found to correlate negatively with one pair of networks: the basal ganglia network with the orbitofrontal network ($p = 0.04$).

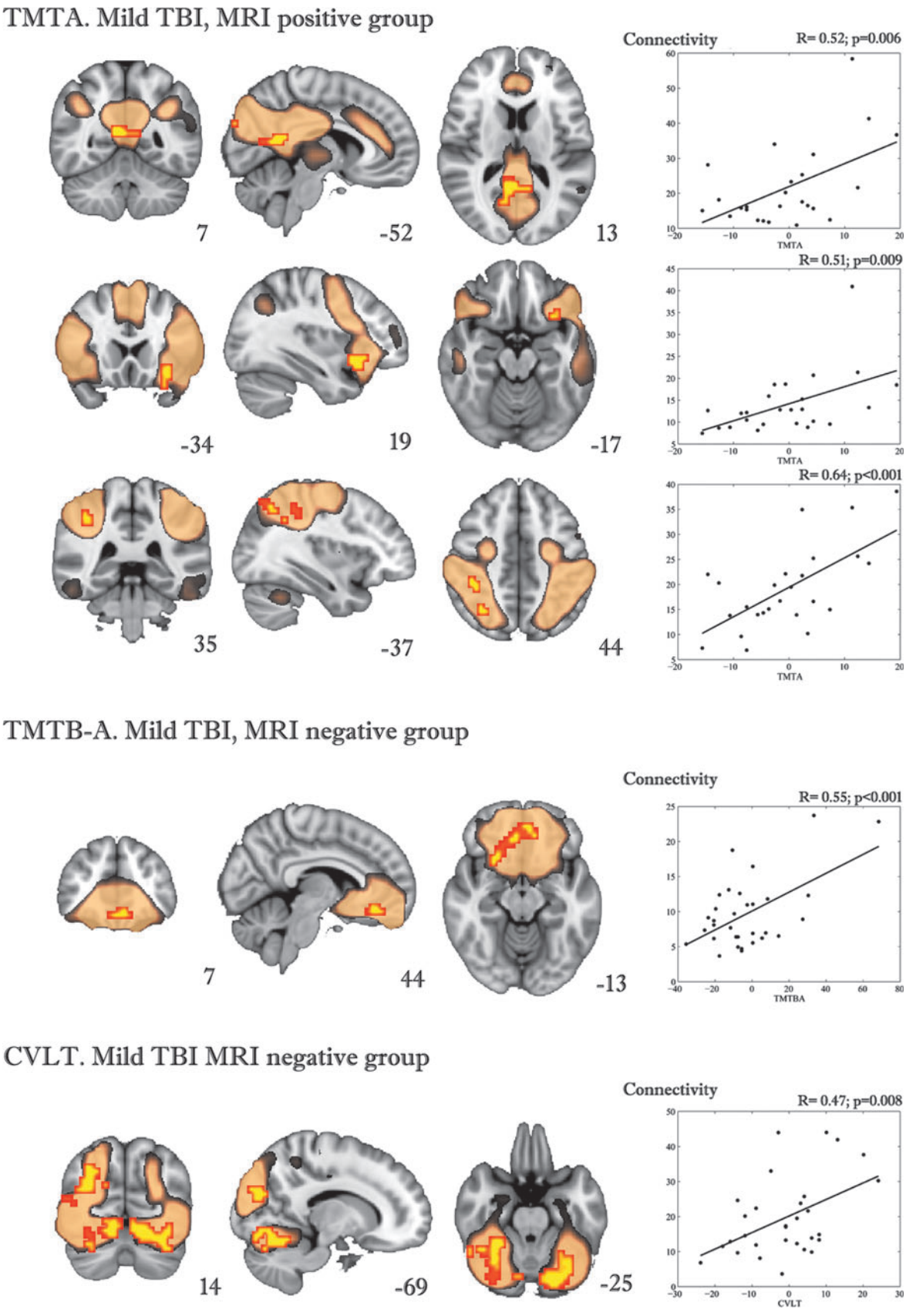

FIG. 6. Increases in connectivity related to cognitive measures in the mild traumatic brain injury (mTBI), MRI negative group. Trail Making Test A (TMT-A), CT/MRI positive mTBI: **(a)** default mode network (DMN); **(b)** salience network; **(c)** dorsal attention network. TMTB-A, CT/MRI negative mTBI: orbitofrontal network. California Verbal Learning Test–(CVLT), CT/MRI negative mTBI: occipito-cerebellar network. In red-yellow: positive correlations with the cognitive test. Scatter plots show individual mean values for connectivity within the significant areas in relation to the de-meaned results of the cognitive tests. Color image is available online at www.liebertpub.com/neu

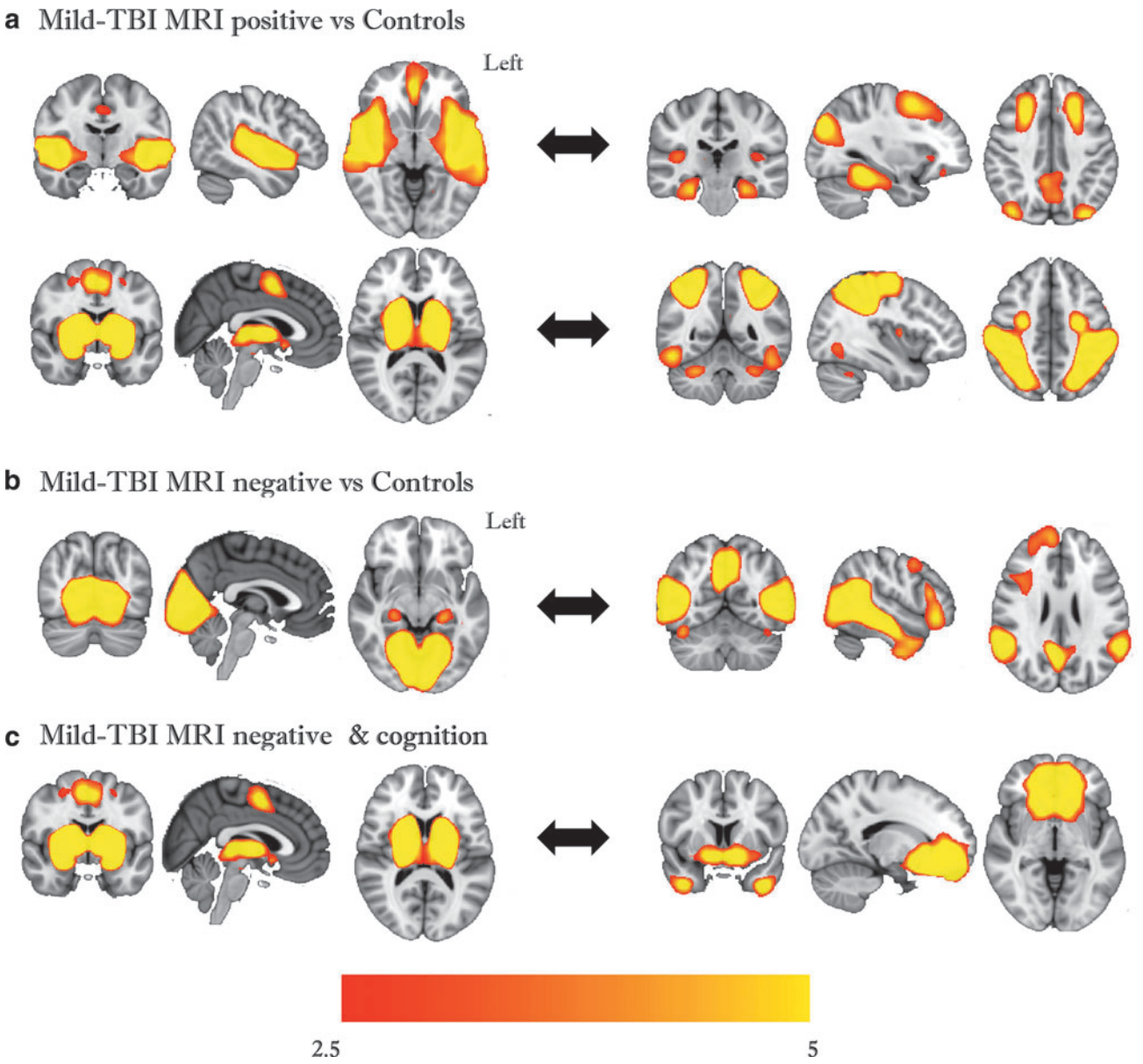

FIG. 7. Temporal interactions between resting-state networks (RSNs). **(a,b)** Pairs of networks with reduced functional connectivity in mild traumatic brain injury (mTBI) patients positive/negative versus controls. **(c)** Negative correlation between a pair of networks and the executive function measure. Color image is available online at www.liebertpub.com/neu

Discussion

To our knowledge, this is the first study to relate alterations in early resting-state functional connectivity to long-term postconcussive symptoms and cognitive outcome in a large and clinically well-defined mTBI sample. The main findings of this study are that: 1) patients with mTBI in the semiacute stage, with CT/MRI scans either positive or negative, have alterations in the connectivity of the most representative RSNs that are associated with cognitive performance at 6 months after injury; 2) patients with CT/MRI negative scans show reduced RSN connectivity that predicts postconcussive symptoms; and 3) each subgroup of mTBI patients presents a different pattern of network interaction alterations.

Previous resting-state studies have discovered both reductions and increases in connectivity in some networks after mTBI. In agreement with our study, decreases in DMN functional connectivity have been found in mTBI and postconcussive patients.[6,8] In addition, some authors have reported increases in this network in the rostral anterior cingulate and ventrolateral prefrontal cortex.[6]

Zhou and coworkers[7] showed reduced connectivity in the posterior cingulate and parietal cortex together with an increase in the connectivity in the medial prefrontal cortex. The increased connectivity of the posterior and anterior brain nodes of the DMN correlated positively with neurocognitive dysfunction. In our study, when the whole group of mTBI patients was taken together, we found reductions in connectivity mainly in the frontal areas of the DMN, the orbitofrontal network, the frontoparietal networks bilaterally, and the left parietal dorsal attentional network. Only the visual network was found to have increased connectivity. Alterations in these resting-state networks might interfere with several

cognitive functions, as they have been described as being involved in processes such as internal focus of attention, social cognition, inhibition, memory, divided attention, emotion, and language.[5]

Similarly, Stevens and coworkers[15] also assessed alteration in resting-state connectivity in mTBI patients with negative MRI scans. They studied the DMN, cognitive control networks, motor networks, and visual processing networks, finding increases and decreases in resting-state connectivity in all the studied networks. Discrepancies between our study and theirs might be explained in terms of sample sizes and the time interval between injury and performance of scanning in the mTBI patients. Our patients were scanned within 3–18 days of their injury, whereas Stevens and coworkers scanned their patients 13–136 days from injury. The longer window between injury and scan could account for the differences in the studies, as the patients were at different stages in their biological recovery from injury. Moreover, comparison of their results with ours was confounded not only by the sample differences, but also by differences in the method of analysis. Although both studies used ICA to define the networks, we restricted our analysis to within the masks of the networks, whereas Stevens and coworkers examined the connectivity differences in brain areas out of the network masks or across the whole brain.

Shumskaya and coworkers[14] studied functional connectivity at rest in the most common resting-state networks reported in the literature using ICA. The patients examined were a homogeneous sample of MRI positive mTBI patients with fronto-occipital impact injuries scanned in subacute stage. They found reductions in connectivity in the motor-striatal network and the frontoparietal network. Although their sample of patients showed deficits in some behavioral and cognitive areas compared with the control group, they did not find specific correlations with the behavioral and cognitive scores and functional connectivity within the affected RSNs.

When we compared each of the mTBI groups separately (CT/MRI positive vs. negative scans) to the healthy control group, with a few exceptions, the connectivity reductions found corresponded to the same networks that emerged from the whole-group comparison analysis. One exception is that the reductions in the orbitofrontal network for the mTBI negative MRI group were greater in extent than those for the mTBI MRI positive group. In contrast, the increased connectivity found when the visual network in the whole mTBI group was compared with the control group was solely attributed to the mTBI negative group.

With regard to the behavioral measures, we found a relationship between postconcussive symptoms at 6 months measured using the RPQ, and reductions in the connectivity of several resting-state networks in mTBI patients with MRI negative scans. The pattern of decreased connectivity that was predominantly correlated with the symptomatology was observed in posterior brain regions involving parieto-occipital areas, with the exception of a reduction in the anterior cingulate. It is particularly interesting that we found that a reduction in the visual network connectivity was correlated with behavioral symptomatology, whereas this network showed increased connectivity in the group comparisons performed between the mTBI negative group and the control group. This increased connectivity might be interpreted as compensation for injury, although we cannot tell to what extent this increase results in more efficient brain functioning, as behavioral symptoms remained present 6 months after injury. We did not find results in the network connectivity of the CT/MRI positive group of patients associated with behavioral symptoms. We suspect that this may be because of the difference in sample size we have between the mTBI MRI positive and negative groups, but it may also be a result of the heterogeneous distribution of the focal brain lesions of the mTBI CT/MRI positive group.

In addition to behavioral symptoms, we found associations among attention, executive and memory performance, and the connectivity of some networks of the two mTBI groups. In the mTBI CT/MRI positive group of patients, performance in attention and processing speed were found to be related to increases in connectivity in the DMN, the salience network, and the dorsal attentional network. Interestingly, the DMN has been shown to correlate negatively with the salience network and the dorsal attention network in healthy subjects,[24] and the DMN is associated with successful attentional response in TBI patients with different levels of severity.[25,26] Our results involving the DMN in the cognitive tasks could be also understood within the recently explored idea stating that the DMN is not only a ''task negative'' network that deactivates during goal-directed tasks, but also an active network contributing to task performance.[27–29] For example, in recent articles by Vatansever and coworkers, the authors revealed how the DMN actively interacts between various large scale connectivity networks, possibly through global integration of the information, when increasing the environmental demand of a cognitive task such as working memory.[29,30] In the mTBI CT/MRI negative group, increases in connectivity in the orbitofrontal network were related to executive function. This measure involves attention and inhibition, working memory, and mental flexibly. These cognitive skills rely on frontal circuitries, including orbitofrontal connections, especially when inhibition is involved. Increases in connectivity of the occipito-cerebellar network in this last group of patients were also found to be related to good learning performance. Overall, in the absence of control cognitive testing data, we can only presume that these increases in connectivity would favor successful performance as compensation is made for the effect of the reduced connectivity found in networks closely involved in attention and executive functions.

The interaction among resting-state networks is thought to be critical for cognition, suggesting that an imbalance between the connectivity dynamics of different networks can alter cognitive function and behavior.[31] Our study has used a novel approach to explore how brain functional resting-state networks interact. We believe that this increases understanding of how the brain produces complex behaviors. As an example, previous studies using other methodologies have found that the alteration in the network interaction between the DMN and the salience network was associated with cognitive alterations after TBI.[10,32] We found reduced interactions in both CT/MRI positive and negative mTBI groups compared with controls in several pairs of networks. For the CT/MRI mTBI positive group, dorsal and ventral attentional networks were found to have reduced connectivity with the basal ganglia and the auditory network, respectively, when compared with the control group. These alterations in the interactions between the main attentional networks could provide further information as to how patients increase connectivity in the dorsal attentional network when performing an attentional cognitive test. On the other hand, the CT/MRI mTBI negative group followed a different pattern of reduced network interactions involving the dorsal and ventral visual stream and the primary visual network. Further, in this group, the reduced interactions found between the basal-ganglia and orbitofrontal networks were associated with executive performance. The orbitofrontal, basal ganglia, and visual networks in the CT/MRI negative mTBI group of patients seemed to play an important role in behavior and cognitive performance. The orbitofrontal

cortex is the neocortical extension of the limbic system, and the medial division of the orbitofrontal circuit projects to basal ganglia structures and, therefore, is involved in the determination of the appropriate environmentally elicited behavioral responses. Lesions in this area can result in behavioral disinhibition and emotional lability.[33] Moreover, the connectivity of the basal ganglia with the frontal cortex and with posterior visual areas have been reported to have a role in successful attention shifting (van Schouwenburg).[34]

It is of note that in our study we used nonparametric statistics based on permutation testing with a threshold-free cluster enhancement (TFCE) method to correct for multiple comparisons. In the TFCE method, combined with permutation testing using the FSL randomize function, the cluster-level threshold is not defined ''*a priori*'' as criticized in the recent fMRI literature.[35] This TFCE method combined with nonparametric permutation testing is accepted as the recommended approach for cluster-level inference for neuroimaging studies.[36]

Conclusion

In conclusion, despite the questions that remain to be clarified, the overall findings of our study show widespread alterations of functional connectivity within and between resting- state networks in the semiacute phase after mTBI, with significant relationships to long-term symptoms as well as to behavioral and cognitive outcomes. We further demonstrate some conserved and some different patterns of altered functional connectivity in those mTBI patients with focal lesions on CT and/or MRI versus those without such visible lesions. These results support the use of functional connectivity from rsfMRI as an early biomarker for mTBI diagnosis and outcome prediction, specifically for the development of persistent postconcussive syndrome.

Acknowledgments

This study was supported by National Institutes of Health (NIH) grants NS069409 and NS069409-02S1 (principal investigator [PI]: G.T.M.) and NS60776 (PI: P.M.) and Department of Defense United States Army Medical Research Acquisition Activity W81XWH-13-1-0441 (PI: G.T.M.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke (NINDS) or the NIH.

Author Disclosure Statement

No competing financial interests exist

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