Title

MEG Working Memory N-Back Task Reveals Functional Deficits in Combat-Related Mild Traumatic Brain Injury

Journal

Cerebral Cortex, 29(5)

ISSN

1047-3211

Authors

Huang, Ming-Xiong
Nichols, Sharon
Robb-Swan, Ashley
et al.

Publication Date

2019-05-01

DOI

10.1093/cercor/bhy075

Peer reviewed

Cerebral Cortex, May 2019;29: 1953–1968

doi: 10.1093/cercor/bhy075
Advance Access Publication Date: 13 April 2018
Original Article

ORIGINAL ARTICLE

MEG Working Memory N-Back Task Reveals Functional Deficits in Combat-Related Mild Traumatic Brain Injury

Ming-Xiong Huang[1,2], Sharon Nichols[3], Ashley Robb-Swan[2], Annemarie Angeles-Quinto[2], Deborah L. Harrington[1,2], Angela Drake[4], Charles W. Huang[5], Tao Song[2], Mithun Diwakar[6], Victoria B. Risbrough[1,7,8], Scott Matthews[9], Royce Clifford[1,7,8], Chung-Kuan Cheng[10], Jeffrey W. Huang[11], Anusha Sinha[12], Kate A. Yurgil[1,8,13], Zhengwei Ji[2], Imanuel Lerman[1], Roland R. Lee[1,2] and Dewleen G. Baker[1,7,8]

[1]Radiology, Research, and Psychiatry Services, VA San Diego Healthcare System, San Diego, CA 92161, USA, [2]Department of Radiology, University of California, San Diego, CA 92093, USA, [3]Department of Neuroscience, University of California, San Diego, CA 92093, USA, [4]Cedar Sinai Medical Group Chronic Pain Program, Beverly Hills, CA 90211, USA, [5]Department of Bioengineering, University of California, San Diego, CA 92093, USA, [6]Department of Radiology and Biomedical Imaging, University of California, San Francisco, CA 94131, USA, [7]Department of Psychiatry, University of California, San Diego, CA 92093, USA, [8]VA Center of Excellence for Stress and Mental Health, San Diego, CA 92161, USA, [9]ASPIRE Center, VASDHS Residential Rehabilitation Treatment Program, San Diego, CA 92110, USA, [10]Department of Computer Science and Engineering, University of California, San Diego, CA 92093, USA, [11]Columbia University, New York, NY 10027, USA, [12]California Institute of Technology, Pasadena, CA 91125, USA and [13]Loyola University New Orleans, LA 70118, USA

Address correspondence to Ming-Xiong Huang, Radiology Imaging Laboratory, University of California, San Diego, 3510 Dunhill Street, San Diego, CA 92121, USA. Email: mxhuang@ucsd.edu

Abstract

Combat-related mild traumatic brain injury (mTBI) is a leading cause of sustained cognitive impairment in military service members and Veterans. However, the mechanism of persistent cognitive deficits including working memory (WM) dysfunction is not fully understood in mTBI. Few studies of WM deficits in mTBI have taken advantage of the temporal and frequency resolution afforded by electromagnetic measurements. Using magnetoencephalography (MEG) and an N-back WM task, we investigated functional abnormalities in combat-related mTBI. Study participants included 25 symptomatic active-duty service members or Veterans with combat-related mTBI and 20 healthy controls with similar combat experiences. MEG source–magnitude images were obtained for alpha (8–12 Hz), beta (15–30 Hz), gamma (30–90 Hz), and low-frequency (1–7 Hz) bands. Compared with healthy combat controls, mTBI participants showed increased MEG signals across frequency bands in frontal pole (FP), ventromedial prefrontal cortex, orbitofrontal cortex (OFC), and anterior dorsolateral prefrontal cortex (dlPFC), but decreased MEG signals in anterior cingulate cortex. Hyperactivations in FP, OFC, and anterior dlPFC were associated with slower reaction times. MEG activations in lateral FP also negatively correlated with performance

on tests of letter sequencing, verbal fluency, and digit symbol coding. The profound hyperactivations from FP suggest that FP is particularly vulnerable to combat-related mTBI.

Key words: blast brain injury, frontal pole, magnetoencephalography, traumatic brain injury, working memory

Introduction

Combat-related traumatic brain injury (TBI), mainly due to blast exposure to improvised explosive devices (IED), is a leading cause of sustained physical, cognitive, emotional, and behavioral deficits in military service members and Veterans. The signature TBI associated with combat has changed from penetrating injuries sustained during the Vietnam war to blast-induced TBI in contemporary warfare. At the same time, new equipment for body protection has increased the survival rate after TBI on the battlefield. Thus, understanding the sequelae of blast TBI is of increasing importance.

Of TBIs in which blast was the main cause in active-duty military personnel and Veterans wounded in combat in Iraq and Afghanistan, the majority (89%) were mild TBIs (mTBI) (MacGregor et al. 2011). However, the pathophysiology of blast mTBI is not completely understood and the long-term effects of mTBI in general are controversial. Identifying and assessing neuropathological, cellular, cognitive, emotional, behavioral, and neurological consequences of blast TBI have been challenging (DePalma and Hoffman 2018). Although blast TBI may be another subtype of TBI, different from blunt TBI (Fischer et al. 2014; Young, Rule, Bocchieri, Walilko, et al. 2015; DePalma and Hoffman 2018), there is consensus that blast TBI has some unique injury mechanisms (Young, Rule, Bocchieri, and Burns 2015).

In the majority of individuals with mTBI symptoms resolve within days postinjury (Bigler 2008). Yet, postconcussive symptoms (PCS) can persist 3 months postinjury or longer, indicating chronic sequelae (McInnes et al. 2017). Estimates of the prevalence of persistent PCS vary widely, particularly in Veterans with mTBI, with at least 3 enduring symptoms reported in 7.5–40% of patients (Schneiderman et al. 2008; Terrio et al. 2009; Morissette et al. 2011; Cooper et al. 2015). Among the persistent PCS in individuals with blast mTBI, the majority of the systems are in the cognitive domain (e.g., executive function, attention, working-memory) (McInnes et al. 2017). Currently, optimal rehabilitation treatments for the cognitive deficits in blast mTBIs are not fully developed, in part due to insufficient information about the loci and mechanisms of the injury. This highlights the need for neuroimaging techniques that are sensitive to the cognitive effects of blast exposure on the brain and to the efficacy of therapeutic interventions aimed at improving functional capacity.

Complaints of cognitive deficits are common in Veterans and active-duty military personnel with mild TBI. While deficits exist in several cognitive domains, many complaints and deficits suggest problems in working memory (WM) (Nyberg et al. 2003; McAllister et al. 2006; Newsome et al. 2008), which disrupts the ability to maintain information in the face of competing stimuli and manipulate it in accord with current goals (Baddeley 1986; Glahn et al. 2005). WM is one of the fundamental mechanisms of executive functioning that mediate complex skills. Like most complex behaviors, WM tasks activate a large-scale network of cortical (frontal, parietal, and temporal) and subcortical (e.g., basal ganglia) regions (Mesulam 1998; Smith and Jonides 1998; Glahn et al. 2005). The frontal lobes, including dorsolateral prefrontal cortex (dlPFC), orbitofrontal cortex (OFC) areas, and medial prefrontal cortex, and related circuitry (e.g., subcortical white matter, basal ganglia, thalamus) support WM and other executive functions that are particularly vulnerable to TBI (Bigler 1999; McDonald et al. 2002; McAllister et al. 2006). Thus, WM and other executive deficits are prominent cognitive sequelae of TBI. To date, the brain mechanisms underlying WM deficits in TBI have been largely studied using functional magnetic resonance imaging (fMRI). Many fMRI studies have compared brain activation during WM tasks in mild, moderate, and severe TBI patients and in healthy subjects (Christodoulou et al. 2001; Scheibel et al. 2003; Chen et al. 2004; Perlstein et al. 2004; McAllister et al. 2006; Newsome, Scheibel, Hunter, et al. 2007; Newsome, Scheibel, Steinberg, et al. 2007; Sanchez-Carrion et al. 2008; Hillary et al. 2010; Cazalis et al. 2011; Kasahara et al. 2011; Turner et al. 2011; Medaglia et al. 2012, 2015; Bryer et al. 2013; Sinopoli et al. 2014; Gillis and Hampstead 2015; Newsome et al. 2015; Wylie et al. 2015; Manktelow et al. 2017). However, the results have been mixed in TBI (Bryer et al. 2013) with most studies reporting hyperactivation in prefrontal cortices, but some studies reporting hypoactivation in prefrontal cortices, or both hyper- and hypoactivation in different prefrontal regions. Although the number of fMRI WM studies showing aberrantly increased PFC activation outnumbers the other 2 types, the reason(s) for the discrepant findings is unclear. Furthermore, WM fMRI studies in combat-related TBI have been rare (Graner et al. 2013; Newsome et al. 2015), and among those, only increased activation in the caudate nucleus has been reported (Newsome et al. 2015), which is surprising since the frontal lobes are particularly vulnerable to injury (Wilde et al. 2005) due to 1) their large size and location at the front of the cranium, and 2) the acceleration and deceleration of the brain as it is shaken backwards and forwards in response to impact, causing the frontal lobe to collide with the inside of the skull cavity (El Sayed et al. 2008; Phillips et al. 2017).

The N-back task is one of the most frequently used WM paradigms (Gevins and Cutillo 1993) to investigate the neural basis of WM processes. Meta-analyses indicate that the WM network consists of 6 reliably activated cortical regions (Owen et al. 2005; Phillips et al. 2017): 1) bilateral rostral prefrontal cortex (rPFC) including frontal pole (FP, BA 10), ventromedial prefrontal cortex vmPFC, and orbitofrontal cortex (OFC, BA 11); 2) bilateral dlPFC (BA 9, 46); and 3) bilateral ventrolateral prefrontal cortex (vlPFC) or frontal operculum (BA 45,47); 4) bilateral medial posterior parietal cortex (PPC), including the precuneus, and the inferior parietal lobules (approximate BA7,40); 5) bilateral premotor cortex (BA 6, 8); and 6) dorsal cingulate/medial premotor cortex, including supplementary motor area (SMA; BA 32, 6). The cerebellum is also consistently activated during WM.

In contrast to the rich WM mTBI literature using fMRI, few studies have used electromagnetic based techniques to assess WM abnormalities in mTBI. Magnetoencephalography (MEG) directly measures the electromagnetic aspects of neuronal activity with excellent temporal resolution (milliseconds) and high spatial resolution (~3 mm at the cortical level, versus centimeters in EEG) (Leahy et al. 1998). The excellent temporal resolution of MEG also enables analysis of signals from different frequency bands separately, rendering it a potentially powerful technique for studying WM deficits in combat-related mTBI. To our knowledge, there have been only a few EEG studies of TBI

Table 1A Demographic characteristics and correct rates during N-back tests in the control and blast mTBI groups

	Control, N = 20		mTBI, N = 25		t-test	MWU-test
	Mean	SD	Mean	SD	P-value	P-value
Age	27.95	3.85	26.76	5.75	0.412	
Years of education	12.55	0.76	13.00	1.89	0.286	
Months postinjury			10.36	7.21		
1-Back correct rate (%)	98.12	1.97	96.81	2.65		0.08
2-Back correct rate (%)	90.45	5.07	88.72	6.20		0.34
3-Back correct rate (%)	80.01	5.23	76.95	6.15		0.09
1-Back reaction time (s)	0.479	0.084	0.630	0.145	<0.001*	
2-Back reaction time (s)	0.629	0.139	0.742	0.177	0.02*	
3-Back reaction time (s)	0.722	0.174	0.836	0.238	0.08	

*Statistically significant ($P < 0.05$).

using WM protocols, and no MEG studies. Using EEG recordings during a WM task, Bailey et al. (2017) showed higher gamma-band connectivity in both subjects with TBI and major depressive disorder (MDD) following TBI. In a separate EEG study that examined the WM maintenance, parietal–occipital upper alpha-band signals were also reduced in MDD and TBI–MDD groups, but not in the TBI-only group (Bailey et al. 2014).

The present study used MEG to investigate neuronal mechanisms underlying WM abnormalities in individuals with combat-related mTBI. Participants included active-duty service members or Veterans who had combat-related mTBI and healthy control active-duty service members or Veterans with similar combat experiences. Several modern MEG preprocessing and source imaging approaches were used to obtain source images of the WM responses in different frequency bands. Based on previous studies, we predicted abnormal MEG signals in the PFC of combat-related mTBI patients, owing to the vulnerability of this region to blast and the key functional roles that it plays in executive functions. Relationships between MEG WM activations and measures of executive functioning were also examined to determine the characteristics of the abnormal MEG WM signals with respect to these deficits in combat-related mTBI.

Methods and Materials

The study protocol was approved by institutional review boards of the VA San Diego Healthcare System and Naval Health Research Center at San Diego. All participants gave written informed consent prior to study procedures. The informed consent followed the ethical guidelines of the Declarations of Helsinki (sixth revision, 2008).

Research Subjects

Demographic characteristics of participants are listed in Table 1A. Study participants included 25 individuals (all males) who had a chronic or subacute blast mTBI with persistent PCS for an average duration of 10.36 months between the incident and MEG exam. Participants with documented blast mTBI (based on corroborating information available from charts) were US active-duty military service members or Operation Enduring Freedom (OEF)/Operation Iraqi Freedom (OIF) Veterans with injuries caused by blast exposure during combat or military training. We also recruited 20 healthy control subjects (all males) with combat experience (active-duty military service members or OEF/OIF Veterans), but without significant history of concussion based on self-report. There were no significant group differences in age or education.

All participants with mTBI were evaluated in a clinical interview to assess the nature of their injuries and persistent PCS. The diagnosis of mTBI was based in part on standard VA and Department of Defense (DOD) diagnostic criteria (The Management of Concussion/mTBI Working Group 2009): 1) loss of consciousness (LOC) < 30 min or transient confusion, disorientation, or impaired consciousness immediately after the blast-related trauma; 2) post-traumatic amnesia (PTA) < 24 h; 3) an initial Glasgow Coma Scale (GCS) (Teasdale and Jennett 1974) between 13 and 15 (if available). Since the GCS assessment was often not available in theater, individuals with missing GCS, but who met other inclusion criteria, were also enrolled. As for the duration of LOC in mTBI participants, 28% of participants reported that they were altered/dazed, 44% reported LOC for 1 minute or less, 24% reported 2–15 min LOC, and 4% reported 15–30 min. Regarding the duration of PTA, 36% of mTBI participants reported 0–15 min PTA, 52% reported 16–30 min PTA, and 12% reporting 31 min–24 h PTA. The majority of participants experienced one mTBI (76%), with 20% reporting 2–3 mTBIs, and 4% reporting 4–5 mTBIs. Few participants provided GCS information since GCS was not recorded or accessible for most blast mTBI participants who received their injury in theater.

The clinical interview also assessed persistent PCS and neurological deficits in 21 categories (Table 1B), modified slightly from the Head Injury Symptom Checklist (HISC) (McLean et al. 1984). Only participants with persistent symptoms in at least three of the categories were enrolled into the blast mTBI group. The number of PCS endorsed ranged from 4 to 10 (mean = 6.56; standard deviation = 1.61). Table 1B lists the percentages of the mTBI participants and controls that endorsed each symptom. Though controls were asked about these symptoms, they did not endorse having a TBI. Hence, symptoms in control subjects were unrelated to TBI.

Exclusion criteria for study participation were as follows: 1) history of other neurological, developmental or psychiatric disorders (e.g., brain tumor, stroke, epilepsy, Alzheimer disease, or schizophrenia, bipolar disorder, or diagnosis of learning disability); 2) diagnosis of either full or partial post-traumatic stress disorder (PTSD) based on a Clinician Administered PTSD scale score (CAPS) ≥ 30 total score (Weathers et al. 1999), 3) diagnosis of MDD prior to the mTBI; 4) endorsement of substance or alcohol abuse according to DSM-V criteria within the 6 months prior to the study, based on a clinical interview; 5) history of metabolic or other diseases known to affect the central nervous system (see (Dikmen et al. 1995) for similar criteria); 6) extensive metal dental hardware (e.g., braces and large metal dentures; fillings were acceptable) or other metal objects in the head, neck, or face areas that cause artifacts in the MEG data, not removable during preprocessing; 7) currently taking

Table 1B Percentage of subjects showing individual symptoms in mTBI | control groups

Headaches		Dizziness		Fatigue	
88.00%	5.00%	60.00%	5.00%	40.00%	10.00%
Memory Difficulty		Irritability		Anxiety	
84.00%	15.00%	64.00%	15.00%	56.00%	0%
Trouble with sleep		Hearing difficulties		Blurred vision other visual difficulties	
64.00%	5.00%	60.00%	10.00%	8.00%	0%
Personal Changes		Apathy		Lack of spontaneity	
20.00%	5.00%	4.00%	0%	0%	0%
Affective Lability		Depression		Trouble concentrating	
8.00%	5.00%	20.00%	5.00%	8.00%	0%
Bothered by noise		Bothered by light		Coordination/ balance problems	
0%	0%	4.00%	5.00%	16.00%	10.00%
Motor difficulty		Difficulty with speech		Numbness/ tingling	
4.00%	0%	0%	5.00%	12.00%	0%

medications (e.g., some sedative neuroleptics and hypnotics) known to alter the power of brain rhythms (Niedermeyer and Lopes da Silva 2005); 8) suicidal ideation as evaluated using the Beck Depression Inventory (BDI-II), that is, any participant reporting a score of "2" or "3" on the BDI-II: item 9 (suicidal thoughts or wishes), confirmed in follow-up risk assessment; and 10) previous diagnosis of attention deficit hyperactivity disorder (ADHD) or learning disorders.

For mTBI participants who were taking medications that might globally change brain activity such as neuroleptic sedatives, antidepressants, and hypnotics (Niedermeyer and Lopes da Silva 2005), we sought permission from treating physicians to discontinue the medications for 5 half-lives prior to MEG exams. If the treating physician denied the request, the participant was not enrolled in the study. No controls were taking medications. Past history of drug and alcohol use was asked about in a detailed screening interview. Participants with previous substance abuse were excluded from the study. Participants were asked to refrain from using alcohol or other substances the night before the MEG scan.

Neuropsychological Exams

Neuropsychological tests focused on the assessment of executive functions [Kaplan Executive Function System (D-KEFS) Trail Making Test and Verbal Fluency Test (Delis et al. 2001)], and processing speed [Symbol Search and Digit Symbol Coding subtests from the Wechsler Adult Intelligence Scale (WAIS) (Wechsler 1997, 2008)], which are sensitive to cognitive decline in mTBI (see cited references in (Robb Swan et al. 2015)). The D-KEFS Trail Making Test has a visual scanning condition and then requires participants to connect circles as quickly as possible in numerical, alphabetical, and alternating numerical/alphabetical orders. The alternating numerical/alphabetical orders subtest was used as a measure of cognitive flexibility in visual-motor sequencing. The D-KEFS Verbal Fluency Test requires participants to generate words as quickly as possible beginning with particular letters (Letter Fluency), in specified semantic categories (Category Fluency) and then while shifting between semantic categories (Category Switching) (Delis et al. 2001). The Trail Making and Verbal Fluency tests are sensitive to subtle cognitive-shifting deficits. As for processing speed, the WAIS Symbol Search subtest requires participants to scan a target group and search a group of symbols, indicating whether one of the target symbols appears in the search group. In the WAIS Digit Symbol Coding subtest, the participant fills in boxes below digits with symbols that are paired with them in a key at the top of the page. Both of these subtests are timed. Scaled scores from each subtest were combined to create an overall Processing Speed Index. The WAIS III was administered to most participants, although a subset of participants completed the WAIS IV version. Sensitivity analyses were performed removing the WAIS IV and the tests for group differences were unchanged.

The above neuropsychological tests were embedded within a larger test battery that was 2–2.5 h in length. To ensure optimal performance by participants, the testing battery was structured to include breaks and the psychometrists testing the individual queried participants throughout the session about fatigue, headaches, and other PCS to safeguard the participants well-being and the quality of the data collected. All tests were performed in a single session, within one week of the MEG/MRI session. One participant was excluded from analyses of the neuropsychological data owing to potentially invalid data as indicated by the Test of Memory Malingering (Tombaugh 1996). All scores were age-corrected scaled scores based on normative data provided by the test publishers.

N-Back Working Memory Task

Participants underwent MEG recordings while performing an N-back WM task. The task entails on-line monitoring, updating, and manipulation of remembered information. During the task, the participant was required to monitor a series of letters (both upper and lower case) presented for 500 ms in the middle of the screen. A fixation cross was presented during the 3000 ms interstimulus interval. The participant was instructed to respond only when a letter was presented that matched (i.e., target) the one presented in trials previously, while not to responding to the unmatched stimuli (nontarget). Three load conditions were used (1-back, 2-back, and 3-back) (Fig. 1), which place increasing demands on WM processes. About 50 trials per load condition were collected for each participant. Performance was recorded using an MEG-compatible response pad, in which index finger blocks-and-unblocks a laser-beam. The outputs of the response pad including reaction times (RTs) were recorded in the MEG file. The percent correct responses to target and nontarget stimuli was measured.

MEG Data Acquisition and Signal Preprocessing to Remove Artifacts

MEG responses to the N-back WM task were collected using the VectorView™ whole-head MEG system (Elekta-Neuromag, Helsinki, Finland) with 306 MEG channels. Participants were seated in upright position inside a multilayer magnetically shielded room (IMEDCO-AG) (Cohen et al. 2002) at the UCSD MEG Center. Data were sampled at 1000 Hz and were run through a high-pass filter with a 0.1 Hz cut-off, and a low-pass filter with a 330 Hz cut-off. Eye blinks and eye movements were monitored using 2 pairs of bipolar electrodes with one pair placed above and below the left eye, and the other pair placed on the 2 temples. Heart signals were monitored with another pair of bipolar electrodes. Precautions

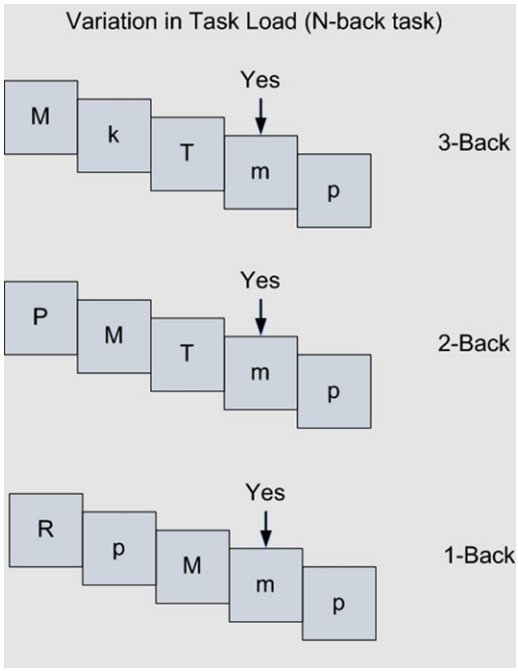

Figure 1. N-back working memory task.

were taken to ensure head stability; foam wedges were inserted between the subject's head and the inside of the unit, and a Velcro strap was placed under the subject's chin and anchored in superior and posterior axes. Head movement across different sessions was about 2–3 mm on average.

MEG sensor waveforms in raw (unaveraged) format were first run through MaxFilter, also known as signal space separation (Taulu, Kajola, et al. 2004; Taulu, Simola, et al. 2004; Song et al. 2008), to remove external interferences (e.g., magnetic artifacts due to metal objects, strong cardiac signals, environment noises). Next, residual artifacts near the sensor array due to eye movements and residual cardiac signals were removed via Independent Component Analysis using Fast-ICA (http://research.ics.aalto.fi/ica/fastica/) (Hyvarinen 1999; Hyvarinen and Oja 2000). The waveforms associated with top independent components (ICs) were examined by an experienced MEG data analyst, along with ECG and EOG signals. ICs associated with eye blinks, eye movements, heartbeats, and other artifacts were removed.

Structural MRI, MEG–MRI Registration, BEM Forward Model for MEG

Structural MRI of the subject's head was collected using a General Electric 1.5 T Excite MRI scanner. The acquisition contains a standard high-resolution anatomical volume with a resolution of $0.94 \times 0.94 \times 1.2\,mm^3$ using a T1-weighted 3D-IR-FSPGR pulse sequence. Scanner-related imaging distortions were corrected using a gradient nonlinearity correction approach (Jovicich et al. 2006). To co-register the MEG with MRI coordinate systems, 3 anatomical landmarks (i.e., left and right preauricular points, and nasion) were measured for each subject using the Probe Position Identification system (Polhemus, USA). By identifying the same 3 points on the subject's MR images using MRILAB (Elekta/Neuromag), a transformation matrix involving both rotation and translation between the MEG and MR coordinate systems was generated. To increase the reliability of the MEG–MR coregistration, approximately 120 points on the scalp were digitized with the Polhemus system, in addition to the 3 landmarks, and those points were coregistered onto the scalp surface of the MR images. The T1-weighted images were also used to extract the brain volume and innermost skull surface (SEGLAB software developed by Elekta/Neuromag). Realistic Boundary Element Method (BEM) head model was used for MEG forward calculation (Mosher et al. 1999; Huang et al. 2007). The BEM mesh was constructed by tessellating the inner skull surface from the T1-weighted MRI into ~6000 triangular elements with ~5 mm size. A cubic source grid with 5 mm size covering cortical and subcortical GM areas was created. Such a source grid was used for calculating the MEG gain (i.e., lead-field) matrix, which leads to a grid with ~10 000 nodes covering the whole brain. Then, the source grid was combined with the BEM mesh in the MRI coordinate for the BEM forward calculation.

Other conventional MRI sequences typical for identifying structural lesions in TBI participants were also performed: 1) Axial T2*-weighted; 2) Axial fast spin-echo T2-weighted; and 3) Axial FLAIR; These conventional MRIs were carefully reviewed by a Board-certified neuroradiologist (R.R.L.) to determine if the subject had visible lesions on MRI.

Covariance Matrix of Single Trials and MEG Source Magnitude Imaging Using Fast-VESTAL

Following the preprocessing step, N-back MEG sensor-waveform datasets were run through band-pass filters for different frequency bands: alpha band (8–12 Hz), beta band (15–30 Hz), gamma band (30–80 Hz), and low-frequency band (1–7 Hz, delta + theta). Each data set was then divided into trials, each with 2.5-s duration (−500 to 1500 ms with respect to the stimulus onset). In the present study, we focus on the trials associated with target stimuli.

Different from the conventional MEG approach in which sensor waveforms are averaged with respect to the onset of the stimuli, we calculated the sensor covariance matrices for individual trials. Then for each frequency band, a total sensor-waveform covariance matrix of the target condition was calculated by averaging across the covariance matrices from individual trials for the target stimuli. We averaged the covariance matrices across trials. This approach prevents potential signal cancellation across trials due to nontime-lock nature of the WM signals (see Discussion). Using the total covariance matrix, voxel-wise MEG source magnitude images that cover the whole brain were obtained for each subject, and each frequency band, following the Fast-VESTAL procedure (see Method in (Huang, Huang, et al. 2014) and Appendix in (Huang et al. 2016)). An Objective Prewhitening Method was applied to remove correlated environmental noise and objectively select the dominant eigen-modes of sensor-waveform covariance matrix (Huang, Huang, et al. 2014).

Voxel-wise Group Statistical Analyses for MEG Source Magnitude Images

In all participants, voxel-wise whole brain MEG source magnitude images obtained from Fast-VESTAL were first spatially coregistered to the MNI-152 (Grabner et al. 2006) brain-atlas template using a linear affine transformation program, FLIRT, in the FSL software package (Smith et al. 2004; Woolrich et al. 2009). Then in MNI-152 space, the MEG source magnitude images were spatially smoothed using a Gaussian kernel with 5 mm full width half maximum (FWHM), followed by a logarithmic transformation using FSL. Next, voxel-wise group statistical analysis was performed to detect group differences in brain activation during the MEG N-back task.

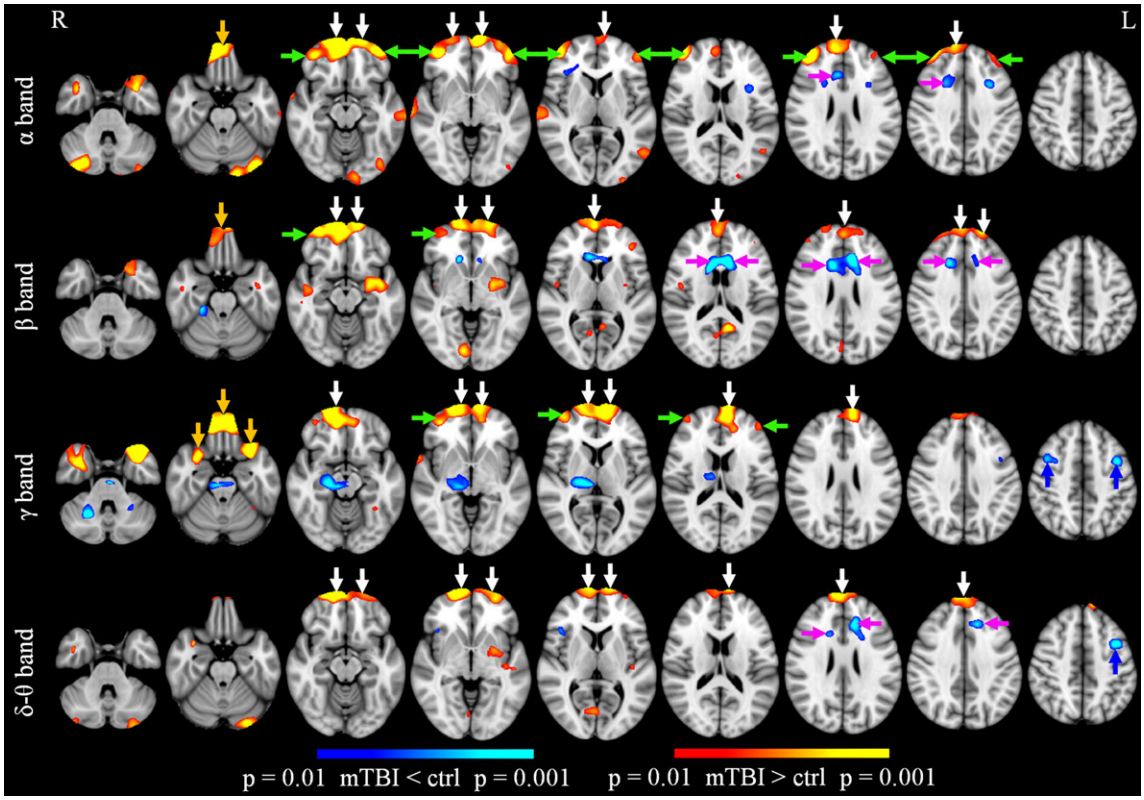

Figure 2. MEG hyper- and hypoactivations revealed by working memory N-back task in combat-related mTBI versus control subjects. MEG signals in 1-, 2-, and 3-back conditions were treated as repeat measures. Different rows were for different frequency bands. White arrows: hyperactivations from rPFC; green arrows: hyperactivations from anterior aspect of dlPFC; orange arrows: hyperactivations from vmPFC and OFC. Magenta arrows: hypoactivations from ACC; blue arrows: hypoactivations from posterior aspect of dlPFC.

For each frequency band, a voxel-wise repeated measure ANOVA was performed to create *F*-value maps for examining the group differences (i.e., mTBI vs. control groups), with 1-, 2-, and 3-back conditions treated as repeated measures. Family-wise error across voxels was corrected using standard cluster analysis for the *F*-value maps to control for family-wise errors at a corrected $P < 0.01$ level, using "3dFWHMx" and "3dClustSim" functions in the latest version of AFNI (http://afni.nimh.nih.gov). A mask that contained the statistically significant clusters was created, and then applied to the *F*-value maps to create the corrected group statistical maps for the MEG source magnitude images. In addition, performance measures (e.g., RT) that showed significant group difference during the N-back task was correlated with the voxel-wise MEG source magnitude in order to examine the relationship between the performance variables and MEG activations.

Another goal was to study the neuronal correlates of potential cognitive dysfunctions observed using behavioral measures in mTBI versus control subjects. Specifically, we investigated whether inferior performance on the neuropsychological exams was associated with abnormal activation during the N-back task in the mTBI versus control groups. Voxel-wise correlation analyses were also performed to examine the association of N-back source images and neuropsychological scores. All subjects from both mTBI and control groups were combined together for the correlation analyses. In each frequency band, the MEG source images in the MNI-152 space (following the spatial smoothing and logarithm transformation) were formed into 3 4D datasets: Dimensions 1–3 represent the *x*-, *y*-, and *z*-coordinates, and the fourth dimension represents all the subjects by combining both mTBI and control groups together. A total of 12 datasets were created for 1-, 2-, and 3-back conditions, and for 4 frequency bands. Next, along the fourth dimension, voxel-wise repeated measure correlation analyses (Bakdash and Marusich 2017) were performed between MEG source images and each of the neuropsychological scores. For each frequency band, the 1-, 2-, and 3-back conditions were treated as repeated measures in such analyses. In this study, we only examined the neuropsychological scores that showed statistical group differences, an approach similar to our previous studies (Robb Swan et al. 2015; Huang et al. 2017). The repeated measure correlation analyses created *r*-value correlation maps, and cluster analysis was used to control for family-wise errors at a corrected $P < 0.01$ level for the *r*-value maps, similar to the correction procedure for the *F*-value maps.

Results

Hyper- and Hypoactivations revealed by MEG Source Magnitude Images in Combat mTBI

Table 1A shows performance accuracy for the target stimuli during the N-back tests in the combat-related mTBI and control groups. The percent correct responses decreased as task difficulty increased from 1-back to 3-back in both groups. Due to a ceiling effect, the percent correct responses did not follow the Gaussian distribution. Therefore, the rank-based nonparametric Mann–Whitney *U* test (MWU) was used to assess groups differences in accuracy. Though performances of the mTBI group tended to be worse than the control group across all memory

load conditions, no significant group differences were found in the percent correct responses for any of the load conditions.

Table 1A also shows the mean RTs for correct responses during the N-back tests in both groups. The mTBI group showed significantly longer RTs than the control group for the 1-back and 2-back conditions, but not the 3-back condition, which showed a nonsignificant trend for group differences.

Figure 2 displays the main findings of hyper- and hypoactivations during the WM N-back task in the mTBI group relative to the control group, for the target stimuli. Group differences for the alpha, beta, gamma, and low-frequency bands are displayed in different rows. All the significant group findings are summarized in Table 2 with respect to different cortical and subcortical regions.

Abnormalities in frontal regions: Hyper activation refers to greater activation in combat mTBI than in controls, and hypoactivation to lower activation in combat mTBI than controls. Compared with the controls, combat mTBI participants demonstrated hyperactivations across all frequency bands in part of rostral prefrontal cortex (rPFC), particularly in frontal pole (FP, Brodmann Area or BA 10). The anterior aspect of the dorsolateral prefrontal cortex (dlPFC, BA 9 and 46) and the ventromedial prefrontal cortex (vmPFC) showed hyperactivations in alpha, beta, and gamma bands for the mTBI group. In the gamma band, bilateral orbitofrontal cortex (OFC) showed hyperactivations in mTBI group. In contrast, mTBI participants showed hypoactivations in anterior cingulate cortex (ACC, BA 24) mainly in the beta band, but to a lesser extent in alpha and low-frequency bands as well. In addition, hypoactivation in the posterior aspect of the dlPFC was found bilaterally in the gamma band and in the left hemisphere in the low-frequency band.

MEG WM activations also showed abnormalities in other brain regions. *Abnormalities in Temporal Regions*: In alpha, beta, and gamma bands, temporal pole areas showed hyperactivations during the WM N-back task in combat-related mTBI versus control subjects. Lateral temporal areas also showed hyperactivations in alpha band. In beta band, left amygdala showed hyperactivations as well. In contrast, the right parahippocampal gyrus showed hypoactivation in alpha and gamma bands. *Abnormalities in Occipital Regions*: Medial occipital cortex showed hyperactivations in beta and low-frequency bands, whereas lateral occipital cortices showed hyperactivations in alpha band. *Abnormalities in Subcortical Regions*: Left insular cortex, putamen, and globus pallidus showed hyperactivations in both beta and low-frequency bands. In gamma band, hypoactivation was seen in right thalamus. *Abnormalities in Cerebellum*: Posterior lobe of cerebellum showed hyperactivation in alpha and low-frequency bands, whereas right anterior lobe of cerebellum showed hypoactivation.

Importantly, tests for group differences in the different frequency bands were repeated using only the trials with correct responses. The results from the group comparisons are displayed in the Supplementary Materials (Fig. S1). The group differences based on the analyses of correct trials were highly similar to the group tests that were based on all target trials (Fig. S1 vs. Fig. 2). Some subtle differences are likely due to the lower signal-to-noise ratio when the analyses are conducted on fewer trials.

Table 2 Brain areas showing hyper- (↑) and hypo- (↓) MEG activations for different frequency bands during working memory N-back task in combat-related mTBI versus control subjects

Brain regions	α	β	γ	δ–θ
R FP	↑	↑	↑	↑
R dlPFC, anterior	↑	↑	↑	
R dlpFC, posterior			↓	
R vmPFC	↑	↑	↑	
R OFC, posterior			↑	
R ACC	↓	↓		↓
R temporal pole	↑		↑	↑
R lateral temporal	↑			
R parahippocampal gyrus		↓	↓	
R medial occipital		↑		↑
R thalamus			↓	
R cerebellum, anterior			↓	
R cerebellum, posterior	↑			↑
L FP	↑	↑	↑	↑
L dlPFC, anterior	↑		↑	
L dlPFC, posterior			↓	↓
L vmPFC	↑	↑	↑	
L OFC, posterior			↑	
L ACC		↓		↓
L temporal pole	↑	↑	↑	
L lateral temporal	↑			
L medial occipital		↑		
L lateral occipital	↑			
L insular cortex		↑		↑
L putamen		↑		↑
L globus pallidus		↑		↑
L cerebellum, posterior	↑			↑

The major clusters were highlighted in bold. Abbreviations: FP, frontal pole; dlPFC, dorsolateral prefrontal cortex; vmPFC, ventrolateral prefrontal cortex; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex.

Activation Maps of the N-Back Tasks

Figure 3 shows the task activation maps for the target stimuli in both the combat mTBI and control groups. In each group, the MEG activations during N-back tasks were compared with "empty room" MEG data, which were collected when no subjects were in the MEG scanner. Repeated measures ANOVA were used to assess the effect of the 1-, 2-, and 3-back MEG activation over the empty room data. In order to have sufficient dynamic range for the color scale, we adopted highly conservative significance thresholds for the activation maps. Although the activation maps in Figure 3 do not directly test for group differences, marked differences between the mTBI and control groups are evident in some regions upon visual inspection of these maps. For example, the FP area clearly showed stronger and more spatially distributed activations in the mTBI than in the control group across all frequency bands (white arrows in Fig. 3). In both groups, posterior parietal activation was strong across all frequency bands. It is also interesting that both groups showed activation in the posterior aspect of dlPFC with apparently stronger activation in control group than in mTBI group, especially in gamma and low-frequency bands (blue arrows in Fig. 3). Activations maps in Figure 3 are highly consistent with previous fMRI studies (see reviews in (Owen et al. 2005; Phillips et al. 2017)).

MEG Activations Correlate With RT

Correlations were conducted between the MEG WM activations and RTs during the 1-back and 2-back conditions, in which the mTBI group showed longer RTs than the control group. Only MEG signals for the target trials with correct responses were used in these analyses. The voxel-wise MEG source magnitude

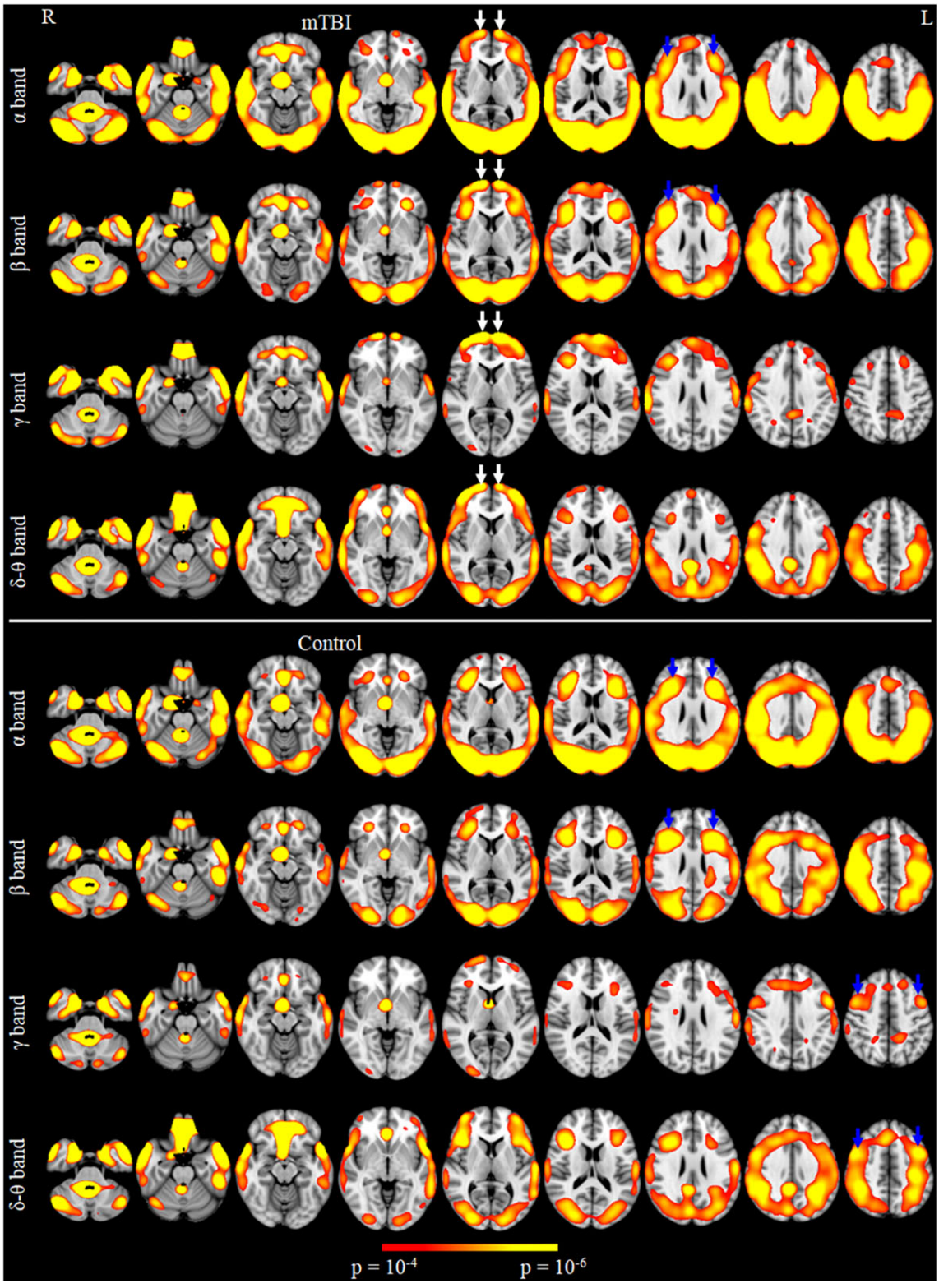

Figure 3. MEG activation maps during N-back tasks in combat-related mTBI versus controls. In each group, MEG activations were compared with "empty room" MEG data using repeated measure ANOVA. White arrows: FP area clearly showed apparently stronger activations in mTBI group than in control group across all frequency bands. Blue arrows: both group showed activation in the posterior aspect of dlPFC.

images from both combat-related mTBI and control subjects were pooled together. As shown in Figure 4, the vast majority of the significant correlations were positive, such that higher MEG activations significantly correlated with longer RTs. In the 1-back condition, RT positively correlated with: 1) alpha-band activations in bilateral FP, 2) beta-band activations in bilateral

OFC, 3) gamma-band activations in bilateral FP and left pars opercularis (BA 44, Broca's area), and 4) low-frequency activations in left FP, anterior left dlPFC, and left hippocampus. In the 2-back condition, RT positively correlated with: 1) alpha-band activations in the anterior left dlPFC and posterior left superior temporal gyrus (Wernicke's area); 2) beta-band activations in the right inferior temporal lobe and anterior left dlPFC; 3) gamma-band activations in the anterior left dlPFC, left primary motor cortex, and left cuneal cortex; and 4) low-frequency activations in the left caudate and left primary motor cortex.

In the right panel of Figure 4, 3 representative scatter plots from left FP (1-back alpha band), right OFC (1-back beta-band), and mid-line FP (1-back gamma-band) areas show significant positive correlations between MEG activation and RT in mTBI and control subjects. For the data in these plots, the mTBI group showed significant MEG hyperactivation (*y* axis) and longer RTs (*x* axis) relative to the control group. Thus, slower RTs in mTBI were associated with hyper activation in these PFC areas.

Neuropsychological Test Performance

Table 3 shows the mean (SD) performances for each group on the neuropsychological tests. The mTBI group performed significantly worse than the control group on 3 of the measures, namely Number–Letter Sequencing subtest of the Trail Making Test, $t(40.356) = 4.111$, $P = 0.000$; Letter Fluency, $t(39.309) = 2.105$, $P = 0.042$; and Digit Symbol Coding, $t(38.568) = 2.458$, $P = 0.019$.

Correlation Between MEG N-Back Activations and Neuropsychological Performance

The left panel of Figure 5 displays the results of voxel-wise correlation analyses that examined the neuronal correlates of WM N-back source images in MEG and neuropsychological scores. The voxel-wise MEG source magnitude images from both combat mTBI and control subjects were pooled together, and repeated measure correlations were performed with the 1-, 2-, and 3-back conditions treated as repeated measures (see Methods and

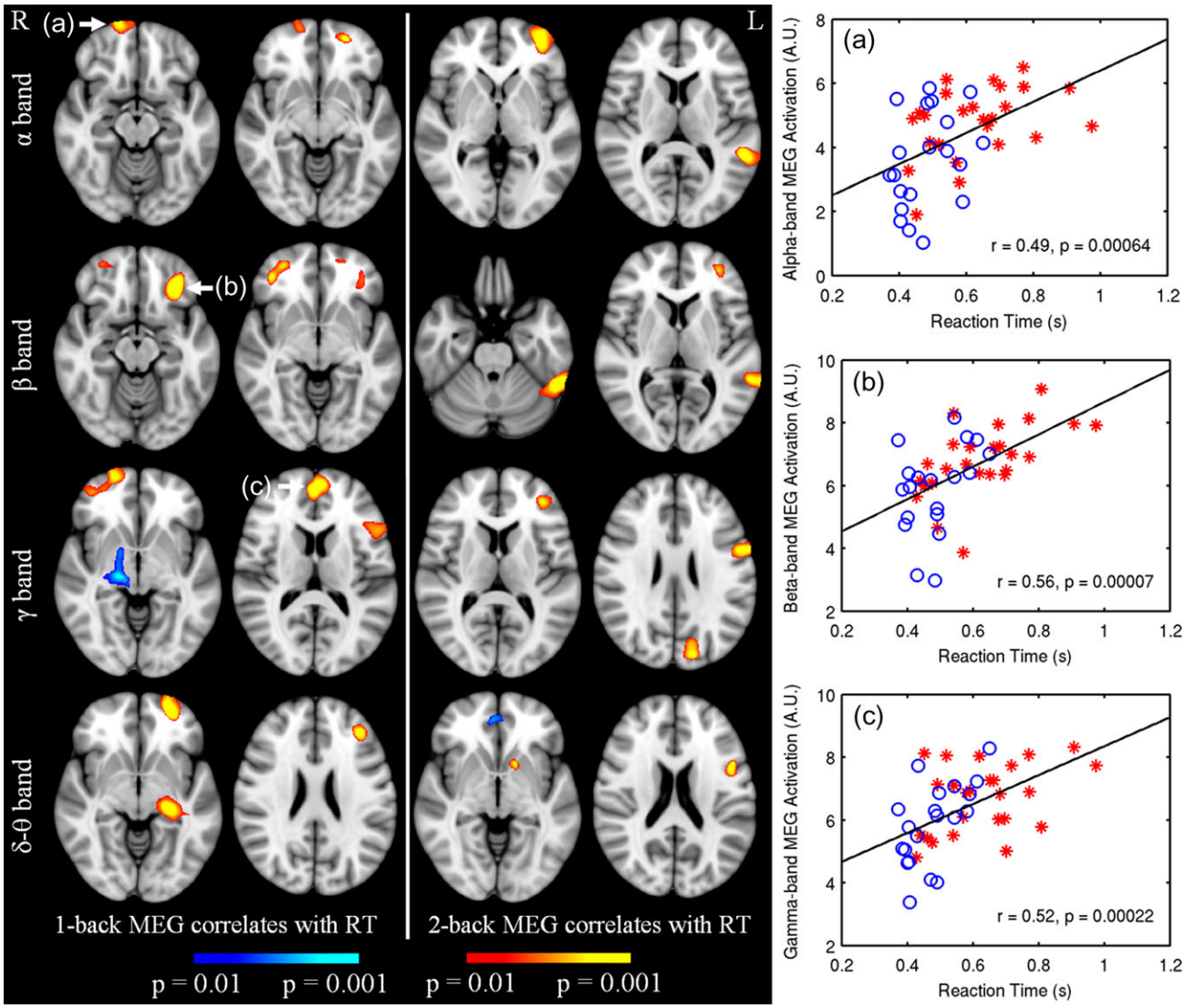

Figure 4. MEG activations correlate with reaction time measures. Columns 1 and 2 in the left panel of show that MEG WM activations correlate with RT measure during 1-back task. Columns 3 and 4 in the left panel show the MEG-RT correlation result during 2-back task. *Right panel*: 3 representative scatter plots showing significant positive correlations between MEG activation and RT for mTBI (red stars) and control subjects (blue circles). The plots are for the 3 areas (a), (b), and (c) in which the locations are indicated by white arrows in the left panel.

Table 3 Neuropsychological test performance in the control and blast mTBI groups

	Control, N = 20		mTBI, N = 25			
	Mean	SD	Mean	SD	P-value	Cohen's d
D-KEFS trail making test						
Visual scanning	10.55	2.16	10.00	3.10	0.488	0.21
Number sequencing	10.40	2.90	10.64	1.68	0.744	−0.10
Letter sequencing	11.30	2.52	10.08	2.02	0.087	0.53
Number-letter sequencing	11.25	1.45	8.88	2.39	0.000*	1.20
Motor speed	11.40	1.57	11.16	2.23	0.675	0.12
D-KEFS verbal fluency test						
Letter fluency	10.90	3.18	8.96	2.94	0.042*	0.63
Category fluency	11.75	3.14	10.48	2.69	0.160	0.43
Category switching	11.40	2.50	9.88	2.83	0.063	0.57
WAIS						
Symbol search	11.05	4.22	10.52	2.42	0.621	0.15
Digit symbol coding	10.45	2.80	8.48	2.50	0.019*	0.74
Processing speed index	103.90	17.08	96.92	11.72	0.129	0.48

*Statistically significant ($P < 0.05$).

Group differences on the measures reported in the table were tested using independent *t*-tests.

Neuropsychological analysis used scaled scores (mean=10, standard deviation = 3). The WAIS Processing Speed Index is the sum of the scaled scores of Symbol Search and Digit Symbol Coding to create a composite standard score (mean = 100, standard deviation = 15).

Materials). We focused on the 3 neuropsychological tests that showed significant group differences, namely, 1) Number-Letter Sequencing subtest of the Trail Making Test (scaled score); 2) Letter Fluency (scaled score), and 3) Digit Symbol Coding (scaled score). Figure 5 plots the findings for some representative regions.

Across all frequency bands, significant negative correlations were found between activations in lateral aspects of the FP and all 3 of the neuropsychological test scores. In the alpha band, significant negative correlations were found between activations of the anterior right dlPFC and Number-Letter Sequencing scores. In the gamma band, significant positive correlations were found between left thalamus activations and Number-Letter Sequencing scores.

Representative scatter plots from the lateral aspect of the FP areas (Fig. 5) show significant negative correlations between MEG activation and neuropsychological scores in mTBI and control subjects. In these plots, the mTBI group showed significant MEG hyperactivation (*y*-axis) and poorer performance in neuropsychological exams (*x*-axis) relative to the control group. These findings suggest that MEG hyperactivation in FP contributed to the poorer executive functions and processing speed in mTBI patients.

Discussion

In the present study, aberrant activations during WM were revealed for the first time in combat-related mTBI using MEG source magnitude imaging. In comparison with a well-matched healthy control group with combat exposures, the combat-related mTBI group was predominantly characterized by hyperactivations in areas of the prefrontal cortex including FP, dlPFC, vmPFC, and OFC, but hypoactivations in ACC. In addition, MEG hyperactivations in FP, OFC, and anterior dlPFC were associated with slower RTs during the WM task. Furthermore, MEG activations from the lateral FP area were associated with worse performance on neuropsychological tests that measure processing speed and executive functions (i.e., letter sequencing, verbal fluency, and digit symbol coding). These findings suggested that aberrant neuronal activity in combat-related mTBI, especially in the prefrontal cortex, was functionally significant, relating to individual differences in cognitive proficiency.

MEG PFC Abnormalities Consistency With Previous fMRI Studies

The hyperactivations in different PFC regions, especially dlPFC, during the N-back task were consistent with many previous fMRI studies of WM in TBI (McAllister et al. 1999, 2001, 2006; Christodoulou et al. 2001; Scheibel et al. 2003; Perlstein et al. 2004; Kasahara et al. 2011; Turner et al. 2011; Medaglia et al. 2012; Gillis and Hampstead 2015; Phillips et al. 2017). Hyperactivation of the dlPFC in individuals with TBI may signify a stronger engagement of cognitive-control processes that are involved in WM (McAllister et al. 1999; Scheibel et al. 2003; Perlstein et al. 2004; Turner et al. 2011; Medaglia et al. 2012; Gillis and Hampstead 2015; Phillips et al. 2017). Still, the nature of PFC hyper activation remains debated (Gillis and Hampstead 2015) and may be due to: 1) a transient and natural recruitment of existing resources or 2) functional reorganization that results in the engagement of neuronal resources that do not normally support WM performance (Hillary 2011). Different mechanisms have been suggested to explain such phenomena, such as compensatory mechanisms that maximize cognitive functioning, a lowered threshold for recruiting additional cognitive resources, and cognitive fatigue (see (Hillary 2008, 2011) for reviews). Our results suggest over-recruitment of the PFC in TBI may partly represent the unmasking of latent resources.

MEG rPFC Hyperactivations and Their Functional Correlates

Among all the abnormalities in the PFC, the profound hyper activation in rPFC (mainly the FP, but also vmPFC, and OFC) revealed by the present study suggests that the aberrant rPFC is an important feature of combat-related mTBI. These findings are consistent with current knowledge about the function of the FP, which is engaged during more complicated tasks, for example, when the application of one cognitive operation (such as a rule) on its own is not sufficient to solve the problem as a

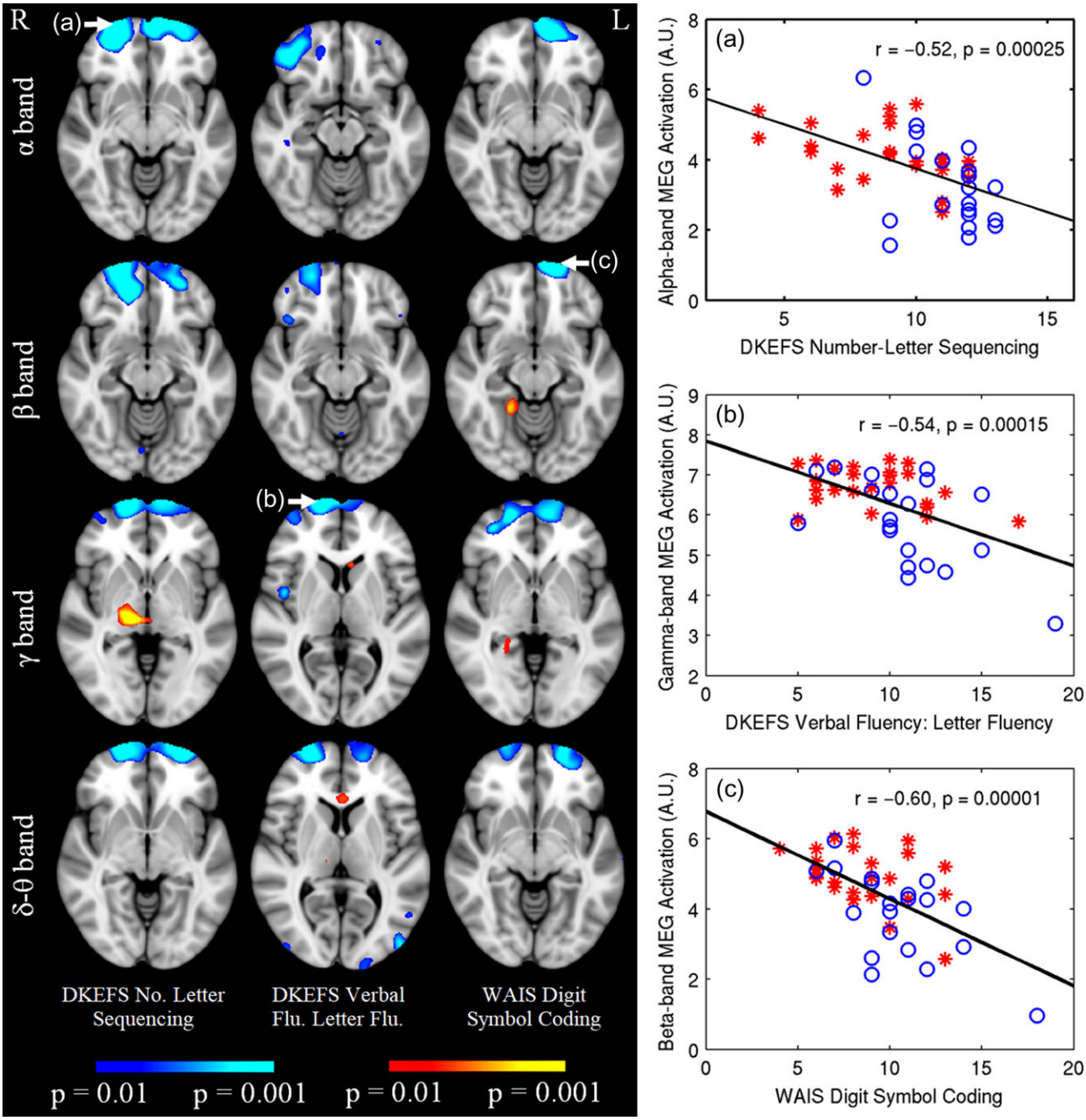

Figure 5. Left panel: MEG N-back signals correlate with 3 neuropsychological scores in a combined group with combat-related mTBI and control subjects. Blue-cyan color: significant negative correlations; red-yellow color: significant positive correlations. *Right panel*: 3 representative scatter plots showing significant negative correlations between MEG activation and neuropsychological scores for mTBI (red stars) and control subjects (blue circles). The plots are for the 3 areas (a), (b), and (c) in which the locations are indicated by white arrows in the left panel.

whole and the integration of the results of 2 or more separate cognitive operations is required to fulfill the higher behavioral goal (Owen et al. 2005). The N-back task is a perfect example of such a procedure, requiring simultaneous monitoring of a series of stimuli, ongoing adjustment of that information to incorporate recently presented items and reject temporally distant stimuli, the drawing of comparisons, and the consequent switching of attention between various items in the series. Multiple related cognitive operations can only be carried out successfully if they are coordinated, and the coordination of information processing and information transfer between multiple operations across supramodal cortex is an important aspect of BA10 function (Owen et al. 2005).

FP can be further divided into 2 subdivisions: the lateral FP1 and medial FP2. FP1 is involved in cognition, working memory and perception (see review in (Bludau et al. 2014)) and is particularly important for organized behavior, action planning, and the management of multiple goals based on information from episodic and short-term memory (Bludau et al. 2014). In contrast, area FP2 is mainly involved in emotional and social cognition

(Bludau et al. 2014). These functional distinctions between the lateral and medial FP are compatible with our findings that aberrant FP (i.e., FP1) activation in mTBI was related to slower WM (RT) and poorer processing speed and executive functioning. These results also provide compelling evidence to suggest that the cognitive sequelae of combat-related brain injury is largely due to altered FP functioning.

We are not aware of any previous fMRI studies of WM in TBI reporting abnormalities in FP and other regions in rFPC. We believe this is, at least in part, due to the substantial susceptibility to artifacts in fMRI signals from the rPFC regions (Hutton et al. 2002; Cusack et al. 2003). Distortions in rPFC regions (e.g., FP, vmPFC, and OFC) are usually about 10 mm or larger (Cusack et al. 2003). Although a number of techniques have been used to correct for such serious distortions (see review in (Cusack et al. 2003)) the decrease in statistical power of the fMRI signal remains a major concern. However, the absence of FP abnormalities in previous fMRI studies of WM may also be because the vast majority of the research was not performed in combat-related mTBI. That raises the question whether FP abnormalities during WM are unique to combat-related mTBI participants. Regardless of the underlying reasons, we believe that our finding of profound hyperactivations in rPFC in combat-related mTBI is a significant contribution to the mTBI functional imaging literature and also supports the sensitivity of MEG source imaging task-activated protocols that engage more complex cognitive operations.

Hypoactivation in ACC Region

In the present study we also found hypoactivation of the ACC in combat-related mTBI in alpha, beta, and low-frequency bands. This finding is consistent with previous fMRI studies in TBI that also showed reduced ACC activation (Cazalis et al. 2006), although some fMRI studies of WM reported increased ACC activation in TBI (Cazalis et al. 2011). We believe the inconsistency may be due to the differences in experimental conditions of the studies (e.g., differences in WM stimuli such as pictures versus letters, with spatial location information or not, complexity in the stimuli). The exact role of the ACC in executive functions remains debated, with prominent theories suggesting a role in cognitive control, including error detection, conflict monitoring and adjustment, and/or task switching (Botvinick et al. 2004; Kerns et al. 2004; Ullsperger and von Cramon 2004; Carter and van Veen 2007; Hyafil et al. 2009; Cazalis et al. 2011). Activity in the ACC is also described in relation to changes in effort, complexity, or attention (Callicott et al. 1999; Owen 2000; Owen et al. 2005). Thus, in the present study, it is possible that compared with our control subjects, our participants with combat-related mTBI were not able to activate the ACC as much, due to deficits in conflict monitoring, attention, and complexity management, which may not have been sufficiently engaged by our neuropsychological testing.

MEG Source Imaging Approaches Differ From the Conventional Approaches

In the present study, we used several nonconventional approaches in analyzing the MEG N-back responses. First, instead of the conventional approach of trial-averaging for the MEG sensor waveforms (Hamalainen et al. 1993), we calculated the covariance matrix for each target trial and then averaged these single-trial based covariance matrices to form an overall sensor waveform covariance matrix. Such an approach is similar to the method we used in analyzing resting-state MEG signals in mTBI (Huang et al. 2012, 2017; Huang, Nichols, et al. 2014).

The conventional approach of trial-averaging the sensor waveform is designed to enhance the signal-to-noise ratio (SNR) of the neuronal responses that are time-locked to the stimuli. However, direct trial-averaging of the sensor waveform may cause signal cancellations among different trials for those neuronal responses that are not fully time-locked to the stimuli. We believe the cognitive components of the N-back responses are typically not fully time-locked to the stimuli (i.e., the onset of the letters). In fact, the N-back responses may be on-going since the brain needs to not only update, but also constantly maintain the WM information. Our single-trial-based covariance matrix approach is impervious to the signal cancellation across different trials. We believe this was the main reason that we can, for the first time, reveal differences in activation during WM in mTBI using MEG.

The application of Fast-VESTAL (Huang, Huang, et al. 2014) in the present study is another important factor that allows the detection of MEG WM deficits in mTBI. Fast-VESTAL can create high-resolution MEG sources images for 100% correlated sources, uncorrelated sources, and anywhere in between (Huang, Huang, et al. 2014). Our findings of WM abnormalities in combat-related mTBI further highlights the strength of this method.

Military Trainings and Blast Exposures

A strength of the present study was the use of a control group who had combat exposure without experiencing brain injuries. All military personnel undergo basic training, and attend infantry school for different lengths of time depending on their future occupational specialty. Thus, the control group and mTBI group would have had similar exposures to combat training and artillery training. We have recruited deployed military controls with similar training and experience who most adequately match on all levels of demographics. These controls did not report any TBI incidence nor postconcussive symptoms following any blast exposure. In future studies, we plan on incorporating the QCuBe measure to collect further detail on the blast exposure, including distance from blast and semi-quantification of the immediate symptoms following blast exposure (Petrie et al. 2014).

Additionally, all of these participants (mTBI and control) have had resting-state MEG exams. Injured brain tissues generate a pathological low-frequency neuronal magnetic signal at 1–4 Hz that can be measured by resting-state MEG. The automated MEG source imaging approach for localizing abnormal low-frequency slow-waves in mTBI participants was about 90% successful (Huang et al. 2012; Huang, Nichols, et al. 2014). For the participants in this study, each of the mTBI participants had abnormal low-frequency low waves during their resting state session, while the military controls did not have the pathological slow waves.

Limitations and Conclusions

There are several limitations of the present study that warrant consideration. First, the spatial resolution and localization accuracy of MEG are limited for subcortical areas. Second, although we preprocessed the MEG data through both MaxFilter and ICA to remove artifacts from the heartbeats, eye-movement, and eye-blinks, the impact of residual artifacts may not be totally negligible. Third, not excluding previous nonblast concussions such as sport-, fall- or other concussions was another limitation

of the present study. Despite these limitations, the present study using MEG source imaging technique revealed aberrant brain activations during performance of a WM task in individuals with combat-related mTBI. Compared with the controls, combat mTBI participants showed hyper activation in FP, anterior dlPFC, vmPFC and OFC areas, but hypoactivation in ACC. In addition, MEG hyperactivations in FP, OFC, and anterior dlPFC also positively correlated with longer RT during the WM tasks. Furthermore, the MEG activations from the lateral aspect of the FP area also negatively correlated with scores from neuropsychological exams of executive functioning and processing speed. To our knowledge, the abnormalities in FP have not been reported previously in fMRI WM studies. The profound MEG hyperactivations from FP and their association with cognitive sequelae suggest that this region may be particularly vulnerable to combat-related mTBI.

We believe that the main findings in the present study are due to blast-induced TBI. For example, to our knowledge hyperactivation in FP and anterior aspect of the dlPFC has not been reported in previous WM neuroimaging studies in blast-induced TBI. However, we cannot rule out the confounding factors of previous concussions unrelated to blast. Future MEG studies involving civilians with mTBI due to motor vehicle accidents, sport injuries, and falls plus civilian controls without blast exposure are needed to assess this issue. In the future we will also be assessing the aberrant functional connectivity in mTBI using MEG responses to the N-back stimuli, similar to our recent functional connectivity study in mTBI using resting-state MEG (Huang et al. 2017).

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

Funding

Supported in part by Merit Review Grants from the U.S. Department of Veterans Affairs to M.X. Huang (I01-CX000499, I01-RX001988, MHBA-010-14F, NURC-022-10F, NEUC-044-06S), R.R. Lee, D.L. Harrington (I01-CX000146), and The U.S. Naval Medical Research Center's Advanced Medical Development program (Naval Medical Logistics Command Contract #N62645-11-C-4037, for MRS-II (D.G. Baker, M.A. Geyer, M.X. Huang, V.B. Risbrough).

Notes

We acknowledge the MRS-II administrative core, Anjana Patel, Andrew De La Rosa, and members of the MRS-II Team, including logistic coordinators, clinician–interviewers, and data collection staff. We thank staff at the VA San Diego Healthcare System and the Veterans Medical Research Foundation (VRMF). We also thank the participating Marines and Veteran volunteers for their military service and participation in this study. Furthermore, we thank 3 anonymous reviewers for their constructive critiques and suggestions which substantially strengthened the paper. *Conflict of interest*: none declared.

References

Baddeley A. 1986. Working memory. New York: Oxford University Press.

Bailey NW, Rogasch NC, Hoy KE, Maller JJ, Segrave RA, Sullivan CM, Fitzgerald PB. 2017. Increased gamma connectivity during working memory retention following traumatic brain injury. Brain Inj. 31:379–389.

Bailey NW, Segrave RA, Hoy KE, Maller JJ, Fitzgerald PB. 2014. Impaired upper alpha synchronisation during working memory retention in depression and depression following traumatic brain injury. Biol Psychol. 99:115–124.

Bakdash JZ, Marusich LR. 2017. Repeated measures correlation. Front Psychol. 8:456.

Bigler ED. 1999. Neuroimaging in pediatric traumatic head injury: diagnostic considerations and relationships to neurobehavioral outcome. J Head Trauma Rehabil. 14:406–423.

Bigler ED. 2008. Neuropsychology and clinical neuroscience of persistent post-concussive syndrome. J Int Neuropsychol Soc. 14:1–22.

Bludau S, Eickhoff SB, Mohlberg H, Caspers S, Laird AR, Fox PT, Schleicher A, Zilles K, Amunts K. 2014. Cytoarchitecture, probability maps and functions of the human frontal pole. NeuroImage. 93(Pt 2):260–275.

Botvinick MM, Cohen JD, Carter CS. 2004. Conflict monitoring and anterior cingulate cortex: an update. Trends Cogn Sci. 8: 539–546.

Bryer EJ, Medaglia JD, Rostami S, Hillary FG. 2013. Neural recruitment after mild traumatic brain injury is task dependent: a meta-analysis. J Int Neuropsychol Soc. 19:751–762.

Callicott JH, Mattay VS, Bertolino A, Finn K, Coppola R, Frank JA, Goldberg TE, Weinberger DR. 1999. Physiological characteristics of capacity constraints in working memory as revealed by functional MRI. Cereb Cortex. 9:20–26.

Carter CS, van Veen V. 2007. Anterior cingulate cortex and conflict detection: an update of theory and data. Cogn Affect Behav Neurosci. 7:367–379.

Cazalis F, Babikian T, Giza C, Copeland S, Hovda D, Asarnow RF. 2011. Pivotal role of anterior cingulate cortex in working memory after traumatic brain injury in youth. Front Neurol. 1:158.

Cazalis F, Feydy A, Valabrègue R, Pélégrini-Issac M, Pierot L, Azouvi P. 2006. fMRI study of problem-solving after severe traumatic brain injury. Brain Inj. 20:1019–1028.

Chen JK, Johnston KM, Frey S, Petrides M, Worsley K, Ptito A. 2004. Functional abnormalities in symptomatic concussed athletes: an fMRI study. NeuroImage. 22:68–82.

Christodoulou C, DeLuca J, Ricker JH, Madigan NK, Bly BM, Lange G, Kalnin AJ, Liu WC, Steffener J, Diamond BJ, et al. 2001. Functional magnetic resonance imaging of working memory impairment after traumatic brain injury. J Neurol Neurosurg Psychiatry. 71:161–168.

Cohen D, Schlapfer U, Ahlfors S, Hamalainen M, Halgren E. 2002. New six-layer magnetically-shielded room for MEG. In: Nowak HHJ, Giebler F, editors. Proceedings of the 13th International Conference on Biomagnetism. Jena, Germany: VDE Verlag. p. 919–921.

Cooper DB, Bunner AE, Kennedy JE, Balldin V, Tate DF, Eapen BC, Jaramillo CA. 2015. Treatment of persistent post-concussive symptoms after mild traumatic brain injury: a systematic review of cognitive rehabilitation and behavioral health interventions in military service members and veterans. Brain Imaging Behav. 9:403–420.

Cusack R, Brett M, Osswald K. 2003. An evaluation of the use of magnetic field maps to undistort echo-planar images. NeuroImage. 18:127–142.

Delis DC, Kaplan E, Kramer JH. 2001. Delis-Kaplan Executive Function System. San Antonio, TX: The Psychological Corporation.

DePalma RG, Hoffman SW. 2018. Combat blast related traumatic brain injury (TBI): decade of recognition; promise of progress. Behav Brain Res. 340:102–105.

Dikmen SS, Ross BL, Machamer JE, Temkin NR. 1995. One year psychosocial outcome in head injury. J Int Neuropsychol Soc. 1:67–77.

El Sayed T, Mota A, Fraternali F, Ortiz M. 2008. Biomechanics of traumatic brain injury. Comput Methods Appl Mech Eng. 197:4692–4701.

Fischer BL, Parsons M, Durgerian S, Reece C, Mourany L, Lowe MJ, Beall EB, Koenig KA, Jones SE, Newsome MR, et al. 2014. Neural activation during response inhibition differentiates blast from mechanical causes of mild to moderate traumatic brain injury. J Neurotrauma. 31:169–179.

Gevins AS, Cutillo BC. 1993. Neuroelectricevidence for distributed processing in human working memory. Electroencephalogr Clin Neurophysiol. 87:128–143.

Gillis MM, Hampstead BM. 2015. A two-part preliminary investigation of encoding-related activation changes after moderate to severe traumatic brain injury: hyperactivation, repetition suppression, and the role of the prefrontal cortex. Brain Imaging Behav. 9:801–820.

Glahn DC, Ragland JD, Abramoff A, Barrett J, Laird AR, Bearden CE, Velligan DI. 2005. Beyond hypofrontality: a quantitative meta-analysis of functional neuroimaging studies of working memory in schizophrenia. Hum Brain Mapp. 25:60–69.

Grabner G, Janke AL, Budge MM, Smith D, Pruessner J, Collins DL. 2006. Symmetric atlasing and model based segmentation: an application to the hippocampus in older adults. Med Image Comput Comput Assist Interv. 9:58–66.

Graner J, Oakes TR, French LM, Riedy G. 2013. Functional MRI in the investigation of blast-related traumatic brain injury. Front Neurol. 4:16.

Hamalainen MS, Hari R, Ilmoniemi RJ, Knuutila J, Lounasmaa OV. 1993. Magnetoencephalography—theory, instrumentation, and applications to noninvasive studies of the working human brain. Rev Mod Phys. 65:413–497.

Hillary FG. 2008. Neuroimaging of working memory dysfunction and the dilemma with brain reorganization hypotheses. J Int Neuropsychol Soc. 14:526–534.

Hillary FG. 2011. Determining the nature of prefrontal cortex recruitment after traumatic brain injury: a response to Turner. Front Syst Neurosci. 5:24.

Hillary FG, Genova HM, Medaglia JD, Fitzpatrick NM, Chiou KS, Wardecker BM, Franklin RG, Wang J, DeLuca J. 2010. The nature of processing speed deficits in traumatic brain injury: is less brain more? Brain Imaging Behav. 4:141–154.

Huang M-X, Harrington DL, Robb Swan A, Angeles Quinto A, Nichols S, Drake A, Song T, Diwakar M, Huang CW, Risbrough VB, et al. 2017. Resting-state magnetoencephalography reveals different patterns of aberrant functional connectivity in combat-related mild traumatic brain injury. J Neurotrauma. 34:1412–1426.

Huang CW, Huang M-X, Ji Z, Swan AR, Angeles AM, Song T, Huang JW, Lee RR. 2016. High-resolution MEG source imaging approach to accurately localize Broca's area in patients with brain tumor or epilepsy. Clin Neurophysiol. 127:2308–2316.

Huang M-X, Huang CW, Robb A, Angeles A, Nichols SL, Baker DG, Song T, Harrington DL, Theilmann RJ, Srinivasan R, et al. 2014. MEG source imaging method using fast L1 minimum-norm and its applications to signals with brain noise and human resting-state source amplitude images. NeuroImage. 84:585–604.

Huang M-X, Nichols S, Baker DG, Robb A, Angeles A, Yurgil KA, Drake A, Levy M, Song T, McLay R, et al. 2014. Single-subject-based whole-brain MEG slow-wave imaging approach for detecting abnormality in patients with mild traumatic brain injury. NeuroImage Clin. 5:109–119.

Huang M-X, Nichols S, Robb A, Angeles A, Drake A, Holland M, Asmussen S, D'Andrea J, Chun W, Levy M, et al. 2012. An automatic MEG low-frequency source imaging approach for detecting injuries in mild and moderate TBI patients with blast and non-blast causes. NeuroImage. 61:1067–1082.

Huang MX, Song T, Hagler DJ Jr, Podgorny I, Jousmaki V, Cui L, Gaa K, Harrington DL, Dale AM, Lee RR, et al. 2007. A novel integrated MEG and EEG analysis method for dipolar sources. NeuroImage. 37:731–748.

Hutton C, Bork A, Josephs O, Deichmann R, Ashburner J, Turner R. 2002. Image distortion correction in fMRI: a quantitative evaluation. NeuroImage. 16:217–240.

Hyafil A, Summerfield C, Koechlin E. 2009. Two mechanisms for task switching in the prefrontal cortex. J Neurosci Off J Soc Neurosci. 29:5135–5142.

Hyvarinen A. 1999. Fast and robust fixed-point algorithms for independent component analysis. IEEE Trans Neural Netw. 10:626–634.

Hyvarinen A, Oja E. 2000. Independent component analysis: algorithms and applications. Neural Netw. 13:411–430.

Jovicich J, Czanner S, Greve D, Haley E, van der Kouwe A, Gollub R, Kennedy D, Schmitt F, Brown G, Macfall J, et al. 2006. Reliability in multi-site structural MRI studies: effects of gradient non-linearity correction on phantom and human data. NeuroImage. 30:436–443.

Kasahara M, Menon DK, Salmond CH, Outtrim JG, Tavares JV, Carpenter TA, Pickard JD, Sahakian BJ, Stamatakis EA. 2011. Traumatic brain injury alters the functional brain network mediating working memory. Brain Inj. 25:1170–1187.

Kerns JG, Cohen JD, MacDonald AW, Cho RY, Stenger VA, Carter CS. 2004. Anterior cingulate conflict monitoring and adjustments in control. Science. 303:1023–1026.

Leahy RM, Mosher JC, Spencer ME, Huang MX, Lewine JD. 1998. A study of dipole localization accuracy for MEG and EEG using a human skull phantom. Electroencephalogr Clin Neurophysiol. 107:159–173.

MacGregor AJ, Dougherty AL, Galarneau MR. 2011. Injury-specific correlates of combat-related traumatic brain injury in operation Iraqi freedom. J Head Trauma Rehabil. 26:312–318.

Manktelow AE, Menon DK, Sahakian BJ, Stamatakis EA. 2017. Working memory after traumatic brain injury: the neural basis of improved performance with methylphenidate. Front Behav Neurosci. 11:58.

McAllister TW, Flashman LA, McDonald BC, Saykin AJ. 2006. Mechanisms of working memory dysfunction after mild and moderate TBI: evidence from functional MRI and neurogenetics. J Neurotrauma. 23:1450–1467.

McAllister TW, Saykin AJ, Flashman LA, Sparling MB, Johnson SC, Guerin SJ, Mamourian AC, Weaver JB, Yanofsky N. 1999. Brain activation during working memory 1 month after mild traumatic brain injury: a functional MRI study. Neurology. 53:1300–1308.

McAllister TW, Sparling MB, Flashman LA, Guerin SJ, Mamourian AC, Saykin AJ. 2001. Differential working memory load effects after mild traumatic brain injury. NeuroImage. 14:1004–1012.

McDonald BC, Flashman LA, Saykin AJ. 2002. Executive dysfunction following traumatic brain injury: neural substrates and treatment strategies. NeuroRehabilitation. 17:333–344.

McInnes K, Friesen CL, MacKenzie DE, Westwood DA, Boe SG. 2017. Mild traumatic brain injury (mTBI) and chronic cognitive impairment: a scoping review. PLoS One. 12:e0174847.

McLean A Jr, Dikmen S, Temkin N, Wyler AR, Gale JL. 1984. Psychosocial functioning at 1 month after head injury. Neurosurgery. 14:393–399.

Medaglia JD, Chiou KS, Slocomb J, Fitzpatrick NM, Wardecker BM, Ramanathan D, Vesek J, Good DC, Hillary FG. 2012. The less BOLD, the wiser: support for the latent resource hypothesis after traumatic brain injury. Hum Brain Mapp. 33:979–993.

Medaglia JD, McAleavey AA, Rostami S, Slocomb J, Hillary FG. 2015. Modeling distinct imaging hemodynamics early after TBI: the relationship between signal amplitude and connectivity. Brain Imaging Behav. 9:285–301.

Mesulam MM. 1998. From sensation to cognition. Brain. 121(Pt 6):1013–1052.

Morissette SB, Woodward M, Kimbrel NA, Meyer EC, Kruse MI, Dolan S, Gulliver SB. 2011. Deployment-related TBI, persistent postconcussive symptoms, PTSD, and depression in OEF/OIF veterans. Rehabil Psychol. 56:340–350.

Mosher JC, Leahy RM, Lewis PS. 1999. EEG and MEG: forward solutions for inverse methods. IEEE Trans Biomed Eng. 46: 245–259.

Newsome MR, Durgerian S, Mourany L, Scheibel RS, Lowe MJ, Beall EB, Koenig KA, Parsons M, Troyanskaya M, Reece C, et al. 2015. Disruption of caudate working memory activation in chronic blast-related traumatic brain injury. NeuroImage Clin. 8:543–553.

Newsome MR, Scheibel RS, Hunter JV, Wang ZJ, Chu Z, Li X, Levin HS. 2007. Brain activation during working memory after traumatic brain injury in children. Neurocase. 13:16–24.

Newsome MR, Scheibel RS, Steinberg JL, Troyanskaya M, Sharma RG, Rauch RA, Li X, Levin HS. 2007. Working memory brain activation following severe traumatic brain injury. Cortex. 43:95–111.

Newsome MR, Steinberg JL, Scheibel RS, Troyanskaya M, Chu Z, Hanten G, Lu H, Lane S, Lin X, Hunter JV, et al. 2008. Effects of traumatic brain injury on working memory-related brain activation in adolescents. Neuropsychology. 22:419–425.

Niedermeyer E, Lopes da Silva FH. 2005. Electroencephalography: basic principles, clinical applications, and related fields. Philadelphia, Baltimore, New York, London, Buenos Aires, Hong Kong, Sydney, Tokyo: Lippincott Williams & Wilkins.

Nyberg L, Marklund P, Persson J, Cabeza R, Forkstam C, Petersson KM, Ingvar M. 2003. Common prefrontal activations during working memory, episodic memory, and semantic memory. Neuropsychologia. 41:371–377.

Owen AM. 2000. The role of the lateral frontal cortex in mnemonic processing: the contribution of functional neuroimaging. Exp Brain Res. 133:33–43.

Owen AM, McMillan KM, Laird AR, Bullmore E. 2005. N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. Hum Brain Mapp. 25:46–59.

Perlstein WM, Cole MA, Demery JA, Seignourel PJ, Dixit NK, Larson MJ, Briggs RW. 2004. Parametric manipulation of working memory load in traumatic brain injury: behavioral and neural correlates. J Int Neuropsychol Soc. 10:724–741.

Petrie EC, Cross DJ, Yarnykh VL, Richards T, Martin NM, Pagulayan K, Hoff D, Hart K, Mayer C, Tarabochia M, et al. 2014. Neuroimaging, behavioral, and psychological sequelae of repetitive combined blast/impact mild traumatic brain injury in Iraq and Afghanistan war veterans. J Neurotrauma. 31:425–436.

Phillips NL, Parry L, Mandalis A, Lah S. 2017. [Formula: see text] Working memory outcomes following traumatic brain injury in children: a systematic review with meta-analysis. Child Neuropsychol J Norm Abnorm Dev Child Adolesc. 23:26–66.

Robb Swan A, Nichols S, Drake A, Angeles A, Diwakar M, Song T, Lee RR, Huang M-X. 2015. Magnetoencephalography slow-wave detection in patients with mild traumatic brain injury and ongoing symptoms correlated with long-term neuropsychological outcome. J Neurotrauma. 32:1510–1521.

Sanchez-Carrion R, Fernandez-Espejo D, Junque C, Falcon C, Bargallo N, Roig T, Bernabeu M, Tormos JM, Vendrell P. 2008. A longitudinal fMRI study of working memory in severe TBI patients with diffuse axonal injury. NeuroImage. 43:421–429.

Scheibel RS, Pearson DA, Faria LP, Kotrla KJ, Aylward E, Bachevalier J, Levin HS. 2003. An fMRI study of executive functioning after severe diffuse TBI. Brain Inj. 17:919–930.

Schneiderman AI, Braver ER, Kang HK. 2008. Understanding sequelae of injury mechanisms and mild traumatic brain injury incurred during the conflicts in Iraq and Afghanistan: persistent postconcussive symptoms and posttraumatic stress disorder. Am J Epidemiol. 167:1446–1452.

Sinopoli KJ, Chen J-K, Wells G, Fait P, Ptito A, Taha T, Keightley M. 2014. Imaging "brain strain" in youth athletes with mild traumatic brain injury during dual-task performance. J Neurotrauma. 31:1843–1859.

Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, et al. 2004. Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage. 23(Suppl 1):S208–S219.

Smith EE, Jonides J. 1998. Neuroimaging analyses of human working memory. Proc Natl Acad Sci USA. 95:12061–12068.

Song T, Gaa K, Cui L, Feffer L, Lee RR, Huang M. 2008. Evaluation of signal space separation via simulation. Med Biol Eng Comput. 46:923–932.

Taulu S, Kajola M, Simola J. 2004. Suppression of interference and artifacts by the Signal Space Separation Method. Brain Topogr. 16:269–275.

Taulu S, Simola J, Kajola M. 2004. MEG recordings of DC fields using the signal space separation method (SSS). Neurol Clin Neurophysiol. 2004:35.

Teasdale G, Jennett B. 1974. Assessment of coma and impaired consciousness. A practical scale. Lancet. 2:81–84.

Terrio H, Brenner LA, Ivins BJ, Cho JM, Helmick K, Schwab K, Scally K, Bretthauer R, Warden D. 2009. Traumatic brain injury screening: preliminary findings in a US Army Brigade Combat Team. J Head Trauma Rehabil. 24:14–23.

The Management of Concussion/mTBI Working Group. 2009. VA/DoD clinical practice guideline for management of concussion/mild traumatic brain injury. J Rehabil Res Dev. 46:CP1–CP68.

Tombaugh TN. 1996. Test of memory malingering (TOMM). New York, NY: Multi-Health Systems, Inc.

Turner GR, McIntosh AR, Levine B. 2011. Prefrontal compensatory engagement in TBI is due to altered functional engagement of existing networks and not functional reorganization. Front Syst Neurosci. 5:9.

Ullsperger M, von Cramon DY. 2004. Neuroimaging of performance monitoring: error detection and beyond. Cortex J Devoted Study Nerv Syst Behav. 40:593–604.

Weathers FW, Ruscio AM, Keane TM. 1999. Psychometric properties of nine scoring rules for the clinican-administered posttraumatic stress disorder scale. Psychol Assess. 11:124–133.

Wechsler D. 1997. WAIS-III Wechsler adult intelligence scale. San Antonio, TX: The Psychological Corporation.

Wechsler D. 2008. WAIS-IV Wechsler adult intelligence scale. San Antonio, TX: The Psychological Corporation.

Wilde EA, Hunter JV, Newsome MR, Scheibel RS, Bigler ED, Johnson JL, Fearing MA, Cleavinger HB, Li X, Swank PR, et al.

2005. Frontal and temporal morphometric findings on MRI in children after moderate to severe traumatic brain injury. J Neurotrauma. 22:333–344.

Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, Beckmann C, Jenkinson M, Smith SM. 2009. Bayesian analysis of neuroimaging data in FSL. NeuroImage. 45:S173–S186.

Wylie GR, Freeman K, Thomas A, Shpaner M, OKeefe M, Watts R, Naylor MR. 2015. Cognitive improvement after mild traumatic brain injury measured with functional neuroimaging during the acute period. PLoS One. 10:e0126110.

Young LA, Rule GT, Bocchieri RT, Burns JM. 2015. Biophysical mechanisms of traumatic brain injuries. Semin Neurol. 35: 5–11.

Young L, Rule GT, Bocchieri RT, Walilko TJ, Burns JM, Ling G. 2015. When physics meets biology: low and high-velocity penetration, blunt impact, and blast injuries to the brain. Front Neurol. 6:89.