Evidence of disrupted functional connectivity in the brain after combat-related blast injury

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ABSTRACT

Non-impact blast-related mild traumatic brain injury (mTBI) appears to be present in soldiers returning from deployments to Afghanistan and Iraq. Although mTBI typically results in cognitive deficits that last less than a month, there is evidence that disrupted coordination of brain activity can persist for at least several months following injury (Thatcher et al., 1989, 2001). In the present study we examined whether neural communication may be affected in soldiers months after blast-related mTBI, and whether coordination of neural function is associated with underlying white matter integrity. The investigation included an application of a new time–frequency based method for measuring electroencephalogram (EEG) phase synchronization (Aviyente et al., 2010) as well as fractional anisotropy measures of axonal tracts derived from diffusion tensor imaging (DTI). Nine soldiers who incurred a blast-related mTBI during deployments to Afghanistan or Iraq were compared with eight demographically similar control subjects. Despite an absence of cognitive deficits, the blast-related mTBI group exhibited diminished EEG phase synchrony of lateral frontal sites with contralateral frontal brain regions suggesting diminished interhemispheric coordination of brain activity as a result of blast injury. For blast injured (i.e., blast-related mTBI) soldiers we found that EEG phase synchrony was associated with the structural integrity of white matter tracts of the frontal lobe (left anterior thalamic radiations and the forceps minor including the anterior corpus callosum). Analyses revealed that diminished EEG phase synchrony was not the consequence of combat-stress symptoms (e.g., post-traumatic stress and depression) and commonly prescribed medications. Results provide evidence for poor coordination of frontal neural function after blast injury that may be the consequence of damaged anterior white matter tracts.

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Introduction

Injury from explosive blast is a prominent feature of contemporary combat (DePalma et al., 2005). Although protective armor and acute medical intervention allow soldiers to survive explosions, a growing number of veterans will have disability stemming from blast-related brain damage (Warden, 2006). Estimates of the prevalence of blast-induced brain injury vary (Carlson et al., 2010) but a study employing clinician assessments and informant reports indicated that about a fifth of individuals in a Brigade Combat Team sustained traumatic brain injuries due to blast during a 1-year deployment to the Iraq War (Terrio et al., 2009). To date, few studies have systematically investigated the effects of blast injury on brain function and the well-being of soldiers (Ling et al., 2009; Taber et al., 2006). There is evidence that survivors of blasts on the battlefield have mostly incurred mild traumatic brain injury (mTBI) (Belanger et al., 2009; Hoge et al., 2008; McCrea et al., 2008; Schneiderman et al., 2008). The predominance of mTBI in returning soldiers from wars may be due to the fatal nature of explosive blasts with more powerful pressure waves (e.g., 56 to 76 psi) that hemorrhage air-filled organs (e.g., lung) and cause death (see DePalma et al., 2005; Mayorga, 1997; Wolf et al., 2009 for reviews). Thus, there is a limit to the wave strength that impacts the brain in soldiers who survive the explosion. Recent modeling of explosive blasts has provided evidence that a pressure wave resulting in 50% mortality due to damage to the lung results in head injury approximately equivalent to that of an mTBI associated with sports concussion (Moore et al., 2009). In the present study we sought to use quantitative analyses of resting state electroencephalograms (EEGs) to characterize the effects of explosive blast on soldiers who were deployed to conflicts in Afghanistan and Iraq. Because

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diffuse axonal injury and the resulting disruption in neural communication are thought to be primary effects of blast-related mTBI (Taber et al., 2006), we were specifically interested in whether deficient functional synchronization of EEG activity was evident in soldiers who had experienced explosive blasts perhaps reflecting compromised connective fibers that support communication between brain regions.

mTBI (i.e., concussion) typically results in cognitive deficits immediately after the brain injury (Barr and McCrea, 2001); however impairment appears to be short-lived and nearly undetectable a month post injury (see McCrea et al., 2009 for a review). A recent systematic and detailed analysis concluded that mTBI resulted in essentially no cognitive impairment on neuropsychological indices 3 months after injury (Belanger et al., 2005). Yet, it is an open question whether mTBI due to explosive blast is unique and results in behavioral, cognitive, and emotional disruption that is distinct from other mTBIs (e.g., sports concussion). An initial study has provided evidence that the cognitive sequelae of blast injury is essentially the same as that of other mTBIs, and that what is most predictive of longer terms cognitive deficits from brain injury is injury severity and not mechanism of injury (Belanger et al., 2009). Because the brain can adapt and compensate for damage, and assessment of cognitive functions is largely determined through behavior, neuropsychological evaluation may fail to capture phenomena that characterize neural damage. In an initial effort to identify the effects of blast-related mTBI we applied direct measures of neural function and structure to a sample of veterans of combat in Iraq and Afghanistan who were injured by explosive blast.

To date there have been only a handful of studies specifically examining the effects of explosive blast on the human brain. Clinical EEG readings of blast injured individuals have been found to be similar to those of individuals who incurred other forms of closed-head injury (Cramer et al., 1949; Fabing, 1947). A more recent EEG study of older veterans who reported blast-exposure predominantly as part of World War II and conflicts in Korea and Vietnam revealed higher scores on a EEG-based discriminant index designed for identification of mTBI (Trudeau et al., 1998). The discriminant index was defined most strongly by abnormalities in EEG phase coherence over frontal brain regions consistent with axonal injury in these areas (Thatcher et al., 1989). Studies of animals have revealed blast-related cognitive dysfunction (Cernak et al., 2001) and depressed EEG signals (Axelsson et al., 2000). Post-mortem and experimental animal studies document white matter hemorrhages, degeneration of nissl bodies, microglial activation, and diffuse axonal injury after exposure to explosive blasts (Kaur et al., 1995, 1997a,b; Svetlov et al., 2009). The evidence suggests that diffuse injury to axons results from high velocity impact of any origin and involves widespread damage to the brainstem, cerebellum, corpus callosum, and parasagittal white matter of the cortex (Meythaler et al., 2001). But the most direct effects of blast injury on the connective fibers of the brain are thought to occur in the corpus callosum, the corticomedullary junction, and frontotemporal areas (Taber et al., 2006) and thus may affect interhemispheric neural communication involving frontal brain regions. Recent advances in computational techniques for analysis of functional magnetic resonance imaging data, magneto-encephalography, and electroencephalographic data have allowed examination of the dynamic synchronization of neural activity across brain regions (Bullmore and Sporns, 2009; Georgopoulos et al., 2007). The functional consequence of blast-related disruption of connective fibers in the brain may well be expressed in terms of poorly coordinated activity across neural structures that is measured through examining the phase synchronization of neural signals (Aviyente et al., 2010; Lachaux et al., 1999).

Individuals who have suffered a mTBI by any mechanism are often found to have normal generalized neuropsychological function and clinical EEGs (Nuwer et al., 2005); however quantitative analysis of EEG data has yielded indices of frequency power, synchrony between brain regions, and asymmetry that can discriminate individuals with mTBI from individuals without brain trauma months after injury (Thatcher et al., 1989). mTBI has also been associated with diminished and delayed event-related potentials in the EEG (Gaetz et al., 2000; Gaetz and Weinberg, 2000; Solbakk et al., 2005) and anomalous time–frequency EEG profiles (Slobounov et al., 2002). Therefore, it appears that direct measures of neural function may be sensitive to the effects of mTBI while behavioral measures of cognitive performance may fail to capture altered brain function due to the injury.

Because of prior evidence for disruption of synchronization in frontal brain activity following mTBI (Thatcher et al., 1989, 2001) we examined whether frontal interhemispheric neural communication may be affected in subjects with mTBI after blast injury and whether interhemispheric coordination of neural function was associated with underlying white matter integrity. To this end, we collected resting state EEG and diffusion tensor imaging data from 9 veterans with blast-related mTBI and 8 demographically similar control subjects. The similarity in the phase of EEG signals from distant electrodes was analyzed as an index of functional synchronization, while fractional anisotropy (FA) of white matter tracts was analyzed as a measure of structural connectivity (i.e., white matter integrity). Because the frontal temporal brain regions appear vulnerable to the effects of blast injury (Taber et al., 2006), we examined interhemispheric phase synchrony of fronto-temporal scalp electrodes (i.e., sites F7 and F8) with several contralateral electrodes. Unique aspects of the analysis included the first systematic application of a new time–frequency based method for measuring phase synchronization (Aviyente et al., 2010) and examination of the relationship between the functional synchronization and structural connectivity of the human brain after exposure to explosive blast.

Method

Participant Characteristics

Nine individuals who had been exposed to explosive blasts during military deployments to Afghanistan or Iraq were compared with 8 healthy controls of similar age and gender. Individuals in the blast injured group were recruited from the Traumatic Brain Injury/Polytrauma clinic at the Veterans Affairs Medical Center in Minneapolis, Minnesota. Recruitment letters were sent to individuals who had recently been referred through the clinic for neuropsychological evaluations. Participants from the control group were recruited through internet advertisements (e.g., Craig's List) and were not necessarily veterans and had not been deployed. Interested individuals were screened through a telephone interview prior to enrollment. Exclusion criteria for all subjects were contraindications to MRI scan, age under 18 or over 60, current medical-condition-related or substance-induced psychotic disorder, alcohol or substance abuse in the past month, severe neurological or psychiatric illness, significant risk of homicide or suicide, current major depressive episode, and an unstable medical condition affecting brain function. Controls were additionally excluded if they had a history of an affective disorder, alcohol or substance dependence, any use of psychotropic medication, moderate to severe brain injury, or concussions with reported effects lasting over 24 h. All subjects were male and Caucasian. Please refer to Table 1 for a summary of participant characteristics and supplemental materials for additional details. The Institutional Review Boards for the Minneapolis VA Medical Center and University of Minnesota approved the study protocol and determined that the investigational procedures were in compliance with the Health Insurance Portability and Accountability Act (HIPAA).

Background and blast-related information was obtained through questionnaires and a review of medical records. Table 2 includes descriptions of the most significant and clearly described explosive

Table 1
Characteristics of participants.

Variable	Blast group N = 9 Mean (SD)	Controls N = 8 Mean (SD)	t-Test Statistic (df)	p Value
Age (years)	33.7 (7.67)	30.3 (8.00)	0.90 (15)	n.s.
Percent male	100	100	N/A	N/A
Handedness (R/L)	7/2	7/1	N/A	N/A
Years of education	13.7 (1.00)	14.6 (1.92)	1.27 (15)	n.s.
Estimated IQ[a]	112 (13.70)	115 (13.89)	0.49 (14)	n.s.
NSI total score	31.1 (16.59)	5.9 (8.08)	4.06 (15)	0.002
PCL-C total score	42.7 (17.49)	21.1 (4.02)	3.59 (15)	.006
BDI-II total score	14.2 (9.78)	2.3 (2.61)	3.53 (15)	.006

Note. SD = Standard Deviation. IQ = Intelligence Quotient. Estimated IQ was derived from the tables in Jeyakumar et al. (2004) using Vocabulary and Block Design subtests. NSI = Neurobehavioral Symptom Inventory. PCL-C = Posttraumatic Stress Disorder Checklist-Civilian version. BDI-II = Beck Depression Inventory, second edition. Analyses were adjusted for unequal variances when indicated.
[a] Analyses completed on only 8 participants in the blast group.

blasts experienced by subjects. Subjects were classified as blast-injured through reference to the Diagnostic criteria for Mild TBI by the American Congress of Rehabilitation Medicine Special Interest Group on Mild Traumatic Brain Injury and the concussion grading system by the American Academy of Neurology (both reported in Ruff et al., 2009) All blast injured subjects were exposed to blasts that threw them, significantly damaged or threw heavy military vehicles they were riding in, or injured the occupants of the vehicle. All individuals in the blast group had been deployed to either Iraq or Afghanistan and had been exposed to at least one blast explosion. Four individuals were exposed to greater than one explosion (ranging from 2 to 40). Four individuals also reported receiving blows to the head as the result of either their person or their vehicle being displaced by the blast. Estimated mean number of months since the blast injury was 32.7 (SD = 9.26).

Subjects completed a battery of neuropsychological tests designed to assess memory and executive functions. The age-adjusted scaled score of WAIS-III Vocabulary and WAIS-III Block Design also were used to calculate an estimated IQ score. Please see supplemental information for a detailed description of the neuropsychological assessment. Research staff were not blind to group membership of subjects. Subjects also completed questionnaires for the assessment of symptoms following mild brain injury (Neurobehavioral Symptom Inventory [NSI]; Cicerone and Kalmar, 1995), current depressive symptomatology (Beck Depression Inventory-Second Edition [BDI-II]; Beck et al., 1996), and post-traumatic stress symptomatology (Post-traumatic Stress Disorder Checklist-Civilian version [PCL-C]; Weathers et al., 1991).

Electrophysiological data collection and preprocessing

Electroencephalograms (EEG) were recorded using a 64-channel Biosemi Active Two EEG system (http://www.biosemi.com) while subjects were at rest with eyes closed. Electrodes were embedded in an elastic cap and placed on the head to conform to 10-10 nomenclature. Vertical electro-oculograms (VEOG) recorded from above and below the right eye and horizontal electro-oculograms (HEOG) recorded from outer ocular canthi were used to measure eye-movements. EEG signals were digitized at a rate of 512 Hz with 0.5 Hz low frequency and a 60-Hz notch filters. Following data collection, recordings from scalp sites were re-referenced to linked earlobes. Segments of EEG with obvious non-neurogenic signals were deleted after visual inspection aided by amplitude threshold criterion applied to each electrode signal. Brief time periods of non-neurogenic noise in one or two electrodes were interpolated from adjacent electrode signals using spherical spline interpolation (Perrin et al., 1989). Independent component analysis (ICA) using FastICA algorithm (Hyvarinen and Oja, 2000) was then applied to eliminate artifacts from eye-movements, cardiac and muscle

Table 2
Descriptions of blast events and subsequent post-concussive symptoms.

Subject	Blast event	Reported symptoms	LOC	PTA
1	A rocket propelled grenade exploded 20 yards away and the blast wave threw the subject against a barrier.	Disorientation (several seconds), momentarily dazed.	None	None
2	IED detonated on passenger side while subject was driving a truck. Vehicle was armored and engulfed in a ball of flames. Penetrating shrapnel in extremities.	Ongoing tinnitus, described by others as confused for several minutes.	None	None
3	1) Mortar round detonated 10 feet away from subject behind a barrier. Felt pressure wave and was stunned.	(1) Tinnitus, sensitivity to light and noise.	(1) None	(1) Possibly 1 min
	2) IED detonated approximately 16 meters away and the vehicle was thrown up onto two wheels.	(2) Tinnitus	(2) None	(2) None
4	Subject was a passenger in an 8-ton armored vehicle that ran over a land mine and was thrown into the air.	Headache, tinnitus, nauseous for 24 to 36 h, blurred vision, tingling in legs, poor coordination for 3 h.	Yes, for unknown period	None
5	Subject was a gunner in a Humvee that was hit by an IED detonation followed by a mortar blast. Blast wave forced his body against the inner walls of the vehicle.	Headache, tinnitus, blurred vision, impaired concentration.	None	Yes, for unknown period
6	Subject was a gunner in a Humvee that swerved when an IED was detonated. Shrapnel was lodged in his helmet and sunglasses. Subject was thrown around in the turret.	Tinnitus, slightly stunned, medic said he was okay to return to duty.	None	None
7	Subject was in vehicle that drove over a mine in order to detonate it. Blast propelled the subject upward in the vehicle.	Headache, tinnitus.	5 s	None
8	Subject was in vehicle that was hit by an IED. Others in the vehicle were injured.	Headache, dazed for several minutes, tinnitus for a few days, hearing loss on right side.	Unknown	None
9	Subject was in a vehicle that was attacked. 28 IED's were detonated within 2 ft of the vehicle over a 3-h period.	Headache, dazed and confused for 15 min.	None	None

LOC—loss of consciousness; PTA—post-traumatic amnesia; IED—improvised explosive device; Humvee—High Mobility Multipurpose Wheeled Vehicle.

activity, and other non-neurogenic sources. Prior to the ICA, principal component analysis (PCA) was applied to the covariance matrix of EEG signals to reduce the number of independent components (ICs). Principal components were retained to account for more than 99.5% of the variance in the original data. ICA was then applied to the reconstituted signals and all resulting ICs were carefully inspected in terms of their topography, frequency power spectrum, and time course. ICs showing

high temporal correlations with VEOG or HEOG signals and typical topography of high frontal polar activity were identified as eye-movement artifacts. ICs characterized by pattern of periodic deflections in the component time series, persistent activity throughout the recordings, low-frequency (<3 Hz) peak power spectrum, and a unilateral or bilateral posterior topography were identified as cardiac artifacts. Muscle artifacts were characterized by spectra with broad peak from 20 to greater than 60 Hz, topography showing prominent activity restricted to marginal electrodes close to facial muscles, and periods of high frequency activations (McMenamin et al., 2010). Brief periods of muscle artifact were removed (i.e., less than 1/5 of the entire time-series), while longer periods of muscle artifact characterized by ICs were removed through elimination of the IC from the reconstituted signal. After preprocessing with ICA and artifact removal, EEG recordings were reconstituted from the remaining ICs, subsampled to 128 Hz, and segmented into 4-s epochs with 50% of adjacent segments.

EEG phase synchrony analysis

Time-varying complex energy time frequency distribution (TFD) of all the epoched EEG signals were obtained using a recently introduced method employing the reduced interference distribution (RID) Rihaczek distribution (Aviyente et al., 2010). The RID-Rihaczek distribution computes a complex TFD with uniform time–frequency resolution, avoiding the trade-off between time and frequency resolution inherent to wavelet analysis. In the present analyses we defined a high resolution complex TFD of all epochs of the EEG signals, the phase spectrum of the signals and the phase differences between two electrode signals were computed. To avoid spurious phase synchrony between scalp electrodes due to volume conduction (Srinivasan et al., 2007), only electrodes more than 12 cm apart were selected and used for phase synchrony estimation. Phase-synchrony was computed using a phase locking value (PLV) (Lachaux et al., 1999) which represented the average difference in phase-synchrony between a pair of electrodes across epochs. The PLV is normalized so that values near 1 indicate highly similar phase between electrodes across trials and values near 0 indicate almost entirely unrelated phase between electrodes across trials. Thus, PLV was used to quantify the synchrony between EEG signals of distant electrode pairs independently for each time–frequency point on the TFD. To compute EEG phase synchrony for various frequency bands,

PLVs in delta (1–3 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta-1 (13–20 Hz), beta-2 (21–30 Hz), and gamma (31–64 Hz) ranges were averaged. To compare the average PLVs between subjects with mTBI and control subjects, non-parametric Wilcoxon ranksum tests were conducted for the phase synchrony indices of pairs of distant electrodes in all frequency bands.

DTI acquisition and processing

Diffusion weighted images were acquired on a 1.5T Philips Achieva (Andover, MA, USA) scanner using a multichannel head coil. Head movement was minimized by placing cushions around the partici-pant's head. A 3-plane localizer was used for orientation and prescription of 3D scans. Diffusion weighted images were collected with a single-shot spin echo planar diffusion sequence of 55 axial slices covering the entire cerebrum and as much of the cerebellum as possible. Thirty-three images were collected at each slice location, 32 of which had diffusion gradients applied in non-collinear direction with $b = 800$ s/mm^2 msocom2 and one with no diffusion gradient ($b = 0$). Additional acquisition parameters for the diffusion sequence were: TR = 7200 ms, TE = 80 ms, Flip Angle = 90 deg. Acquisition voxel size was $2.14 \times 2.14 \times 2.5$ mm^3.

The diffusion weighted images were corrected for eddy current distortions, and fractional anisotropy (FA) values were calculated at each brain voxel using FSL software (Smith et al., 2004) Each FA map was nonlinearly registered to a standard FA image (Mori et al., 2005), and the inverse of this transformation was used to register regions of interest (ROIs) defined in that standard space (Hua et al., 2008; McCrea et al., 2009) back to each subject's space. Four of these ROIs were chosen for the current analysis: forceps major (posterior corpus callosum), forceps minor (anterior corpus callosum), left anterior thalamic radiations, and right anterior thalamic radiations. These regions are shown in Fig. 3. FA values of voxels contained within each ROI were averaged.

Results

Clinical and cognitive characteristics of blast injured and control samples

Blast and control groups were of similar age, gender composition, handedness, educational backgrounds, and intelligence (see Table 1). The
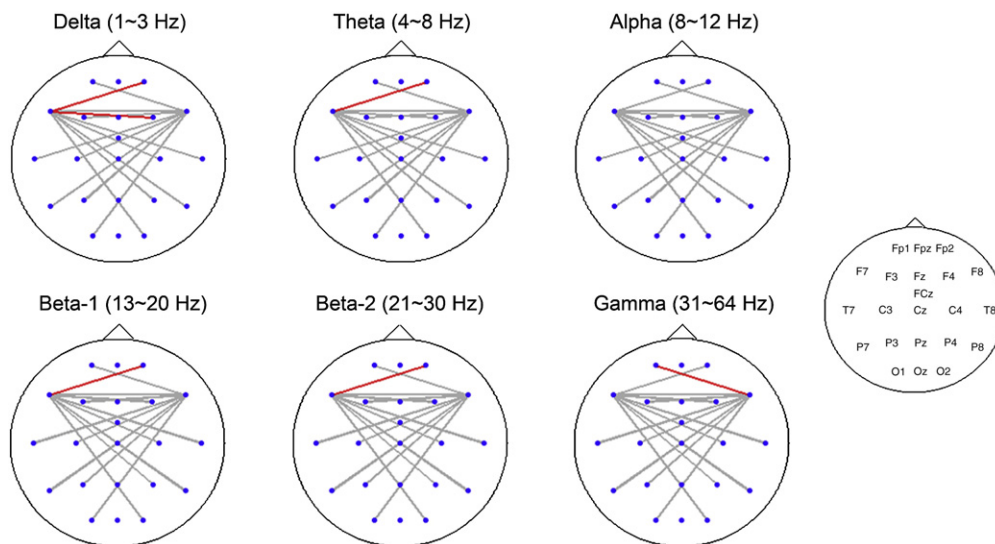

Fig. 1. Topographical representation of EEG phase synchrony results for comparisons of blast-related mTBI and control groups. Phase synchrony was computed for 15 interhemispheric electrode pairings (gray and red lines) involving lateral frontal scalp electrodes (i.e., F7 and F8) in six frequency bands. Red lines indicate a tendency for blast-related mTBI subjects to show reduced phase synchrony between sites (*p* < 0.05).
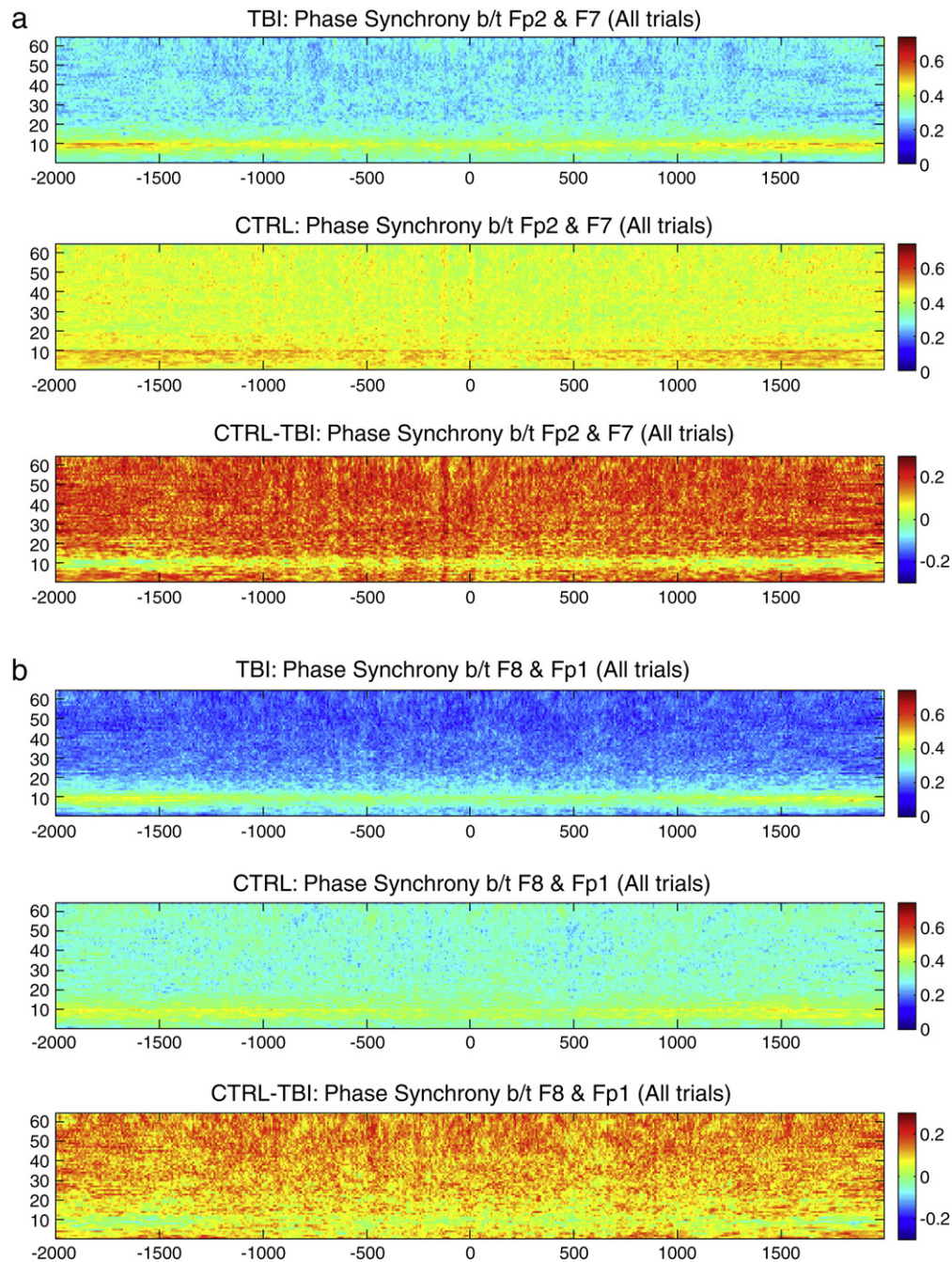
Fig. 2. Average EEG phase synchrony surfaces for blast-related mTBI (TBI) and control groups for 4-s resting state epochs (i.e., trials). *x*-Axis is time in milliseconds with "0" representing the midpoint of the epoch, *y*-axis is frequency (Hz), colors represent between electrode phase synchrony values or their difference (CTRL-TBI). (a) Phase synchrony between the left lateral frontal (F7) and right orbital frontal (Fp2) regions, and (b) the same for right lateral frontal (F8) and left orbital frontal (Fp1) regions. Note diminished phase synchrony in the blast-injured mTBI group for most frequencies with the exception of the alpha band (10 Hz).

blast exposed group had elevated levels of current postconcussive symptoms as measured by the NSI, moderate levels of post-traumatic stress symptomatology but on average below the commonly used PCL-C cut-off for a diagnosis of post-traumatic stress disorder, and mild levels of depressive symptomatology on the BDI-II. The control group had lower scores than the blast injured group on the NSI, PCL-C, and BDI-II. Analysis of cognitive functioning in the blast injured group using a repeated-measures ANOVA with group (blast injured, control) as between subjects factor and neuropsychological index as a within subject factor of primary neuropsychological indices (see Supplemental Table 1 for listing) failed to reveal a group main effect ($F_{1,13} = 0.03$, ns) or an interaction of group

and index ($F_{16,208} = 0.51$, ns). Exploratory univariate analyses also failed to reveal differences between the blast injured and control groups on any neuropsychological index, consistent with mTBI due to blast injury not resulting in enduring measurable cognitive dysfunction.

Between region synchronization of functional brain activity: EEG phase locking

Preliminary analyses of group differences in EEG phase synchrony between distant electrodes revealed diminished phase locking in the blast injured group over anterior portions of the brain and only between

regions of opposite cerebral hemispheres. The two most affected sites resided over homologous regions of the lateral prefrontal cortex (F7, F8). To more fully examine the pattern of diminished EEG synchronization involving the lateral prefrontal electrode sites we tested the hypothesis that blast injury caused inter-hemispheric communication dysfunction involving lateral frontal cortex by specifically testing for group effects in phase locking between each lateral frontal site (F7, F8) and eight electrodes distributed across the opposite cerebral hemisphere. Compared to the control group, the blast injured group showed a tendency toward reduced interhemispheric EEG phase synchrony between frontal sites (see Fig. 1). Blast injured individuals showed diminished EEG synchrony between the left lateral frontal electrode site (F7) and the right orbital frontal electrode (Fp2) for several frequency bands (Cohen's d used to represent effect size) (delta: $p<.05$, $d=1.19$; theta: $p<.05$, $d=1.07$; beta-1: $p<.05$, $d=1.06$; beta-2: $p<.05$, $d=1.20$), and right lateral electrode (F4) in delta ($p<.05$, $d=1.00$). The blast injured group also had diminished reduced EEG synchrony between the right lateral frontal electrode (F8) and the left orbital frontal electrode (Fp1) for gamma frequencies ($p<.05$, $d=.92$). Fig. 2 depicts the phase synchrony surfaces for locking values for the blast mTBI and control groups between F7 and Fp2 and between F8 and Fp1. Although the pattern of group differences generally replicated across electrodes, frequency bands, and both lateral frontal electrode sites, the probability of Type I error cannot be ignored given group comparisons over 15 electrode pairs and 6 frequency bands, thus findings suggest only a tendency toward interhemispheric communication reduction in subjects with blast-related mTBI.

Poor synchronization of brain function after blast and white matter structures: association between EEG phase locking and DTI fractional anisotropy (FA)

To test whether the interhemispheric frontal phase synchrony decrement noted in the blast injured group was associated with the integrity of white matter structures thought to be affected in brain injury, we computed Pearson correlations for the frontal interhemispheric functional connectivity EEG indices with the mean fractional anisotropy (FA) of four major white matter tracts related to frontal interhemispheric communication: forceps major, forceps minor, and the right and left anterior thalamic radiations. Fig. 3 depicts the tracts characterized in the analysis. To control for multiple comparisons the criterion p-value was set at .0028. FA of the forceps minor in blast injured subjects was associated with beta-2 frequency phase synchrony between F7 and Fp2 electrode pairs ($r=0.91$, $p=0.002$). FA of the left anterior thalamic radiations in blast injured subjects was also associated with beta-2 frequency phase synchrony between F7 and F4 ($r=0.90$, $p=0.003$) and between F8 and F3 ($r=0.92$, $p=0.001$), as well as for gamma frequency phase synchrony between F8 and F3 ($r=0.91$, $p=0.002$). There were no associations between EEG synchronization indices and measures of integrity of frontal interhemispheric white matter tracts in the control group. Fig. 4 presents scattergrams depicting the four significant associations in the blast injured group and the absence of associations in the control group. The pattern of neural function–structure associations in the blast injured group provides evidence for poor functional synchronization of frontal brain regions after blast-related mTBI being attributable to the structural integrity of white matter tracts serving the frontal lobes. Blast injured and control groups failed to differ in the fractional anisotropy for the four tracts of interest (all t's<1.44 and all p's>0.2) suggesting that although explosive blast may compromise white matter tracts, the disruption is more sensitively detected through the use of EEG phase synchrony measures.

EEG phase locking and demographic, clinical and cognitive characteristics

We conducted a set of statistical tests to determine whether diminished inter-hemispheric functional synchrony was associated
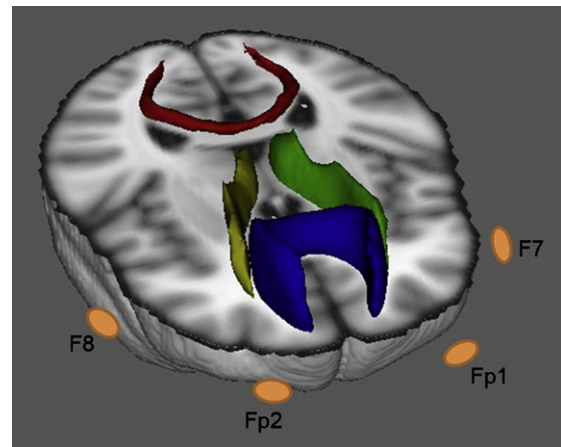

Fig. 3. Four white matter tract regions of interest (ROIs) were chosen for the current analysis: forceps major (red), forceps minor (blue), left anterior thalamic radiations (green), and right anterior thalamic radiations (yellow). Approximate positions for electrodes at F8, Fp2, Fp1, and F7 that demonstrated decreased EEG phase synchronization in the blast-related mTBI group are shown relative to tract-based ROIs. Only the three anterior tract ROIs and not the forceps major were associated with decreased EEG phase synchrony in blast-related mTBI soldiers.

with the demographic, clinical or cognitive status of subjects. Supplemental materials detail these ancillary analyses. EEG phase synchrony measures failed to be associated with age and the NSI, BDI-II, and PCL-C suggesting that self-reports on these symptom questionnaires do not reflect direct neural consequences of mTBI due to blast injury. Within the blast injured group a clinician-based diagnosis of post-traumatic stress disorder (PTSD) and prescribed antidepressant or sleep medication were associated with only greater EEG phase synchrony in select bands and frequencies that were different from those associated with blast mTBI, thus demonstrating that psychopathology or treatment did not result in the blast-related decrement in EEG phase synchrony. Similarly, diminished EEG phase synchrony was not associated with poorer neuropsychological performance. In addition to cognitive recovery typically occurring within a month of mTBI, it is possible that because EEG data were gathered during an eye's closed resting state the identified phase synchrony abnormalities are associated with a tonic brain state and are unrelated to active brain states elicited by neuropsychological tasks.

Conclusions

In the present study we applied a novel time–frequency based method (Aviyente et al., 2010) for characterizing synchronization of EEG signals gathered from blast injured soldiers and demographically similar control subjects. Despite an absence of deficits on neuropsychological indices, the blast injured group exhibited diminished EEG phase synchrony of lateral frontal sites with contralateral frontal brain regions suggesting diminished interhemispheric coordination of brain activity as a result of blast injury. For soldiers who had mild TBI as a result of explosive blasts we found that the EEG phase synchrony of lateral frontal electrodes with frontal regions of the opposite cerebral hemisphere was associated with measures of white matter structural integrity (fractional anisotropy) of frontal white matter tracts (forceps minor and anterior thalamic radiation) but not an inter-hemispheric white matter tract in posterior brain regions (forceps major). Thus, the present analysis yielded evidence for frontal axonal tract structural integrity correlating with poor synchronization of neural function after blast injury.
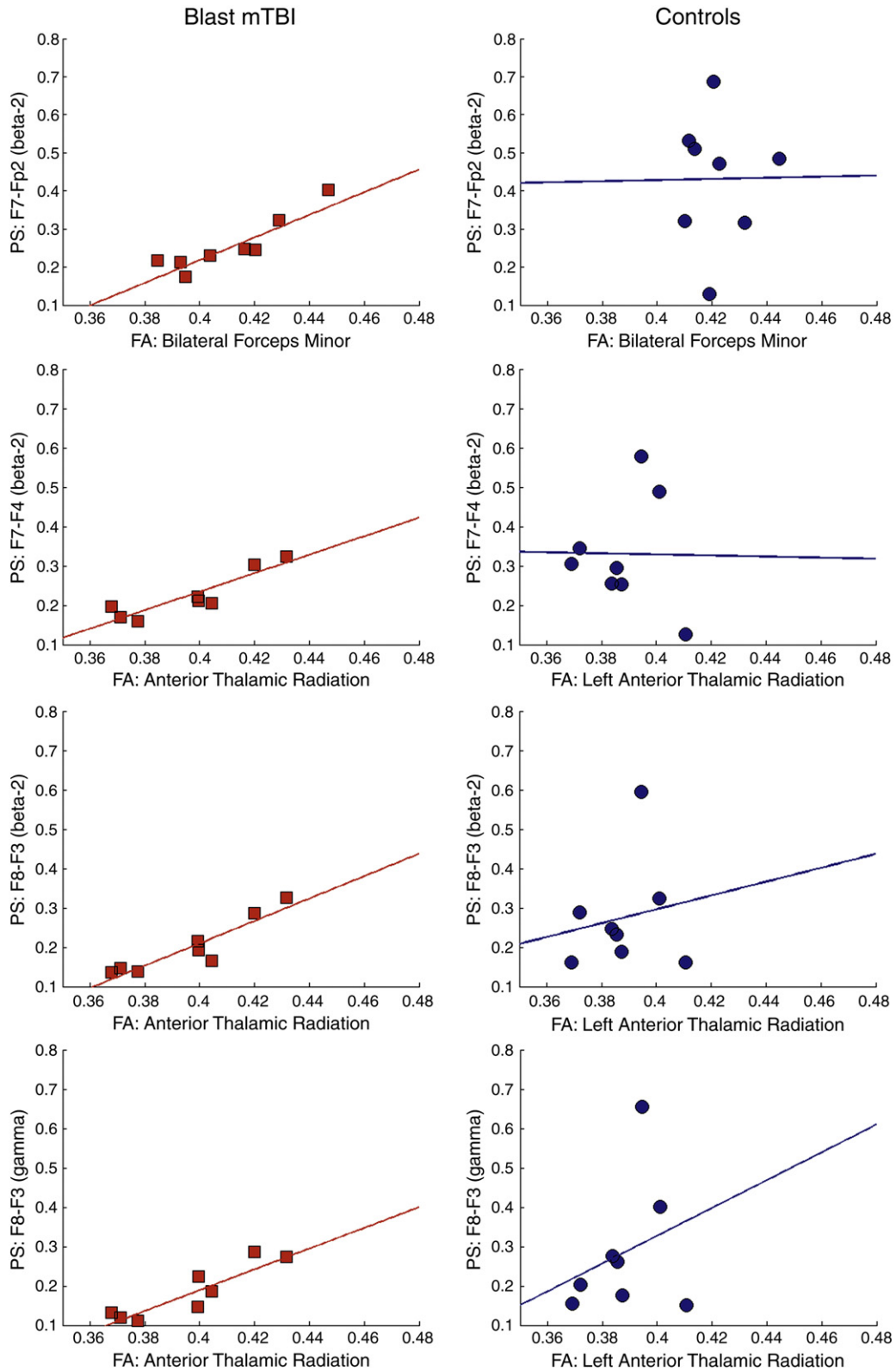
Fig. 4. Fractional anisotropy (FA) of the forceps minor and the left anterior thalamic radiations derived from diffusion tensor imaging (DTI) contrasted with the EEG phase synchrony (PS) between lateral frontal electrode sites (F7, F8) and opposite hemisphere frontal electrodes for beta and gamma frequency bands.

Absent from the findings was any indication of cognitive impairment in the blast-injured group. Studies suggest any cognitive impairment immediately after mTBI is brief and undetectable a month post injury. Because subjects had received only a mild TBI and it had occurred many months prior to study participation, an absence of cognitive impairment is expected. The mechanism by which cognitive functioning recovers after mTBI is unclear. Because the brain is an adaptive organ with dynamic interactions between neural structures it is reasonable to suppose that with damage the brain adopts an alternative neural pathway to complete a cognitive function. Thus, it is

possible that in the studied sample there is an absence of functional impairment despite aberrant EEG phase synchrony than is associated with frontal white matter tract integrity.

Because this investigation focused on explosive blasts that injured soldiers in combat actions there are additional consequences to deployment and the blast events that can confound clear description of the effect of explosions on brain structure and function. Analyses testing possible confounds of diminished EEG phase synchronization in blast injured soldiers failed to yield evidence that factors other than blast mTBI explained the effect. EEG phase synchrony values were uncorrelated with self-reported PTSD and depression symptoms, and a formal diagnosis of PTSD was associated with greater phase synchrony for frequency bands and nonfrontal sites unrelated to phase synchrony effects associated with blast mTBI (see supplemental material). Thus, diminished phase synchrony in blast injured subjects could not be attributable to symptomatology indicative of mental disorders; however, the use of a behaviorally matched control group for PTSD and depression symptoms would better allow establishment of differences due to blast TBI. Prescription of antidepressants and sleep medications were associated with a tendency toward increased phase synchrony in different frequency bands at different electrode sites than the effect associated with blast injury, hence the findings appear unrelated to prescribed medication. Although clinical symptomatology and prescribed medications appeared not to be confounds, the present study is limited by a small sample size and control subjects that were not necessary military veterans and had not been deployed. Nevertheless, the blast injured and control groups were of comparable age, gender, IQ, and handedness and thus findings are unlikely to be the consequence of demographic influences.

The diminished EEG phase synchrony that was associated with the fractional anisotropy of frontal white matter tracts may be the functional expression of a pathological change in tissue. Because the association is most evident in the blast mTBI group, it suggests that structural alteration to the forceps minor and anterior thalamic radiation reduces the EEG phase synchrony of the regions innervated by these white matter tracts. If the association of phase synchrony with white matter tract integrity was evident in both groups then one could argue that the association has little to do with blast injury and instead reflects normative associations between white matter structure and brain function as reflected in the EEG. Also, evidence that the diminished phase synchrony is observed in a similar pattern for both hemispheres between frontal polar and opposite frontal lateral regions indicates that the effect is unlikely due to volume conduction or spurious recording characteristics. Though the presence of potential confounding factors and small sample size need to be taken into consideration, the results of the present study provide important initial evidence for measures of neural function being sensitive to the effects of blast injury despite normal cognitive functioning as assessed by an array of neuropsychological tests. If the present findings are replicated in larger and more definitive studies, serial complex EEG monitoring of combat veterans, together with DTI evaluations, may be necessary elements in the care of military personnel who have experienced non-impact, blast-related mTBI. The value of such testing would also depend on how functional and structural brain measures are associated with clinical variables, treatment response, and recovery.

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Conflict of interest statement

The authors do not have any actual or potential conflict of interest including financial or personal that could inappropriately influence, or be perceived to influence, their work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.neuroimage.2010.09.007.

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