



Post-traumatic stress disorder and chronic hyperconnectivity in emotional processing[☆]

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ARTICLE INFO

Keywords:

Post-traumatic stress disorder
Amygdala
Prefrontal cortex
Implicit emotional face processing
Magnetoencephalography (MEG)
Functional connectivity
Military combat soldiers

ABSTRACT

Post-traumatic stress disorder (PTSD) is associated with heightened responses to threatening stimuli, particularly aggression-related emotional facial expressions. The stability over time of this neurophysiological ‘hyperactive’ threat response has not been determined. We studied implicit emotional face processing in soldiers with and without PTSD at two time-points (roughly 2 years apart) using magnetoencephalography to determine the response of oscillations and synchrony to happy and angry faces, and the reliability of this marker for PTSD over time. At the initial time-point we had 20 soldiers with and 25 without PTSD; 35 returned for follow-up testing 2 years later, and included 13 with and 22 without PTSD. A mixed-effects analysis was used. There were no significant differences (albeit a slight reduction) in the severity of PTSD between the two time-points. MEG contrasts of the neurophysiological networks involved in the processing of angry vs. happy faces showed that the PTSD group had elevated oscillatory connectivity for angry faces. Maladaptive hypersynchrony in PTSD for threatening faces was seen in subcortical regions, including the thalamus, as well as the ventromedial prefrontal cortex, cingulum gyri, inferior temporal and parietal regions. These results are generally consistent with prior studies and our own, and we demonstrate that this hyperconnectivity was stable over a two year period, in line with essentially stable symptomatology. Together, these results are consistent with the theory that hypervigilance in PTSD is driven by bottom-up, rapid processing of threat-related stimuli that engage a widespread network working in synchrony.

1. Introduction

Post-traumatic stress disorder (PTSD) is a severe psychiatric illness that can develop after direct exposure to, or witnessing, a traumatic life-threatening event. It is characterised by emotional dysregulation, hyper-arousal, avoidance of trauma reminders (but elevated perception of) and re-experiencing of traumatic episodes (American Psychiatric Association 2013). In the general population, the incidence of PTSD is around 5–10% (Kessler et al. 2005), but its prevalence is significantly higher in military veterans (Boulos and Zamorski 2013; Gates et al. 2012). As well as the primary positive psychiatric symptoms, secondary sequelae are often evident and are seen as deficits in cognitive domains, such as inhibition (Leskin and White 2007), executive functions

(Jenkins et al. 2000), and attention (Shucard et al. 2008). Emotional processing, both in oneself and in response to others, is also altered, particularly in relation to the perception of hostile and threatening expressions (Aupperle et al. 2012; Badura-Brack et al. 2018; Dalgleish et al. 2003).

To assess threat processing, studies have used threat-related facial expressions, and those with PTSD display heightened neurophysiological activation to angry or fearful faces (Badura-Brack et al. 2018; Bruce et al. 2013; Cisler et al. 2013; Fonzo et al. 2013; Matthews et al. 2011). Although imaging studies report abnormal activity in PTSD (Morey et al. 2009; Tsoory et al. 2007), less is known about how PTSD impacts the *network dynamics* of emotional processing. Neural connectivity is the basis of communication in the brain (Fries 2005), and

[☆] This work was supported by funding from CIMVHR (Contract #: W7714-145967).

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fast, bottom-up brain responses, which are crucially altered in PTSD, are not captured by neuroimaging paradigms of positron emission tomography (PET) or functional magnetic resonance (fMRI) due to their limited time resolution. In contrast, magnetoencephalography (MEG) has a time resolution of milliseconds, while still maintaining good spatial resolution; MEG directly captures neurophysiological interactions of brain function. In the last few years, a number of studies employed MEG to investigate the time course of brain activation evoked by emotionally salient stimuli in PTSD (Adenauer et al. 2010, 2011; Badura-Brack et al. 2018; Catani et al. 2009; Khanna et al. 2017; Todd et al. 2015). These studies have highlighted that the neurophysiological processing of emotional and threat-related information in PTSD is altered compared to both trauma-exposed and trauma-unexposed individuals; however, network connections involved has been less often assessed.

Band-limited, frequency-specific interactions within and among brain areas provide a way to assess circuitry dynamics and the networks they form – these are known to play critical roles in the spatial-temporal organisation of information that underlies cognitive processing (Buzsáki and Watson 2012; Fries 2005; Varela et al. 2001). Electrophysiological techniques (such as MEG) have been instrumental in this area, due to their exquisite temporal resolution and ability to resolve oscillatory synchronisation and large- and small-scale interactions among regions of the brain (Palva and Palva 2011).

Abnormal inter-regional synchrony, and therefore *communication*, has been noted in a number of psychiatric conditions, and understanding these altered networks has contributed to knowledge of these disorders and the associated impacts on cognition (Montez et al. 2009; Tewarie et al. 2013). In PTSD, we have shown that increased synchronisation during resting-state recordings distinguished PTSD from combat-exposed control soldiers, and was related to behavioural sequelae as well as symptom severity in PTSD (Dunkley et al. 2014). We also found that the PTSD group showed heightened threat responses, including over-connectivity, compared to a group of trauma-exposed but healthy control soldiers for angry but not for happy faces, with increases in node strength and clustering in the right amygdala and medial prefrontal cortex, that correlated with anxiety and depression (Dunkley et al. 2016), two hallmarks of PTSD. These studies suggested abnormal synchrony across the brain might be a marker of the impact on cognitive processing in the disorder.

Here, we investigated the stability over time of our previously observed connectivity features when viewing threatening stimuli, focusing on the role of inter-regional oscillatory phase synchrony, in soldiers with PTSD. We retested a subset of the soldiers, both with a diagnosis of PTSD and without, from our original cohort after a two-year interval. Behavioural evaluations of their symptoms were obtained and the neuroimaging protocols were repeated. Given our previous findings in these two groups of soldiers (Dunkley et al. 2014, 2015, 2016) and other literature in this field, we predicted and explicitly set out to test that broad-band synchrony (2–20 Hz) in the ‘fear circuit’ 100–200 ms after stimulus presentation would remain enhanced in the PTSD group when perceiving angry faces (especially the insula and amygdala, and other connected nodes).

2. Materials and methods

2.1. Participants

20 Canadian Armed Forces soldiers diagnosed with PTSD (all male, mean age = 37.67, SD = 1.39) and 25 combat-exposed soldiers without PTSD (all male, mean age = 33.97, SD = 0.98) were recruited to participate in this longitudinal study, including those who participated in the original study as part of Dunkley et al. 2016 in Phase I. In the follow up phase, Phase II, participants were scanned approximately 2 years later, and were a subset of the original cohort, with 13 PTSD and 22 control soldiers returning, thus a total of 80 separate datasets were

analysed in this study.

All participants were initially approached by a military clinician if they wished to participate. All had normal or corrected-to-normal visual acuity and gave prior written informed consent after details about the study were given. All procedures were approved by the Hospital for Sick Children and Canadian Armed Forces Research Ethics Boards.

Inclusion criteria for the PTSD group were: a clinical diagnosis of PTSD at a Canadian operational trauma stress support centre (OTSSC) as determined by a psychiatrist or psychologist specialised in trauma-related mental health injuries; PTSD symptoms present between 1 and 4 years prior to taking part in the study; regular mental health follow-ups; and current PTSD check-list (PCL-Military version) scores of > 50, indicating the presence of moderate to severe PTSD.

The diagnosis was determined through a comprehensive, semi-structured interview with a clinician based on DSM-IV-TR diagnostic criteria (American Psychiatric Association 2013), along with Canadian Armed Forces (CAF) standardized psychometric testing. All participants in the PTSD group were recruited from one of the CAF OTSSCs. There was usually more than one DSM-IV-TR ‘A1’ stressor-related criterion identified as a traumatic event contributing to the development of PTSD (direct personal experience of an event that involves actual or threatened death or injury), with a diagnosis related to operational exposure. Control soldiers were combat-exposed, frontline troops in similar military roles, and selected from cohorts of comparable rank, education level, handedness and military experience. An additional inclusion criterion applied to both groups was no history of a traumatic brain injury (TBI), as screened by a psychiatrist through a review of their electronic health record, telephone interview, and administration of the Defence and Veteran’s Brain Injury Centre (DVBIC) screening tool.

Exclusion criteria for both groups included ferrous metal inside the body or implanted medical devices that might be MRI contraindications or interfere with MEG data acquisition; seizures or other neurological disorders; certain ongoing medications (anticonvulsants, and/or benzodiazepines, or other GABA antagonists) known to directly or significantly influence brain oscillations. As this was a naturalistic study, we accepted PTSD participants undergoing treatment including evidenced-based psychotropic medication(s), such as selective serotonin reuptake inhibitors (SSRIs), serotonin-norephedrine reuptake inhibitors (SNRIs), and Prazosin, and did not ask them to refrain from taking their medications prior to the study, due to ethical concerns regarding the withdrawal of medication in this population.

2.2. Cognitive-behavioural evaluation

All subjects completed short cognitive-behavioural assessments, including the Generalized Anxiety Disorder 7-item Scale - GAD7 (Spitzer et al. 2006), Patient Health Questionnaire - PHQ9 (Kroenke et al. 2001), the Brief Trauma Questionnaire - BTQ (Schnurr et al. 2002) and the Post Traumatic Stress Disorder Check List - PCL (Weathers et al. 2013). They also completed the Positive and Negative Affect Schedule PANAS (Watson et al. 1988) and the State Trait Anxiety Inventory - STAI (Spielberger et al. 1983).

2.3. Task procedure

Participants completed an implicit emotional face processing task, the identical procedure used in the initial study (Dunkley et al. 2016). Emotional stimuli comprised of happy or angry faces taken from the NimStim set of facial expressions (Tottenham et al. 2009; <http://www.macbrain.org/resources.htm>) were rapidly presented to participants. Participants were explicitly instructed to ignore the faces and concentrate on the border/frame around the faces, which would be one of two colours (blue or purple). They were directed to press a button as quickly as possible each time their defined target colour was displayed, which they were told during the pre-scan practice run and reminded of before the experimental run. These target trials were included to

maintain the participants' attention and comprised 25% of the total trial count (sometimes referred to as 'catch trials'). Target trials were only used for the analysis of reaction time to behaviourally categorise participants' responses to emotional faces, and only *correct* (i.e., no response) no-go trials were used in the imaging analysis; the rationale for this was to avoid large evoked motor responses which occur to the target trials and would obscure more subtle cognitive activity related to implicit face processing.

The experimental protocol was programmed using Presentation® software (www.neurobs.com) and projected via a back projection screen (42 w × 32 h cm) placed 78 cm from the participants' eyes. The stimuli were foveal, with a size of 7.4w × 9 h cm (with a 2 cm thick border), and subtended ~14 × 16° of visual angle. This protocol lasted for 2–3 min.

2.4. MEG data acquisition

MEG data were collected inside a magnetically-shielded room on a CTF Omega 151 channel system (CTF Systems, Inc., Coquitlam, Canada) at 600 Hz with third-order spatial gradient noise cancellation applied, at the Hospital for Sick Children. Throughout the run, head position was continuously recorded by three fiducial coils placed on the nasion and left and right pre-auricular points. Sensor time series data were visually inspected and significant artefacts related to head-motion resulted in the removal of a trial from subsequent analysis. This visual inspection was supplemented by head-movement recordings to confirm such observations, with trials displaying > 5 mm head motion being excluded from subsequent analysis (any potential system-related artefacts were investigated before any experimental MEG data was recorded, with bad channels being omitted from any recordings).

After the MEG session, anatomical 3T MRI images were acquired (Magnetom Tim Trio, Siemens AG, Erlangen, Germany) in an adjacent suite, which were T1-weighted magnetic resonance images using high-resolution 3D MPRAGE sequences on a 12 channel head coil. MEG data were coregistered to the MRI structural images using the reference fiducial coil placements.

2.5. MEG processing

This study used a seed-based approach to categorise connectivity, where the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al. 2002) was used to identify 90 sources (seeds) in cortical and subcortical regions. Defining the source space solution to these locations provides reasonable coverage of anatomically-parcellated regions and has shown reliability in studying large-scale network dynamics for functional connectivity analyses (Doesburg et al. 2013; Dunkley et al. 2016). These coordinates defined locations for time-series to be extracted and analysed. These standardized coordinates were unwarped from Montreal Neurological Institute (MNI) space, and broadband (2–20 Hz) time-series from these 90 voxels were reconstructed using an implementation of the Synthetic Aperture Magnetometry (SAM) scalar beamformer based on a single-sphere head model, with noise normalization implemented by conversion of the signal from physical units (Ampere-meter) to pseudo-z. A beamformer is a type of adaptive spatial filter, or inverse source modeling method, that minimizes total brain power (i.e., suppresses the contribution of signal from areas beyond the region-of-interest), whilst being optimally sensitive to activity in a given brain location (in this case, each of the 90 AAL seed locations). Individual weight vectors were applied to each sensor measurement and summated to derive estimated source activity at the seed location. This output, often called a virtual electrode or virtual sensor, can be envisaged as source-level signals (that is, from the brain), and are analogous to what one might expect if there were a sensor in that particular cortical location. Furthermore, because MEG beamformers are spatial filters, they are robust at the suppression of artefacts (Muthukumaraswamy 2013). These time-series were then

filtered into the broadband range of 2–20 Hz, based on our previous data and predictions for this study (Dunkley et al. 2016).

The instantaneous phase of each sample from the filtered time-series bins was calculated using the Hilbert Transform. Each time-series of the instantaneous phase estimate for the 2–20 Hz bin of the filtered waveforms was then used to estimate functional connectivity by calculating the cross-trial weighted Phase Lag Index (wPLI (Lau et al. 2012)). The wPLI was derived for each phase angle time-series from the degree of phase synchronisation for every sample point between all pairwise combinations of the pre-defined seed regions. In other words, the wPLI estimates the (delayed or phase-shifted) *regularity* or *consistency* of the phase angle of the oscillating time-series from two brain regions; brain regions that oscillate together are thought to be 'communicating-through-coherence' (Fries 2005) (16), and in this fashion, the brain is transferring information between areas. The wPLI ranges between 0 and 1, and these values quantify the degree of phase-synchronisation between two sources ('0' being out of phase, or no phase relationship; '1' being phase-synchronised, or oscillating in perfect harmony), which is referred to as *functional connectivity*.

90 × 90 weighted undirected adjacency matrices with wPLI values acting as edge weights for all sources were constructed at each sample point. For the generation of statistically-thresholded functional connectivity images, the elementwise mean baseline (–500 to 0 ms) adjacency matrix wPLI value was subtracted from the 'active window' (the 100–200 ms matrix averaged over time, given our previous findings), to give a baseline-corrected estimate of synchrony for each connection/edge specifically related to face processing. Group (PTSD and control) and time point (Phase I & Phase II) factors were entered into a linear mixed effects model (wpli~isptsd + (isphasetwo + 1|id)).

2.6. Connectivity analysis

Statistical analyses were performed on the resulting baseline-corrected matrices using the Network Based Statistic (NBS; (Zalesky et al. 2012)) implementing a Mixed-Effects model (NBS-ME). Multiple comparison correction was implemented using clustering of graph components based on the NBS-extent method. NBS first applies an initial univariate threshold to each analysed edge. The topological distribution of connectivity components, defined as contiguous groups of nodes connected by suprathreshold connections, is then obtained. Group membership (PTSD or control) is then shuffled and the extent of the largest component which occurs in this surrogated data is recorded, and this process is repeated 5000 times to generate a null distribution. The ranking of connectivity components from the unshuffled data in the surrogate distribution is used to determine statistical confidence; as the surrogate distribution considers the largest connectivity component that could occur, assuming the null hypothesis, across the entire analysed network. This approach controls for false positives due to multiple comparisons at any threshold. In the present analysis, the initial univariate threshold was set and tested at moderate t-value ranges of 1.5 to 3 (Zalesky et al. 2010). Functional brain networks were visualized using BrainNet Viewer (Xia et al. 2013).

3. Results

The neuroimaging data were analysed using a mixed effects model, such that we could determine if the follow-up data from the subset of participants who returned at Phase 2 differed on any of the metrics as a function of time of testing, as well as interactions between factors. Importantly, there were no significant differences in the behavioural or neuroimaging measures between the participants in Phase 1 and Phase 2.

3.1. Cognitive-behavioural measures

Cognitive-behavioural measures were compared using appropriate

Table 1
Cognitive-behavioural measures. Scores are median or mean (depending on the statistical test used), with standard deviation or interquartile range (25% and 75% percentile) shown in brackets, respectively.

	PTSD: M (SD)	Control: M (25%, 75%)	Test statistic
<i>n</i>	13	22	
GAD7	14 (8, 17.5)	1 (0, 2)	$U = 4, p < 0.001$
PHQ9	15 (7.5, 18.5)	1 (0.5, 18.5)	$U = 7, p < 0.001$
PCL	64 (17, 21)	19 (37.5, 68)	$U = 4, p < 0.001$
BTQ	3.39 (1.04)	2.94 (1.47)	$t(29) = -0.92, p = 0.37$
PANAS + Pre	26.46 (9.28)	30.87 (11.62)	$t(29) = 1.1, p = 0.28$
PANAS – Pre	18 (12.5, 21)	10 (10, 11)	$U = 12, p < 0.001$
PANAS + Post	24.15 (12.38)	29.867 (9.73)	$t(29) = 1.37, p = 0.188$
PANAS – Post	16 (12, 20)	10 (10, 11)	$U = 36.5, p < 0.001$
STAI Pre	13 (11, 14.5)	15 (13, 15)	$U = 65.5, p < 0.001$
STAI Post	13 (10.5, 14)	14 (13, 15)	$U = 76, p = 0.09$

GAD7, Generalized Anxiety Disorder 7-item Scale; PHQ9, Patient Health Questionnaire; PCL, Post-Traumatic Stress Disorder Check List; BTQ, Brief Trauma Questionnaire; PANAS, Positive and Negative Affect Schedule + Positive Affect, – Negative Affect, Pre scan, Post Scan; STAI, State Trait Anxiety Inventory.

Table 2
Behavioural measures (reaction time and accuracy) for the emotional faces task completed in the MEG, showing no group differences or effects of emotion, and close-to-ceiling accuracy.

	Mean response time (ms)			Accuracy		
	CTRL	PTSD	t-stat	CTRL	PTSD	t-stat
GoHappy	220.42	231.23	-1.620	95%	97%	-0.760
GoAngry	222.79	235.85	-1.870	96%	96%	-0.660
NogoHappy				98%	99%	0.165
NogoAngry				98%	99%	-0.393

tests based on data normalcy. There was slight but non-significant decrease in PTSD symptom severity for the PTSD group between time Phase I (PCL mean = 64, SD = 7.3) and Phase II (mean = 57, SD = 16.3), $t(11) = 1.24, p = 0.24$ (one of the returning soldiers had a missing PCL score in Phase I).

Test-statistics and p-values for the additional cognitive-behavioural measures are reported in Table 1. When compared to the non-PTSD group, PTSD soldiers had increased levels of anxiety ($U = 4, p < 0.001$), depression ($U = 7, p < 0.001$), and PTSD symptoms

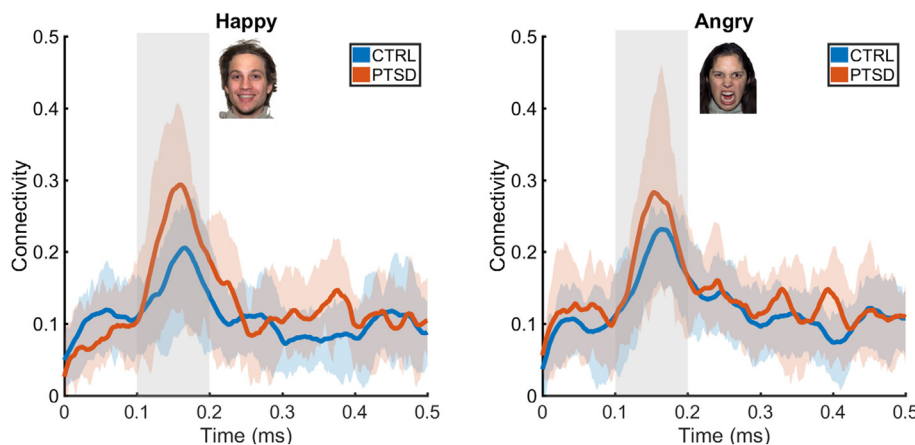


Fig. 1. Whole-brain functional connectivity time-series for PTSD (red line) and control (blue line) groups, for Happy (left) and Angry (right) conditions. Opaque lines denote group means, shading indicates ± 1 standard error bars. Mean levels of connectivity were greater in PTSD for both happy vs. angry faces, but the difference in connectivity was only significant for the angry faces (active time window of interest 100–200 ms denoted by grey bounding box). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

($U = 4, p < 0.001$), but crucially, not self-reported exposure to traumatic events ($t(29) = -0.92, p = 0.37$). The PTSD group also reported greater levels of pre-test negative affect on the Positive and Negative Affect Schedule (PANAS) (pre $U = 12, p < 0.001$; post $U = 36.5, p < 0.001$), but no significant difference in (pre- ($t(29) = 1.1, p = 0.28$) or post-test positive affect ($t(29) = 1.37, p = 0.188$)). There was a significant difference in the State Trait Anxiety Inventory pre ($U = 65.5, p = 0.036$), but not post ($U = 76, p = 0.09$) measures.

There were no differences ($p > 0.05$) in accuracy or reaction time on the go trials, as a function of emotion or group, or any differences in accuracy between groups (see Table 2).

3.2. MEG functional connectivity

Both types of emotional faces elicited increases in mean connectivity across the entire functional network, with relatively elevated synchrony in the PTSD group compared to controls. These responses peak around 150 ms post-presentation (Fig. 1) - the timing of this event-related synchrony is consistent with our previous study. Evaluating connectivity over the 100-200 ms time window, the NBS-ME model revealed a main effect of group, with significant increased synchrony in the PTSD group for the implicit perception of angry faces ($p = 0.04$ corrected, initial supra-threshold $t = 3.0$; Fig. 2) - these effects were concentrated in the right thalamus and other deep grey matter structures, such as the caudate and hippocampus, with extensive interactions in orbital frontal, ventro-medial prefrontal cortex (vmPFC), regions as well as the right temporal-parietal junction (TPJ) (see Table 2). No significant main effect was detected in the happy condition even when we relaxed control over the false positive rate by testing at a number of initial suprathreshold t-statistic levels from $t = 1.5$ to $t = 3$, in 0.5 steps (with initial $t = 1.5, p = 0.92$, to initial $t = 3.0, p = 0.89$). Moreover, there was no significant main effect of time point, which suggests that connectivity did not significantly change, and was inherently stable between scanning time points, in line with no significant change in PTSD symptom severity measured by the PCL (Table 3).

When we examined the overall connectivity within the ‘angry network’ (nodes and connections derived from the group contrasts in Fig. 1), soldiers with PTSD exhibited greater levels of connectivity on average, for both the happy and angry emotional faces when compared with their trauma-exposed, non-PTSD peers; however, the effect was far larger for the angry faces (Fig. 3). It is interesting to note, as well, that the level of synchrony did not immediately return to baseline after stimulus offset in the group with PTSD, suggesting persistent hyperarousal and synchrony related to the perception of threat that was not apparent to the happy faces. We also examined the concomitant evoked response, by condition, and at the whole-brain and ‘Angry network’ level to elucidate the interplay between event-related evoked and phase synchrony measures. We found differential effects, whereby evoked



Angry - PTSD > CONTROL 2-20 Hz wPLI, 100-200 ms

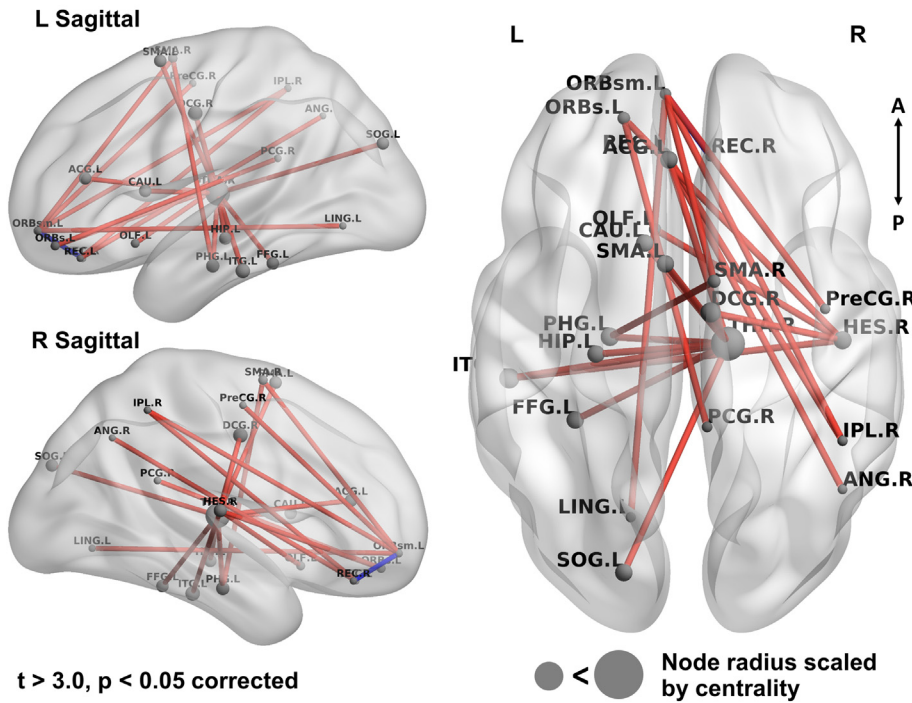


Fig. 2. Soldiers with PTSD exhibited increases in brain synchrony when viewing angry faces ($t > 3.0$, $p < .05$ corrected), which was stable over recording sessions. This network of hyperconnectivity included the left hippocampal area, the thalamus, caudate, orbital frontal areas as well as parietal association areas (for a list of areas, see Table 2). The size of the nodes indicates the relative centrality of the node (larger = greater centrality).

$t > 3.0$, $p < 0.05$ corrected

Table 3
Nodes at which soldiers with PTSD exhibited increases in brain synchrony when viewing angry faces ($p < 0.05$), in descending order of eigenvector centrality (arbitrary units).

Centrality	Area
0.6606	Thalamus_R
0.2841	Temporal_Inf_L
0.2841	Cingulum_Mid_R
0.2402	ParaHippocampal_L
0.2307	Heschl_R
0.2106	Caudate_L
0.2106	Fusiform_L
0.2106	Occipital_Sup_L
0.2106	Hippocampus_L
0.2106	Cingulum_Ant_L
0.2106	Supp_Motor_Area_L
0.0928	Supp_Motor_Area_R
0.0819	Frontal_Sup_Orb_L
0.0736	Olfactory_L
0.051	Frontal_Med_Orb_L
0.0261	Cingulum_Post_R
0.0183	Parietal_Inf_R
0.0163	Lingual_L
0.0163	Rectus_R
0.0163	Precentral_R
0.0065	Rectus_L
0.0021	Angular_R

responses were temporally concomitant with increased synchrony, but also present in the absence of inflated connectivity, suggesting a degree of independence in these measures (see Supplementary Materials, Fig. S1).

4. Discussion

We examined brain connectivity via MEG in soldiers with and

without PTSD, ~75% of whom returned for a follow-up assessment after a two-year interval. We found that the results between the two time-points were remarkably stable, and found no significant differences over the two-year period in the measures of PTSD, on the behavioural measures of the emotional faces task, nor any differences in the MEG connectivity. The results remained highly significant in terms of group differences, with the soldiers with PTSD still showing the signs and symptoms of the disorder. The task behavioural measures did not differentiate the two groups, as all participants performed near ceiling, but there were significant effects in the neuroimaging. The lack of behavioural differences was not unexpected, as the task was easy, the targets were non-face and non-emotional, and both groups performed very well. However, the implicit presentation of emotional stimuli still triggers, automatically, processing of the emotions in the brain, and this processing differed significantly between groups, even though both had comparable combat exposure. The soldiers with PTSD showed increased connectivity, in the broadband 2–20 Hz response (encompassing theta through low beta ranges), to the emotional faces, that was significant only to the angry faces. This hyperconnectivity is consistent with prior studies and the model of hyperarousal in the presence of threatening stimuli in PTSD.

The increased connectivity network to angry faces included significant involvement of regions critical to emotional processing, such as the vmPFC areas (e.g., (Khanna et al. 2017; Levens et al. 2014)) and the right TPJ, important for social cognitive functions (Krall et al. 2015; Young et al. 2010). A number of functional neuroimaging studies have investigated the neural underpinnings of emotional difficulties in PTSD and have suggested that atypical modulation within and between the amygdalae and the vmPFC may be the cause (Badura-Brack et al. 2018; Bruce et al. 2013; Shin et al. 2004, 2005). We did not find abnormal connectivity involving the amygdalae (unlike our preceding study - discussed below) but did show the increases in the ventromedial, orbital frontal regions, posterior cingulate cortex, and right parietal regions consistent with previous work (Dunkley et al. 2016). Other studies using non-explicit emotional face processing tasks have shown

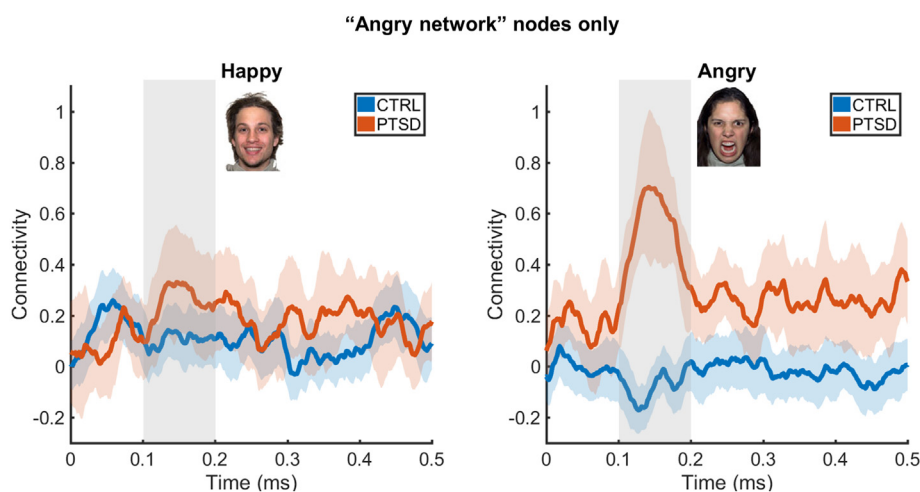


Fig. 3. “Angry network” mean connectivity time-series for PTSD (red line) and control (blue line) groups, derived from the nodes and connections found to be significantly different using NBS-ME (network shown in Fig. 2). Opaque lines denotes group means, shading indicates ± 1 standard error. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

increased prefrontal activation patterns (Bruce et al. 2013; Bryant et al. 2008; Fani et al. 2012), which would also be consistent with the hypothesised fear circuitry model of fronto-limbic disinhibition in PTSD. Bryant et al. (2008) proposed that the fronto-limbic model in PTSD of disinhibition and attentional control may be applicable only to conscious threat perception.

A recent study reported that rapid and elevated amygdala oscillatory responses occur in veterans with PTSD when witnessing threatening faces (Badura-Brack et al. 2018). In light of this study, the absence of any amygdalae synchrony in the work reported here could be due to a number of factors, statistical or physiological in origin. Firstly, the lack of significant amygdala activity may be due to a reduction in statistical power from participant attrition, as only 65% of the original cohort with PTSD returned for Phase II, driving an increase in Type II errors. Moreover, imaging deep sources (e.g. amygdalae) with MEG is attainable given sufficient signal-to-noise (i.e. trial number and participants), which would have been adversely affected here and explain the lack of effects observed this time. Secondly, the absence might be explained in part by physiological changes related to the non-significant reduction in symptom severity between the two time-points.

The involvement of the TPJ in this hyperconnected network to angry faces further suggests that in the soldiers with PTSD there is increased involvement in interpreting the angry faces, to determine the social-cognitive value. This would seem to be unnecessary in a lab testing environment and may support more broadly the hyperarousal model for any stimuli seen as threatening. The fact that the increased connectivity did not return to baseline after the stimulus off-set reinforces the notion of their maintaining a heightened arousal level and elevated and ongoing threat scanning.

The over-connectivity covered the broadband 2–20 Hz, which straddles the alpha range (8–12 Hz) – this particular rhythm is known to underlie long-range connectivity and integration in the brain (Palva and Palva 2011), playing a particularly important role in visual working memory processing (Palva et al. 2010). This is consistent with numerous reports of memory impairments in PTSD to stress-induced stimuli (e.g., (Paunovic et al. 2002)), as well as the involvement of the left hippocampus in this hyperconnected network (Dunkley et al. 2014; Thomaes et al. 2009), an area important in experiential memory, and implicated in our earlier resting-state study, where it, was found to be hypersynchronous, and the degree to which it was connected to other areas was directly related to PTSD symptomatology. Thus, we speculate that the presentation of emotional faces could invoke mnemonic processing and contribute to the increased arousal.

This study should, however, be interpreted with a number of caveats. First, it is difficult to entirely disentangle trial-wise, phase-locked evoked responses from true synchronised, induced oscillatory responses – the evoked component could drive the connectivity measure and

spuriously inflate the PLI estimate. The only decisive resolution, however, would be to implement an experimental design that lacks any component that might drive evoked responses (Palva and Palva 2012). Unfortunately, whilst every consideration was made in the experimental design and analyses to capture veridical electrophysiological connectivity and minimise the spurious contribution of confounding (e.g. evoked) factors, this was not possible and the data presented should be considered with this in mind.

4.1. Conclusions

This study used a longitudinal design of soldiers with and without PTSD, and we found increased connectivity to angry faces in the soldiers with PTSD, that was nevertheless stable over multiyear time points, in conjunction with no mean reduction in PTSD severity. This hyperconnected network included brain areas involved in emotional and social cognitive processing, and may also suggest links with the memory impairments seen in PTSD to emotional stimuli. Importantly, as we found no differences between testing periods, we have established that this neurophysiological effect is stable over time in a PTSD population, and therefore might constitute a reliable biomarker to aid in prognosis and the assessment of treatment efficacy.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nicl.2018.07.007>.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was funded by Defence Research and Development Canada (DRDC) (Contract #: W7719-135182/001/TOR) and CIMVHR (W7714-145967) with support from the Canadian Forces Health Services. The authors would like to thank Marc Lalancette and Amanda Robertson for help in the data collection. The authors acknowledge the tremendous help of Drs. Paul Sedge and Richard Grodecki for identifying, recruiting and confirming diagnosis in the military cohorts.

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