Research Paper

Altered resting-state amygdala functional connectivity in men with posttraumatic stress disorder

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Background: Converging neuroimaging research suggests altered emotion neurocircuitry in individuals with posttraumatic stress disorder (PTSD). Emotion activation studies in these individuals have shown hyperactivation in emotion-related regions, including the amygdala and insula, and hypoactivation in emotion-regulation regions, including the medial prefrontal cortex (mPFC) and anterior cingulate cortex (ACC). However, few studies have examined patterns of connectivity at rest in individuals with PTSD, a potentially powerful method for illuminating brain network structure. **Methods:** Using the amygdala as a seed region, we measured resting-state brain connectivity using 3 T functional magnetic resonance imaging in returning male veterans with PTSD and combat controls without PTSD. **Results:** Fifteen veterans with PTSD and 14 combat controls enrolled in our study. Compared with controls, veterans with PTSD showed greater positive connectivity between the amygdala and insula, reduced positive connectivity between the amygdala and hippocampus, and reduced anticorrelation between the amygdala and dorsal ACC and rostral ACC. **Limitations:** Only male veterans with combat exposure were tested, thus our findings cannot be generalized to women or to individuals with non–combat related PTSD. **Conclusion:** These results demonstrate that studies of functional connectivity during resting state can discern aberrant patterns of coupling within emotion circuits and suggest a possible brain basis for emotion-processing and emotion-regulation deficits in individuals with PTSD.

Introduction

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric disorder characterized by symptoms of re-experiencing, hyperarousal, emotional numbing and avoidance;[1] however, exact brain mechanisms involved in the generation of PTSD symptoms or in PTSD pathophysiology have yet to be elucidated. Converging neuroimaging research points to a potentially critical role for disrupted emotion neurocircuitry in individuals with PTSD, and whereas many studies have delineated patterns of activations during face viewing or symptom provocation (for a review, see Shin and Liberzon[2]), relatively few have examined patterns of connectivity in the brains of patients with PTSD at rest, a potentially powerful method for illuminating brain network structure.[3,4] Most PTSD neuroimaging studies to date have described abnormalities in emotion-generation regions, such as the amygdala or insula, and emotion-regulation regions, including the anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC). This is consistent with the known role of the amygdala as a key region in threat detection,[5] fear conditioning[6] and emotional salience,[7] and of the mPFC as a modulatory region interconnected with limbic structures[8] and involved in emotion regulation.[9] Taken together, functional magnetic resonance imaging (fMRI) studies of individuals with PTSD suggest patterns of hyperactivation of the amygdala and insula to emotion-related stimuli and corresponding hypoactivation of ventromedial prefrontal and rostral anterior cingulate cortices.[2] This pattern of amygdala hyperactivity and mPFC hypoactivity was recently confirmed by a meta-analysis of 15 PTSD neuroimaging studies[10] and is generally understood to reflect a lack of regulatory control over emotion in individuals with PTSD.

Studies of functional connectivity, however, can provide additional and potentially more direct information about regulatory relationships between the mPFC and amygdala. The amygdala has tight structural connections and reciprocal feedback loops with the mPFC and orbitofrontal cortex[11] as well as with the dorsolateral PFC[12] and ACC.[13] As amygdala

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hyperactivity tends to coincide with mPFC hypoactivity in healthy individuals,[9,14] recent studies have begun to investigate task-related functional connectivity between these regions. Roy and colleagues[15] reported functional connectivity of the amygdala with ventral medial prefrontal regions (including the medial frontal gyrus and rostral ACC), insula, thalamus and striatum at rest and anticorrelations with the dorsal ACC, superior frontal gyrus, bilateral middle frontal gyrus and posterior cingulate cortex (PCC), interpreted as dissociations between the emotion production network and the cognitive or affect regulation network. In individuals with PTSD, amygdala connectivity during task-based studies has yielded inconsistent findings. For instance, a [^{15}O]-H_2O positron emission tomography (PET) study of recently traumatized individuals found positive functional connectivity between the amygdala and ACC in response to trauma scripts.[16] In contrast, a different PET study reported anticorrelation between the amygdala and ACC during neutral (but not trauma) scripts, and reductions in the strength of this connectivity in individuals with PTSD.[17] It is noteworthy, however, that regardless of the direction of the connectivity identified (positive connectivity v. anticorrelation), most studies have found diminished strength of connectivity in individuals with PTSD compared with controls.[2,16,18,19] These relationships might potentially be better assessed, however, through connectivity analyses at rest, without the confounds of tasks that may be biased to elicit amygdala activity or provoke PTSD symptoms.

Functional MRI studies of individuals with PTSD have also demonstrated aberrant activity in the insula, an area responsible for interoception,[20] disgust,[21] emotion processing,[22] emotion recall[9] and anticipation of aversive stimuli.[19] The amygdala and insula are structurally interconnected,[23] and early PET studies reported increased insula activity in response to trauma script–driven imagery in individuals with PTSD,[24] though in some studies no more than in combat-exposed controls.[25] Recent fMRI studies have reported greater insula activation in anticipation of negative images[19] and negative emotional faces[18] in individuals with PTSD as well as enhanced coupling between the insula and amygdala during negative emotion induction in healthy volunteers[26] and during symptom provocation in recently traumatized individuals.[16] Etkin and Wager's meta-analysis[10] also suggests coactivation of the right amygdala and insula across studies, and collectively these studies offer evidence of strong anatomic and functional links between the amygdala and insula during emotion processing.

Finally, the hippocampus, a medial temporal lobe region adjacent to the amygdala that is implicated in declarative memory,[27] contextual memory[28] and fear conditioning,[29] has been an important area of interest in PTSD research. Functional neuroimaging studies of the hippocampus in individuals with PTSD have yielded conflicting findings, with some showing hyperactivity and some showing hypoactivity.[2] A quantitative meta-analysis, however, suggested overall reduced hippocampal activity in individuals with PTSD.[10] Additionally, it has been hypothesized that the hippocampus integrates context into emotional memories and modulates amygdala activity according to context,[30] a function that might be disrupted in individuals with PTSD.

Recent studies have begun to investigate resting-state connectivity in individuals with PTSD and have reported alterations in subcortical[31] and default network connectivity.[32–35] Resting-state connectivity offers a powerful way to assess intrinsic connections between brain networks,[3,4,36] which in turn have been linked to important functions such as processing speed[37] and cognitive flexibility[38] in health and in disease. Resting-state amygdala connectivity may have particular relevance for the study of mood and anxiety disorders, as it has been reported to be altered in individuals with generalized anxiety disorder (GAD),[39] social phobia,[40] major depressive disorder (MDD)[41,42] and bipolar disorder;[43] however, amygdala connectivity at rest in individuals with PTSD has not been studied. Given hypotheses that the mPFC exerts regulatory control over the amygdala, we hypothesized anticorrelations between the mPFC and amygdala, and positive connectivity between the amygdala and insula. Based on heightened emotion reactivity and diminished emotion control in individuals with PTSD, we hypothesized enhanced positive connectivity between the amygdala and insula, and reduced anticorrelation between the PFC and amygdala in patients with PTSD. Finally, we have recently proposed that PTSD is associated with failure to contextualize emotional memory,[30,44] and given the hippocampal role in contextual processing,[28] we hypothesized reduced positive connectivity between the amygdala and hippocampus in patients with PTSD compared with combat controls.

Methods

Participants

We recruited study participants from among veterans returning from deployments to Afghanistan (Operation Enduring Freedom [OEF]) and Iraq (Operation Iraqi Freedom [OIF]) who had a current PTSD diagnosis and were seeking treatment at the VA Ann Arbor. All participants in the PTSD group met DSM-IV criteria for current (past month) PTSD, as assessed via the Clinician Administered PTSD Scale (CAPS).[45] No psychiatric diagnoses were allowed among participants in the control group. Participants were screened for comorbid disorders using the Mini-International Neuropsychiatric Interview (MINI).[46] Patients with PTSD were notified at their initial VA Ann Arbor visit of the opportunity to participate in research, and all interested, eligible participants were included in the study. Combat controls (veterans of OEF and OIF without PTSD) were recruited from the community via advertisement.

After a complete description of the study was provided to the participants, written informed consent was obtained. Participants in both groups were exposed to the same conditions. All procedures were carried out between August 2008 and July 2010. The study was approved by the Institutional Review Boards of the University of Michigan Medical School and the Ann Arbor Veterans Affairs Healthcare System. All procedures were carried out in accordance with the Declaration of Helsinki, as adopted and promulgated by the National Institutes of Health.

Resting-state paradigm

Participants underwent structural MRI (sMRI) and fMRI scanning that included both emotion regulation and conditioning tasks (reports forthcoming) and resting-state procedures. Resting-state scans always occurred before tasks. Participants were positioned in the scanner with their heads comfortably restrained to reduce head movement. Heart rate and respiration measurements were acquired for removal of physiologic noise in the imaging process. Participants lay supine in the fMRI scanner and wore glasses with built-in mirrors (NordicNeuro Laboratories) to view the projected stimuli inside the scanner. A black fixation cross on a white background was displayed in the centre of the screen for 10 minutes (300 volumes). Participants were instructed to relax and keep their eyes open and fixed on the cross. A pulse oximeter was attached to the participant's finger, allowing us to record their cardiac activity. In addition, participants wore a pressure belt around the abdomen, allowing us to record their respiratory activity. Both the cardiac and respiratory signals were synchronized to the fMRI data and were collected so that these physiologic variations could be removed in a regression analysis.[47]

Data acquisition

Scans were collected on a 3 T General Electric Signa EXCITE scanner. After participants were positioned in the scanner, a T_1-weighted low-resolution structural image was prescribed approximately parallel to the anterior commissure–posterior commissure line: gradient recall echo sequence (GRE), repetition time (TR) 250 ms, echo time (TE) 5.7 ms, flip angle (FA) 90°, 2 averages, field of view (FOV) 22 cm, matrix 256 × 256, slice thickness 3 mm, 40 axial slices to cover the whole brain. This was similar to the prescription of the functional acquisitions. Functional images were acquired with a T_2*-weighted reverse spiral acquisition sequence (GRE sequence, TR 2000 ms, TE 30 ms, FA 90°, FOV 22 cm, matrix 64 × 64, slice thickness 3 mm with no gap, 40 axial slices to cover the whole brain), which has been shown[48] to minimize signal drop-out in regions, such as the ventral striatum and orbitofrontal cortex, that are vulnerable to susceptibility artifact. The intermediate template and fMRI images were acquired using a GE Quadrature sending and receiving head coil. The 4 initial volumes were discarded from each run to allow for equilibration of the scanner signal. A high-quality T_1-weighted sMRI scan was obtained using a 3-dimensional (3-D) volume inversion recovery fast spoiled gradient recall echo (IR-FSPGR) protocol (TR 12.3 ms, TE 5.2 ms, FA 9°, inversion time 650 ms, FOV 26 cm, matrix 256 × 256 for in-plane resolution of 1 mm, slice thickness 1 mm with no gap, 160 contiguous axial slices to cover the whole brain). The sMRI scans were acquired with an 8-channel GE phase array receiving head coil.

Preprocessing of fMRI data

An initial series of preprocessing steps was carried out. First, we removed k-space outliers in raw data that were 2 standard deviations (SD) away from the mean and substituted them with the average value from neighbouring voxels. Next, we used a B_0 field map in the reconstruction of the images to remove the distortions that resulted from magnetic field inhomogeneity.[49] The variance owing to physiologic responses (i.e., cardiac and respiratory sources) was removed using regression.[47] The data were then slice-time corrected using local sinc interpolation[50] and realigned using MCFLIRT in FSL.[51] We performed additional preprocessing and image analysis in SPM5 (Wellcome Trust Centre for Neuroimaging). First we coregistered the high-resolution T_1 images to the functional images. Second, T_1 images were normalized to the scalped T_1 template, and the functional volumes were normalized to the Montreal Neurological Institute (MNI) template using a similar transformation matrix. Third, images were smoothed using an isotropic 5 mm full-width at half-maximum Gaussian kernel.

Data analysis

Resting-state functional connectivity measures low-frequency spontaneous blood oxygen level–dependent (BOLD) oscillations (0.01–0.10 Hz band);[36] thus, the time course for each voxel was band-pass filtered in this range. Amygdala seed regions of interest (ROIs) from cytoarchitectonically determined probabilistic maps of the human basolateral and centromedial amygdala, adapted from Etkin and colleagues.[39] These subregions were combined to form a single, whole amygdala seed. We extracted the spatially averaged time series from right and left amygdala ROIs for each participant. A global-signal regressor was added to the model to remove nonspecific global sources of noise associated with BOLD fMRI scanning, consistent with a number of recent resting-state studies that also used global-signal regression.[15,36] Note that global-signal regression raises certain methodologic issues, which are mentioned in the Discussion section. Pearson product–moment correlation coefficients were calculated between average time courses in the amygdala "seed" ROIs and all other voxels of the brain resulting in a 3-D correlation coefficient image (r-image). Both positive correlations and anticorrelations were computed. These r-images were then transformed to z scores using a Fisher r-to-z transformation.

Z score images from the individual functional connectivity analyses were entered into second-level random-effects analyses (1-sample and 2-sample t tests) implemented in SPM5. Second-level maps were thresholded at $p < 0.005$, uncorrected, extent threshold $k = 20$. In addition, we conducted ROI analysis with small volume correction. A priori ROIs, including the hippocampus, ACC and insula, were used as masks, as these regions are of interest in individuals with PTSD.[2,10] Images were thresholded using a voxel-wise threshold of $p < 0.005$, uncorrected, with a minimum cluster size of 4 connected voxels for hippocampus clusters and 6 connected voxels for ACC or insula clusters. These combinations of activation threshold and cluster size were determined using AlphaSim[52] to correspond to a false-positive rate of $p < 0.05$, corrected for multiple comparisons within ROIs.

Using the thresholds and cluster sizes defined above, the corrected voxel-wise probabilities are as follows: hippocampus,

$p < 0.00097$; ACC, $p < 0.0003$; and insular cortex, $p < 0.00045$. Only the activations within the ROIs that survived the volume and voxel correction criteria were extracted and used for further analysis. Connectivity foci were labelled by comparison with the neuroanatomical atlas by Talairach and Tournoux.[53] Reported voxel coordinates correspond to standardized MNI space. To assess for correlations with symptom severity, CAPS scores were added as regressors in a separate whole-brain analysis of connectivity between the amygdala and ROIs.

Results

We scanned 30 veterans from OEF and OIF with (n = 15) or without (n = 15) a current PTSD diagnosis. Imaging data from 1 control participant was lost owing to scanner malfunction, leaving a final sample of 15 patients with PTSD and 14 combat controls. All CAPS scores in the PTSD group were greater than or equal to 50 (mean 75.9, SD 17.2), and CAPS scores in the control group (mean 10.9, SD 7.7) were significantly lower than in the PTSD group (t_{26} = 12.9, $p < 0.001$). Seven patients in the PTSD group met diagnostic criteria for depression and 1 had comorbid panic disorder, assessed by the MINI.[46] There were no other current Axis I or Axis II disorders in the PTSD group. Two patients with PTSD were using trazodone as a sleep aid; no other psychiatric medications were permitted. All participants were right-handed men between 21 and 37 years old. The mean age of the participants was 27.3 (SD 4.5) years in the PTSD group and 26.6 (SD 3.3) years in the control group (t_{26} = 0.477, p = 0.64). Groups did not differ by race (n = 28, χ^2_5 = 4.18, p = 0.52), marital status (n = 28, χ^2_3 = 6.27, p = 0.099) or level of education (n = 26, χ^2_3 = 5.39, p = 0.15).

Combat control group

The right amygdala seed showed positive connectivity with a number of regions, including the left amygdala, bilateral periamygdala and bilateral hippocampus, and anticorrelation with the dorsal mPFC, dorsal ACC, precuneus, lateral PFC and inferior parietal cortex (Table 1 and Fig. 1).

The left amygdala seed showed positive connectivity with the right amygdala, periamygdala, bilateral hippocampus and middle temporal gyrus, and anticorrelation with the dorsal ACC, rostral ACC, lateral PFC, inferior parietal cortex and precuneus (Table 1 and Fig. 1).

Table 1: Activation results from single-group whole brain voxel-wise analysis (part 1 of 2)

Contrast map; brain region	Cluster size	MNI coordinate			*Z* score analysis
		x	*y*	*z*	
Right amygdala positive connectivity in combat controls					
Left amygdala/bilateral periamygdala/bilateral hippocampus	3506	27	–6	–18	7.22
Right amygdala anticorrelations in combat controls					
Right postcentral gyrus	54	30	–36	42	4.63
Midcingulate cortex/posterior cingulate cortex	120	0	–30	33	4.38
Precuneus (anterior)	57	–15	51	36	4.26
Right inferior parietal cortex	65	45	–45	36	4.14
Right middle frontal gyrus	45	30	9	66	4.07
Anterior cingulate cortex/superior medial frontal gyrus	80	–3	39	12	4.03
Left angular gyrus	81	–33	–57	42	4.02
Calcarine gyrus	57	3	–81	9	4.01
Right superior orbital frontal gyrus	66	24	63	0	3.98
Left superior frontal gyrus	170	–33	57	12	3.91
Left supplementary motor area	24	–18	0	66	3.85
Right superior frontal gyrus	155	18	36	39	3.77
Precuneus (posterior)	75	9	–84	45	3.76
Cuneus	31	6	–75	21	3.44
Left middle frontal gyrus	21	–45	18	48	3.37
Left inferior parietal cortex	35	–57	–57	42	3.36
Left amygdala positive connectivity in combat controls					
Left amygdala/bilateral periamygdala/bilateral hippocampus	890	–24	–6	–18	6.78
Right amygdala/periamygdala/hippocampus	736	36	12	–36	4.63
Right thalamus	20	12	–21	–9	3.81
Left inferior orbital frontal gyrus	44	–39	36	–15	3.76
Left amygdala anticorrelations in combat controls					
Precuneus/midcingulate cortex	889	12	–69	45	4.68
Left middle frontal gyrus	237	–27	48	36	4.61
Right middle frontal gyrus	525	30	63	3	4.54
Anterior cingulate cortex/superior medial frontal gyrus	231	0	36	12	4.38
Right inferior parietal cortex	131	48	–51	36	4.31
Right superior frontal gyrus	117	27	30	36	3.62
Supplementary motor area	43	6	24	60	3.58

Posttraumatic stress disorder group

The right amygdala seed showed positive connectivity with a number of regions, including the left amygdala, periamygdala, bilateral hippocampus and bilateral insula, and anticorrelation with the dorsal mPFC, rostral ACC and inferior parietal cortex (Table 1 and Fig. 1).

The left amygdala seed showed positive connectivity with the right amygdala, periamygdala, bilateral hippocampus and bilateral insula, and anticorrelation with the dorsal ACC, lateral PFC, inferior parietal cortex and precuneus (Table 1 and Fig. 1).

Comparison of PTSD versus combat control groups

Compared with combat controls, patients with PTSD showed greater positive connectivity between the right amygdala seed and the right insula/superior temporal gyrus (MNI coordinates $x, y, z = 48, -39, 21$; $k = 26$; z score = 3.38; $p < 0.005$) and reduced anticorrelation between the right amygdala seed and the dorsal ACC (MNI coordinates $x, y, z = -12, 24, 30$; $k = 24$; z score = 3.32; $p < 0.005$; Table 2 and Fig. 1). Compared with patients with PTSD, combat controls showed greater positive connectivity between the right amygdala seed and the left hippocampus (MNI coordinates $x, y, z = -30, -21, -9$; $k = 20$; z score = 3.48; $p < 0.005$) and left inferior orbital frontal gyrus (MNI coordinates $x, y, z = -30, 36, -9$; $k = 22$; z score = 3.45; $p < 0.005$; Table 2 and Fig. 1). No other group differences were observed.

Compared with combat controls, patients with PTSD showed greater positive connectivity between the left amygdala seed and the right insula (MNI coordinates $x, y, z = 54, 0, -3$; $k = 28$; $Z = 3.56$; $p < 0.005$) and reduced anticorrelation between the right amygdala seed and the rostral ACC (MNI coordinates $x, y, z = 6, 36, 12$; $k = 20$; $Z = 3.64$, $p < 0.005$; Table 2 and Fig. 1). Combat controls did not show greater connectivity than patients with PTSD in any significant clusters ($k > 20$).

Within the PTSD group, symptom severity, as measured by the CAPS, was not significantly associated with connectivity between the amygdala and ROIs.

Discussion

In this study, we investigated patterns of resting-state functional connectivity of the amygdala in whole brain analyses, comparing military combat veterans deployed to Iraq or Afghanistan (OEF/OIF) with PTSD versus OEF/OIF combat

Table 1: Activation results from single-group whole brain voxel-wise analysis (part 2 of 2)

Contrast map; brain region	Cluster size	MNI coordinate			*Z* score analysis
		x	*y*	*z*	
Left amygdala anticorrelations in combat controls (continued)					
Right superior frontal gyrus	20	27	9	63	3.41
Right amygdala positive connectivity in patients with PTSD					
Left amygdala/insula/bilateral periamygdala/hippocampus	2198	27	–6	–18	6.76
Right inferior temporal gyrus	216	48	–57	–3	4.39
Right postcentral gyrus	23	63	–3	21	3.68
Left superior temporal gyrus	36	–60	–24	3	3.53
Right amygdala anticorrelations in patients with PTSD					
Left supplementary motor area/middle frontal gyrus	275	–24	6	57	4.10
Calcarine gyrus	346	–12	–69	6	3.91
Anterior cingulate cortex/superior medial frontal gyrus	43	15	60	3	3.81
Left superior parietal cortex	20	–21	–51	66	3.64
Right superior frontal gyrus	94	36	9	57	3.63
Right middle frontal gyrus	22	30	57	18	3.54
Precuneus	55	0	–69	42	3.49
Left angular gyrus/inferior parietal cortex	23	–39	–63	42	3.23
Left amygdala positive connectivity in patients with PTSD					
Left insula/periamygdala/hippocampus	999	–27	–6	–15	6.43
Right amygdala/periamygdala/hippocampus/insula	316	21	–6	–27	5.00
Right superior temporal gyrus	550	54	3	–3	4.72
Left amygdala anticorrelations in patients with PTSD					
Right supplementary motor area	103	12	24	60	4.34
Right superior frontal gyrus	93	21	42	15	4.27
Left superior parietal cortex	70	–12	–66	42	4.27
Precuneus	340	24	–36	33	3.93
Left middle frontal gyrus/anterior cingulate cortex	207	–9	36	15	3.89
Right angular gyrus/inferior parietal cortex	25	48	–51	39	3.88
Right middle frontal gyrus	88	39	24	33	3.82
Left precentral gyrus	23	–27	–3	48	3.38
Left angular gyrus/inferior parietal cortex	34	–45	–63	39	3.38

MNI = Montreal Neurological Institute; PTSD = posttraumatic stress disorder.

controls. To our knowledge, this is the first examination of resting-state amygdala connectivity in patients with PTSD, and we found greater positive connectivity between the amygdala and insula, reduced positive connectivity between the amygdala and hippocampus, and reduced anticorrelation between the amygdala and dorsal and rostral ACC in patients with PTSD. These findings suggest abnormalities in emotion generation and regulation circuits that may contribute to the pathophysiology of PTSD and demonstrate that studies of functional connectivity of the amygdala may be used to discern aberrant patterns of coupling within these circuits.

Consistent with previous resting-state functional connectivity studies[14,15] and effective connectivity studies,[17,54] we found anticorrelations between the amygdala and medial prefrontal regions, including the dorsal and rostral ACC, consistent with our a priori hypothesis. Whereas resting-state functional connectivity analyses examine correlations (i.e., between activity in the seed ROI and other brain regions), and thus do not allow for inferences about causal relationships, other lines of evidence support an inhibitory role of the dorsal mPFC in relation to amygdala activity. Animal studies of fear conditioning establish mPFC involvement in the extinction of conditioned fear,[55] and in humans, a recent Granger causality analysis of a face-processing task indicated an inhibitory influence from the rostral ACC to the amygdala,[56] supporting the idea that the rostral ACC might be suppressing amygdala activity. Resting-state amygdala connectivity studies also reveal anticorrelations between the dorsal mPFC and amygdala in low-anxious individuals and, interestingly, a lack of coupling in high-anxious individuals.[14] Similarly, Gilboa and colleagues[17] reported that during neutral emotion conditions, the ACC exerts an inhibitory influence on the amygdala, and this relation is diminished in individuals with PTSD. These studies provide accumulating, albeit indirect, support for interpreting anticorrelations between the mPFC and amygdala as an inhibitory relationship. If the ACC and mPFC play an important inhibitory role in modulating amygdala signal, our findings of a weaker anticorrelation between the amygdala and ACC might reflect diminished capacity of the medial prefrontal regions in individuals with PTSD to suppress amygdala activity. This is indeed consistent with a theory of diminished top–down regulation of the amygdala by emotional regulatory circuits.[10] Such a lack of inhibition from dorsal medial regions to the amygdala can be interpreted as a deficit in automatic emotion regulation[10] or a lack of cognitive control over emotion.[54] It is noteworthy that individuals with PTSD demonstrate both of these deficits in clinical settings.[57] Moreover, training patients with PTSD in emotion-regulation strategies has been shown to reduce negative emotional responses and normalize PFC responses to aversive stimuli.[58]

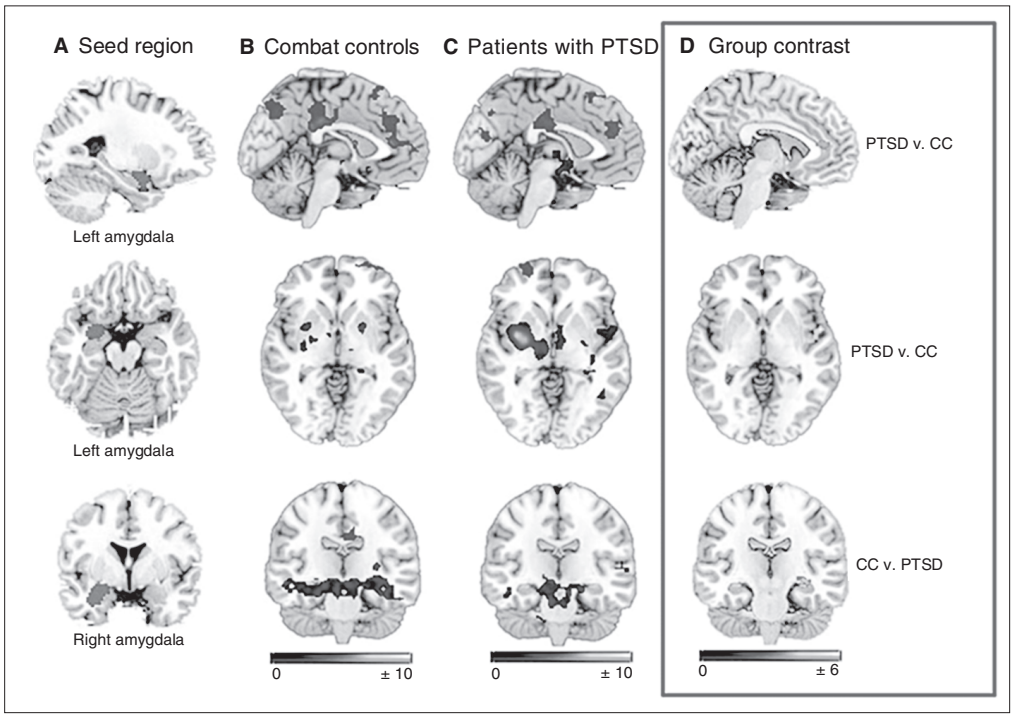

Fig. 1: Functional connectivity analysis. Patients with posttraumatic stress disorder (PTSD; **C**) compared with combat controls (CC; **B**) showed reduced anticorrelation between the left amygdala (seed region shown in **A**) and the rostral anterior cingulate cortex (**D** top), increased positive connectivity between the left amygdala and right insula (**D** middle) and reduced positive connectivity between the right amygdala and left hippocampus (**D** bottom). Slices displayed at Montreal Neurological Institute coordinates $x = 3$ (**top row**), $z = 1$ (**middle row**) and $y = -18$ (**bottom row**). Activations are corrected for multiple comparisons within regions of interest.

We also found increased functional connectivity between the bilateral amygdala and the right insula. Similar findings of enhanced connectivity between the insula and amygdala were previously reported in studies of recently traumatized individuals during symptom provocation.[16] In individuals with PTSD, insula activation has been associated with trauma recall[21] and viewing fearful faces,[18] and co-occurring increases in insula and amygdala activity were found to be greater in individuals with PTSD than combat-exposed or non–combat exposed controls during trauma reminders[59] and fear acquisition.[60] The amygdala and insula have strong structural connections[61] and exhibit high functional connectivity at rest.[62] Hyperconnectivity of the amygdala and anterior insula could indicate stronger anticipation of negative events, as seen in the PTSD symptom of hyperarousal, and hyperconnectivity between the posterior insula and amygdala could indicate a tighter functional link between visceral perception and emotional response, as seen in re-experiencing symptoms. Indeed, insula responsivity to negative images in individuals with PTSD has previously been shown to be positively correlated with hyperarousal symptoms,[19] re-experiencing symptoms[63] and flashback intensity.[64] Given these findings, our finding of increased amygdala–insula functional connectivity at rest might suggest a role for maladaptive coupling of emotion and visceral sensation in PTSD symptoms.

We have also observed reduced positive connectivity between the amygdala and left hippocampus in patients with PTSD. This is in contrast to several recent studies showing increased amygdala–hippocampus correlations during symptom provocation or induction of negative mood states. For example, patients with PTSD show exaggerated activity in both the amygdala and hippocampus while recollecting negative autobiographical memories[65] and while viewing negative pictures,[66] and the degree of this hyperactivity correlated with symptom severity.[66] On the other hand, studies using neutral tasks more often report decreased hippocampal activity in individuals with PTSD.[67] One study found that amygdala hyperactivity during fear extinction and hippocampal hypoactivity during extinction recall was associated with a behavioural deficit in extinction recall in individuals with PTSD.[68] Thus, existing findings suggest divergent patterns of amygdala–hippocampus connectivity in fear or safety contexts. A failure to properly contextualize threat and safety signals, or integrate corrective information into fear schemas, may be relevant to the development of PTSD symptomatology. Weakened connection strength between the amygdala and hippocampus during times of safety, as suggested by the present study, could contribute to these deficits.

Table 2: Activation results from 2-group comparison

Contrast map; brain region	Cluster size	MNI coordinate			*Z* score analysis
		x	*y*	*z*	
Right amygdala PTSD > combat controls					
Right insula/superior temporal gyrus	26	48	–39	21	3.38
Dorsal anterior cingulate cortex*	24	–12	24	30	3.32
Right amygdala combat controls > PTSD					
Left hippocampus	20	–30	–21	–9	3.48
Left inferior orbital frontal gyrus	22	–30	36	–9	3.45
Left amygdala PTSD > combat controls					
Rostral anterior cingulate cortex*	20	6	36	12	3.64
Right insula	28	54	0	–3	3.56
Left amygdala combat controls > PTSD					
No clusters greater than 20 voxels					

MNI = Montreal Neurological Institute; PTSD = posttraumatic stress disorder.
*Indicates reduced anticorrelation in the PTSD group.

To our knowledge, there have been 4 previous studies of resting-state connectivity in individuals with PTSD, all using a thalamus or PCC/precuneus seed for connectivity analysis. Bluhm and colleagues[32] reported reduced functional connectivity between the PCC/precuneus and amygdala in individuals with PTSD; however, in a more recent study with acutely traumatized individuals, they found PCC/precuneus-to-amygdala connectivity to be positively correlated with PTSD symptoms.[33] In our study, the amygdala was less anticorrelated with the PCC/precuneus in patients with PTSD than controls, though the extent of this activation was small (less than 10 voxels). A recent connectivity modelling analysis found a strong functional link between the PCC and the amygdala in healthy individuals.[15] Disruptions in this connection may point to default network disturbances in individuals with PTSD, a hypothesis that merits further research.

The patterns of amygdala connectivity at rest that we found in patients with PTSD are consistent with the idea that this disorder involves a functional dissociation between dorsal regions (including the PCC, dorsal ACC, dorsal mPFC and dorsolateral PFC) involved in effortful emotion regulation and ventral regions (amygdala, subgenual ACC and insula) involved in emotion experience.[69] Our findings of enhanced connectivity in emotion-experience regions (amygdala and insula) and reduced connectivity between these regions and emotion-regulation regions suggest plausible mechanisms involved in exaggerated emotional responses and apparent regulation dysfunction seen in patients with PTSD.

Limitations

Our study has several limitations. First, we tested male veterans with combat exposure, and thus generalizations to women or to individuals with non–combat related PTSD cannot be made. Second, our PTSD sample included 7 participants with comorbid major depression. Though depression commonly co-occurs with PTSD in veteran populations (up to 80% by some estimations[70]), our inclusion of depressed participants may render some of our effects attributable to the presence of depression independently or in interaction with PTSD. Of note, our results were not affected by the removal of participants with current comorbid depression. Therefore, we retained depressed participants in our final analysis. Third, we have interpreted our findings under the assumption that both groups responded similarly to the scanning environment. It is possible, however, that patients with PTSD experienced higher levels of anxiety during the scan, potentially contributing to differential patterns of connectivity. Fourth, our methods are essentially correlational and

thus do not allow for inferences about causal relationships. Future studies should use methods designed to probe effective connectivity (e.g., dynamic causal modelling and path analytic methods, such as mediation analysis) to further clarify causal interrelationships between the amygdala and emotion-production and -regulation regions. Finally, we used global regression to remove global sources of noise and interpreted negative correlations to represent anticorrelated brain regions. Global regression averages whole brain activity at every time point and factors out this value from the time series of every voxel. It is particularly helpful to interpret within-group effects; if global signal is not removed, every region in the brain appears to be massively correlated with every other region.[71] Thus, global regression could be considered a strength of our study insofar as it allows analysis of within-group effects as well as between-group effects. However, our use of global regression is a limitation in that there is continued controversy about whether global regression introduces artifactual anticorrelation.[71] Recently, however, it had been convincingly argued that global regression does not introduce large-scale artifacts, and globally regressed connectivity maps more accurately depict the known anticorrelations between functional networks in the brain.[72,73] In sum, recent resting-state functional connectivity studies of the amygdala have used global regression and identified networks proposed to be anticorrelated with the amygdala (for example, see Roy and colleagues[15]), thus we have adopted an approach that is consistent with existing practices in the literature.

Conclusion

Enhanced amygdala coupling with emotion-production regions including the insula, and reduced amygdala coupling with emotion-regulation and contextualization regions, including the hippocampus and the dorsal and rostral ACC, was observed in patients with PTSD. These findings suggest that abnormalities in emotion generation and regulation circuits may contribute to PTSD pathophysiology and demonstrate that studies of functional connectivity of the amygdala during the resting state may be used to discern aberrant patterns of coupling within these circuits.

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