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Neurobiological correlates of distinct PTSD symptom profiles during threat anticipation in combat veterans

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Abstract

Background—Previous research in posttraumatic stress disorder (PTSD) has identified disrupted ventromedial prefrontal cortex (vmPFC) function in those with versus without PTSD. It is unclear whether this brain region is uniformly affected in all individuals with PTSD, or whether vmPFC dysfunction is related to individual differences in discrete features of this heterogeneous disorder.

Methods—In a sample of 51 male veterans of Operation Enduring Freedom/Operation Iraqi Freedom, we collected functional magnetic resonance imaging data during a novel threat anticipation task with crossed factors of threat condition and temporal unpredictability. Voxelwise regression analyses related anticipatory brain activation to individual differences in overall PTSD symptom severity, as well as individual differences in discrete symptom subscales (re-experiencing, emotional numbing/avoidance, and hyperarousal).

Results—The vmPFC showed greater anticipatory responses for safety relative to threat, driven primarily by deactivation during threat anticipation. During unpredictable threat anticipation, increased PTSD symptoms were associated with relatively greater activation for threat vs. safety. However, simultaneous regression on individual symptom subscales demonstrated that this effect was driven specifically by individual differences in hyperarousal symptoms. Furthermore, this analysis revealed an additional, anatomically distinct region of the vmPFC in which re-experiencing symptoms were associated with greater activation during threat anticipation.

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Supplementary information: Supplementary methods and results; 7 figures; 4 tables; 1 movie

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Dr. Grupe had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosures and Conflicts of Interest

Dr. Davidson serves on the board of directors for the following non-profit organizations: The Mind and Life Institute and Healthy Minds Innovations. Dr. Grupe, Mr. Wielgosz, and Dr. Nitschke report no conflicts of interest or financial disclosures.

Conclusions—Increased anticipatory responses to unpredictable threat in distinct vmPFC subregions were uniquely associated with elevated hyperarousal and re-experiencing symptoms in combat veterans. These results underscore the disruptive impact of uncertainty for veterans, and suggest that investigating individual differences in discrete aspects of PTSD may advance our understanding of underlying neurobiological mechanisms.

Keywords

posttraumatic stress disorder (PTSD); veterans; hyperarousal; re-experiencing; ventromedial prefrontal cortex (vmPFC); functional magnetic resonance imaging (fMRI); uncertainty; unpredictability

Introduction

Exposure to traumatic and life-threatening combat events leads to a diagnosis of posttraumatic stress disorder (PTSD) in approximately 14% of combat veterans (Schell & Marshall 2008), and subthreshold symptoms are observed in many veterans who do not meet full diagnostic criteria. The broad range of PTSD symptoms observed in response to trauma, and the diverse clinical manifestations of the disorder, challenge the view that PTSD is a monolithic, categorical entity. As such, increased understanding of the neurobiological mechanisms underlying maladaptive responses to trauma may benefit not by contrasting groups of individuals with and without a categorical diagnosis, but rather by investigating continuous variability in different features of this heterogeneous disorder (Insel *et al.* 2010).

Recent neuroimaging studies in combat veterans have identified relationships between elevated hyperarousal symptoms and reduced amygdala volume (Pietrzak *et al.* 2015), and between re-experiencing symptoms and disrupted hippocampal resting-state connectivity (Spielberg *et al.* 2015). Additionally, in civilian trauma survivors performing an emotional Stroop task, increased hyperarousal symptoms were associated with reduced medial prefrontal cortex (mPFC)-amygdala functional connectivity, and re-experiencing symptoms were associated with altered hippocampus-insula connectivity (Sadeh *et al.* 2014). These studies implicate brain regions commonly identified in neuroimaging studies of PTSD – the amygdala, hippocampus, and mPFC (Etkin & Wager 2007; Milad *et al.* 2009; Hayes *et al.* 2012; Admon *et al.* 2013) – while suggesting that this circuitry may not be uniformly affected across all manifestations of PTSD. Instead, these brain regions may show distinct alterations corresponding to the relative dominance of particular symptoms.

To date, few studies have related specific dimensions of PTSD symptomatology to task-based fMRI activation, with one study investigating functional connectivity during emotional processing (Sadeh *et al.* 2014) and a second relating state (rather than trait) symptomatology to brain activation during script-driven imagery (Hopper *et al.* 2007). A particularly relevant but largely unexplored task in which to apply this analytic strategy is threat anticipation under conditions of uncertainty (Grupe & Nitschke 2013). Exposure to threatening stimuli, such as mild electric shock, is a robust and ecologically valid stressor, and concurrent manipulations of uncertainty can illuminate individual differences of relevance for clinical anxiety that are not observed under conditions of certainty (Lissek *et al.* 2006; Grillon *et al.* 2009). The anticipation of unpredictable threat should in particular

target hypervigilance and hyperarousal symptoms, which are especially prevalent in veteran populations: one study reported equivalent levels of hypervigilance in veterans *without* PTSD as in civilian trauma survivors *with* PTSD (Kimble *et al.* 2013). Although maintaining a constant state of vigilance is adaptive in unpredictable and dangerous combat zones, this tendency is maladaptive for veterans returning to objectively safe, non-combat environments, and may contribute to other symptoms of hyperarousal such as disrupted sleep, increased startle responsivity, irritability, and difficulty concentrating (Wilson *et al.* 2001).

The current study investigated relationships between task-based functional activation and continuous variability in discrete PTSD symptoms related to combat trauma. We collected fMRI data from 51 combat-exposed veterans using a novel paradigm that orthogonally manipulated threat of shock and temporal predictability. In contrast to fear conditioning and extinction studies, cue-outcome associations were explicitly provided to minimize learning and memory demands. We related individual differences in different symptom clusters to anticipatory activation on a voxelwise basis within the dorsal and ventral mPFC (dmPFC/vmPFC), amygdala, and hippocampus, the regions most frequently implicated in neuroimaging studies of PTSD. We hypothesized that elevated symptomatology would be associated with increased dmPFC activation during threat anticipation and decreased vmPFC activation during safe anticipation (Etkin & Wager 2007; Milad *et al.* 2009; Hayes *et al.* 2012). The specific role of the amygdala and hippocampus during prolonged periods of threat and safe anticipation is less clear (Mechias *et al.* 2010; Satpute *et al.* 2012), precluding specific directional hypotheses for these regions.

Methods and Materials

Participants

Operation Enduring Freedom/Operation Iraqi Freedom veterans were recruited through community and online advertisements, and in collaboration with veterans' organizations, the Wisconsin National Guard, and the Madison VA Hospital. Following complete study description, written informed consent was obtained. A team of clinically trained interviewers administered the Clinician-Administered PTSD Scale (CAPS; Blake et al. 1990) and Structured Clinical Interview for DSM-IV (SCID; First et al. 2002) with supervision from a licensed clinical psychologist (JBN). Exclusionary conditions included substance dependence within the past 3 months and current or past bipolar, psychotic, or cognitive disorders. Although participants were assigned to one of two groups, we analyzed data based on continuous variability in symptoms irrespective of group. Individuals in the control group were free of current Axis I disorders and had very low PTSD symptoms (CAPS scores < 10). Individuals in the posttraumatic stress symptoms (PTSS) group had PTSD symptoms occurring at least monthly with moderate intensity, and CAPS scores ≥ 20. Current major depression or dysthymia was not exclusionary in the PTSS group. Current treatment with psychotropic medications (other than benzodiazepines or beta-blockers) or maintenance psychotherapy was permitted if treatment was stable for 8 weeks prior to the beginning of the study (see Supplementary Table 1 for complete participant characteristics).

A total of 58 veterans were enrolled, but due to the small number of eligible females (N=4) analyses were conducted on male participants only. Two participants could not tolerate the shock and 1 was excluded due to excessive motion, resulting in a final sample of 51 subjects, 16 of whom met full PTSD diagnostic criteria. Of the other 35 veterans, 18 met diagnostic criteria for 1 or 2 of the CAPS subscales; 17 did not meet criteria for any subscales and were enrolled in the control group (see Supplementary Figure 1 for symptom distributions).

Procedure

During a pre-MRI visit, a series of 200-ms shocks between 0.5-5.5 milliamps were delivered to the participant's right ventral wrist to identify a stimulus rated as “very unpleasant, but not painful” (a “3” on the 0-5 scale). Participants then received task instructions, underwent a simulated MRI session, and completed self-report measures.

The MRI scan took place within 2 weeks of this visit. A single shock was delivered to confirm shock calibration levels, and a novel threat anticipation task (Figure 1A; Movie 1) was delivered using PsychoPy 2 (Peirce 2007). Participants were instructed on cue-outcome contingencies during the simulated MRI session and again immediately before the fMRI scan.

Each trial began with a 2-s presentation of a blue or yellow square, indicating threat of shock or safety from shock (counterbalanced). Next, the same color clock appeared for 4-10 s. On predictable trials, a red mark appeared in a random location and the anticipation period ended when a slowly rotating hand reached this mark. On unpredictable trials, no red mark appeared and participants could not predict the end of the anticipation period. On 12/42 threat trials, a 200-ms electric shock was delivered concurrently with a neutral tone. On the remaining threat trials and all safe trials, the anticipation period concluded with the same 200-ms tone only. Participants rated the unpleasantness of the shock on 75% of shock trials and anticipatory anxiety on 33% of no-shock trials. Trials were separated by a 5-9 s inter-trial interval.

Each of 3 task runs lasted 8:00 and consisted of 24 trials. The scan included 42 threat trials and 30 safe trials, resulting in the same number of non-reinforced threat and safe trials (Schiller *et al.* 2008). For each of the 4 conditions, there were twice as many trials with long (8-10 s) as short anticipation durations (4-6 s).

Magnetic resonance imaging data collection

MRI data were collected on a 3T X750 GE Discovery scanner using an 8-channel head coil and ASSET parallel imaging with an acceleration factor of 2. Data collected included 3 sets of echo planar images during the threat anticipation task (240 volumes, TR=2000, TE=20, flip angle=60°, field of view=220 mm, 96×64 matrix, 3-mm slice thickness with 1-mm gap, 40 interleaved sagittal slices), a T1-weighted anatomical image for functional data registration (“BRAVO” sequence, TR=8.16, TE=3.18, flip angle=12°, field of view=256 mm, 256×256 matrix, 156 axial slices), and field map images. Visual stimuli were presented using Avotec fiberoptic goggles, auditory stimuli were presented binaurally using Avotec headphones, and behavioral responses were recorded using a Current Designs button box.

Electrodermal activity was recorded from the distal phalanges of participants' third and fourth fingers using Ag/AgCl electrodes (see Supplementary Methods).

FMRI data processing and analysis

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Preprocessing steps included removal of the first 4 volumes, motion correction using MCFLIRT, removal of non-brain regions using BET, spatial smoothing using a Gaussian kernel with 5mm FWHM, grand-mean intensity normalization, and high-pass temporal filtering.

First-level modeling of task data included predictors for threat and safe cues, each anticipation condition (unpredictable threat [uThreat], predictable threat [pThreat], unpredictable safe [uSafe], predictable safe [pSafe]), shocks, tones, and the shock/anxiety rating periods. A double-gamma hemodynamic response function was convolved with a boxcar function with duration equivalent to each stimulus presentation; for the anticipation period, this regressor thus varied between 4-10 s (Grupe *et al.* 2013). The first-level design matrix also included 6 motion parameters, first- and second-order motion derivatives, and a confound regressor for each time point with > .9 mm framewise displacement (Siegel *et al.* 2014). Autocorrelation of time series data was corrected using FILM (Woolrich *et al.* 2001). Functional images were resampled to $2mm^3$ isotropic voxels and registered to high-resolution T1 images and then Montreal Neurological Institute template space using FLIRT and FNIRT (http://www.fmrib.ox.ac.uk/fsl/fnirt/index.html).

Although participants were initially assigned to separate groups based on overall symptoms, we sought to identify neural correlates of continuous variability in PTSD symptoms irrespective of group. We thus regressed uThreat vs. uSafe contrast estimates on total CAPS symptom scores across all 51 participants. Next, we conducted simultaneous multiple regression of uThreat vs. uSafe contrast estimates on each of the three DSM-IV CAPS subscales: re-experiencing, emotional numbing/avoidance, and hyperarousal. This analysis accounts for shared variance across symptom clusters, and highlights unique variance in brain activation associated with specific symptoms above and beyond shared effects.

Primary analyses were conducted using small-volume correction over an anatomically defined mask including the mPFC, amygdala, and hippocampus. The amygdala and hippocampus were defined using the Harvard-Oxford anatomical atlas with a 50% maximum likelihood cutoff (Desikan *et al.* 2006). The mPFC was defined using the Wake Forest University PickAtlas (Maldjian *et al.* 2003) and consisted of medial portions of Brodmann Areas 9, 10, 11, 12, 24, 25, and 32 anterior to y=0 (Motzkin *et al.* 2011; Shackman *et al.* 2011). Secondary voxelwise analyses were carried out across the whole brain. Cluster threshold correction was applied to *a priori* masked regions and across the whole brain using a voxelwise threshold of $p < 0.005$, resulting in corrected significance of $p < 0.05$. All unthresholded statistical maps were uploaded to the NeuroVault.org database, and are available at http://neurovault.org/collections/1104/.

Results

Anticipatory anxiety ratings and skin conductance responses

Self-report and skin conductance data demonstrated that our novel task was effective in robustly eliciting anticipatory anxiety and physiological arousal, with greater self-reported anxiety ratings and skin conductance responses for threat relative to safe trials (Figure 1B-C; Supplementary Results). Neither anxiety ratings nor phasic skin conductance responses were related to PTSD symptoms (Supplementary Results). There was, however, a positive relationship between *tonic* skin conductance during the task and overall PTSD symptom severity ($r(45) = 0.42$, $p = 0.003$; Figure 1D) as well as scores on each CAPS subscale (re-experiencing: $r(45) = 0.43$, $p = 0.003$; avoidance/numbing: $r(45) = 0.32$, $p = 0.03$; hyperarousal: $r(45) = 0.41$, $p = 0.004$). Speaking to the specificity of this relationship to trauma-related symptoms, although Beck Anxiety Inventory (BAI) scores were also correlated with tonic skin conductance ($r(45) = 0.30$, $p = 0.043$), multiple regression analysis showed that tonic skin conductance levels were uniquely predicted by total CAPS scores ($t(44) = 2.18$, $p = 0.035$) and not BAI scores ($t(44) = 0.08$, $p = 0.94$).

Overall task activation and relationships with skin conductance responses

For *a priori* regions of interest, greater activation for uThreat vs. uSafe was observed across the dmPFC, whereas greater activation for uSafe vs. uThreat – resulting from relative deactivation during threat anticipation – was observed in the vmPFC and in clusters spanning bilateral hippocampus and amygdala (Figure 2). Across the rest of the brain, the contrast of uThreat vs. uSafe showed activation consistent with previous instructed threat anticipation studies (Mechias *et al.* 2010; Grupe *et al.* 2013) (Figure 2, Supplementary Tables 2-3); results were highly similar for the pThreat vs. pSafe contrast.

Regression of uThreat vs. uSafe brain activation on skin conductance responses showed that elevated skin conductance responses were associated with increased anticipatory brain activation in an expansive network of threat-responsive regions (Supplementary Figure 2, Supplementary Table 4). Notably, the right dorsal amygdala – which did not show a main effect of threat condition – also showed this positive correlation with skin conductance responses.

Relationships between PTSD symptoms and activation in the mPFC, amygdala, and hippocampus

We next regressed brain activation during unpredictable anticipation on overall CAPS scores. Within the *a priori* masked region, CAPS scores were positively correlated with uThreat vs. uSafe activation in the left pregenual anterior cingulate cortex (pACC), at the dorsal and anterior edge of the vmPFC cluster that showed deactivation for uThreat (Figure 3A).

Qualifying this relationship, however, simultaneous regression on the three CAPS subscales demonstrated that this relationship was driven specifically by hyperarousal symptoms, which were positively correlated with uThreat vs. uSafe activation in an overlapping pACC cluster (Figure 3B). Furthermore, this simultaneous regression revealed an additional, more

anterior/ventral vmPFC cluster (corresponding to BA10) in which uThreat vs. uSafe activation was positively associated with re-experiencing symptoms (Figure 3C). In each of these vmPFC regions, relationships with PTSD symptoms were driven by responses to uThreat and not uSafe; in other words, higher symptoms were associated with *less vmPFC deactivation* during unpredictable threat anticipation (Supplementary Figure 3). Analogous regressions for predictable trials indicated that the pACC relationship with hyperarousal symptoms was specific to unpredictable trials, whereas a similar (but uncorrected) relationship between BA10 activation and re-experiencing symptoms was seen for predictable trials (Supplementary Results; Supplementary Figure 4).

Within the *a priori* masked region, hyperarousal symptoms were positively correlated with uThreat vs. uSafe activation in a small, uncorrected cluster spanning the left posterior amygdala and anterior hippocampus (MNI coordinates: [−22, −7, −17]; 26 voxels). There were no threat-responsive dmPFC regions within the masked region that showed a relationship with total CAPS symptoms or any CAPS subscales. Furthermore, avoidance/numbing symptoms were unrelated to activation anywhere within the *a priori* masked region.

To address the possibility that vmPFC relationships with continuous symptom measures may have reflected categorical differences in veterans with high and low levels of PTSD symptoms, we conducted an additional regression analysis within the group of 34 subjects with elevated PTSD symptoms (see *Methods: Participants*). Additionally, to address the possibility that current use of psychotropic medications may have affected our results, we repeated regression analyses within the 39 medication-free participants. Finally, we ran a regression analysis including Beck Anxiety scores as a covariate to test whether the same effects would be observed when controlling for non-trauma-specific anxiety symptomatology. In each of these 3 cases, we identified highly similar small-volume-corrected vmPFC clusters that were associated with hyperarousal and re-experiencing symptoms (Supplementary Figures 5-7).

Relationships between PTSD symptoms and BOLD activation: whole-brain results

Outside of the *a priori* small-volume-corrected mask, total CAPS scores were positively correlated with uThreat vs. uSafe activation in lateral occipital cortex and occipital poles (Figure 4A). Additionally, emotional numbing/avoidance symptoms were negatively correlated with uThreat vs. uSafe activation in an anterior and very superior aspect of the right medial frontal gyrus (Figure 4B).

Discussion

Using a novel unpredictable threat anticipation task in a large sample of trauma-exposed combat veterans, we observed altered vmPFC responses to threat vs. safety in veterans with elevated PTSD symptoms. Critically, this finding was expanded upon when considering variability in individual symptom clusters. The vmPFC cluster (corresponding to pACC) that showed a relationship with overall PTSD symptoms was actually related more specifically to hyperarousal symptoms. Furthermore, the analysis of individual symptom clusters revealed an additional vmPFC region (corresponding to BA10) in which activation was uniquely associated with re-experiencing symptoms. Thus, distinct PTSD symptom clusters were

associated with functional alterations to distinct vmPFC subregions during unpredictable threat anticipation.

The presence of unique associations with distinct vmPFC regions is not surprising, given the functional heterogeneity of this region. The vmPFC is central to an array of diverse processes including self-reference, default mode function, mentalizing, prospection, memory retrieval, reward processing and valuation, autonomic control, fear inhibition, and safety learning, to name a few (Roy *et al.* 2012). Nonetheless, the extant PTSD literature has largely emphasized this region's role in safety learning and fear inhibition, and has not examined how its functional heterogeneity may be related to diverse symptoms of PTSD. Analytic strategies that treat PTSD as a unitary construct could have the consequence of smoothing across anatomically proximal (yet functionally distinct) regions that may be associated with different symptoms. Although our results warrant replication before strong conclusions can be made, they offer the intriguing possibility that examining continuous variability in distinct symptom clusters could paint a more nuanced picture of vmPFC dysfunction in different manifestations of PTSD.

Perigenual aspects of the cingulate cortex -- including the pACC region associated here with hyperarousal symptoms -- are centrally involved in threat appraisal and corresponding regulatory control of peripheral physiological response systems (Thayer *et al.* 2012; Gianaros & Wager 2015). A speculative possibility is that disrupted function of this region may be associated with poorer autonomic control of heart rate or other peripheral physiological response systems, leading to the specific relationship we observed with hyperarousal symptoms. Notably, in a study of civilian trauma survivors using an analogous analytic strategy with functional connectivity data, hyperarousal symptoms were associated with altered functional connectivity between the amygdala and a similar pACC region during an emotional Stroop task (Sadeh *et al.* 2014).

The relationship between re-experiencing symptoms and anticipatory activation in BA10 is interesting given this region's role – along with the hippocampus – in episodic autobiographical memory (Svoboda *et al.* 2006) or projecting the self into the past or future (Tulving 2002; Buckner & Carroll 2007). Re-experiencing symptoms of PTSD have previously been linked to altered hippocampus functional connectivity during the emotional Stroop task (Sadeh *et al.* 2014) and at rest (Spielberg *et al.* 2015). We did not identify a relationship between re-experiencing and task-based hippocampus activation, and it is unclear how altered BA10 function in the current study is related to these previously identified relationships between hippocampal connectivity and re-experiencing symptoms.

Activity in the vmPFC and other nodes of the default-mode network (DMN) is typically elevated at rest, and shows transient task-related deactivation (Raichle *et al.* 2001). In the current study, we saw deactivation in the vmPFC and across the DMN for threat vs. safe anticipation (Figure 2A). Associations with hyperarousal and re-experiencing symptoms were primarily driven by the threat condition, meaning that greater symptoms were associated with less vmPFC deactivation during threat anticipation. This pattern of responses – similar to that observed across the DMN during negative picture viewing in major depressive disorder (Sheline *et al.* 2009) – suggests an inability to flexibly modulate

activation within this region to reflect changing task conditions in the larger context of threat (Daniels *et al.* 2010; Sripada *et al.* 2012; Garfinkel *et al.* 2014). The observation of less vmPFC *deactivation* to instructed threat may appear at odds with previous observations of reduced vmPFC *activation* to learned safety in PTSD (Milad *et al.* 2009; Rougemont-Bücking *et al.* 2011). One important distinction is that these previous studies found vmPFC hypoactivation for previously-reinforced cues that were subsequently extinguished; by explicitly instructing our participants about cue-outcome contingencies that are never reversed, we may have tapped into distinct neurobiological processes in the current study. These discrepancies aside, a consistent finding across these studies is that PTSD is associated with *undifferentiated* vmPFC activation across conditions of safety and threat, whether learned or instructed, a message that resonates with recent fMRI studies linking PTSD to overgeneralization of threat responses (Morey *et al.* 2015) or deficient context-appropriate modulation of vmPFC, amygdala, and hippocampus activation (Garfinkel *et al.* 2014).

We did not identify relationships between PTSD symptoms and activation in the amygdala or hippocampus, both of which showed *deactivation* during threat anticipation. Although these regions are not consistently implicated in instructed threat anticipation studies (Mechias *et al.* 2010), the robust deactivation to threat in the amygdala was somewhat surprising, given this region's canonical role in the expression of fear and anxiety (notably, in the dorsal amygdala we observed increasing activation to threat in participants with stronger skin conductance responses; Supplementary Figure 2). An important consideration in interpreting this effect is the time course of amygdala involvement. The amygdala responds phasically to threat cues but does not continue to respond in the absence of new information about threat (Mechias *et al.* 2010; Grupe *et al.* 2013); to the contrary, deactivation to sustained periods of threat has been observed in at least 4 prior studies using prolonged anticipatory periods (for review, see McMenamin et al. 2014). Additional work is needed to clarify the functional significance of this sustained deactivation and to investigate relationships with the frequently observed amygdala hyperactivation in PTSD (Etkin & Wager 2007).

Because we focused exclusively on male combat veterans, further research is needed to determine whether findings generalize to female veterans or civilian trauma survivors. Future research is also needed in a no-trauma control group to characterize normative behavioral and neural responses on this novel task. An additional limitation of the current study is that our inclusion criteria targeted distinct ranges of CAPS scores, excluding those veterans with scores between 10-20. Although effects involving the entire sample were still observed in a group of 34 veterans with elevated PTSD symptoms (Supplementary Figure 5), future work adopting this approach should include veterans across the entire range of PTSD symptoms. Finally, nearly 25% of participants were on psychotropic medications at the time of scanning, although the exclusion of these participants resulted in the same results despite a reduced sample size (Supplementary Figure 6).

In summary, individual differences in hyperarousal and re-experiencing symptoms showed unique relationships with distinct regions of the vmPFC during the anticipation of unpredictable threat. These results provide a fruitful example of investigating individual

differences in discrete dimensions of PTSD, and suggest that similar approaches may shed new light on neurobiological mechanisms of this heterogeneous disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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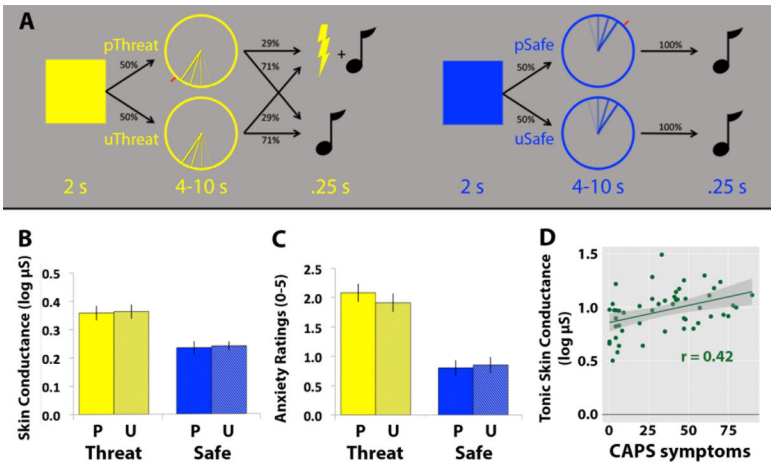

Figure 1. Threat anticipation paradigm, skin conductance responses, and self-reported anxiety

(A) Schematic of the threat anticipation paradigm (see also Movie 1). (B) Across all participants, skin conductance responses showed a main effect of threat, with elevated responses during threat vs. safety. (C) Self-reported anxiety, collected at the conclusion of the anticipatory period on a subset of trials, revealed elevated anxiety ratings for threat vs. safe trials. A significant Threat x Predictability interaction reflected greater (threat – safe) differences for predictable relative to unpredictable trials ($F(1,50) = 12.18$, $p = 0.001$; Supplementary Results). (D) Posttraumatic stress disorder symptoms, measured using the Clinician-Administered PTSD Scale (CAPS), were positively correlated with tonic skin conductance. Notes: P=predictable, U=unpredictable; error bars represent standard error of the mean; shaded area indicates 95% confidence interval.

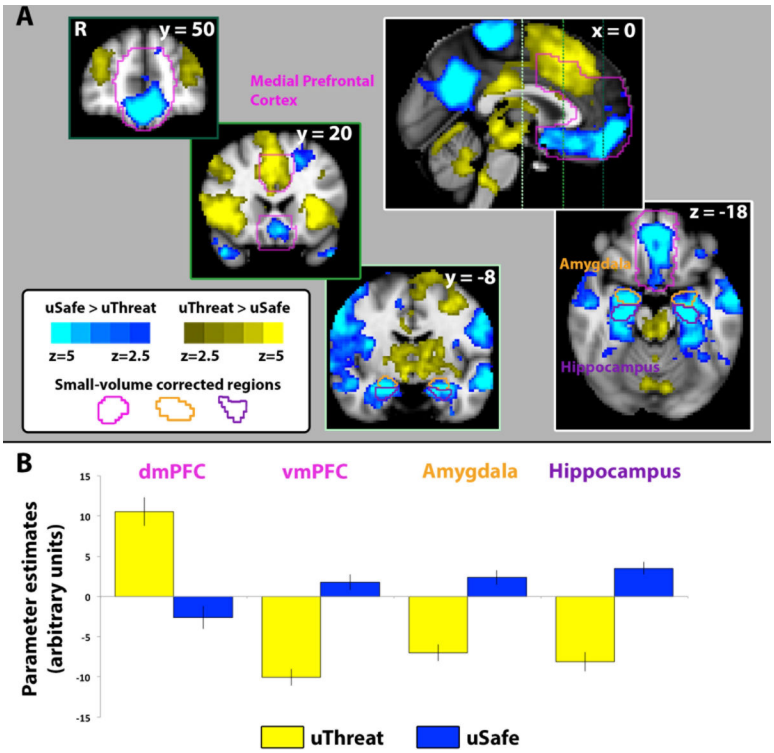

Figure 2. Overall task effects across the whole brain and in *a priori* regions of interest

(A) Results of a whole-brain corrected, voxelwise paired *t* test of unpredictable threat (uThreat) vs. unpredictable safe (uSafe) trials, with *a priori* regions of interest outlined. (B) Within the medial prefrontal region of interest, greater anticipatory activation for threat vs. safe trials was seen in the dorsomedial prefrontal cortex (dmPFC), whereas deactivation for threat vs. safe was seen in the ventromedial PFC (vmPFC). A similar pattern of deactivation for threat relative to safe trials was seen in the amygdala and hippocampus. Error bars reflect standard errors of the mean.

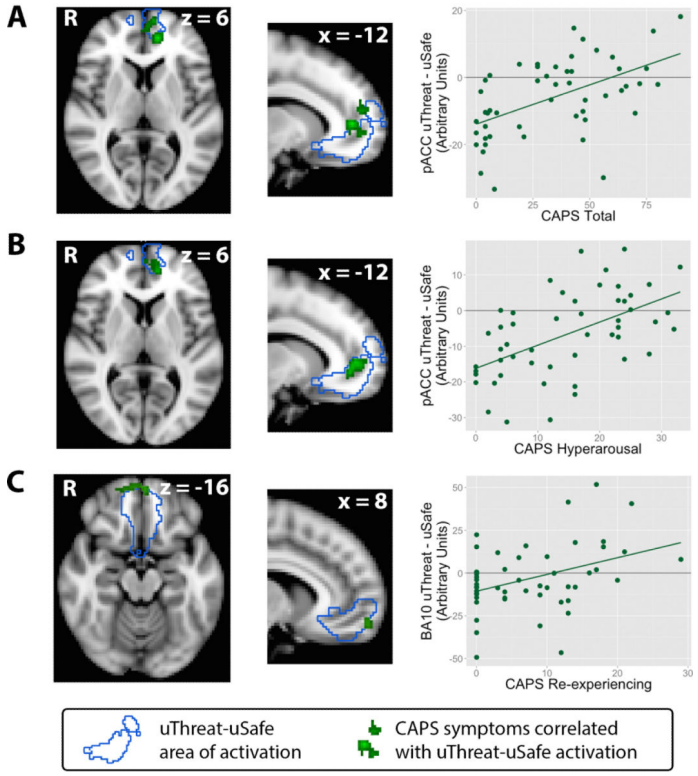

Figure 3. Relationships between PTSD symptoms and threat vs. safe activation in the ventromedial prefrontal cortex

(A) Total scores on the Clinician-Administered PTSD Scale (CAPS) were positively correlated with anticipatory uThreat vs. uSafe activation in the left pregenual anterior cingulate cortex (pACC, in green), in a region of the ventromedial prefrontal cortex (vmPFC) that showed deactivation for uThreat vs. uSafe. (B) Simultaneous multiple regression of uThreat vs. uSafe activation on all 3 CAPS subscales demonstrated that this relationship was driven by individual differences in hyperarousal symptoms. (C) The same regression analysis revealed a cluster at the ventral and anterior edge of the vmPFC (BA10) in which activation was positively correlated with re-experiencing symptoms. Scatter plots illustrate relationships between symptom scores and average contrast estimates across each cluster, and do not represent independent statistical tests. Notes: All clusters are small-volume-corrected, $p < 0.05$; uThreat = unpredictable threat; uSafe = unpredictable safe.

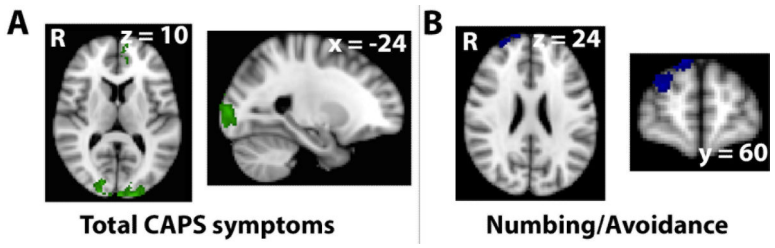

Figure 4. Whole-brain relationships with PTSD symptoms

(A) Across the whole brain, total PTSD symptoms on the Clinician-Administered PTSD Scale (CAPS) were positively correlated with uThreat vs. uSafe activation in bilateral occipital poles. (B) Simultaneous regression of uThreat vs. uSafe activation on all 3 CAPS subscales revealed an inverse relationship between emotional numbing/avoidance symptoms and activation in an anterior and very superior aspect of the right medial frontal gyrus. Notes: All clusters are small-volume-corrected, $p < 0.05$; uThreat = unpredictable threat; uSafe = unpredictable safe.