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Perceived threat bias and reduced hippocampal volume in combat veterans

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Abstract

Reduced hippocampal volume is frequently observed in posttraumatic stress disorder (PTSD), but the exact psychological processes associated with these alterations remain unclear. Given the role of the hippocampus in contextual representations of threat and memory, we investigated relationships between retrospectively reported combat exposure, perceived threat, and hippocampal volume in trauma-exposed veterans. T1-weighted anatomical images were obtained from a sample of 52 male veterans with a broad range of PTSD symptoms during MRI scanning. Hippocampal volume was estimated using automatic segmentation tools in FreeSurfer. An index of Perceived Threat Bias (PTB) was calculated, reflecting the degree of discordance between subjective perceptions of threat while deployed and self-reported combat exposure. Hippocampal volume was regressed on PTB and PTSD symptoms on the Clinician-Administered PTSD Scale (CAPS). Results indicated that perceived threat bias was unrelated to overall CAPS symptoms, but was positively associated with CAPS avoidance/numbing symptoms and symptoms of depression, anxiety, and worry. Elevated PTB was associated with smaller hippocampal volume for individuals with a wide range of PTSD symptoms. Hippocampal volume was also inversely related to avoidance/numbing CAPS symptoms. These results indicate that volume of the hippocampus, a region involved in contextual threat processing and memory, is related to recalled associations between traumatic events and accompanying subjective threat appraisals. Future research should clarify the precise temporal milieu of these effects and investigate whether individual differences in hippocampal structure and function contribute to exaggerated threat appraisal at the time of trauma, or in subsequently biased memories or appraisals of traumatic events.

Introduction

Reduced hippocampal volume is consistently observed in posttraumatic stress disorder (PTSD), with meta-analyses revealing these reductions across different trauma types and demographic groups.[1–3] The magnitude of this reduction, however, is quite modest: the largest meta-analysis to date (consisting of nearly 1,900 subjects) revealed an effect size of $d = 0.17$ for the comparison of participants with PTSD vs. trauma-exposed controls.[4] Exposure to trauma alone, even in the absence of PTSD pathology, can be associated with volumetric reductions, as hippocampal volume in trauma-exposed controls falls between that of individuals with PTSD and unexposed controls.[1,2] These meta-analytic findings of modest effect sizes and reduced post-trauma hippocampal volume in the absence of PTSD symptoms underscore the need for updated models of what is reflected in post-trauma structural alterations of the hippocampus.

A separate line of research has investigated psychological and psychosocial risk factors to help explain why different individuals exposed to similar traumatic events experience divergent long-term trajectories.[5] This question, however, may be based on a faulty premise: just because two individuals are exposed to the same external circumstances does not mean that they experienced the "same" trauma. The interpretation and meaning of these traumatic experiences will vary widely across individuals based on biological and psychological predispositions, past experiences, and current environmental factors. Indeed, subjectively *perceived threat* – fear or worry about one's safety and well-being during and after exposure to trauma – is one of the best predictors of PTSD[5–7] and other mood and anxiety disorders,[8,9] and mediates the relationship between combat exposure and PTSD symptoms in multiple veteran samples.[10–12] Although the DSM-5 no longer requires the experience of "fear, helplessness, or horror" for a PTSD diagnosis, the relationship between subjective threat appraisals and maladaptive responses to trauma underscores the importance of identifying neurobiological mechanisms of this psychological characteristic.

The hippocampus is a prime candidate brain region that may be related to subjectively perceived threat, due to its central role in the contextual processing of threat[13] and the aforementioned evidence for structural alterations to the hippocampus following trauma exposure. Two studies of healthy, older adults[15,16] identified an inverse relationship between hippocampal volume and the related construct of perceived stress, or the degree to which individuals appraise daily life events as being stressful, overwhelming, and uncontrollable. However, no studies to our knowledge have directly examined the relationship between perceived threat following combat trauma and hippocampus structure.

A fundamental issue unaddressed by prior studies of perceived deployment threat is that deployment environments are often associated with high levels of genuine threat, in which case extreme levels of perceived threat would reflect contextually appropriate, adaptive responses. Whereas hypervigilance toward threat in new recruits during a safe baseline period predicts the eventual development of PTSD, the opposite is true immediately prior to and during deployment, where increased threat avoidance is associated with later PTSD.[17,18] It is not the case that particular behavioral profiles are universally adaptive or maladaptive; rather, a defining characteristic of maladaptive threat responding is incongruence between a specific context/environment and one's response.[19] Examining perceived threat independent of the degree of trauma exposure may fail to distinguish adaptive from pathological modes of threat processing. In considering risk for psychopathology, the critical factor may not be absolute levels of perceived threat, but *relative biases* in perceived threat for individuals exposed to similar levels of traumatic events.

To that end, we introduce here a novel measure of "perceived threat bias" (PTB), which quantifies the relative congruence or incongruence of retrospectively reported combat exposure and perceived threat during deployment. In a sample of 52 combat-exposed veterans with a wide range of PTSD symptoms, we investigated relationships between PTB and symptoms of PTSD,

depression, anxiety, and trait worry. We tested the hypothesis that elevated PTB would be associated with reductions in hippocampal volume, while also investigating hippocampal volume as a function of severity of discrete PTSD symptom clusters. To investigate the specificity of relationships to the hippocampus, analogous analyses were conducted for the amygdala, due to its role in threat perception[20] and observations of smaller amygdala volume in PTSD.[1,21]

Methods

Participants

We recruited veterans of Operation Enduring Freedom/Operation Iraqi Freedom through community and online advertisements and in collaboration with veterans' organizations, the Wisconsin National Guard, and the Madison VA. Following complete study description, written informed consent was obtained. A team of clinically trained interviewers administered the Clinician-Administered PTSD Scale (CAPS)[22] and Structured Clinical Interview for DSM-IV (SCID)[23] with supervision from a licensed clinical psychologist (JBN). Exclusionary conditions included substance dependence within the past 3 months and lifetime bipolar, psychotic, or cognitive disorders.

Participants were enrolled either into a combat-exposed control (CEC) group or a posttraumatic stress symptoms (PTSS) group (Table 1). Participants in the CEC group had no current Axis I disorder and very low PTSD symptoms (CAPS scores < 10) and did not meet diagnostic criteria for any CAPS subscales. Participants in the PTSS group had PTSD symptoms occurring at least monthly with moderate intensity and CAPS scores ≥ 20 and met diagnostic criteria for at least 1 of 3 CAPS subscales. Current treatment with psychotropic medications (other than benzodiazepines or beta-blockers) or maintenance psychotherapy was permitted if treatment was stable for 8 weeks.

A total of 58 veterans met eligibility criteria and were enrolled, but due to the small number

of female veterans (n=4) we analyzed data from men only. Data from 2 participants were excluded due to excessive motion that prevented accurate delineation of white/gray matter boundaries. The final sample consisted of 36 PTSS subjects – 17 of whom met full PTSD diagnostic criteria and 19 who met diagnostic criteria for 1 or 2 of the CAPS subscales – and 16 veterans enrolled in the CEC group. We previously reported on relationships between PTSD symptoms and fMRI activation in an overlapping sample.[24]

Data collection

In a pre-MRI visit, participants completed self-report measures including the Combat Exposure Scale (CES),[25] subscales of the Deployment Risk and Resiliency Inventory (DRRI),[5] the Beck Anxiety Inventory (BAI),[26] Beck Depression Inventory (BDI-II),[27] and Penn State Worry Questionnaire.[28] Participants took part in an MRI scan within the subsequent 40 days. 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. Brain structure was assessed through the collection of T1-weighted anatomical images with 1-mm isotropic voxels ("BRAVO" sequence, TR=8.16, TE=3.18, flip angle=12°, field of view=256 mm, 256x256 matrix, 156 axial slices). Self-reported PTSD symptoms were assessed on the day of the MRI scan using the PTSD Checklist-Military Version (PCL-M).[29]

Perceived threat bias calculation

The CES[25] is a 7-item Likert scale assessing the frequency of different wartime stressors (example items: "Did you ever go on combat patrols or have other dangerous duty?", "Were you ever under enemy fire?"). The DRRI[5] includes 17 scales characterizing environmental, psychosocial, and psychological factors before, during, and after deployment. Among these scales is "perceived threat" (or "combat concerns"), a 15-item Likert scale reflecting veterans' cognitive or subjective appraisals of combat-related danger (example items: "I thought I would

never survive", "I was concerned that my unit would be attacked by the enemy").

We used these two scales to create an index of perceived threat bias (PTB; Figure 1). Both scales were approximately normally distributed across the entire sample (Figure 1A). To calculate PTB, we first z-normalized each measure to put each on a unit normal distribution, $N(0,1)$. PTB was operationalized as the difference between normalized perceived threat and normalized combat exposure scores (PTB = z(perceived threat) – z(combat exposure)). Owing to this normalization procedure, and assuming a linear relationship between these measures, a PTB score of 1.0 reflects a 1 SD increase in perceived threat based on a given level of combat experience (Figure 1B).

FreeSurfer processing

Cortical reconstruction and volumetric segmentation was performed using the FreeSurfer image analysis suite (stable release version 5.3.0; http://surfer.nmr.mgh.harvard.edu/). Processing included motion correction, skull removal, intensity normalization, registration, segmentation of subcortical white and deep gray matter structures, white matter and pial surface tessellation, and cortical surface parcellation. Segmentation quality was visually assessed and manually edited as necessary (http://freesurfer.net/fswiki/Edits). Automated segmentation of the bilateral hippocampus and amygdala was conducted for each subject, a procedure that compares favorably with labor-intensive manual segmentation.[30] Hippocampus subfields were not investigated as it is unclear whether automatic subfield segmentation allows for reliable volumetric measurements, particularly without T2 images.[31]

Data analysis

To better understand our novel PTB measure and assess its convergent and discriminant validity with existing measures, we related PTB scores to scores on the CAPS, BAI, BDI, and PSWQ. For each measure we calculated Pearson correlation coefficients with PTB across the

sample as a whole and within the PTSS group alone. For the CAPS we examined correlations with total scores and each subscale. For each measure we calculated correlations with each component of PTB separately (CES and DRRI scores).

We calculated correlations between total hippocampal and amygdalar volume and PTB scores to explore the neuroanatomical correlates of this novel measure. Correlations were conducted across the entire sample and within the PTSS group alone. We again calculated correlations between structural volume and CES/DRRI scores separately.

We correlated hippocampal and amygdalar volume with total CAPS symptoms in an attempt to replicate previously observed volumetric reductions, and with each CAPS subscale to explore relationships with specific symptom clusters. We also compared hippocampal volume between the CEC group (N=16) and participants with a PTSD diagnosis (N=17).

Finally, we conducted exploratory voxelwise brain morphometry analyses to relate PTB and CAPS symptoms to structural changes within the anatomically constrained hippocampus and amygdala, and across the whole brain (Supplementary Methods).

Results

Clinical and self-report measures

Demographics and symptom data are provided in Table 1. The PTSS group had higher scores on the CAPS, BAI, BDI, and PSWQ. The groups did not differ on self-reported combat exposure on the CES, but the PTSS group did report elevated DRRI perceived threat.

Perceived threat bias and relationships with PTSD symptoms and self-report data

Perceived threat bias (PTB) was operationalized as the relative difference, in standardized units, between self-reported combat exposure and perceived threat while deployed (see Methods). Consistent with previous research,[9,11] the CEC group showed a robust linear relationship between combat exposure and perceived threat ($r(14) = 0.65$, $p = 0.0061$), a pattern

that was absent in the PTSS group ($r(34) = -0.04$, $p = 0.83$; significant difference in correlation between groups, Fisher's $Z = 2.52$, $p = 0.011$; Figure 1B).

Across the full sample, there was no relationship between PTB and total CAPS scores ($r(50) = 0.16$, $p = 0.25$; Figure 2A). PTB was correlated with emotional numbing/avoidance CAPS symptoms ($r(50) = 0.33$, $p = 0.01$; Figure 2B), but not with re-experiencing or hyperarousal symptoms (rs < 0.07, ps > 0.6). PTB was correlated with self-reported PTSD symptoms on the PCL ($r(50) = 0.33$, $p = 0.019$) and PCL avoidance/numbing ($r(50) = 0.39$, $p = 0.0045$).

Across the entire sample and within the PTSS group alone, PTB was robustly correlated with depression symptoms on the BDI ($r(50) = 0.48$, $p < 0.001$), anxiety symptoms on the BAI ($r(50) = 0.44$, $p = 0.0010$), and trait worry on the PSWQ ($r(50) = 0.49$, $p < 0.001$; Figure S1). These correlations were primarily driven by individual differences in perceived threat (Tables S1-S2); however, within the PTSS group, self-reported combat exposure was *negatively* related to anxiety, depression, and worry scores in the PTSS group alone. Correlations with these symptom measures in the PTSS group were numerically (but not statistically) higher for PTB vs. perceived threat alone (Table S2).

These analyses revealed a range of coherence and divergence between self-reported combat exposure and perceived threat while deployed, which we operationalized as a measure of perceived threat bias (PTB). PTB was related to symptoms of anxiety, depression, and worry, but by no means redundant with these symptoms, suggesting that this measure has discriminant validity and that it may show unique relationships with neurobiological features.

<u>Perceived threat bias is associated with smaller hippocampal volume</u>

Across all participants, elevated PTB was associated with reduced hippocampal volume ($r(50) = -0.29$, $p = 0.039$; Figure 3a). This relationship remained significant when adjusting hippocampal volume for total estimated intracranial volume ($r(50) = -0.29$, $p = 0.035$). Although the PTSS and

CEC groups both showed inverse relationships (r = -0.25 and -0.41, respectively), a significant correlation was only seen when combining across all participants.

We next assessed correlations between hippocampal volume and the subcomponents of PTB. Hippocampus volume was not significantly correlated with self-reported combat exposure ($r(50) = 0.12$, $p = 0.40$; Figure 3b) or perceived threat ($r(50) = -0.22$, $p = 0.11$; Figure 3c), although PTB and perceived threat did not differ statistically in their relationship with hippocampal volume (William's $t(50) = 0.57$, $p = 0.57$).

PTSD avoidance/numbing symptoms are associated with smaller hippocampal volume

There was not a significant relationship between total PTSD symptoms on the CAPS and reduced hippocampal volume ($r(50) = -0.21$, $p = 0.14$; PTSS subjects: $r(34) = -0.30$, $p = 0.079$; Figure 4a). Similarly, there was no between-group difference in hippocampal volume between the CEC group and individuals diagnosed with PTSD ($t(31) = 0.73$, $p = 0.47$). We also investigated hippocampal volume in relation to specific CAPS symptom clusters. Hippocampal volume was most strongly related to avoidance/numbing symptoms (Figure 4c), with a non-significant relationship across all subjects ($r(50) = -0.27$, $p = 0.052$) and a significant relationship in the PTSS group ($r(34) = -0.36$, $p = 0.032$). Similar (and more robust) correlations with hippocampal volume were observed for PCL symptom scores (Figure S2).

Individual differences in PTB and CAPS avoidance/numbing symptoms were each associated with smaller hippocampal volume, and the two measures were correlated with one another. To see whether these factors explained shared or unique variance in hippocampal volume, we conducted a simultaneous linear regression in the full sample of hippocampal volume on PTB and avoidance/numbing symptoms. Neither PTB ($t(49) = -1.57$, $p = 0.12$) nor avoidance/numbing symptoms ($t(49) = -1.39$, $p = 0.17$) accounted for unique variance in hippocampal volume. The two factors together accounted for 8.2% of the variance in hippocampal volume ($F(2,49) = 3.26$,

$p = 0.047$, multiple $R^2 = .118$, adjusted $R^2 = .082$).

Analysis of amygdalar volume in relation to perceived threat bias and PTSD symptoms

There were no relationships between amygdala volume and PTB ($r(50) = 0.13$, $p = 0.35$) or either PTB subcomponent (CES: $r(50) = -0.17$, $p = 0.22$; perceived threat: $r(50)$ -0.02, $p = 0.91$). There were also no relationships between amygdala volume and total CAPS symptoms ($r(50) = -0.10$, $p = 0.49$) or individual CAPS symptom clusters (rs < 0.18, ps > 0.2).

Whole-brain structural correlates of perceived threat bias and PTSD symptoms

As exploratory analyses, we regressed voxelwise brain morphometry (VBM) maps on PTB and CAPS scores (Figure S3). Consistent with ROI-based volumetric analyses, at $p < 0.1$ (small volume correction within the amygdala/hippocampus), PTB was inversely correlated with local gray matter volume in the left mid/posterior hippocampus. This relationship was driven by perceived threat, which was associated with reduced gray matter volume across much of the left hippocampus ($p < 0.05$). Combat Exposure Scale scores were *positively* associated with gray matter volume in the right posterior hippocampus at $p < 0.1$. CAPS scores were not associated with VBM in the hippocampus or amygdala, and there were no clusters that survived whole-brain correction for PTB, its subcomponents, or CAPS scores.

Discussion

The relationship between combat experiences and maladaptive psychological outcomes has been shown to be mediated by subjective perceptions of threat,[5,10,11] but little is known about underlying neurobiological mechanisms. Here, we introduce the novel construct of perceived threat bias (PTB), which reflects relative discrepancies between self-reported combat experiences and subjectively perceived threat while deployed. In a sample of male OEF/OIF veterans with a wide range of PTSD symptoms, PTB was associated with avoidance/numbing symptoms of PTSD, as well as depression and anxiety symptoms and trait worry. In addition,

elevated PTB was associated with reduced hippocampal volume, suggesting that this brain region may play an important role in differential threat appraisals in the aftermath of combat exposure or other traumatic events.

Previous research on perceived threat has underscored the importance of this factor in conferring risk for trauma-related psychopathology.[8–11] We suggest that individual differences in perceived threat should be considered in relation to levels of combat or other trauma exposure, as the *mismatch* between trauma exposure and subjective threat appraisals may be a better indicator of maladaptive responding than absolute levels of perceived threat.[19,32] In more symptomatic veterans, PTB showed numerically higher correlations with anxiety, depression, and worry scores than perceived threat alone. Further, PTB but not perceived threat was significantly associated with hippocampal volume, though these two correlations did not differ statistically. Although perceived threat was shown in previous work with combat veterans to be associated with persistent changes in functional connectivity between the amygdala and dorsomedial PFC, a circuit important for threat processing and expression,[20,33] we did not observe any relationships here with amygdala volume.

We also did not observe a relationship between overall PTSD symptoms and hippocampal volume, or a difference in volume between combat-exposed controls and participants diagnosed with PTSD. Our relatively small sample size precludes strong conclusions from these null results, particularly given the modest effect size of reduced hippocampal volume in PTSD.[4] Work from van Rooij and colleagues[34] further suggests that a simple comparison of combat-exposed veterans with and without PTSD may obfuscate important individual differences reflected in hippocampal volume. These authors reported no baseline volumetric difference between combat-exposed controls and PTSD patients who remitted following subsequent trauma-focused treatment, but smaller hippocampal volume in treatment-resistant PTSD patients. The authors concluded that reduced hippocampal volume may be a risk factor for persistent PTSD, consistent

with a classic report of smaller hippocampal volume in healthy identical twins of Vietnam veterans with PTSD.[35] Inflated subjective perceptions of threat – which we found to be associated with smaller hippocampal volume – may be one factor that contributes to the persistence of PTSD. This is a particularly intriguing possibility given that we identified significant relationships between avoidance/numbing symptoms of PTSD and both PTB and hippocampal volume, and that avoidance plays a central role in the persistence of fear memories.[36]

Recognizing that perceived threat bias as defined here is a somewhat broad construct, we offer two accounts of psychological processes that may be involved. First, elevated PTB may reflect biased threat appraisals during deployment. High correlations between PTB and self-reported anxiety, depression, and worry suggest a relationship with previously described cognitive or attentional biases in mood and anxiety disorders. For example, PTB may reflect attentional bias to threat, observed in laboratory studies of PTSD and anxiety disorders.[37] These disorders are also associated with interpretation bias, or a tendency to interpret ambiguous information as threatening.[38] Although interpretation and attentional biases may be adaptive in unpredictable and potentially dangerous deployment contexts, such biases may subsequently predispose veterans to develop psychopathology in objectively safe non-combat settings.[32]

Alternatively, different individuals may appraise events similarly at the time of exposure, but retrospectively recall or report on these experiences very differently.[39] For example, elevated PTB could be driven in part by underreporting of combat experiences. The positive relationship between PTB and CAPS avoidance symptoms lends support to the possibility that avoidance of aversive combat memories leads to underreporting (consistent with this avoidance account, we observed somewhat paradoxical *inverse* associations between CES scores and symptoms of anxiety and depression in more symptomatic veterans; Table S2). Conversely, individuals with negative PTB may retrospectively minimize perceptions of threat experienced during deployment. Notably, memories for specific traumatic events and perceived threat to one's life are not fixed

and indelible, but evolve as traumatic events become more distal, particularly for individuals with elevated PTSD symptoms.[40,41]

These evolving memories – which largely depend on the integrity of the hippocampus – make it impossible to discern the sequence of events resulting in reduced hippocampal volume observed years after these veterans returned from combat. To adjudicate between the accounts laid out above (or other alternatives), it will be important to conduct longitudinal research on subjectively perceived threat that includes both neuroimaging and behavioral outcomes. Baseline measures of hippocampal integrity could be related to behavioral indices of hypervigilance and threat avoidance before, during, and after combat exposure, allowing one to test whether reduced hippocampal function or structure is associated with contextually inappropriate, maladaptive behavior based on current levels of threat exposure.[32] Assessing perceived threat *during* deployment[7] in concert with objective indices of threat exposure (based on official military records)[17] would reveal whether PTB assessed after combat reflects retrospective biases in perceived threat or reported combat exposure.

Such research may also reveal whether reduced hippocampal volume predisposes individuals to perceive events as more threatening, or whether subjective perceptions of threat during deployment *contribute* to hippocampal damage, perhaps via chronic alterations to HPA axis output. Animal models of chronic psychosocial stress have shown that stress-induced elevations in glucocorticoids cause cellular damage to the hippocampus[42] observable at the macroscopic level.[43] Human neuroimaging studies have found that basal plasma cortisol levels are inversely correlated with hippocampal volume,[44,45] and that chronic stress is associated with reduced hippocampal volume 20 years later.[15] Structural alterations of the hippocampus – whether a predisposing risk factor or resulting from combat exposure and elevated perceived threat – would have deleterious consequences for hippocampal-dependent processes such as appropriate threat contextualization[13,46] and pattern separation ability.[47] The inability to ground threatening

stimuli or fear memories in appropriate contexts may contribute to excessive avoidance of people, places, or things that bear resemblance to trauma-related stimuli, consistent with the relationship between avoidance symptoms and smaller hippocampal volume.

Limitations and future directions

A major limitation of this work is that the PTB index was constructed from two retrospective self-report measures, each of which was collected on average 5 years after deployment, and each of which is subject to response and recall biases. In addition to retrospective reporting errors, individuals with equivalent CES scores may have experienced objectively different amounts of combat trauma, as these events can vary in their duration and severity. The presence of PTSD symptomatology may also systematically influence retrospective reporting, leading to inflated recall of perceived threat (as discussed above) and over- *or* under-reporting of combat events. Notably, CES scores in the PTSS group were positively correlated with re-experiencing symptoms, which could reflect heightened estimates of combat exposure due to flashbacks or nightmares (or vice versa). These factors further confound any inferences of causality and underscore the need for longitudinal studies and the incorporation of military records (or at least concurrent self-report measures) to determine combat exposure. Additionally, because our sample consisted entirely of male OEF/OIF veterans, these results may not generalize to females or veterans of other conflicts.

Owing to the manner in which it was created, the PTB index is currently optimized for use in a research context. Its reliance on regression modeling is advantageous for detecting statistical signal, but the resultant values are not absolute and must be interpreted relative to the specific sample being investigated. For translational purposes, a valuable future extension of this work would be to investigate whether a valid PTB metric can be developed that is invariant of the specific sample, accompanied by the establishment of normative population distributions (for both

combat and non-combat trauma), such that this index could be incorporated into routine clinical assessment.

Summary

In summary, we have described a new construct for research on combat trauma, perceived threat bias (PTB). PTB was associated with PTSD symptoms, self-reported anxiety, depression, and worry, and smaller hippocampal volume. These results suggest that hippocampus is an important neural substrate for individual differences in subjective appraisals of threat, which have previously been shown to influence the development of PTSD and other mood and anxiety disorders. Future research on this construct that utilizes longitudinal neurobiological, behavioral, and symptom-based measures will elucidate the temporal milieu of events that result in elevated threat appraisal following trauma exposure, and may help prevent the emergence of trauma-related psychopathology.

References

1. Karl, A. *et al.* A meta-analysis of structural brain abnormalities in PTSD. *Neurosci. Biobehav. Rev.* **30,** 1004–1031 (2006).
2. Woon, F. L., Sood, S. & Hedges, D. W. Hippocampal volume deficits associated with exposure to psychological trauma and posttraumatic stress disorder in adults: A meta-analysis. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **34,** 1181–1188 (2010).
3. Kühn, S. & Gallinat, J. Gray matter correlates of posttraumatic stress disorder: A quantitative meta-analysis. *Biol. Psychiatry* **73,** 70–74 (2013).
4. Logue, M. W. *et al.* Smaller Hippocampal Volume in Posttraumatic Stress Disorder: A Multisite ENIGMA-PGC Study: Subcortical Volumetry Results From Posttraumatic Stress Disorder Consortia. *Biol. Psychiatry* **83,** 244–253 (2018).
5. King, L. A., King, D. W., Knight, J. & Samper, R. E. Deployment Risk and Resilience Inventory: A collection of measures for studying deployment-related experiences of military personnel and veterans. *Mil. Psychol.* **18,** 89–120 (2006).
6. Blanchard, E. B. *et al.* The impact of severity of physical injury and perception of life threat in the development of post-traumatic stress disorder in motor vehicle accident victimsj. *Behav. Res. Ther.* 529–534 (1995).
7. Lancaster, C. L., Cobb, A. R., Lee, H. & Telch, M. J. The Role of Perceived Threat in the Emergence of PTSD and Depression Symptoms During Warzone Deployment. *Psychol. Trauma Theory Res. Pract. Policy* **8,** 528–534 (2016).
8. King, L. A., King, D. W., Bolton, E. E., Knight, J. A. & Vogt, D. S. Risk factors for mental, physical, and functional health in Gulf War veterans. *J. Rehabil. Res. Dev.* **45,** 395–407 (2008).

9. Mott, J. M., Graham, D. P. & Teng, E. J. Perceived threat during deployment: Risk factors and relation to axis I disorders. *Psychol. Trauma Theory Res. Pract. Policy* **4,** 587–595 (2012).

10. Vogt, D. S. & Tanner, L. R. Risk and resilience factors for posttraumatic stress symptomatology in Gulf War I veterans. *J. Trauma. Stress* **20,** 27–38 (2007).

11. Vogt, D. S. *et al.* Predeployment, deployment, and postdeployment risk factors for posttraumatic stress symptomatology in female and male OEF/OIF veterans. *J. Abnorm. Psychol.* **120,** 819–831 (2011).

12. King, D. W., King, L. A., Gudanowski, D. M. & Vreven, D. L. Alternative representations of war zone stressors: Relationships to posttraumatic stress disorder in male and female Vietnam veterans. *J. Abnorm. Psychol.* **104,** 184–195 (1995).

13. Liberzon, I. & Abelson, J. L. Context Processing and the Neurobiology of Post-Traumatic Stress Disorder. *Neuron* **92,** 14–30 (2016).

14. Smith, M. E. Bilateral hippocampal volume reduction in adults with post-traumatic stress disorder: A meta-analysis of structural MRI studies. *Hippocampus* **15,** 798–807 (2005).

15. Gianaros, P. J. *et al.* Prospective reports of chronic life stress predict decreased grey matter volume in the hippocampus. *NeuroImage* **35,** 795–803 (2007).

16. Zimmerman, M. E. *et al.* Perceived Stress Is Differentially Related to Hippocampal Subfield Volumes among Older Adults. *Plos One* **11,** e0154530 (2016).

17. Wald, I. *et al.* Attention to Threats and Combat-Related Posttraumatic Stress Symptoms. *JAMA Psychiatry* **70,** 401–409 (2013).

18. Wald, I. *et al.* Attention bias away from threat during life threatening danger predicts PTSD symptoms at one-year follow-up. *Depress. Anxiety* **28,** 406–411 (2011).

19. Shechner, T. & Bar-Haim, Y. Threat Monitoring and Attention-Bias Modification in Anxiety and Stress-Related Disorders. *Curr. Dir. Psychol. Sci.* **25,** 431–437 (2016).

20. van Wingen, G. A., Geuze, E., Vermetten, E. & Fernández, G. Perceived threat predicts the neural sequelae of combat stress. *Mol. Psychiatry* **16,** 664–671 (2011).

21. Morey, R. A. *et al.* Amygdala volume changes in posttraumatic stress disorder in a large case-controlled veterans group. *Arch. Gen. Psychiatry* **69,** 1169–1178 (2012).

22. Blake, D. D. *et al.* A clinician rating scale for assessing current and lifetime PTSD: The CAPS-1. *Behav. Ther.* **13,** 187–188 (1990).

23. First, M. B., Spitzer, R. L., Gibbon, M. & Williams, J. B. W. *Structured clinical interview for DSM-IV-TR axis I disorders, research version, patient edition (SCID-I/P)*. (Biometrics Research, New York State Psychiatric Institute, 2002).

24. Grupe, D. W., Wielgosz, J., Davidson, R. J. & Nitschke, J. B. Neurobiological correlates of distinct post-traumatic stress disorder symptom profiles during threat anticipation in combat veterans. *Psychol. Med.* **46,** 1885–1895 (2016).

25. Keane, T. M. *et al.* Clinical evaluation of a measure to assess combat exposure. *Psychol. Assess.* **1,** 53–55 (1989).

26. Beck, A. T., Epstein, N., Brown, G. & Steer, R. A. An inventory for measuring clinical anxiety: Psychometric properties. *J. Consult. Clin. Psychol.* **56,** 893–897 (1988).

27. Beck, A. T., Steer, R. A., Ball, R. & Ranieri, W. F. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J. Pers. Assess.* **67,** 588–597 (1996).

28. Meyer, T. J., Miller, M. L., Metzger, R. L. & Borkovec, T. D. Development and validation of the Penn State Worry Questionnaire. *Behav. Res. Ther.* **28,** 487–495 (1990).

29. Blanchard, E. B., Jones-Alexander, J., Buckley, T. C. & Forneris, C. A. Psychometric properties of the PTSD checklist (PCL). *Behav. Res. Ther.* **34,** 669–673 (1996).

30. Morey, R. A. *et al.* A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *NeuroImage* **45,** 855–866 (2009).

31. Wisse, L. E. M., Biessels, G. J. & Geerlings, M. I. A Critical Appraisal of the Hippocampal Subfield Segmentation Package in FreeSurfer. *Front. Aging Neurosci.* **6,** 127–134 (2014).

32. Wald, I. *et al.* Attention to Threats and Combat-Related Posttraumatic Stress Symptoms. *JAMA Psychiatry* **70,** 401–409 (2013).

33. van Wingen, G. A., Geuze, E., Vermetten, E. & Fernández, G. The neural consequences of combat stress: Long-term follow-up. *Mol. Psychiatry* **17,** 116–118 (2012).

34. van Rooij, S. J. H. *et al.* Smaller hippocampal volume as a vulnerability factor for the persistence of post-traumatic stress disorder. *Psychol. Med.* **45,** 2737–2746 (2015).

35. Gilbertson, M. W. *et al.* Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat. Neurosci.* **5,** 1242–1247 (2002).

36. Foa, E. B. & Kozak, M. J. Emotional processing of fear: Exposure to corrective information. *Psychol. Bull.* **99,** 20–35 (1986).

37. Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J. & van Ijzendoorn, M. H. Threat-related attentional bias in anxious and nonanxious individuals: A meta-analytic study. *Psychol. Bull.* **133,** 1–24 (2007).

38. Yoon, K. L. & Zinbarg, R. E. Interpreting neutral faces as threatening is a default mode for socially anxious individuals. *J. Abnorm. Psychol.* **117,** 680–685 (2008).

39. Coles, M. E. & Heimberg, R. G. Memory biases in the anxiety disorders: Current status. *Clin. Psychol. Rev.* **22,** 587–627 (2002).

40. Southwick, S. M., Morgan, A., Nicolaou, A. L. & Charney, D. S. Consistency of memory for combat-related traumatic events in veterans of Operation Desert Storm. *Am. J. Psychiatry* **154,** 173–177 (1997).

41. Engelhard, I. M., van den Hout, M. a & McNally, R. J. Memory consistency for traumatic events in Dutch soldiers deployed to Iraq. *Memory* **16,** 3–9 (2008).

42. Magariños, A. M., McEwen, B. S., Flügge, G. & Fuchs, E. Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *J. Neurosci.* **16,** 3534–3540 (1996).

43. Uno, H. *et al.* Neurotoxicity of glucocorticoids in the primate brain. *Horm. Behav.* **28,** 336–348 (1994).

44. Starkman, M. N., Gebarski, S. S., Berent, S. & Schteingart, D. E. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biol. Psychiatry* **32,** 756–765 (1992).

45. Lupien, S. J. *et al.* Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat. Neurosci.* **1,** 69–73 (1998).

46. Hayes, J. P. *et al.* Reduced hippocampal and amygdala activity predicts memory distortions for trauma reminders in combat-related PTSD. *J. Psychiatr. Res.* **45,** 660–669 (2011).

47. Kheirbek, M. A., Klemenhagen, K. C., Sahay, A. & Hen, R. Neurogenesis and generalization: A new approach to stratify and treat anxiety disorders. *Nat. Neurosci.* **15,** 1613–1620 (2012).

Tables

Table 1: Demographic, clinical, and self-report data for the posttraumatic stress symptoms (PTSS) and combat-exposed control (CEC) groups.

	PTSS (N = 36)		CEC (N = 16)		Independent samples *t* test	
	Mean	SD	Mean	SD	*t*(50)	*p* value
Age	30.8	6.8	30.1	5.3	-0.36	0.72
Years since Deployment	4.8	2.7	5.5	3.0	-0.81[a]	0.42
CAPS Total	46.7	18.1	3.7	2.5	9.42	< 0.001
CAPS B (Re-experiencing)	10.7	6.9	0.3	1.0	5.97	< 0.001
CAPS C (Numbing/Avoidance)	16.7	9.9	0	0	6.70	< 0.001
CAPS D (Hyperarousal)	19.3	7.1	3.4	2.7	8.59	< 0.001
Combat Exposure Scale	19.8	8.4	15.9	9.3	1.51	0.14
DRRI: Perceived Threat	50.1	8.1	41.6	10.3	3.22	0.0023
Beck Depression Inventory	26.9	8.7	17.8	6.4	3.78	< 0.001
Beck Anxiety Inventory	36.3	9.3	24.5	4.1	4.85	< 0.001
Penn State Worry Questionnaire	45.8	16.3	32.3	12.8	2.94	0.0050

NOTES: [a] One subject in the PTSS group was missing data for Years since Deployment. CAPS = Clinician-Administered PTSD Scale; DRRI = Deployment Risk and Resilience Inventory.

Figures

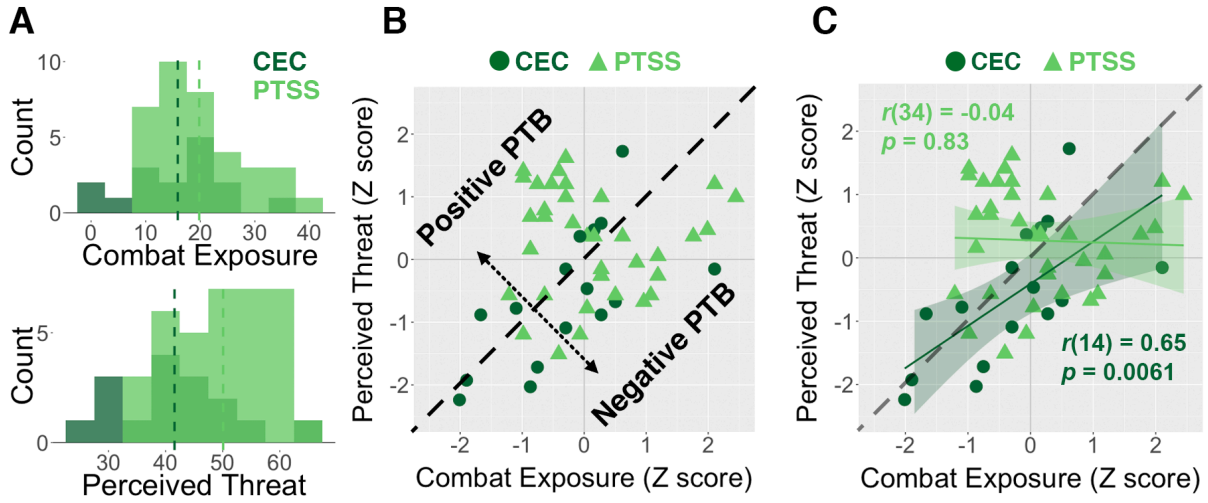

Figure 1: (A) Overlapping histograms show the distributions of self-reported combat exposure and perceived threat for the posttraumatic stress symptoms (PTSS) and combat-exposed control (CEC) groups. Each of these measures was approximately normally distributed across the entire sample, although the PTSS group had elevated perceived threat relative to the CEC group ($t(50) = 3.22$, $p = 0.0023$). Dashed lines indicate mean values for each group. (B) A plot of combat exposure vs. perceived threat, with the dashed line indicating zero perceived threat bias (PTB). Individuals above this line had a positive PTB, with elevated perceived threat relative to the amount of self-reported combat experience, whereas individuals below the line had a negative PTB. (C) Individuals in the CEC group showed a strong and positive correlation between combat experience and perceived threat, whereas this relationship was absent for individuals in the PTSS group. Shaded areas indicate 95% confidence intervals.

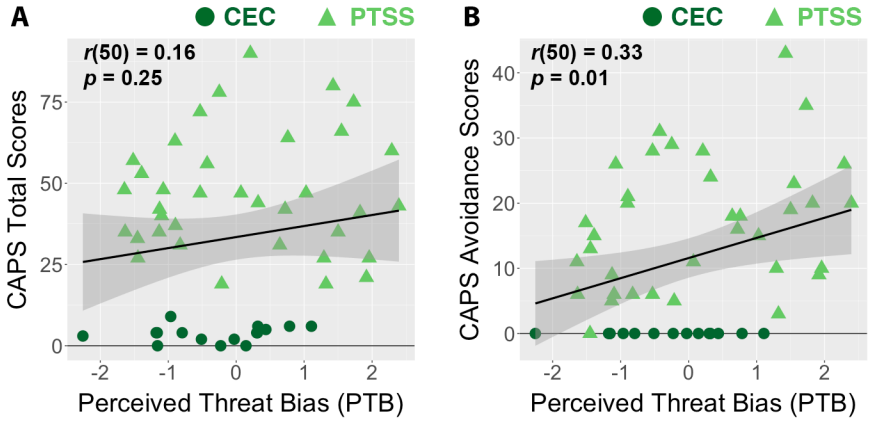

Figure 2: (A) Perceived threat bias (PTB) showed no relationship with overall scores of PTSD on the Clinician-Administered PTSD Scale (CAPS) across the entire sample or in the posttraumatic stress symptoms (PTSS) group alone ($r(34) = 0.04$, $p = 0.82$). (B) There was a significant and positive correlation between PTB and CAPS avoidance/numbing symptoms across the entire sample, and a non-significant correlation in the PTSS group ($r(34) = 0.32$, $p = 0.06$).

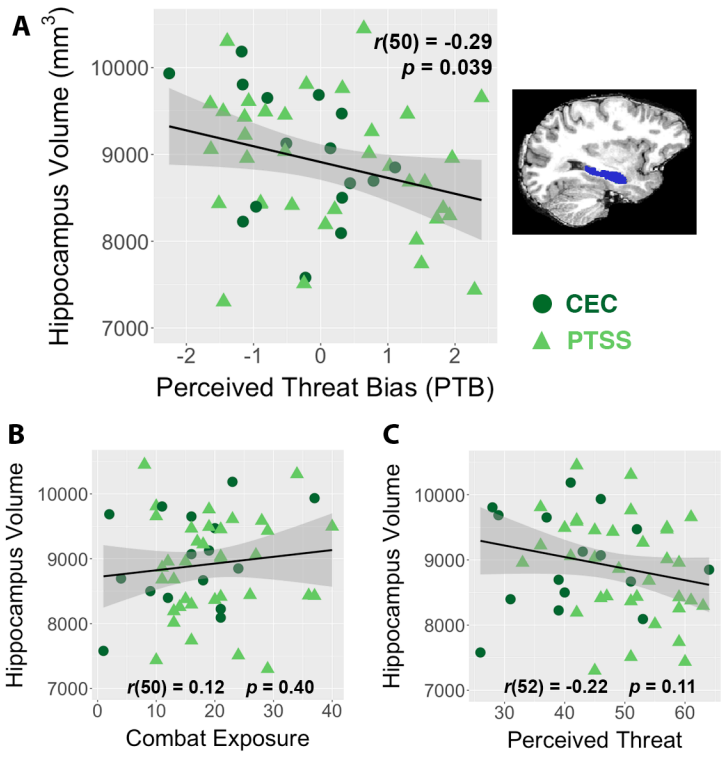

Figure 3: (A) Across all participants, higher perceived threat bias (PTB) scores were negatively correlated with bilateral hippocampal volume. Hippocampal volume was unrelated to combat exposure (B) and perceived threat scores (C). Shaded areas indicate 95% confidence intervals.

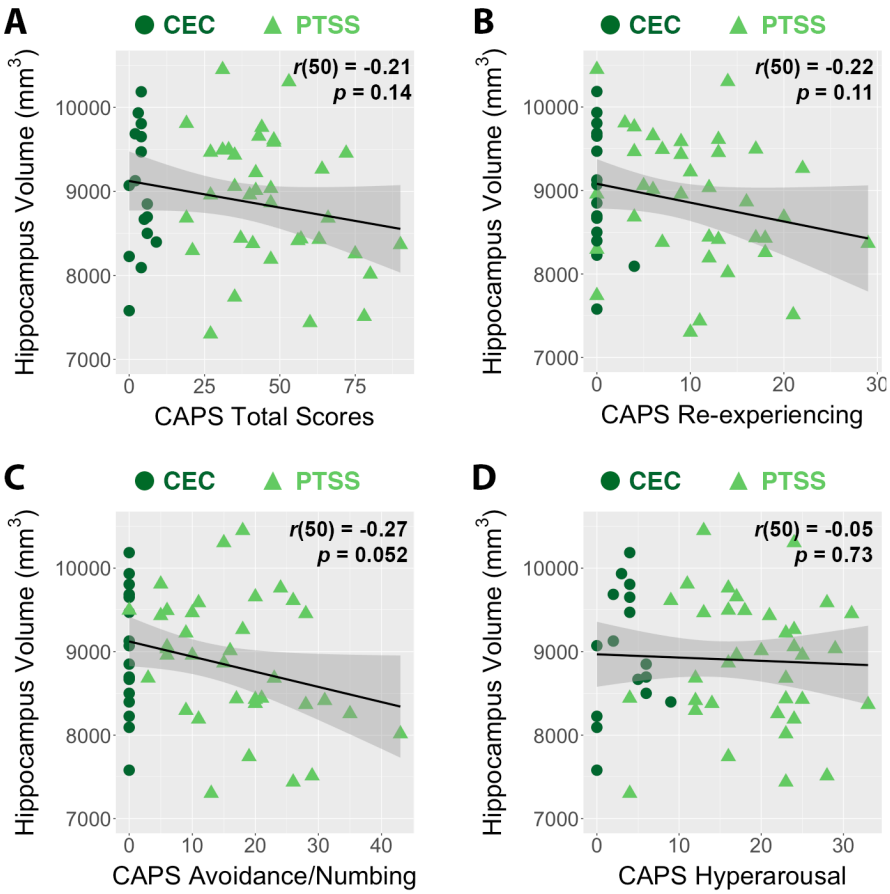

Figure 4: Overall PTSD symptom severity (A), as assessed by the Clinician-Administered PTSD Scale (CAPS), showed no significant relationship with bilateral hippocampal volume across the entire group or for subjects in the posttraumatic stress symptoms (PTSS) group alone ($r(34) = -0.30$, $p = 0.079$). Hippocampal volume was inversely correlated with CAPS avoidance/numbing symptoms (C) in the entire sample and the PTSS group alone ($r(34) = -0.36$, $p = 0.032$). Hippocampal volume was not related to re-experiencing (B) or hyperarousal symptoms (D). Shaded areas indicate 95% confidence intervals.