Amygdala volume changes with posttraumatic stress disorder in a large case-controlled veteran group

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Abstract

Context—Smaller hippocampal volumes are well established in posttraumatic stress disorder (PTSD), but the relatively few studies of amygdala volume in PTSD have produced equivocal results.

Objective—To assess a large cohort of recent military veterans, with PTSD and trauma-exposed controls, with sufficient power to definitively assess the effect of PTSD on volumetric changes in the amygdala and hippocampus, as well as the contribution of illness duration, trauma load, and depressive symptoms.

Design—Case-controlled design with structural MRI and clinical diagnostic assessments. Important potential confounds of alcohol use, depression, and medication use were statistically controlled.

Setting—Durham VA Medical Center located in central North Carolina (USA) in proximity to major military bases.

Patients—Ambulatory patients (n=200), recruited from a registry of military service members and veterans serving after 11-September 2001, consisting of a group with current PTSD (n=99) and trauma-exposed comparison group without PTSD (n=101).

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Main Outcome measure—Amygdala and hippocampus volumes computed from automated segmentation of high-resolution structural MRI acquired at 3 Tesla.

Results—Smaller volume was demonstrated in the PTSD compared to non-PTSD groups for the left amygdala ($p = .002$), right amygdala ($p = .01$), and left hippocampus ($p = .02$), but not for the right hippocampus ($p = .25$). Amygdala volumes were not associated with PTSD chronicity, trauma load, or severity of depressive symptoms.

Conclusions—These results provide clear evidence of an association between smaller amygdala volume and PTSD. The lack of correlation between trauma load or illness chronicity and amygdala volume suggests that either a smaller amygdala represents a vulnerability to developing PTSD, or the lack of a dose-response relationship with amygdala volume. Our results may trigger a renewed impetus for investigating structural differences in the amygdala, its genetic determinants, its environmental modulators, and the possibility that it reflects an intrinsic vulnerability to PTSD.

INTRODUCTION

The amygdala is perhaps the most strongly implicated brain structure in the pathophysiology of posttraumatic stress disorder (PTSD). Prevalent models of anxiety have focused on an amygdalocentric neurocircuitry\(^1\) that is critical in fear response, conditioning, generalization\(^2,3,4\), and facilitates the response to stressful experiences\(^5\). Functional MRI studies\(^6-9\) have shown that individuals with PTSD have an exaggerated amygdala response to emotional stimuli when compared to controls. Animal studies have demonstrated changes in amygdala morphology with chronic stress\(^10\), evident primarily in the growth of dendritic spines. Experimental studies of amygdala volume in mice and humans have shown an association between smaller amygdala volumes, increased levels of fear conditioning, and an exaggerated glucocorticoid response to stress\(^11-13\). However, efforts to find evidence of an association between amygdala volume and PTSD in humans have produced equivocal results\(^14-15\). Our goal was to reinvestigate amygdala volume changes in PTSD by addressing some of the potential methodological issues contributing to inconclusive findings.

The hippocampus has been the overwhelming focus of prior studies of morphological change in PTSD. These studies have demonstrated a clear association between smaller hippocampal volume and PTSD\(^15-16\). In contrast, the relatively few studies of amygdala volume differences in PTSD have yielded mixed results (see Table 1). Two meta-analyses that included amygdala volumetry showed inconsistent differences between trauma-exposed participants with and without PTSD. The first meta-analysis found lower volumes with small effect sizes\(^17\) in both the left (effect size $= -.22$) and right amygdala ($-.18$)\(^15\), but only after restricting analysis to the subset of studies that produced a homogeneous sample. The second meta-analysis demonstrated only a trend association ($p = .06$), with a small effect size ($Hedges’ g = -.29$), between smaller left amygdala volume and PTSD patients as compared to trauma-unexposed healthy controls\(^14\). The interpretation of these data is complicated by several methodological issues and limitations. For instance, the first meta-analysis\(^15\) included a group with intrusive memories but lacked a diagnosis of PTSD, the use of varied manual segmentation protocols, differences in raters across studies, scanners with lower spatial resolution and field strengths than current standards, heterogeneous trauma type (combat, sexual assault, intimate partner violence, etc.), and inclusion of children\(^18\). Although these meta-analyses offer weak support for an association of amygdala volume and PTSD, several individual studies that failed to show significant differences\(^19-20\) suggest caution in this interpretation (see Table 1). Furthermore, the number of studies reporting negative results may be an underestimation of the actual number given the disincentives for publishing negative results.
Our study had three main goals. The first was to assess the association of amygdala volume and PTSD in a large sample of trauma-exposed adults. The effect sizes from several prior studies that yielded marginal p-values for amygdala differences suggest they were underpowered to detect real effects in the population. Therefore, we hypothesized that the PTSD group would show smaller amygdala volume than the trauma-exposed non-PTSD group when powered to adequately control for Type II error. We previously reported the sample size required to demonstrate a significant group difference in amygdala volume derived from automated segmentation using published effect sizes to be at least 55 subjects per group, which is substantially larger than sample sizes used in prior published studies (see Table 1).

The second goal was to gain empirical evidence that might offer clues about factors contributing to PTSD. For example, if trauma exposure, trauma load, or illness chronicity were correlated with amygdala volume, this would be compatible with either an environmental cause for the volume change, or a pre-existing vulnerability that interacts with environmental factors such as trauma load and illness chronicity. Evidence of an association with PTSD in the absence of an association with trauma exposure would allow for the possibility that smaller amygdala volume may represent a vulnerability to PTSD. We hypothesized that a diagnosis of PTSD would be associated with smaller amygdala volume, but that volume would not be correlated with trauma exposure or illness chronicity based on evidence in animals and humans that smaller amygdalae constitute a risk for heightened fear and stress responses.

Our third and final goal was to confirm the established finding of smaller hippocampal volume in our sample of veterans serving after 11-September 2011 using the structural neuroimaging methods we have adopted. These methods included several enhancements such as an automated segmentation approach to better control variability in manual segmentation protocols by human raters, improved signal-to-noise ratio (SNR) provided by 3 Tesla field strength, and higher spatial resolution (1-mm isotropic voxels). For analyses of both structures, we used a multiple regression approach similar to Bremner and colleagues to control for variables such as symptoms of depression, trauma load, duration of PTSD, intracranial volume (ICV), age, medication, and alcohol abuse.

METHODS
Participants
Participants (n=200) were recruited between September 2007 and November 2010 from a registry of military service members and veterans. All participants served since 11-September 2001 and most (77%) served in the Iraq and/or Afghanistan military conflicts. Participants were screened for inclusion/exclusion criteria based on information available in the registry and from subsequent telephone contact where 85% of potential subjects agreed to participate. Important exclusions included major Axis I diagnosis (other than depression), contraindication to MRI, traumatic brain injury, substance dependence, neurological disorders, and age over 55 years. All participants provided written informed consent to participate in procedures reviewed and approved by the Institutional Review Boards at Duke University and the Durham VA Medical Center. Participants completed questionnaires assessing depressive symptoms (Beck Depression Inventory-II, BDI-II), traumatic life events (Traumatic Life Events Questionnaire, TLEQ), combat exposure (Combat Exposure Scale, CES), alcohol abuse (Alcohol Use Disorders Test, AUDIT) current medication use, and were administered (n=191; 96%) the Structured Clinical Interview for DSM-IV (SCID) to assess comorbid Axis I diagnoses. A summary of participants’ demographic and clinical features (see Table 2), indicates groups were matched for age, gender, and race. The PTSD group had greater trauma and combat exposure.
symptoms, alcohol use, and psychotropic medication use. PTSD diagnosis was ascertained with the Clinician-Administered PTSD Scale (CAPS) in 149 (75%) participants, and with the Davidson Trauma Scale (DTS) in 51 (26%) participants. The participants assessed with the DTS were assigned to a diagnostic group based on a cutoff score of 40 that we have previously reported to have high positive (0.95) and negative predictive value (0.85) as compared to clinician-administered interview in a larger sample of veterans serving after 11-September 2001 from the same registry. Initial study participants received the DTS before the study team had completed CAPS training. Major depression was diagnosed (SCID) in 27 (14%) PTSD and two (1%) control participants.

Two participants in the PTSD group and two participants in the non-PTSD group were excluded (total n=4) due to use of mood stabilizers that have previously been reported to have conflicting effects on brain volume. PTSD duration, determined from clinical assessment and defined as the time elapsed between the occurrence of criterion-A trauma and the MRI scan, was available for 91 of 99 participants. For delayed onset of symptoms (n=4), ranging from eight months to one year, PTSD duration was calculated relative to the time of symptom onset.

MRI acquisition

Images were acquired on a General Electric 3 Tesla Signa EXCITE scanner equipped with an 8-channel headcoil. High-resolution T1-weighted whole brain images with 1-mm isotropic voxels using array spatial sensitivity encoding technique (ASSET) and fast spoiled gradient-recall (3D-FSPGR) were acquired axially for all participants. Image parameters were optimized for contrast between white matter, gray matter, and CSF (TR/TE/flip angle=7.484 ms/2.984 ms/12°, FOV=256 mm, 1-mm slice thickness, 166 slices, 256×256 matrix, 1 excitation).

Image analysis

All T1 images were visually inspected (CCH) to assure appropriate quality. Automated segmentation and labeling of the amygdala and hippocampus and estimation of total intracranial volume from participants’ T1 images were performed using the FreeSurfer image analysis suite (version 5.0.0; http://surfer.nmr.mgh.harvard.edu/) and its library tool recon-all. We previously validated FreeSurfer automated segmentation of the amygdala and hippocampus compared to manual tracing. Spatial normalization by affine registration to Talairach space and skull stripping were performed on the T1 images. Registration was checked visually for accuracy (CCH). FreeSurfer segmentation and labeling of subcortical structures was based on a combination of voxel intensity, probabilistic atlas location, and the spatial relationships of voxels to the location of nearby subcortical structures. Using the FreeSurfer library function mri_label2vol and a transformation matrix generated by tkregister2, the segmentation labels were returned to native space. The native-space segmentations were converted to LAS orientation and then the amygdala and hippocampus were extracted using the segmentation labels. The segmentation of the amygdala and hippocampus overlayed on original T1 images (see Figure 1) was visually inspected (ALG, VMB, KSL) slice-by-slice for correct location and shape. All participants passed this inspection process without the need for manual adjustment.

Statistical analysis

We used the general linear model (GLM) to control for potential confounding variables in examining the influence of PTSD diagnosis on regional brain volume. GLM analysis (VMB, RAM, GM, biostatistician HRW) on each brain region (left and right amygdala and hippocampus) included the following covariates: intracranial volume, age, gender, combat exposure, traumatic lifetime events, depressive symptoms, alcohol abuse, duration of PTSD,
and use of serotonergic (n=28) and antipsychotic (n=7) medications (separate covariates) based on reports of increased regional volumes associated with serotonergic\textsuperscript{39} and decreased regional volumes associated with antipsychotic\textsuperscript{40} agents. Alpha was .05 given our \textit{a priori} hypotheses of PTSD volume differences in the amygdala and hippocampus.

Because of expected intercorrelations among the covariates, principal components analysis (PCA) was performed to reduce the data dimensionality. A principal components factor analysis using varimax rotation was conducted on age, PTSD duration, BDI, CES, TLEQ, and AUDIT scores. Selection of specific components to be used in follow-up modeling was confirmed by \textit{parallel analysis}\textsuperscript{41}, which compares the eigenvalues from the principal components analysis with those from a randomly generated dataset of the same size (Monte Carlo PCA for Parallel Analysis, \url{http://edpsychassociates.com/Watkins3.html}). Only the components with eigenvalues higher than the randomly generated dataset were retained and substituted for the individual covariates in follow-up modeling.

Finally, the disparity in trauma load between groups raised the possibility that differences in volume might be associated with magnitude of trauma exposure rather than PTSD illness. Therefore, we repeated the GLM on two subgroups that were matched for combat exposure and lifetime trauma.

\section*{RESULTS}
\subsection*{Demographic Information}
Basic demographic and clinical information is reported by diagnostic group in Table 2.

\subsection*{Volumetry results}
Group means, standard deviations, and the GLM results testing our hypotheses on the effects of PTSD diagnosis, combat exposure, lifetime trauma, illness chronicity, and BDI are summarized in Table 3. The between-group results demonstrated that PTSD diagnosis was associated with smaller volume in the left and right amygdala and the left hippocampus. Right hippocampal volume was not significantly associated with PTSD diagnosis (see Table 3). Combat exposure was not significantly related to left amygdala, right amygdala, or right hippocampal volume, but showed a trend towards significance for left hippocampal volume. Lifetime trauma, illness chronicity, and depressive symptoms were not associated with volume differences in either structure for either hemisphere. Intracranial volume was significantly correlated with bilateral amygdala and hippocampus volumes (all \(p\)-values < .001). The other covariates including AUDIT, medication use, age, gender, BDI, CES, and PTSD duration were not significantly related to either amygdala or hippocampal volumes (data not shown; all \(p\)-values > .05). Results obtained by statistically controlling for medication effects were compared to a secondary analysis that excluded participants on antidepressant (n=28) and antipsychotic medications (n=7). Smaller volumes were confirmed in left amygdala \([F(1,154)=8.67, p = .004]\), right amygdala \([F(1,154)=6.60, p = .01]\), and left hippocampus \([F(1,154)=5.74, p = .02]\). The right hippocampus did not reach statistical significance \([F(1,154)=1.89, p = .17]\).

Duration of PTSD was unavailable in eight PTSD participants, therefore analysis of the whole group omitted this covariate to include 99 participants with PTSD and 101 controls (see Table 4). The differences in volume between PTSD and non-PTSD groups in the right amygdala and left hippocampus were significant but had a higher \(p\)-value than with the inclusion of PTSD duration as a covariate, whereas the volume difference in the left amygdala was at trend level. The volume difference in the right hippocampus was again not significant. Severity of PTSD in patients assessed with the CAPS, controlling for ICV, was negatively correlated with volume of the left amygdala \((r = -.22, p = .03)\), but did not reach
significance in the right amygdala ($r = -0.18, p = 0.09$). As expected, removing ICV as a covariate resulted in a non-significant correlation given the established association between head size and regional brain volume.

Data reduction

Two components, which individually explained 30.3% and 26.9% of the variance, respectively, had eigenvalues greater than 1. Selection of these two components was confirmed by parallel analysis\textsuperscript{41}. The first component was associated with depressive symptoms, traumatic events, longer PTSD duration, older age, and combat exposure. The second component was associated with longer PTSD duration, younger age, alcohol abuse, and less combat exposure. A factor analysis that included intracranial volume showed it had low communality (.33), meaning the extracted components accounted for minimal ICV variance). Consequently, ICV was excluded from the final factor analysis. Results of GLM using covariates of components 1 and 2, intracranial volume, gender, use of serotonergic medication, and use of antipsychotic medication were similar to the original analyses conducted with all covariates. That is, PTSD diagnosis was associated with smaller left and right amygdala volume and left hippocampal volume (see Table 5).

Trauma Load

To account for the possible nonlinear influences of trauma exposure on regional brain volume irrespective of PTSD diagnosis, follow-up analysis conducted with subgroups matched for combat exposure and lifetime trauma exposure (n=122: 76 PTSD and 46 non-PTSD) revealed similar group differences to the primary analytic models (see Table 6). There were significant group differences in left and right amygdala, but a trend significance in the left hippocampus.

COMMENT

Our study establishes diminished amygdala volume in a large cohort of recent military veterans with PTSD compared to trauma-exposed non-PTSD veterans after controlling for depressive symptoms, alcohol use, intracranial volume, medication, PTSD chronicity, and trauma load. In contrast to inconclusive results from prior studies that reported marginal or non-significant effects for amygdala volumetry, we found a significant association between smaller amygdala volume and PTSD. This association was not accounted for by PTSD chronicity, trauma load, severity of depressive symptoms, alcohol use, or medication status. We confirmed findings of smaller volume in the left hippocampus for PTSD compared to trauma-exposed non-PTSD controls, which remained significant after controlling for the same variables used in the amygdala analysis. The laterality of our findings for hippocampus was consistent with a prior meta-analysis\textsuperscript{13} that showed significantly decreased left but not right hippocampal volume in PTSD.

Comparison to previous findings for amygdala volumetry

Although most previous studies reported non-significant findings, some studies showed a trend towards significance for smaller amygdala volumes in PTSD\textsuperscript{21,22,24,26,42}. The investigations of PTSD and amygdala volume with the largest samples included PTSD groups with n=44\textsuperscript{43} and n=28\textsuperscript{44}, but were conducted in children and therefore do not generalize well to adults due to developmental changes in brain structure and connectivity\textsuperscript{45}. All studies of adults had a sample size of fewer than 20 in the PTSD group, with the majority of studies having 15 or fewer participants\textsuperscript{20,22–26}. Consistent with our findings and relevant to the core symptom cluster of re-experiencing symptoms in PTSD, smaller amygdala volume was associated with the presence of cancer-related intrusive recollections in a sample of 76 breast cancer survivors\textsuperscript{18}.
It is worthwhile to consider the factors that may have produced a significant association of decreased amygdala volume given the preponderance of negative findings in prior studies. The small effect sizes observed from the meta-analyses suggest that they were underpowered to detect significant differences. Assessing a large sample size is impractical with manual segmentation, and this motivated our use of an automated segmentation technique. Automated segmentation also: (i) facilitates future replication of these results by other investigators; (ii) diminishes differences among studies due to the use of different protocols for defining anatomical boundaries; (iii) eliminates variability associated with different raters across studies and even a single rater over time; and (iv) removes bias introduced by varied software interfaces that are used in manual tracing (e.g., 3-plane views vs. single plane view). Thus, replication of the present results based on automated segmentation may be achieved with greater fidelity than manual tracing methods.

Although we previously reported that FreeSurfer introduces additional variance compared to the gold standard of hand tracing, this variance would not bias volumetry measures in favor of one group over another.

Rather than matching individuals on a participant-by-participant basis to achieve the large enrollment necessary for this study in a realistic time frame, we used a statistical approach to control for potentially confounding variables (e.g., depressive symptoms, trauma load, duration of PTSD, intracranial volume, age, medication, alcohol abuse). The effects of many of these variables have not previously been tested as covariates in PTSD amygdala investigations. Assembling a PTSD group that is free of depressive symptoms is unlikely to generalize, and moreover new evidence calls into question whether PTSD and depression are distinct entities among individuals exposed to trauma. Notably, there was no significant association between depressive symptoms and amygdala or hippocampal volumes after controlling for PTSD among the other covariates. Larger amygdala volumes have been found in early depression but smaller or null findings have been found in chronic depression. Nevertheless, it is important to exert statistical control for these symptoms. Exclusion of participants taking psychotropic medication has also been the accepted orthodoxy, although leaders in the field of PTSD neuroimaging have argued for their inclusion. Technical concerns in previous studies include the use of older MRI technology such as lower field strength scanners (1.5 or 2.0 Tesla, see Table 1) and lower spatial resolution (≥ 1.5 mm slice thickness). We addressed these issues with a 3 Tesla scanner to improve SNR and 1-mm slice thickness (1 mm isometric) for superior spatial resolution. Based upon our previously reported power calculation for amygdala segmentation with FreeSurfer, a sample size of at least 55 subjects per group is required for the effect sizes we observed.

In addition to these methodological improvements, important demographic and trauma-related characteristics of our sample differ from those of previous studies. In contrast to some earlier studies of veterans with chronic PTSD, our participants were recent military personnel who served largely in military conflicts in Iraq and Afghanistan.

**Associations with functional neuroimaging of amygdala**

Functional differences of the amygdala, particularly in the left hemisphere, during emotion processing were supported by a meta-analysis showing ventral anterior hyperactivation and a dorsal posterior hypoactivation in PTSD. Amygdala engagement during fear conditioning is well established in healthy adults. Thus, amygdala hyperactivity in PTSD may reflect an exaggerated response of fear circuitry and may explain PTSD symptoms such as hypervigilance and hyperarousal. Despite concerns with statistical power, heterogeneity of task design, patient characteristics, imaging modality, and analytic approaches in functional neuroimaging studies of PTSD, these results have been more consistent than results from volumetric studies of the amygdala. Overall, there are numerous reports of greater amygdala...
activation in PTSD\textsuperscript{6–7,52–55}, whereas some others failed to show increased amygdala activation\textsuperscript{56–58}, further obfuscating a coherent hypothesis for amygdala volume differences in PTSD. Indeed, amygdala volume decrease appears to correspond to increased fMRI functional activation in PTSD\textsuperscript{59}. The ventral hyperactive cluster reported by Etkin and Wager\textsuperscript{9} may relate to the basolateral amygdala (described below) and may be relevant to acquired fear responses in PTSD given the role of this region in forming emotional memories\textsuperscript{60–61}.

Pathobiology of PTSD

The amygdala plays a key role in a wide variety of behaviors and mnemonic functions, most critically in modulating negative affect and emotion.\textsuperscript{3, 63, 64} Changes in fear, stress, and anxiety in rats are induced by lesions to subnuclei of the amygdala, notably the BLA, which is needed to form and later express associative fear memories. Evidence primarily from rodent work has led to a model in which the lateral nucleus in the BLA receives aversive and sensory signals that are passed on to the basal nucleus and central nucleus. Fear-associated behaviors are governed by the central nucleus, which provides the major outputs of the amygdala.\textsuperscript{65,66} This model is consistent with the observation that prior to fear conditioning, rats with lesions to the lateral nucleus did not form fear memories, unlike lesions to the basal nucleus.\textsuperscript{67,68} However, following fear conditioning, lesions to the basal nucleus blocked the expression of fear memories, but not the ability to encode those memories.\textsuperscript{69} Thus, fear-related plasticity in the amygdala is essential for fear learning and accompanying fear behaviors.\textsuperscript{11,66}

The effect of persistent and chronic threat-induced hyperexcitation of the amygdala on its volume in humans is yet unclear. Much of our knowledge on the pathobiology of threat and stress effects on the brain, and on the medial temporal lobe in particular, comes from animal models using acute stressors that are qualitatively different from the chronic and/or extreme stressors typically experienced by humans with PTSD. One of several competing theories is based on established findings that the amygdala and hippocampus undergo stress-induced structural remodeling, albeit in very different ways.\textsuperscript{10} Most of the research on stress effects has focused on the hippocampus, with very few studies investigating the amygdala directly.

Smaller amygdala volume: vulnerability or consequence?

The second goal of this study was to gain insight into whether the smaller amygdala volume is a preexisting vulnerability factor for developing PTSD or a consequence of having PTSD. Our data failed to show a correlation of trauma load or PTSD chronicity with lower amygdala volume, suggesting the lack of a dose-response effect for trauma and amygdala volume or that smaller amygdalae might be a risk factor for developing PTSD.

Evidence from prior work in humans and animals demonstrates an association between smaller amygdalae and stronger fear conditioning and stress reactivity, which are considered risk factors for PTSD. Prior work in animals by Yang and colleagues\textsuperscript{11} used recombinant inbred strains of mice that exhibit up to a 2-fold difference in BLA size.\textsuperscript{11} Mice were categorized into groups with small, medium, and large BLA volume and underwent a pavlovian fear-conditioning procedure. The small-BLA mice showed stronger fear conditioning than the medium- and large- BLA mice, and freezing to the conditioned stimulus was significantly correlated with volume of the BLA but not of comparison regions, including the hippocampus, striatum, or cerebellum. Mice were also subjected to a stress condition (forced swim), which was associated with elevated corticosterone level in the small-BLA but not the medium- or large-BLA groups. Nonstressed mice did not differ by corticosterone level. The BLA is a critical site through which corticosterone enhances associative fear memories.
The BLA volume association raises a fundamental question whether a small BLA is the consequence or the cause of stronger fear conditioning. A smaller BLA is unlikely to be a consequence given that chronic threat and stress lead to corticosterone-mediated spinogenesis and dendritic arborization in mice. In clear contradistinction, the hippocampus shows loss of neurons and synaptic connectivity in response to elevated adrenal hormone levels associated with chronic threat and stress. Thus, the smaller volume of the BLA, which constitutes the largest of the amygdalar nuclei, is linked to stronger fear conditioning, chronic threat, and vigilance, leading to a potential increase in BLA volume.  

Evidence from 2 studies in humans builds on the work of Yang and colleagues in mice. Studies in humans not exposed to trauma or chronic threat and stress are especially informative because trauma-induced structural changes in the amygdala are unlikely to have occurred. First, Hartley et al paired colored squares (conditioned stimuli) with mild electrical shock (unconditioned stimuli) and measured the strength of fear acquisition via skin conductance response. The magnitude of the conditioned fear response was correlated with the smaller amygdala volume. Second, Gianaros et al measured mean arterial pressure in response to a stressor (performance-titrated Stroop Color-Word Interference task) and found that a smaller amygdala volume was correlated with stressor-evoked blood pressure reactivity.

To investigate directly whether our finding of decreased amygdala volume in PTSD represents a preexisting vulnerability factor or an acquired sign of the disorder, research studies using prospective, longitudinal design and twin-discordance models are needed. Vulnerability related to genetic or epigenetic effects has been hotly debated, but little empirical evidence is available. Altered serotonin binding in the left amygdala and increased left amygdala activation modulation by the 5HTTLPR serotonin transporter gene have been observed in PTSD. However, evidence showing specific genetic modulation of amygdala volume in PTSD is lacking.

Limitations

We used covariates to control for variables such as depressive symptoms, alcohol abuse, and age. This approach is adequate unless a nonlinear association is present. For instance, trauma exposure may not follow a linear dose response but instead may require a specific threshold beyond which a marked effect on amygdala volume is produced. Secondary analyses that matched groups for trauma exposure were performed to rule out this possibility. Also, our sample consisted of veterans from the Iraq and Afghanistan conflicts, who were mostly men; therefore, owing to these and other sources of selection bias, we urge caution when generalizing these results to other demographic groups. Our sample also showed high levels of combat trauma relative to other trauma types, and it remains unclear whether the type of trauma predicts the magnitude of volume loss in the amygdala. Approximately one-fourth of the sample was diagnosed using the DTS, a self-report measure that has high predictive power compared with the CAPS but may misclassify some subjects. Finally, based on the effect sizes we obtained for the hippocampus, the present sample size may have been insufficient to detect volume differences in the right hippocampus; however, this effect size was consistent with a large meta-analysis.
Conclusions

These results provide robust evidence of an association between a smaller amygdala volume and PTSD. We did not observe correlation between trauma load or illness chronicity with amygdala volume. When considered in the context of previous translational research linking smaller amygdala volume with stronger fear conditioning and stress response, our results are consistent with the theory that a smaller amygdala represents a vulnerability to developing PTSD rather than an outcome of the disorder. The story for amygdala volumetry might be more elusive than that of the hippocampus. For instance, counteracting influences show that, on one hand, smaller amygdala size may be consistent with a vulnerability to PTSD but that elevated corticosterone levels lead to increased amygdala volume on the other hand. Our results may trigger a renewed impetus for investigating structural changes in the amygdala, its genetic determinants, environmental modulators, and the possibility that lower amygdala volume represents an intrinsic vulnerability to PTSD.

Acknowledgments

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References


Figure 1.
Example of automated segmentation of the amygdala and hippocampus using FreeSurfer. The high-resolution structural MRI of a representative subject is displayed in radiological convention with segmentation labels for the amygdala (in blue) and hippocampus (in yellow) as shown in (A) sagittal (left hemisphere), (B) coronal, and (C) axial slices.
### Table 1a. Structural MRI studies of amygdala volume differences in adults with PTSD

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<th>TEC (n)</th>
<th>HC (n)§</th>
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<th>effect size (Cohen’s d)</th>
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</tr>
<tr>
<td>Gurvits 1996</td>
<td>1.5</td>
<td>1.5</td>
<td>.94</td>
<td>combat: Vietnam</td>
<td>CAPS</td>
<td>7</td>
<td>7†</td>
<td>8</td>
<td>29</td>
<td>.53</td>
<td>.07</td>
<td>−.57</td>
<td>−.44</td>
</tr>
<tr>
<td>Lindauer 2004</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
<td>police officers</td>
<td>SCID</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>40</td>
<td>.25</td>
<td>.17</td>
<td>.47</td>
<td>.56</td>
</tr>
<tr>
<td>Lindauer 2005</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
<td>Mixed</td>
<td>SCID</td>
<td>18</td>
<td>14</td>
<td>0</td>
<td>40</td>
<td>.85</td>
<td>.09</td>
<td>.23</td>
<td>−.44</td>
</tr>
<tr>
<td>Rogers 2009</td>
<td>1.5</td>
<td>1.5</td>
<td>.94</td>
<td>Tokyo subway</td>
<td>CAPS (Japanese)</td>
<td>9</td>
<td>16</td>
<td>0</td>
<td>12</td>
<td>.05</td>
<td>.05</td>
<td>.88</td>
<td>.64</td>
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<tr>
<td>Wignall 2004</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
<td>Mixed: ED</td>
<td>CAPS</td>
<td>15</td>
<td>0</td>
<td>11</td>
<td>14</td>
<td>.07</td>
<td>.37</td>
<td>.84</td>
<td>.36</td>
</tr>
</tbody>
</table>

### Table 1b. Structural MRI studies of amygdala volume differences in pediatric PTSD

<table>
<thead>
<tr>
<th>Study</th>
<th>MR field strength (Tesla)</th>
<th>slice thickness (mm)</th>
<th>in-plane resolution (mm²)</th>
<th>Type of trauma</th>
<th>PTSD measure</th>
<th>PTSD (n)</th>
<th>TEC (n)</th>
<th>HC (n)^</th>
<th>Met est.²⁷</th>
<th>Amygdala volume (p)</th>
<th>effect size (Cohen’s d)</th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Bellis 1999</td>
<td>1.5</td>
<td>1.5</td>
<td>.94 × 1.25</td>
<td>child maltreatment</td>
<td>K-SADS</td>
<td>44</td>
<td>0</td>
<td>61</td>
<td>40</td>
<td>NS/NR</td>
<td>.44</td>
<td>.40</td>
<td></td>
</tr>
<tr>
<td>De Bellis 2001</td>
<td>1.5</td>
<td>1.5</td>
<td>.94 × 1.25</td>
<td>child maltreatment</td>
<td>K-SADS</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>29</td>
<td>NS/NR</td>
<td>.56</td>
<td>.29</td>
<td></td>
</tr>
<tr>
<td>De Bellis 2002</td>
<td>1.5</td>
<td>1.5</td>
<td>.94 × 1.25</td>
<td>child maltreatment</td>
<td>K-SADS</td>
<td>28</td>
<td>0</td>
<td>66</td>
<td>783</td>
<td>.62</td>
<td>−11</td>
<td>.08</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CAPS, Clinician-Administered PTSD Scales; ED, emergency department; ES, effect size; est., estimated; HC, healthy control; IPV, intimate partner violence; K-SADS, Schedule for Affective Disorders and Schizophrenia for School-Age; NR, not reported; NS, non-significant; PTSD, posttraumatic stress disorder; SADS, Schedule for Affective Disorders and Schizophrenia; SCID, Structured Clinical Interview for DSM; TEC, trauma-exposed control.

§ For healthy control group, trauma exposure was either not assessed or determined to be absent.
The per group sample size for each study was compared with the sample size estimate based on power=.80, alpha=.05 from Morey et al. A small effect size (based on Woon & Hedges meta-analysis) was used for the amygdala sample size criterion of ES=.1, n=783; ES=.2, n=199; ES=.25, n=121; ES=.3, n=90; ES=.35, n=67; ES=.4, n=51; ES=.45, n=40; ES=.5, n=34; ES=.55, n=29; ES=.6, n=24; ES=.65, n=22; ES=.7, n=19; ES=.75, n=17; ES=.8, n=15; ES=.85, n=14; ES=.9, n=12. per group for manual tracing.

For studies with three groups, the p-values and Cohen's d refer to the PTSD vs. TEC group comparison.

Segmentation was performed by manual tracing for all studies.
### Table 2

**Demographic and Clinical Information by group**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PTSD (n=99)</th>
<th>Non-PTSD (n=102)</th>
<th>Group Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD), y</td>
<td>38.4 (9.9)</td>
<td>37.5 (10.6)</td>
<td>( t = -0.549, p = .58 )</td>
</tr>
<tr>
<td>Gender, No. female (%)</td>
<td>20 (20)</td>
<td>16 (16)</td>
<td>( \chi^2 = .644, p = .42 )</td>
</tr>
<tr>
<td>Race, # Caucasian (%)†</td>
<td>49 (49)</td>
<td>54 (53)</td>
<td>( \chi^2 = .239, p = .67 )</td>
</tr>
<tr>
<td>Beck Depression Inventory, mean (SD)</td>
<td>19.3 (11.0)</td>
<td>5.56 (5.6)</td>
<td>( t = -11.2, p &lt; .001 )</td>
</tr>
<tr>
<td>Combat Exposure Scale, mean (SD)</td>
<td>15.6 (10.1)</td>
<td>8.64 (9.9)</td>
<td>( t = -4.89, p &lt; .001 )</td>
</tr>
<tr>
<td>Trauma Life Events Questionnaire, mean (SD)</td>
<td>14.6 (11.4)</td>
<td>7.41 (8.7)</td>
<td>( t = -5.03, p &lt; .001 )</td>
</tr>
<tr>
<td>Alcohol Use Disorders Identification Test, mean (SD)</td>
<td>4.90 (5.9)</td>
<td>2.97 (3.3)</td>
<td>( t = -2.81, p = .006 )</td>
</tr>
<tr>
<td>PTSD Duration, mean (SD)</td>
<td>8.02 (8.40)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serotonergic medication, # (%)</td>
<td>24 (24)</td>
<td>4 (4)</td>
<td>( \chi^2 = 17.3, p &lt; .001 )</td>
</tr>
<tr>
<td>Antipsychotic medication, # (%)</td>
<td>7 (7)</td>
<td>0 (0)</td>
<td>( \chi^2 = 7.40, p = .007 )</td>
</tr>
</tbody>
</table>

† Participants reported race and ethnicity information according to investigator-defined options.
## Table 3

Volumetry Results by diagnosis and effect of trauma load and illness chronicity

<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>Volume mm³ (mean ± SD)</th>
<th>PTSD</th>
<th>Combat Exposure Scale</th>
<th>Trauma Life Events Questionnaire</th>
<th>PTSD Duration</th>
<th>Beck Depression Inventory</th>
<th>PTSD effect size a Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PTSD</td>
<td>F(1,180)</td>
<td>p</td>
<td>F(1,180)</td>
<td>p</td>
<td>F(1,180)</td>
</tr>
<tr>
<td>Amygdala</td>
<td>L</td>
<td>1810 ± 231</td>
<td>1746 ± 233</td>
<td>10.0</td>
<td>.002</td>
<td>2.26</td>
<td>.13</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1994 ± 257</td>
<td>1894 ± 257</td>
<td>6.83</td>
<td>.010</td>
<td>2.54</td>
<td>.11</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>L</td>
<td>4180 ± 505</td>
<td>4067 ± 421</td>
<td>5.81</td>
<td>.02</td>
<td>3.60</td>
<td>.06</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>4188 ± 469</td>
<td>4129 ± 415</td>
<td>1.36</td>
<td>.25</td>
<td>1.23</td>
<td>.27</td>
</tr>
</tbody>
</table>
Table 4

Effect of diagnosis after omitting PTSD duration from GLM

<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>$F(1,189)$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>3.55</td>
<td>.06</td>
</tr>
<tr>
<td>R</td>
<td>5.51</td>
<td>.02</td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>4.78</td>
<td>.03</td>
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<tr>
<td>R</td>
<td>.920</td>
<td>.40</td>
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</table>
### Table 5

Results for diagnosis using component covariates

<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>F(1,184)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>6.27</td>
<td>.01</td>
</tr>
<tr>
<td>R</td>
<td>6.59</td>
<td>.010</td>
</tr>
<tr>
<td>Hippocampus</td>
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<td></td>
</tr>
<tr>
<td>L</td>
<td>6.76</td>
<td>.01</td>
</tr>
<tr>
<td>R</td>
<td>1.35</td>
<td>.25</td>
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</table>
Table 6

Results of PTSD diagnosis matched for trauma exposure

<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>F(1,110)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td></td>
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<tr>
<td>L</td>
<td>7.97</td>
<td>.006</td>
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<tr>
<td>R</td>
<td>6.54</td>
<td>.02</td>
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<tr>
<td>Hippocampus</td>
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<tr>
<td>L</td>
<td>3.56</td>
<td>.07</td>
</tr>
<tr>
<td>R</td>
<td>1.93</td>
<td>.17</td>
</tr>
</tbody>
</table>