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Amygdala Nuclei Volume and Shape in Military Veterans With Posttraumatic Stress Disorder

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ABSTRACT

BACKGROUND: The amygdala is a subcortical structure involved in socioemotional and associative fear learning processes relevant for understanding the mechanisms of posttraumatic stress disorder (PTSD). Research in animals indicates that the amygdala is a heterogeneous structure in which the basolateral and centromedial divisions are susceptible to stress. While the amygdala complex is implicated in the pathophysiology of PTSD, little is known about the specific contributions of the individual nuclei that constitute the amygdala complex.

METHODS: Military veterans (n = 355), including military veterans with PTSD (n = 149) and trauma-exposed control subjects without PTSD (n = 206), underwent high-resolution T1-weighted anatomical scans. Automated FreeSurfer segmentation of the amygdala yielded 9 structures: basal, lateral, accessory basal, anterior amygdaloid, and central, medial, cortical, and paralaminar nuclei, along with the corticoamygdaloid transition zone. Subregional volumes were compared between groups using ordinary-least-squares regression with relevant demographic and clinical regressors followed by 3-dimensional shape analysis of whole amygdala.

RESULTS: PTSD was associated with smaller left and right lateral and paralaminar nuclei, but with larger left and right central, medial, and cortical nuclei ($p < .05$, false discovery rate corrected). Shape analyses revealed lower radial distance in anterior bilateral amygdala and lower Jacobian determinant in posterior bilateral amygdala in PTSD compared with control subjects.

CONCLUSIONS: Alterations in select amygdala subnuclear volumes and regional shape distortions are associated with PTSD in military veterans. Volume differences of the lateral nucleus and the centromedial complex associated with PTSD demonstrate a subregion-specific pattern that is consistent with their functional roles in fear learning and fear expression behaviors.

Keywords: Amygdala, Amygdala nuclei, Shape analysis, Structural MRI, Trauma, U.S. Military

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The amygdala is one of the brain structures most strongly implicated in the pathophysiology of posttraumatic stress disorder (PTSD). The dominant neurobiological models of trauma-related disorders have focused on an amygdalocentric neurocircuitry that facilitates the response to stressful experiences (1) and is critical for threat response, fear conditioning, extinction, and generalization (2). While the largest studies to date reported smaller volume in PTSD compared with trauma-exposed control subjects without PTSD (3–5), other smaller studies reported larger volume (3–6). Converging evidence from functional magnetic resonance imaging (MRI) studies (7,8) further implicates the amygdala in PTSD with exaggerated amygdala response to emotional stimuli. Although subregion volumetric evidence is lacking in PTSD, research indicates that PTSD-associated differences in functional connectivity of the amygdala may be subregion specific (9–11). On the other hand, several animal studies demonstrate that nuclei-specific hypertrophic changes in the basolateral complex accompany anxiety-like behavior after exposure to chronic but not acute stress (12). Unfortunately, the translational value of animal research is limited by the lack of an adequate model system for PTSD. The present study was motivated by the inconsistent amygdala volume findings in PTSD, which may arise in part from the heterogeneous functions and differential trophic responses of specific amygdala nuclei to trauma and chronic stress, thus mandating more refined measures of structural abnormalities (13).

Shape analysis of the amygdala in a sample of 12 women with PTSD exposed to trauma as children was used to infer smaller basolateral amygdala (BLA) and superficial nuclei compared with 12 trauma-exposed women without PTSD (14). In shape analysis of 69 veterans, PTSD was associated with an indentation to the centromedial amygdala (15). With this limited evidence in humans, we turned to evidence from rodent models of PTSD. Recombinant inbred strains of mice that exhibit up to a twofold difference in size of the BLA

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offer some insight. Small-BLA mice show stronger fear conditioning than medium- and large-BLA mice, and freezing to conditioned stimuli is significantly correlated with volume of the BLA (16). That study also reported that in mice subjected to a forced swim stress condition, significantly elevated corticosterone levels were exhibited in small-BLA but not in medium-BLA or large-BLA mice; nonstressed mice did not differ by corticosterone level. On the other hand, the BLA is a critical site through which corticosterone enhances associative fear memories, and exposure to chronic threat and stress in mice leads to corticosterone-mediated spinogenesis and dendritic arborization (17). Thus, low BLA volume prior to stress exposure is linked to stronger fear conditioning, chronic threat, and vigilance, whereas subsequent stress exposure may lead to an increase in BLA volume (1). This evidence suggests that in the present study we would find smaller basal and lateral amygdala subregions in patients with PTSD compared with trauma-exposed control subjects, assuming similar levels of trauma exposure.

Anatomically, there are clear distinctions between subregions of the amygdala. In humans, PTSD is associated with stronger resting-state functional connectivity of the BLA with the pregenual anterior cingulate cortex, dorsomedial prefrontal cortex, and dorsal anterior cingulate cortex, along with weaker connectivity with the left inferior frontal gyrus (11). This work in humans is generally consistent with extensive investigations in animal models that reveal two broad subdivisions of nuclei from the BLA and the centromedial amygdala complexes, with differential functional connectivity and separable roles in threat processing (18). The BLA, comprising the lateral, basolateral, basomedial, and basoventral nuclei, affectively evaluates sensory information and is a site for integration with cortical association areas that regulate fear and other emotional responses (19). The centromedial amygdala, comprising the central and medial nuclei, is critical for the orchestration of fear responses via connections with the hypothalamus, basal forebrain, and brainstem (18).

Standard in vivo neuroimaging tools to automatically delineate the amygdala into multiple nuclei have been beyond reach until recently (20). The release of FreeSurfer version 6.0 incorporates an ex vivo atlas of amygdala nuclei in publicly available software for exploring amygdala nuclei with unprecedented detail that may be applied to widely available structural MRI data (1-mm isotropic). The new method was developed by scanning postmortem brains at ultrahigh resolution (100–150 μm) using 7T MRI to visualize and label nine amygdala subregions and seven amygdala subnuclei (20). The atlas from these labels was generated with an algorithm based on Bayesian inference.

Accordingly, we hypothesized PTSD to be associated with altered volume of basal, lateral, and accessory basal nuclei compared with those structures in trauma-exposed patients without PTSD. Given their role in fear expression and given that central nucleus lesions increase active avoidance behavior, we hypothesized volumetric differences in central and medial nuclei. On the one hand, competing forces that produce hypertrophy of the basolateral amygdala following stress (12), and on the other hand, the vulnerability to fear conditioning and threat linked to low basolateral volume (1,16), meant that we could not confidently hypothesize the direction of an effect (18). Finally, we hypothesized that reexperiencing symptoms that stem from associations with trauma would be correlated with volume differences in the basal, lateral, and accessory basal nuclei. We predicted that avoidance symptoms, which are related to fear expression, might be associated with volume differences of central and medial nuclei, although we lacked sufficient evidence to inform the direction of the association (21).

METHODS AND MATERIALS

Participants

We enrolled 372 Iraq- and Afghanistan-era United States military service veterans, who were recruited from our local repository (22). PTSD diagnosis was determined with the Clinician-Administered PTSD Scale (23), based on either DSM-IV (Clinician-Administered PTSD Scale for DSM-IV [CAPS-IV]) or DSM-5 (CAPS-5), in 329 (88.4%) of the participants. We previously reported the procedure to convert CAPS-5 scores to CAPS-IV scores (24). Diagnosis in the remaining 43 participants (11.6%) was based on the Davidson Trauma Scale (25), which was available in all subjects (N = 372) and was used to compute symptom cluster scores (reexperiencing, avoidance, hyperarousal) to avoid complications from combining Davidson Trauma Scale, CAPS-IV, and CAPS-5 scores across subjects. Five scans failed FreeSurfer segmentation outright. Among the scans that were successfully segmented, 12 scans did not meet our established quality control protocol for amygdala segmentation (26). Therefore, a total of 17 scans were excluded (Supplemental Methods and Materials), resulting in 355 participants in the final analysis who were grouped into 149 with PTSD and 206 trauma-exposed control subjects without PTSD. Important exclusions included Axis I diagnosis (other than major depressive disorder or PTSD), contraindication to MRI, moderate or severe traumatic brain injury, past alcohol dependence, past substance dependence, current alcohol dependence, current substance dependence, neurological disorders, and age over 65 years. Past alcohol or substance abuse was permissible. Every participant provided written informed consent to participate in procedures reviewed and approved by the Institutional Review Boards at Duke University and the Durham Veterans Affairs Medical Center in North Carolina. Participants' demographic and clinical information are summarized in Table 1. The Supplemental Methods and Materials includes full inclusion and exclusion criteria.

MRI Acquisition

Participants were scanned at Duke University or the Durham Veterans Affairs Medical Center using 1 of 4 scanners: 1) GE Discovery MR750, n = 144 [GE Healthcare, Milwaukee, WI]; 2) GE 3T Signa EXCITE, n = 50; 3) GE 4T LX Nvi, n = 110; and 4) Philips 3T Ingenia, n = 51 [Philips, Best, the Netherlands]. A scanner covariate was included in all volume analyses to control for the effect of scanner model. Histograms of left and right amygdala subregion volumes across the 4 MRI scanners

Table 1. Clinical and Demographic Features by Posttraumatic Stress Disorder (PTSD) Status

Study Variable	Control, Mean	PTSD, Mean	Control, SE	PTSD, SE	Test Statistic, t or χ^2	p Value
Age, Years	38.596	39.960	0.717	0.830	0.647	.647
Gender, Male, *n*, (%)	156 (76%)	121 (81%)			1.514	.218
CAPS	17.211	68.104	1.292	2.554	−17.74	2.2×10^{-16}
DTS Total	15.234	53.088	1.842	3.039	−10.587	2.2×10^{-16}
DTS avoidance	5.294	18.946	0.749	1.293	−9.101	2.2×10^{-16}
DTS reexperiencing	4.000	14.493	0.519	0.988	−9.368	2.2×10^{-16}
DTS hyperarousal	6.029	19.649	0.694	0.998	−11.16	2.2×10^{-16}
BDI-II	6.407	17.139	0.650	1.016	−9.317	5.1×10^{-19}
AUDIT	2.552	3.185	0.171	0.269	−1.754	.035
TLEQ, Child	0.578	0.899	0.075	0.102	−2.603	.01
Trauma Chronicity Index	222.39	384.05	21.17	34.06	−4.029	7.4×10^{-05}
No. of Deployments	1.396	1.427	0.141	0.087	−0.181	.857
CTQ	50.297	51.988	1.272	1.757	−0.587	.558
Race, African American, *n* (%)	91 (43%)	70 (46%)			0.276	.871
Psychiatric Medications[a]	0.097	0.364	0.019	0.037	−6.425	2.94×10^{-11}
DAST	0.552	1.006	0.123	0.211	−1.811	.050
CES	7.183	14.16	0.601	0.885	−6.836	2.93×10^{-11}
TIV	1,486,841	1,480,785	11,462	12,659	−0.082	.972

AUDIT, Alcohol Use Disorders Identification Test; BDI-II, Beck Depression Inventory II; CAPS, Clinician-Administered PTSD Scale; CES, Combat Exposure Scale; CTQ, Child Trauma Questionnaire; DAST, Drug Abuse Screening Test; DTS, Davidson Trauma Scale (DSM-IV); TIV, total intracranial volume; TLEQ, Trauma Life Events Questionnaire.

[a]Psychiatric medication use includes antidepressant, antipsychotics, and mood stabilizers.

for the PTSD and control groups are shown in Supplemental Figures S1 and S2.

Amygdala Subregion Volume

Automated segmentation and labeling of subcortical volumes and estimation of total intracranial volume from T1 images were performed using the FreeSurfer version 6.0 image analysis suite (27) (http://surfer.nmr.mgh.harvard.edu) and its library tool *recon-all*. Amygdala subregion segmentation was performed using the function *segmentHA_T1.sh* (20). Amygdala volumes from the left and right hemispheres were generated in each subject for the basal, lateral, accessory basal, central, medial, cortical, and paralaminar nuclei as well as the corticoamygdaloid transition area, anterior amygdaloid area, and the whole amygdala. Left and right substructures were analyzed separately. Visualization of amygdala subregional segmentation in a representative MRI scan is shown in Figure 1.

Protocols for quality control and image analysis were adapted from the subcortical and hippocampal subfields developed by the ENIGMA (Enhancing Neuroimaging Genetics Through Meta-analysis) Consortium (http://enigma.ini.usc.edu/protocols/imaging-protocols/). We previously analyzed hippocampal subfields from FreeSurfer segmentation of T1 scans acquired from multiple scanners and found that the results were immune to heterogeneity (26). Previously, in Logue *et al.* (5), we used 18 different scanners for subcortical

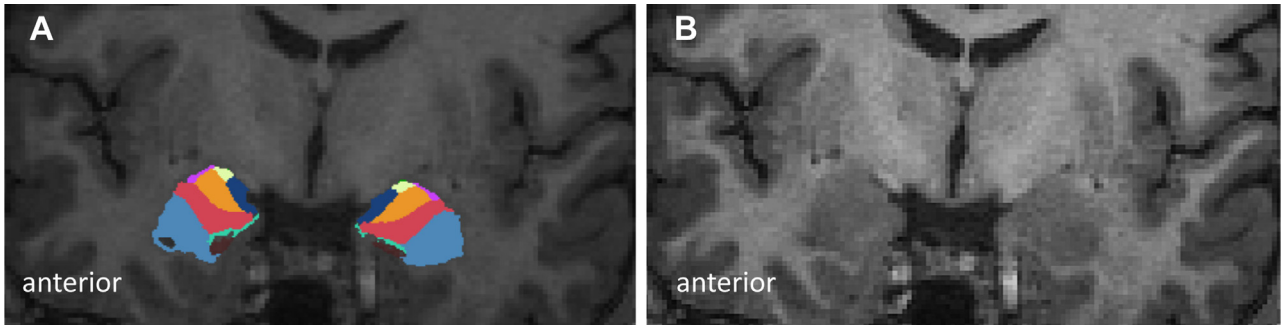

Figure 1. **(A)** FreeSurfer version 6.0 was used to segment 1-mm isotropic structural images to reveal amygdala subregions, which are indicated with color labels (medial nucleus = green, corticoamygdaloid transition area = dark blue, accessory basal amygdala = orange, basal amygdala = red, central amygdala = purple, anterior amygdaloid area = yellow, lateral amygdala = light blue). **(B)** Structural T1 scan provided for reference. Cortical and paralaminar nuclei are not pictured in this view.

segementation and found $p_{heterogeneity}$ = .74 and I^2 = 0. Earlier, Whelan *et al.* (28) demonstrated that intraclass correlations of hippocampal subfields were generally high (>0.85) across 1.5T and 3T scanners, and even higher across different 3T scanners. Volumes for all subregions and subnuclei were normally distributed.

Amygdala Shape

We applied a standard analysis pipeline for subcortical shape developed by the ENIGMA Consortium (29). FreeSurfer segmentation and labels created from the volumetric analysis were used to generate meshes for the amygdala boundary. Using the *Medial Demons* framework, we registered the amygdala shape to matched curvatures and medial features to a precomputed template following Gutman *et al.* (30). The templates and mean medial curves are available as part of the ENIGMA-Shape package. Subject-level vertex information was extracted to perform between-group analyses with regressors for age and gender. We calculated radial distance, a proxy for thickness, and the Jacobian determinant, which indicates local surface area dilation or contraction. Local surface dilation is indicated by a Jacobian determinant >1, whereas a Jacobian determinant <1 indicates local contraction. Smaller-vertex radial distance values indicate concave features, whereas larger values indicate convex features. We applied vertex-wide false discovery rate (FDR) correction to the shape results (31).

Statistical Analysis

The amygdala subregion volumes obtained from FreeSurfer were dependent variables in ordinary-least-squares regression models run separately for each subregion from the left and right hemispheres. The analysis included 20 separate tests (9 subregional and whole-amygdala volumes for left and right hemispheres). FDR correction for multiple testing was applied to control type II errors (31). The following covariates were included in our analysis: whole-amygdala volume, age in years, scanner manufacturer and model, gender per self-report, and intracranial volume (ICV) from FreeSurfer version 6.0. All regressors were entered into the model as continuous measures except for PTSD status, which was dichotomized. We obtained several measures that were not used as covariates in the main analysis because of collinearity with PTSD diagnosis (Table 1): Alcohol Use Disorders Identification Test (32), Combat Exposure Scale, Childhood Trauma Questionnaire (33), Beck Depression Inventory (34), psychotropic medication use based on self-report, and Drug Abuse Screening Test (35). A rationale for including whole-amygdala volume and ICV is provided in the Supplemental Methods and Materials. The results of these analyses are reported as our main findings.

Several clinical variables differed significantly between the PTSD and control groups, including depression symptoms, childhood trauma, psychotropic medication use, combat exposure, alcohol use, and drug abuse. For instance, group differences in depression scores meant that variance in the dependent measure could be attributable to either PTSD or depression symptoms. Inclusion of such a covariate could lead to inconclusive findings because removing the associated variance could alter variance in the dependent variable that is explained by diagnostic groups, as discussed by Miller and Chapman (36) and Kraha *et al.* (37).

The analyses of avoidance, reexperiencing, and hyperarousal symptom clusters were based on the sum of severity and intensity scores from the DSM-IV Davidson Trauma Scale. The analysis was carried out as above with the same ordinary-least-squares regression and regressors.

Trauma Exposure Analysis

Combat exposure was higher in the PTSD group than in the control group. Therefore, we subdivided the control group into a subgroup with very low or no combat exposure (Combat Exposure Scale ≤4) (n = 101) and a subgroup with moderate or high combat exposure (Combat Exposure Scale ≥5) (n = 105) ($t_{249} = -15.273$; $p = 2.2 \times 10^{-16}$), which was similar to the PTSD group ($t_{253} = -0.670$; p = .503).

The chronicity of lifetime trauma exposure was indexed on the number of times and the age at which various trauma types were first experienced to formulate a trauma chronicity index (TCI). Details for calculating the TCI are provided in the Supplemental Methods and Materials. While the TCI was also collinear with PTSD diagnosis, we performed a secondary analysis that included TCI as a covariate, recognizing the inherent limitations of this approach.

RESULTS

Participants' demographic and clinical information are summarized in Table 1. Mean and SEM for all amygdala subregions and the whole amygdala are provided in Supplemental Table S1 for left and right hemispheres in the PTSD and control groups.

Subregion Volume Differences Associated With PTSD

Left hemisphere volumes were significantly different between groups after FDR correction (Figure 2; Table 2) for the lateral nucleus (p_{FDR} = .016, Cohen's d = 0.16), accessory basal nucleus (p_{FDR} = .007, Cohen's d = −0.002), central nucleus (p_{FDR} = .001, Cohen's d = −0.23), medial nucleus (p_{FDR} = .001; Cohen's d = −0.31), cortical nucleus (p_{FDR} = .001, Cohen's d = −0.21), and paralaminar nucleus (p_{FDR} = .016, Cohen's d = 0.25). The left central, medial, and cortical nuclei were larger in the PTSD group than in the control group, whereas the left lateral and paralaminar nuclei were smaller in the PTSD group.

Right hemisphere volumes were significantly different between groups after FDR correction (Figure 2; Table 3) for the lateral nucleus (p_{FDR} = .007, Cohen's d = 0.11), accessory basal nucleus (p_{FDR} = .001, Cohen's d = −0.09), central nucleus (p_{FDR} = .009, Cohen's d = −0.18), medial nucleus (p_{FDR} = .015, Cohen's d = −0.20), cortical nucleus (p_{FDR} = .007, Cohen's d = −0.20), and paralaminar nucleus (p_{FDR} = .006, Cohen's d = 0.25). The right central, medial, and cortical nuclei were larger in the PTSD group than in the control group, whereas the right lateral and paralaminar nuclei were smaller in the PTSD group.

Whole-amygdala volume was the only significant covariate that consistently survived multiple-comparison correction for the subregion analyses, and ICV was the only covariate that

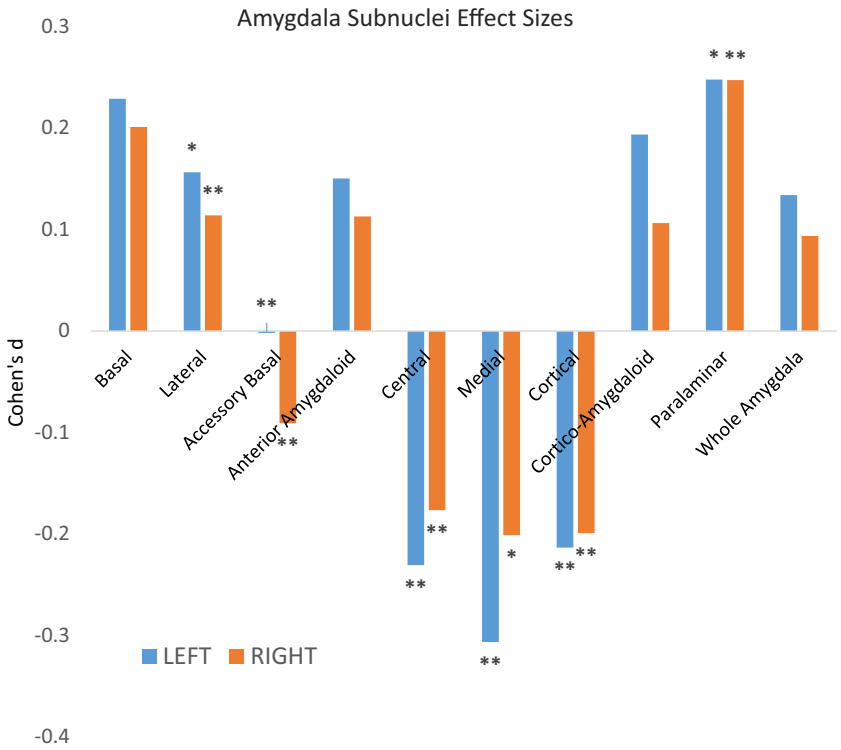

Figure 2. Effect size estimates measured as Cohen's *d* for the left (blue) and right (orange) amygdala subregions. Positive effect size indicates smaller volume in the posttraumatic stress disorder group, and negative effect size indicates larger volume in the posttraumatic stress disorder group. Significance for each structure is indicated for false discovery rate–corrected $^{**}p < .01$ and $^{*}p < .05$.

consistently survived for the whole-amygdala volume analyses. Other covariates that were nominally significant did not survive multiple-comparison correction, except gender for the left and right whole-amygdala volumes and age and scanner for the right corticoamygdaloid transition area. Tables 2 and 3 identify nominally significant covariates for left and right amygdala subregions, respectively.

Effects of High and Low/No Combat Exposure

The comparison of amygdala subregion volumes between the PTSD and low/no-combat subgroup of control subjects (n = 101) was nonsignificant after FDR correction although in a direction consistent with the main findings (Supplemental Tables S2 and S3). The comparison between the PTSD and high-combat subgroup of control subjects (n = 105) survived FDR correction and was consistent with the main findings (Supplemental Tables S4 and S5). See the Supplemental Methods and Materials for details.

Role of Covariates

Amygdala subregion volume results (FDR corrected) based on adding TCI as a covariate were highly consistent (data not presented) with the main results (covariates for whole-amygdala volume, age, gender, scanner, and ICV). However,

Table 2. Left Amygdala Nuclei Comparison Between Participants With Posttraumatic Stress Disorder and Trauma-Exposed Control Subjects With Covariates for Whole-Amygdala Volume, Age, Gender, Scanner Model, and Intracranial Volume (ICV)

	p Value for Covariate									
Amygdala Subregion	Whole Amygdala Volume	Age	Gender	ICV	Scanner	*t*	$p_{uncorrected}$	p_{FDR}	$p_{Bonferroni}$	Cohen's *d*
Basal	4.77×10^{-181}	.692	.622	.816	.153	−0.894	.372	.465	.999	0.23
Lateral	3.01×10^{-104}	.028	.530	.017	.738	−2.607	.010	.016	.191	0.16
Accessory Basal	5.24×10^{-116}	.364	.911	.039	.573	3.007	.003	.007	.057	−0.002
Anterior Amygdaloid	4.26×10^{-47}	.252	.381	.083	.351	−0.654	.513	.57	.999	0.15
Central	6.73×10^{-29}	.531	.711	.318	.133	3.745	.0002	.001	.004	−0.23
Medial	9.73×10^{-14}	.493	.487	.983	.117	3.826	.0002	.001	.003	−0.31
Cortical	4.38×10^{-35}	.995	.877	.039	.231	3.736	.0002	.001	.004	−0.21
Corticoamygdaloid	2.07×10^{-82}	.018	.418	.116	.164	−1.215	.225	.3	.999	0.19
Paralaminar	4.97×10^{-85}	.428	.966	.048	.078	−2.599	.010	.016	.195	0.25
Whole Amygdala	–	.002	1.68×10^{-6}	4.0×10^{-20}	.500	−1.881	.061	.094	.999	0.13

FDR, false discovery rate (Benjamini-Hochberg).

Table 3. Right Amygdala Nuclei Comparison Between Participants With Posttraumatic Stress Disorder and Trauma-Exposed Control Subjects With Covariates for Whole-Amygdala Volume, Age, Gender, Scanner Model, and Intracranial Volume (ICV)

Amygdala Subregion	*p* Value for Covariate									
	Whole-Amygdala Volume	Age	Gender	ICV	Scanner	*t*	$p_{uncorrected}$	p_{FDR}	$p_{Bonferroni}$	Cohen's *d*
Basal	1.45×10^{-189}	.998	.438	.201	.070	−0.727	.468	.550	.999	0.20
Lateral	7.67×10^{-116}	.018	.215	.355	.032	−3.008	.003	.007	.056	0.11
Accessory Basal	6.32×10^{-110}	.136	.989	.961	.307	4.166	.00004	.001	.001	−0.09
Anterior Amygdaloid	5.27×10^{-57}	.567	.214	.034	.200	−0.575	.566	.596	.999	0.11
Central	7.68×10^{-30}	.794	.110	.769	.317	2.883	.004	.009	.084	−0.18
Medial	5.33×10^{-15}	.623	.061	.347	.340	2.685	.008	.015	.152	−0.20
Cortical	4.88×10^{-28}	.968	.571	.908	.345	3.079	.002	.007	.045	−0.20
Corticoamygdaloid	2.39×10^{-96}	1.67×10^{-07}	.596	.543	.0002	−0.212	.832	.832	.999	0.11
Paralaminar	1.52×10^{-85}	.938	.866	.377	.833	−3.203	.001	.006	.03	0.25
Whole Amygdala	–	.007	.001	1.62×10^{-22}	.321	−1.219	.224	.300	.999	0.09

FDR, false discovery rate (Benjamini-Hochberg).

the TCI covariate itself was nonsignificant for all left (*p* values > .22) and right (*p* values > .28) hemisphere subregions. TCI was highly collinear with the grouping variable of PTSD diagnosis ($t_{354} = 4.029$, $p = 7.4 \times 10^{-5}$), which poses challenges in the interpretation of these results (36).

The effect of scanner manufacturer/model was controlled by including a covariate that indicated 1 of the 4 scanners used in the study. Only the right corticoamygdaloid transition area volume was significantly influenced by scanner model ($p < .0005$). However, that subregion did not demonstrate a significant between-group difference in volume ($t_{354} = -0.814$, $p = .489$, Cohen's $d = -0.14$). The left paralaminar nucleus, right basal nucleus, and right lateral nuclei volumes showed nominally significant effects of scanner model ($p < .05$, uncorrected), but none survived FDR correction (Tables 2 and 3).

While both the whole-amygdala volume and ICV were correlated with amygdala subregion volumes, the correlation with whole-amygdala volume was much stronger than the correlation with ICV for each of the amygdala subregions. Thus, variability in amygdala nuclei volume was better predicted by whole-amygdala volume than by ICV. For instance, the correlation between left medial nucleus and left whole-amygdala volume ($r = .451$) was significantly greater than the correlation between left medial nucleus and ICV ($r = .246$) ($z = 3.189$, $p = .001$). The main analysis included covariates for both whole-amygdala volume and ICV, but the latter was nonsignificant for nearly all amygdala subnuclei after FDR correction. As expected, the ICV covariate was highly significant for the whole-amygdala volume analysis (Tables 2 and 3).

Shape Analysis

Three-dimensional shape analysis of whole-amygdala volume was compared between the PTSD group and the trauma-exposed control group without PTSD. Significant differences emerged between groups with regressors included for age, gender, ICV, and scanner. PTSD diagnosis was significantly related to lower radial distance in the left and right anterior amygdala; that is, subjects with PTSD exhibited thinner or concave aspects of the anterior amygdala surface (Figure 3). PTSD diagnosis also related significantly to a lower Jacobian determinant within the posterior aspect of the left and right amygdala; that is, subjects with PTSD exhibited more surface contraction in the posterior aspect of the left and right amygdala.

Subregional Associations With Symptom Clusters and Total Symptoms

Amygdala subregion volume associations with each of the 3 PTSD symptom clusters and total symptom severity scores were tested with regression analysis using the same covariates as the main analysis (Supplemental Table S6). Reexperiencing symptoms showed significant (FDR-corrected) association with left central ($r = .15$, $p_{FDR} = .0002$), left medial ($r = .11$, $p_{FDR} = .02$), and left cortical ($r = .06$, $p_{FDR} = .03$) nuclei and right whole-amygdala ($r = -.12$, $p_{FDR} = .03$) volumes (Supplemental Tables S7 and S8). Avoidance symptoms showed significant (FDR-corrected) association with left lateral ($r = -.12$, $p_{FDR} = .035$), left and right accessory basal (left: $r = .04$, $p_{FDR} = .035$; right: $r = .06$, $p_{FDR} = .035$), left central ($r = .11$, $p_{FDR} = .001$), left medial ($r = .07$, $p_{FDR} = .04$), left and right cortical (left: $r = .04$, $p_{FDR} = .038$; right: $r = .02$, $p_{FDR} = .02$), and right whole-amygdala ($r = -.14$, $p_{FDR} = .05$) volumes (Supplemental Tables S9 and S10). Hyperarousal symptoms showed significant (FDR-corrected) association with left lateral ($r = -.13$, $p_{FDR} = .031$), left and right accessory basal (left: $r = .01$, $p = .31$; right: $r = .04$, $p = .031$), left central ($r = .14$, $p_{FDR} = .0001$), left medial ($r = .09$, $p_{FDR} = .03$), left cortical ($r = .06$, $p_{FDR} = .03$), and right whole-amygdala ($r = .03$, $p_{FDR} = .03$) volumes (Supplemental Tables S11 and S12).

The association of PTSD total symptom scores with amygdala subregion and total amygdala volumes were largely consistent with the main results based on DSM-IV diagnosis. Namely, bilateral central, medial, cortical, and paralaminar nuclei volumes were nominally significant or showed trend-level ($p < .1$) associations with PTSD symptom severity scores, but they did not survive FDR correction.

Effect Size and Power Estimation

Effect size estimates are reported as Cohen's *d* in the last column of Tables 2 and 3. The effect size estimates were used to calculate power to reject the null hypothesis in a post hoc

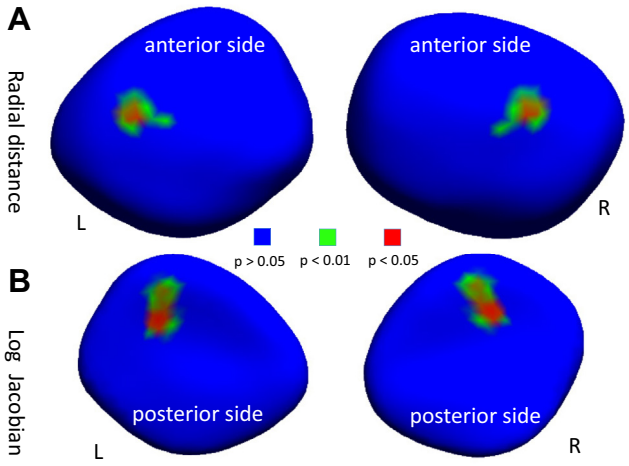

Figure 3. Posttraumatic disorder diagnosis was significantly related to **(A)** lower radial distance in the anterior aspects of the left (L) and right (R) amygdala and **(B)** lower Jacobian determinant in the posterior aspect of the left and right amygdala. Covariates include intracranial volume, age, gender, and scanner model.

fashion. The left medial nucleus had the largest Cohen's *d*, −0.31, which translates with our sample size to 99% power to reject the null hypothesis with a 2-tailed test. Several other nuclei had Cohen's *d* effect-size estimates of ∼0.20, which translates with our sample size to 95% power for 2-tailed tests. The left and right accessory basal nuclei had exceedingly small Cohen's *d* values: −0.002 and −0.09, respectively.

DISCUSSION

The amygdala is a heterogeneous complex whose anatomic origins span 3 divisions that include the olfactory cortex, striatum, and claustrum, which is a gray matter structure that connects cortical with subcortical regions (38). Each division emerges at different points during neurodevelopment and ultimately subserves variegated functions in nonhuman animals. For instance, the lateral and basal nuclei are hypothesized to be a ventromedial extension of the neocortex, the central and medial nuclei are thought to be a ventromedial expansion of the striatum, and the cortical nucleus is thought to be a caudal expansion of the olfactory system. Translation of this knowledge to humans has been limited by the lack of methods to image the amygdala at the subnuclear level. Recent developments in probabilistic atlases that were generated with ultrahigh-power-field ex vivo imaging at ultrahigh resolution have enabled the probabilistic segmentation of subnuclei.

We applied these novel methods to evaluate the morphological and volumetric differences in amygdala subnuclei in military veterans with PTSD. We investigated the association between amygdala substructures and PTSD by quantifying the volume of 9 functionally and cytoarchitecturally discrete subnuclei of the amygdala and the 3-dimensional morphology of the whole amygdala. Diagnosis of PTSD was associated with smaller volumes of bilateral lateral and paralaminar nuclei, but larger volumes of bilateral central, medial, and cortical nuclei. Whole-amygdala volume was consistently and significantly related to volumes for all subregions, but the association with ICV was nonsignificant in a model that included the whole-amygdala volume. The comparison between the PTSD group and the high combat–exposed subgroup of control subjects was consistent with the main findings, which suggests that the group effects were not merely attributable to differential combat exposure. Trauma chronicity, combat exposure, childhood trauma exposure, depression, alcohol use, substance use, and psychotropic medication use were collinear with diagnostic grouping and therefore omitted from the main analyses, but follow-up analyses found that these results were consistent with the main findings and that the covariates did not influence between-group findings. Our shape analysis showed that PTSD was associated with thinner or concave shape on the anterior aspects of the left and right amygdala, as well as greater contraction within the left and right posterior amygdala.

Translational research on the amygdala's role in fear conditioning and extinction provides a framework for understanding the neurobiology of PTSD (21) and the present finding that amygdala shape and volumes of the lateral, central, medial, cortical, and paralaminar nuclei of the amygdala are altered in this disorder. Rodent studies have demonstrated that the lateral nucleus, a subnucleus of the basolateral complex and a sensory hub within the amygdala, plays a key role in fear conditioning (39). The cortical nucleus, another sensory nucleus that receives inputs primarily from the olfactory bulbs, is involved in olfactory fear conditioning (40). However, in some organizational schemes, the cortical nucleus is grouped with the medial nucleus. The medial and central nuclei respond to external traumatic stressors (e.g., predators or noise) and internal systemic stressors (e.g., inflammation or hypoxia), respectively (41). Outputs from these nuclei mediate behavioral (e.g., startle and freezing) and autonomic (e.g., endocrine responses and cardiovascular changes) expressions of fear (42,43). The relatively obscure paralaminar nucleus is dramatically expanded in humans and other primates compared with lower species, particularly rodents, where it is virtually unapparent (44,45). The paralaminar nucleus contains high concentrations of corticotropin-releasing hormone and benzodiazepine receptors, as well as a dense innervation of serotonergic fibers, making it particularly relevant to PTSD. A high concentration of immature cells in the paralaminar nucleus implies heightened neuroplasticity that is potentially susceptible to trauma. Hippocampal inputs and contiguity with the corticoamygdaloid transition area and hippocampal-amygdala transition area implicate the paralaminar nucleus in contextual fear learning (44). It is possible that the neuroplasticity of the paralaminar nucleus might explain the elevated volume of this structure in trauma-exposed control subjects compared with PTSD patients, but this hypothesis will require rigorous investigation in humans or nonhuman primates.

Research in humans has confirmed that the amygdala plays an essential cross-species role in fear conditioning and extinction (46) and that amygdala structure and function are altered in PTSD (3,5,7,8,11), but relatively little is known about the role of specific amygdala nuclei. One exception is humans with BLA lesions who exhibit hypervigilance to fear cues (47) and display passive (e.g., freezing) rather than active avoidance (e.g., running away) (48) responses, which supports a model that implicates the BLA in processing sensory stimuli

and in mediating inhibitory regulation of responses to fear stimuli across species (49). Results of the present study indicate that the lateral nucleus is smaller in PTSD, which is consistent with both human studies that implicate vulnerability for hypervigilance (47) and rodent studies that show increased susceptibility to fear conditioning (16). Interestingly, we found that the high combat–exposed subgroup of control subjects showed significant volume differences compared with the PTSD group, which were not evident in the low/no-combat subgroup of control subjects, further suggesting that the high-combat subgroup of control subjects was driving the main results in the overall control group. Our hypothesis, which is consistent with these results but requires future confirmation, is that the PTSD group had smaller lateral nuclei before trauma, rendering them vulnerable to PTSD. On the other hand, the high-combat control group may have had relatively larger (normal) pretrauma lateral nuclei volumes, thereby conferring resilience to PTSD. Subsequently, combat trauma, which represents significant chronic stress for months to years, may have produced hypertrophy in the lateral nucleus of both PTSD and high-combat groups, but not in the low/no-combat group, which is consistent with evidence from animal models (12,50). Note that the low/no-combat group is likely to be composed of a combination of veterans who are resilient to PTSD and vulnerable to PTSD, which may conflate normal and smaller lateral nuclei volumes in the sample. While less is known about the paralaminar nucleus owing to its virtual absence in rodents, it is possible that a parallel explanation may be applied to the present paralaminar volume findings, pending further investigation. Conversely, the present finding that subnuclei of the centromedial complex (central, medial, cortical) are smaller in PTSD is consistent with the concomitant increase in fear expression associated with PTSD symptoms (51), although recent research also implicates this complex in fear conditioning (52).

Research by Tottenham *et al.* demonstrates that trauma during vulnerable periods early in life can result in amygdala hypertrophy. Specifically, children who experience prolonged institutional rearing, which represent conditions with high psychosocial stress, have larger amygdala volumes than control children (53). Those results are consistent with enhanced spinogenesis and dendritic arborization of the basolateral amygdala complex in rodents exposed to chronic immobilization stress (12,50). Of particular interest in both animal and human studies is that unlike in the hippocampus, the effects of adversity persist in the amygdala for many years after adversity is alleviated (53–55). While PTSD chronicity and the chronicity of trauma exposure tend to be highly correlated, there may be a significant time lag between trauma exposure and the onset of PTSD symptoms. Addressing these questions will require data from large-scale prospective longitudinal studies, such as AURORA (56) and the Brazilian High-Risk Cohort (57).

The present findings provide initial evidence that PTSD is related to local variations in amygdala structure, including thinning of the anterior aspect of the amygdala bilaterally and more constricted vertices at the posterior aspect of the left and right amygdala, which may reflect alterations in gray matter volume. While larger volume appears to be inconsistent with thinner shape and lower radial distance, this is not necessarily the case. Indicators of shape such as radial distance and thinner shape are present at surface locations where there are significant group differences. It is possible, however, that the relatively large remaining surface of the amygdala (blue portions in Figure 2) may be contributing to increased volume, albeit with nonsignificant between-group differences. These surface locations may be compensating for the reduction in radial distance and thinner shape, which happen to be significantly different between groups but occur on a relatively small surface of the amygdala. A limitation of the shape analysis was the lack of an atlas that mapped individual vertices to specific subnuclei, which precluded identifying the specific subnuclei associated with our shape findings.

Strengths and Limitations

Our statistical analyses attempted to quantify the influence of comorbidities such as depression. However, comorbid conditions that segregate with PTSD pose a significant challenge to making robust inferences. An improved study design that includes a non-PTSD psychiatric control group would address this limitation. Furthermore, while our secondary analysis examined effects of low or no combat exposure versus high combat exposure in the control subjects compared with the PTSD group, an improved experimental design with a trauma-unexposed group will be required to study amygdala subregion volume effects specific to trauma exposure. Finally, we were unable to investigate chronicity of PTSD because we lacked consistent reporting and recording about the time of illness onset.

A concern of our analysis pipeline is the heavy reliance of FreeSurfer segmentation on atlas priors. The ultrahigh-resolution (100-μm^3 isotropic) images used in the atlas construction have sufficient contrast to demarcate boundaries of nuclei with high confidence (20). The segmentation of 1-mm isotropic scans depends on this atlas, particularly when the algorithm has insufficient information from image contrast for labeling. Across the cohort, an unintended consequence is that each subject's volume measurement is more similar to every other subject than if ultrahigh-resolution technology were available for in vivo scanning of our subjects. Artificially low variance means that group differences will manifest as smaller effect sizes than the true effect size. However, a lower bound on this reduced variability is imposed by the whole-amygdala segmentation, which is capable of being segmented with fairly high fidelity at the scanning resolution we used. Thus, the variability in nuclei segmentation will be proportional to the variability in whole-amygdala segmentation even if segmentation is 100% atlas driven. However, segmentation in the present study is clearly not 100% atlas driven, given the lack of between-group differences in whole-amygdala volume but the presence of concomitant subregion differences in both directions.

The present sample was composed entirely of military veterans, which limits generalization of results to other demographic groups and trauma types. However, there is no evidence in the literature of sexually dimorphic amygdala volumes. While amygdala volume is ~10% larger in human males than in females, that difference is consistent with an 11.5% larger ICV in males (58). Thus, while no gender differences in

ICV-corrected amygdala volume were identified, this nevertheless leaves open the possibility of sexual dimorphism in amygdala subregion volumes or an interaction of gender and PTSD diagnosis.

The major strengths of this study are the large sample size, which is sufficiently powered to detect the effect sizes we report and to explore underlying heterogeneity related to the level of combat trauma exposure. Equally important is that we shed light on prior equivocal findings by showing that PTSD is associated with both larger and smaller volume of specific amygdala substructures.

Conclusions

Alterations in specific amygdala subnuclear volumes and regional shape distortions are associated with lifetime PTSD in military veterans. Smaller volume in the lateral nucleus, which is implicated in associative fear learning, and larger volumes of the central and medial nuclei, which are implicated in fear expression, are consistent with the differential subregion-specific trophic responses in rodents exposed to chronic restraint stress. We also identify the paralaminar nucleus as smaller in PTSD; this region has not been studied in the rodent fear literature owing to its recent evolutionary expansion. This structure has strong connections with the hippocampus, which is consistently hypotrophic in PTSD. Understanding the role of the amygdala in PTSD will require additional studies in humans and nonhuman primates, given the evolved anatomical structure and functional roles of amygdala subnuclei compared with the extensively researched rodent amygdala.

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