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## Posttraumatic Stress Symptom Persistence Across 24 Years: Association with Brain Structures

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### Abstract

Posttraumatic stress disorder (PTSD) is known to persist, eliciting early medical co-morbidity, and accelerated aging. Although PTSD diagnosis has been found to be associated with smaller volume in multiple brain regions, posttraumatic stress (PTS) symptoms and their associations with brain morphometry are rarely assessed over long periods of time. We predicted that persistent PTS symptoms across ~24 years would be inversely associated with hippocampal, amygdala, anterior cingulate volumes, and hippocampal occupancy (HOC=hippocampal volume/[hippocampal volume+ inferior lateral ventricle volume]) in late middle age. Exploratory analyses examined

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prefrontal regions. We assessed PTS symptoms in 247 men at average ages 38 (time 1) and 62 (time 2). All were trauma-exposed prior to time 1. Brain volumes were assessed at time 2 using 3T structural magnetic resonance imaging. Symptoms were correlated over time ( $r=0.46$   $p<.0001$ ). Higher PTS symptoms averaged over time and symptoms at time 1 were both associated with lower hippocampal, amygdala, rostral middle frontal gyrus (MFG), and medial orbitofrontal cortex (OFC) volumes, and a lower HOC ratio at time 2. Increased PTS symptomatology from time 1 to time 2 was associated with smaller hippocampal volume. Results for hippocampal, rostral MFG and medial OFC remained significant after omitting individuals above the threshold for PTSD diagnosis. Even at sub-diagnostic threshold levels, PTS symptoms were present decades after trauma exposure in parallel with highly correlated structural deficits in brain regions regulating stress responsivity and adaptation.

## Keywords

posttraumatic stress symptoms; PTSD; MRI; neuroimaging; Hippocampus; Amygdala; Prefrontal Cortex; Hippocampal Occupancy; midlife adults; stress; aging

## Introduction

Posttraumatic stress disorder (PTSD) has been linked to stress-induced modulation of brain synaptic circuitry that contributes to adverse psychological health and functioning (Bennett et al. 2016; McEwen et al. 2015; Arnsten 2015). Human and animal studies show that chronic stress is associated with structural brain changes, including changes in spine density, dendritic length, and branching of neurons in multiple brain regions connected with regulating stress responses (McEwen et al. 2016; Davidson and McEwen 2012). Attempts to understand the mechanisms by which PTSD has long-reaching effects have focused on key brain structures implicated in stress and emotion-regulation neurocircuitry such as the hippocampus, amygdala, anterior cingulate cortex (ACC), and prefrontal cortex (PFC) (O'Doherty et al. 2015; Bennett et al. 2016; Fraser et al. 2015). Although few studies have examined neurobiological consequences of PTSD in the context of aging (McEwen and Morrison 2013), reviews of the literature conclude that PTSD is likely to increase risk for accelerated neurodegeneration (Lohr et al. 2015; Wolf and Schnurr 2016; Wolf and Schnurr 2016). Atrophy in stress-response related brain regions, in particular the hippocampus, is considered a risk factor for poorer aging, including dementia (Bangen et al. 2018; Justice et al. 2015; Yaffe et al. 2010; Tanpitukpongse et al. 2017).

Studies examining links between PTSD and brain morphometry have focused on the hippocampus due to its links with the hypothalamic-pituitary-adrenal axis (Bremner and Vermetten 2001; Gilbertson et al. 2002; McEwen 2007). Reviews and meta-analyses find fairly consistent evidence of smaller hippocampal volume in adults with PTSD across a wide range of ages and precipitating events (Logue et al. 2018; O'Doherty et al. 2015; Kühn and Gallinat 2013). Chronic and/or lifetime PTSD also appears to be inversely associated with hippocampal volume (Apfel et al. 2011; Chao et al. 2014). Smaller hippocampal volume also may be a pre-existing risk factor for PTS symptoms (Gilbertson et al. 2002; Kremen et al. 2012). Amygdala and anterior cingulate cortex (ACC) have also been examined in

relation to PTSD due to their putative role in stress processes, fear conditioning and emotion processing (Kim et al. 2011; McEwen et al. 2016; Morey et al. 2016); however, evidence for a relationship is less consistent than for the hippocampus (O’Doherty et al. 2015).

In addition to the hippocampus, glucocorticoid receptors (GR) are highly expressed in PFC regions where aberrant GR signaling may be involved with PTSD (Arnsten et al. 2015; Etkin et al. 2011; Morey et al. 2016; Yang and Liang 2014; McEwen et al. 2016). Stress in animal models has been shown to decrease dendritic arborization and spine density of PFC neurons (Shansky and Morrison 2009) and animal studies indicate that the amygdala, ventral-medial prefrontal cortex and hippocampus comprise the core synaptic circuitry mediating behavior following traumatic events (Bennett et al. 2016). Top-down cognitive functions of the prefrontal cortex appear to be impaired by high levels of catecholamine release during stressful conditions with ongoing stress exposure leading to dendritic atrophy in PFC, dendritic extension in the amygdala, and strengthening of the noradrenergic (NE) system (Arnsten et al. 2015). Adults with PTSD show indications of PFC dysfunction (Arnsten et al. 2015). While the stress hormone cortisol may impact prefrontal regions (Kremen et al. 2010), human research provides a mixed picture regarding associations between PTSD and specific PFC regions of interest (ROI) (McEwen et al. 2016; Y. Li et al. 2017; O’Doherty et al. 2015).

Sub-diagnostic PTS symptoms may be as strongly related to psychological health and cognitive performance as a PTSD diagnosis, yet few studies have examined associations between continuously measured posttraumatic stress (PTS) symptoms and neuroanatomical structures (Franz et al. 2014; Goldberg et al. 2014; Breslau 2009). Focusing only on individuals who cross the threshold for PTSD diagnoses unduly limits attention to the relatively few individuals with diagnoses rather than on those who experienced trauma but do not meet criteria for PTSD and who may, to an unknown degree, still suffer. Because PTS symptoms in the present sample were assessed at two points in time—at average age 38 when this sample was first surveyed and approximately 24 years later when neuroimaging occurred—we had the unique opportunity to examine relationships between PTS symptom persistence and brain structure. Most research has been either cross-sectional or short-term longitudinal rather than covering extended periods of time.

We hypothesized that persistence of PTS symptoms over more than two decades would be associated with smaller hippocampal, amygdala and ACC volumes in late midlife. In addition, we predicted that PTS symptoms would be inversely associated with hippocampal occupancy (HOC), an indicator considered by some as a way of differentiating individuals with a congenitally small hippocampus from those with a small hippocampus relative to the size of the inferior lateral ventricle—potentially an indicator of neurodegeneration (Heister et al. 2011; Jak et al. 2015; Tanpitukpongse et al. 2017). We also hypothesized that volumes in these brain regions would be associated with symptom change from time 1 to time 2; and that findings would remain significant after omitting participants above the diagnostic threshold for PTSD. We examined associations between the PTS measures and specific PFC ROIs identified in a previous study of brain structures associated with neuroimaging of stress responses (Kremen et al. 2010). Given the mixed findings in the literature we considered these exploratory analyses.

Finally we examined the small number of monozygotic (MZ; identical) twin pairs who were discordant for PTSD. Because we did not have pre-trauma MRI data, it is possible that symptom-brain structure associations could represent pre-existing brain differences. If so, those associations would be consistent with brain structure differences being risk factors rather than consequences of PTS symptoms (Gilbertson et al. 2002; Kremen et al. 2012). Comparisons of discordant monozygotic twins allow for stronger inferences about cause versus effect because they control for genes and rearing environment. If the unexposed twin's hippocampal volume does not differ from that of the exposed twin, it would suggest that reduced hippocampal volume is a risk factor. The smaller hippocampus could not be due to exposure in the non-exposed twin, so the inference is that it is due to genetic/familial risk. However, because we do not have baseline MRI measures and the subsample of MZ twins discordant for PTSD is small and thereby underpowered, direction of causality cannot be addressed by this paper.

## Methods and Materials

### Participants

The Vietnam Era Twin Study of Aging (VETSA) is a longitudinal study of risk and protective factors for brain and cognitive aging that has been ongoing since 2002 (Kremen et al. 2006; Kremen et al. 2013). VETSA participants were recruited from the Vietnam Era Twin Registry (VETR), a nationally representative community-dwelling non-patient sample of male-male twin pairs who served in the United States military at some point between 1965 and 1975 (Goldberg et al. 2002; Goldberg et al. 1993). At the initial VETSA recruitment (2002–2008), we randomly recruited participants from the 3322 VETR male twin pairs who had participated in the Harvard Twin Study (Tsuang et al. 2001) and who were between the ages of 50 and 59. VETSA participants are similar in health and lifestyle characteristics to American men in their age range (Schoenborn and Heyman 2009). Although all VETSA participants are veterans, the majority (~72%) reported no combat exposure (Table 1).

The current study focused on the 370 participants who completed the VETSA wave 2 MRI visit (2009–2013) when participants were on average 62 years old ( $SD=2.4$ ; range=56–67), and whose neuroanatomical imaging data passed quality control (Kremen et al. 2013). Analyses were conducted on the 247 participants with MRI data who had also been exposed to trauma since trauma exposure is a prerequisite for PTSD. Data collection occurred at University of California San Diego (UCSD), Boston University, and Massachusetts General Hospital (MGH). Institutional Review Board approval was obtained at all sites and written informed consent was obtained from all individual participants included in the study.

### Measures

**Time 1 PTS symptom measures**—When participants were on average 38 years old (time 1;  $SD=2.7$ ; range 32–44; 1986/1987), they completed a mailed survey in which they reported about combat exposure and PTS symptoms (VETR-PTSD). These archived VETR data were used in the present analyses. The time 1 13-item VETR-PTSD questionnaire

assessed symptoms based on DSM-III-R and has high reliability ( $\alpha=0.89$ ) and validity (Goldberg et al. 2014; Goldberg et al. 1990; Magruder et al. 2014).

**Time 2 PTS symptom measures**—When participants were on average 62 years old (time 2;  $SD=2.4$ ; range=56–67), they were mailed psychosocial questionnaire booklets a month prior to the MRI and brought the completed questionnaire to the test site where they underwent in-depth testing (Kremen et al. 2013). The reliable, valid, 17-item self-report DSM-IV-based PTSD Checklist (PCL) civilian version (Weathers et al. 1993) was part of the booklet. The PCL civilian version was used because we were interested in response to all forms of traumatic exposure not simply those based on military experience. Although the participants are veterans, this is a non-patient community-based sample whose military service occurred over 40 years ago. Many participants reported non-military experiences as their most significant trauma. In an independent study, the VETR-PTSD correlated  $r = 0.90$  with the PCL when administered at the same time (Magruder et al. 2014). In the present sample, the two PTSD measures were correlated  $r = 0.46$ ;  $p < 0.0001$  across the approximately 24 years.

In order to examine persistence of PTS symptoms, we standardized each PTSD measure to a mean of zero and standard deviation of one and then averaged the two scores to create a combined measure (avgPTS symptoms) that is likely to be more reliable than either measure alone and is potentially indicative of persistence/chronicity of PTS symptoms (Chao et al. 2014). If someone had a high score at only one time point, the average would be reduced. Change in PTS symptoms was estimated by subtracting time 2 symptoms from those at time 1 using the standardized PTS measures. We defined presumptive PTSD based on previously developed standard formulas. For the VETR-PTSD scale, each item was first dichotomized (sometimes, often, or very often vs. almost never or never) to indicate severe symptom presence or absence. Cluster severity was then determined based on the number of symptoms associated with a cluster: for re-experiencing at least one severe symptom had to be present; for avoidance, at least three symptoms, and at least two symptoms for arousal. A presumptive diagnosis of PTSD was assigned when all three symptom clusters met severity criteria (Goldberg et al. 1990).

**MRI acquisition and processing**—At time 2, following the on-site test day, eligible twins underwent structural 3T magnetic resonance imaging (MRI) at either UCSD or MGH. Sixty-two percent of the MRI scans were collected at UCSD and 38% of the scans were collected at MGH. T1-weighted images were acquired on a GE 3T Discovery 750 scanner (GE Healthcare, Waukesha, WI, USA) with an eight-channel phased array head coil at UCSD, and a Siemens Tim Trio (Siemens USA, Washington, D.C.) with a 32-channel head coil at MGH (McEvoy et al. 2015). At UCSD, the 3D fast spoiled gradient echo (FSPGR) T1-weighted image protocol was:  $TE=3.164$  msec,  $TR=8.084$  msec,  $TI=600$  msec, flip angle= $8^\circ$ , pixel bandwidth=244.141,  $FOV=25.6$  cm, frequency=256, phase=192, slices=172, slice thickness=1.2 mm. At MGH, the 3D magnetization-prepared rapid gradient echo (MPRAGE) T1-weighted image protocol was:  $TE=4.33$  msec,  $TR=2170$  msec,  $TI=1100$  msec, flip angle= $7^\circ$ , pixel bandwidth=140,  $FOV=25.6$  cm, frequency=256, phase=256, slices=160, slice thickness=1.2 mm.

Raw dicom image files were pre-processed using an automated stream developed by the UCSD Center for Multimodal Imaging Genetics. T1-weighted (T1) structural images were corrected for gradient distortions (Jovicich et al. 2006) and B1 field inhomogeneity (Sled et al. 1998). Corrected images were processed using the FreeSurfer v5.1 software (Fischl 2012) for volumetric segmentation and cortical surface reconstruction and parcellation derived from the Desikan-Killiany atlas (Desikan et al. 2006) to determine regional cortical volumes (Fennema-Notestine et al. 2016; Elman et al. 2017; Kremen et al. 2010). All processed images were visually reviewed for quality. Volumetric segmentation regions were excluded if over- or under-estimated via consensus review, and the cortical surface and related image volumes were visually reviewed and edited for technical accuracy in alignment with standard, objective rules to improve the brain mask (i.e., removing non-brain voxels) and white matter volume (i.e., filling hyperintense white matter lesions). Images with severe scanner artifacts or excessive head motion were excluded due to degraded image quality. All image processing was conducted at UCSD. Twin pairs were assessed on the same scanner to avoid the potential confounding effects of scanner differences.

We created bilateral volumetric variables for amygdala, hippocampus, ACC, and PFC ROIs, then statistically adjusted for scanner (site) and intracranial volume (icv) estimated by FreeSurfer. The nine PFC ROIs examined were: the superior frontal gyrus (SFG), rostral and caudal middle frontal gyrus (MFG), pars orbitalis, pars opercularis, pars triangularis, medial and lateral orbitofrontal cortex (OFC), and frontal pole; these were chosen based on earlier work by Kremen et al. (Kremen et al. 2010). Adjustments for scanner were made to account for differences between the two scanner platforms (Fennema-Notestine et al. 2007).

Hippocampal occupancy was calculated as hippocampal volume/ (hippocampal volume + inferior lateral ventricle volume), first for each hemisphere and then averaged (Heister et al. 2011). Developed and validated based on the unique location of the hippocampus in relation to the inferior lateral ventricle, the HOC is indicative of the size of the hippocampus in relation to the ventricle and has often been interpreted as an indirect cross-sectional estimation of mesial temporal lobe atrophy with increases in the ventricle due to *ex vacuo* dilation (Heister et al. 2011; Jak et al. 2015). In contrast, a small hippocampal volume without an enlarged ventricle may reflect a congenitally small hippocampus. More details on VETSA methods have been reported elsewhere (Fennema-Notestine et al. 2016; Kremen et al. 2013; Brouwer et al. 2017).

**Covariates**—Number of childhood traumatic events rated as severe was assessed retrospectively with the Pennebaker Childhood Trauma Scale administered at time 2 (Pennebaker 1999; Pennebaker and Susman 1988). The combat exposure score assessed at time 1 represents a sum of 18 combat-related experiences during military service and was validated against military record data (Janes et al. 1991; Lyons et al. 1993; Goldberg et al. 1990). Participants entered military service (on average) in 1968 at age 19.38 (SD=1.4); the combat exposure and PTS symptom questionnaires were completed approximately 15 years (SD=2.8) after discharge from military service. These measures, as well as interview rater ratings of traumatic events in response to a diagnostic interview at approximately age 40 (Tsuang et al. 2001) were also used to exclude participants who had not experienced trauma.

Other key covariates were identified based on literature reviews of research on relationships between PTSD and cortical and subcortical brain structures. Childhood SES reflects a commonly used weighted averaging of father's occupation and education during the participant's childhood (<18 years) (Hollingshead and Redlich 1955). Young adult cognitive ability was measured with the well-validated 100-item Armed Forces Qualification Test (AFQT) administered at average age 20 (Bayroff and Anderson 1963; Orme et al. 2001). History of head injury was coded as present if it included any loss of consciousness. Apolipoprotein E (*APOE*) genotype was coded as presence/absence of any  $\epsilon 4$  alleles. Ethnicity was coded as non-Hispanic white versus other. All of these measures were used as covariates in Model 1.

Covariates from time 2 (age 62) included global health, depressive symptoms, smoking, stress, alcohol consumption, and physical activity. Global health counted the presence of 15 major chronic conditions from the Charlson Comorbidity index (Charlson et al. 1994): diabetes, emphysema, asthma, cancer, osteoarthritis, rheumatoid arthritis, stroke, heart attack, heart failure, heart surgery, angina, hypertension, peripheral vascular disease, cirrhosis, and AIDS. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (CESD) (Radloff 1977). Smoking was binary (ever smoked vs. never smoked). Recent adult events/stressful experiences were assessed with the Holmes-Rahe Life Events Inventory on which participants reported whether any of 79 events occurred in the past 24 months at time 2 (Holmes and Rahe 1967). Current alcohol consumption was quantified as: never drank or not currently drinking; 1 or fewer drinks per day; 1–2 drinks per day; > 2 drinks per day in the past two weeks (Franz et al. 2011; Paul et al. 2008). The physical activity measure averaged two questions about frequency of participating in physical fitness and/or walking and hiking in the past month.

### Statistical Analyses

We conducted mixed effects models using SAS 9.4 with PTS symptom measures as the independent variables, and specific time 2 cortical and subcortical ROI volumes and HOC as dependent variables. We ran separate analyses representing two operationalizations of symptom persistence: the first included time 1 PTS symptoms and change in PTS symptoms from time 1 to time 2 as independent variables; the second operationalization of persistence was the average of symptoms from the two timepoints. We report results in terms of a) Model 1, which includes PTS symptom measures and covariates prior to time 1 [ethnicity (non-Hispanic white vs other), young adult general cognitive ability, *APOE* status (presence of any  $\epsilon 4$  allele [ $\epsilon 4+$ ] versus none [ $\epsilon 4-$ ]), head injury (yes, no), number of combat exposures, number of severe childhood traumas, age; and b) Model 2, which includes fully adjusted models that added time 2 covariates measured concurrently with the MRI measures.

We present type III effects; these represent the unique variance accounted for by each variable after controlling for all other variables in the model. All measures were standardized (mean = 0; SD = 1) prior to analyses. All models adjusted for the clustering of twins within pairs by including twin pair ID (i.e., family) as a random effect. Results are reported as two-tailed and significance was set at  $p < 0.05$ . For the nine exploratory PFC regional analyses we applied false discovery rate corrections for multiple comparisons; the

Li and Ji (2005) extension of the Benjamini-Hochberg approach (Benjamini and Hochberg 1995) was used because of the intercorrelations among measures.

## Results

### Descriptive statistics

VETSA MRI participants are predominantly non-Hispanic white (87%). At time 2, when they were approximately age 62, the majority of the men were married (78%), 48% working full-time, 23% were retired and not working, 11% retired but working part-time, 8% were on disability, and the remainder worked part-time. Average education was 13.8 years. Demographic and psychosocial characteristics of the sample are reported in Table 1. There were no significant between-site differences in PTS symptoms or covariates.

The significant correlation between the VETR-PTSD and PCL measures ( $r = 0.46$ ;  $p < 0.0001$ ) indicates moderate levels of symptom stability across these approximately two and a half decades. At time 1, 8.2% ( $N=34$ ) of the sample had symptoms above the cutoff for presumptive PTSD; at time 2, 6.1% of the sample met criteria. Only nine men (2.1%) met criteria at both times. A fifth of the men experienced at least one severe childhood trauma, and 27% experienced at least one combat exposure during military service.

### Associations with hippocampus, amygdala, and ACC measures

In Model 1, adjusted for pre-time 1 covariates, PTS symptoms at time 1 was associated with hippocampal and amygdala volume, and HOC at time 2 (Table 2). Higher levels of symptoms at time 1 were associated with smaller volumes and lower HOC time 2. Change in PTS symptoms from time 1 to time 2 was only associated with hippocampal volume at time 2; a decrease in symptoms was related to larger hippocampal volume. AvgPTS was also significantly associated with hippocampal and amygdala volumes, and HOC at time 2 (Table 3, Model 1).

In the full model (Model 2) that additionally included time 2 covariates, associations between time 1 PTS symptoms with hippocampal volume remained significant while results for hippocampal occupancy and amygdala were attenuated (Online resource Table 1). Similarly, only hippocampal volume remained significant for avgPTS (Table 3; Model 2). No relationships were found between any PTS symptom measure and ACC measures.

### Prefrontal cortex: Exploratory analyses

We only report here results for the PFC analyses that remained significant following corrections for multiple comparisons; corrected p-values are presented in parentheses. With regard to PFC measures, in Model 1, PTS symptoms at time 1 and avgPTS (Tables 2 and 3 respectively) were inversely associated with rostral MFG ( $p=0.007$ ), and medial OFC volumes at time 2 ( $p=0.013$ ). Men with higher symptoms had smaller volumes in these brain regions. In Model 2 results for the rostral MFG remained significant for both PTS measures (Table 3 and Online resource Table 1); associations with medial OFC were no longer significant when corrected. However, both time 1 symptoms and avgPTS now had a



significant relationship with pars triangularis volume. There were no associations between symptom change over time and any PFC measure.

### Omitting participants with presumptive PTSD

We examined associations between PTS symptoms and measures that had been significant in the above analyses after omitting participants with presumptive PTSD (Table 4). Both avgPTS and time 1 PTS symptom associations with hippocampal, rostral MFG, and medial OFC volumes remained significant when individuals with presumptive PTSD diagnoses were excluded. Change in symptoms from time 1 to time 2 was associated with hippocampal and medial OFC volumes; having smaller hippocampal volume at time 2 was related to increases in PTS symptoms.

### Discordant twin analyses

We compared brain volume measures using paired t-tests for the ten pairs of MZ twins discordant for presumptive PTSD at time 1. None of the subcortical, ACC or PFC volumes were significantly different at time 2 (see Online resource Table 2). The median effect size was small (Cohen's *d* of approximately 0.075).

## Discussion

In a nonclinical sample of men spanning two and a half decades, those with higher levels of PTS symptoms had smaller bilateral hippocampal, amygdala, rostral MFG, and medial OFC volumes, as well as lower HOC in late midlife. These associations were significant for both time 1 PTS symptoms (average age 38) and for the average of symptoms at time 1 and time 2 (average age 62). Increase in symptoms was associated with smaller hippocampal volume. Thus having higher symptoms in earlier adulthood and/or sustaining higher levels of symptoms across approximately 24 years was associated with smaller volumes in brain regions known to be involved with key functions such as hypothalamic-pituitary-adrenal axis regulation, stress responsivity, memory, cognition, and top down control of emotion processing (Shin and Liberzon 2010; McEwen et al. 2016; Euston et al. 2012). When participants with putative PTSD were omitted from analyses, associations between symptoms and hippocampal volume, rostral MFG, and medial OFC remained significant. Associations were somewhat attenuated in the full model that also adjusted for current health and stress, though associations with hippocampal volume and rostral MFG remained significant. This study highlights the long-term connection between PTS symptoms and brain structure even at levels below diagnostic thresholds, the importance of evaluating symptom persistence and brain regions beyond the hippocampus, as well as the importance of a life course perspective on relationships between mental health and its neurobiological concomitants (Spiro III et al. 2018). In addition, these results are consistent with growing evidence of associations between persistent PTS symptoms and risk for neurodegeneration (Lohr et al. 2015; Lyons et al. 2013; Yaffe et al. 2010; Justice et al. 2015; Wolf and Schnurr 2016).

Research on the molecular biology underlying stress shows that stress mediators such as corticotropin releasing factor (CRF) are implicated in anxiety and stress disorders and that

stress appears to weaken prefrontal networks (Futch et al. 2017; McEwen et al. 2016; Hauger et al. 2009; Arnsten 2015); CRF is widely expressed throughout the brain, in particular in PFC and hippocampal/amygdala regions. Animal experiments on acquisition, extinction and reactivation of fear behavior may also provide insights that can be translated to humans and PTSD (Bennett et al. 2016). In addition, relationships between trauma, beta amyloid production, and PTSD-like phenotypes have been identified in animal models (Arnsten 2015; Justice et al. 2015) which may provide insight into mechanisms underlying the association between PTS symptoms, stress-related weakening of prefrontal networks, and risk for Alzheimer's disease and related dementias (Futch et al. 2017; Lohr et al. 2015; Yaffe et al. 2010).

As in previous studies of shorter duration and with younger participants (O'Doherty et al. 2015; Logue et al. 2018), higher levels of posttraumatic stress were associated with smaller hippocampal volume. The hippocampus also has been shown to play a key role in dementia risk (Tanpitukpongse et al. 2017) and mild cognitive impairment (Bangen et al. 2018; Jak et al. 2015). Having a small hippocampus, whether due to inheritance or life experience, may be linked to physiological, cognitive and contextual deficits that contribute to a cascade of maladaptive stress responses (Brewin et al. 2010; Fraser et al. 2015; Rutter 2012) with some researchers suggesting that having a smaller hippocampus may be a vulnerability factor for poor recovery (Apfel et al. 2011). Although the HOC index has been examined in studies of mild cognitive impairment and Alzheimer's disease (Heister et al. 2011; Jak et al. 2015; Tanpitukpongse et al. 2017), to our knowledge no studies have reported on the relationship between PTS symptoms and hippocampal occupancy. Longitudinal research is needed to substantiate whether atrophy is occurring or if the hippocampal/inferior lateral ventricle ratio is a congenital state. Similar to dementia findings reported by Tanpitukpongse et al. (2017) HOC did not out-perform hippocampal volume. However, HOC may still be useful as a proxy indicator of hippocampal tissue loss or may contribute additional information about changes in surrounding tissue. The fact that associations with PTS symptoms were more consistent for hippocampal volume than for hippocampal occupancy might suggest that the observed findings are mainly a function of smaller baseline hippocampal volume. Discordant twin studies have found that some features commonly associated with PTSD comprise pre-existing familial/genetic risk factors (i.e., having a small hippocampus) while other features (e.g., fear extinction/conditioning, emotion regulation, pain) were sequelae (Gilbertson et al. 2002; Kremen et al. 2012). Discordant MZ twin designs allow for stronger inferences about risk factors versus sequelae. In this sample there were no differences in brain structure volumes in the discordant twin pairs. Thus, our results are consistent with previous discordant twin findings for hippocampal volume which suggested that smaller volumes were pre-existing, and are therefore, most likely to represent risk factors for, rather than consequences of, PTS symptoms. Given the small number of discordant pairs, however, more research is needed to confirm these results in a larger sample. The association between hippocampal volume and PTS symptom change further suggests that smaller hippocampal volume may be a risk factor for greater persistence of PTS symptoms.

Fewer studies have examined amygdala volume in PTSD compared with studies of the hippocampus (O'Doherty et al. 2015). Here, amygdala volume was associated with both time 1 PTS symptoms and averaged PTS symptoms in the predictive models but these

relationships were attenuated in the full models with the concurrent covariates (current smoking, alcohol consumption, health conditions, depressive symptoms, life events in the past two years). It seems unlikely that current factors or even two-year old events would significantly affect regional brain volumes. Therefore, these probably mostly reflect longstanding characteristics. Thus, the implication is that there is no association of PTS symptoms with amygdala volume over and above these other factors. Multiple studies find inconsistent results for the amygdala—with some studies finding increased amygdala volume and others smaller amygdala, suggesting a potential greater sensitivity to other influences or greater plasticity of the amygdala over time (Kim et al. 2011; Koenigs and Grafman 2009; McEwen et al. 2016; Shin and Liberzon 2010; Lewis et al. 2014; Morey et al. 2016).

The fMRI literature suggests diminished activation of regions of the PFC in PTSD, but structural MRI findings are mixed, as they were in our exploratory analyses (Etkin et al. 2011; Koenigs and Grafman 2009; Kühn and Gallinat 2013; Yang and Liang 2014). PFC exerts top down control of stress reactivity, potentially modulating central responses to stress in the organism (McEwen et al. 2016) and an extensive literature shows the PFC is vulnerable to stress in animals (Arnsten 2015; McEwen and Morrison 2013) and humans (Y. Li et al. 2017; McEwen et al. 2016; Morey et al. 2016). It was surprising then that so few of the a priori identified PFC regions were associated with PTS symptoms despite the fact that previous research found relationships between cortisol and these regions (Kremen et al. 2010). In these analyses, the strongest PFC results were between the measures of averaged PTS or time 1 PTS symptoms with rostral MFG and medial OFC volume. Decreases in rostral MFG efficiency have been found in a study of the effects of re-experiencing related symptoms on brain networks in traumatized veterans (Spielberg et al. 2015). Sharp and Telzer (2017) proposed that poor connectivity between the left rostral MFG and caudate might be a biomarker for high trait anxiety, potentially a risk factor for PTSD. As a subregion of the medial prefrontal cortex, the OFC has been found to play a role in regulating and facilitating emotional learning, including monitoring reward values (Chang and Grace 2018), and is implicated in multiple anxiety disorders. Studies report functional and structural abnormalities in the OFC in patients with obsessive-compulsive disorder and somewhat lower prevalence in panic disorder; findings are mixed in PTSD studies (Shin and Liberzon 2010). Aberrant frontal-amygdala- and frontal-striatal connectivity occurs in multiple anxiety disorders (MacNamara et al. 2016) and a recent animal study found that activation of the OFC appeared to modulate the mPFC-amygdala pathway (Chang and Grace 2018). Pars triangularis became significant in the models that included the concurrent time 2 covariates but more research is needed to understand the relationship between PTS symptoms and pars triangularis. In this instance the pars triangularis became significant in the model with more concurrent covariates suggesting a potential suppression effect by other variables. The pars triangularis does appear to play a role in emotional regulation (Kircher et al. 2013; Kohn et al. 2014) and is associated with stress hormone elevation (Kremen et al. 2010).

## Strengths and limitations

The sample comprised predominantly Caucasian men, so we cannot generalize to women or other racial/ethnic groups. Sex differences have been found in type of trauma and prevalence of PTSD, but it is unknown whether these reflect differences in underlying neurobiology (McEwen and Morrison 2013; O'Doherty et al. 2015). Different PTS symptom measures were used at the two timepoints; when administered concurrently, however, the measures correlated  $r=0.90$  (Magruder et al. 2014). Although the validity of the VETR-PTSD measure has been established, we chose to test participants with the PCL at time 2 since it is the current gold-standard for self-report PTSD measures (Freedy et al. 2010; Magruder et al. 2014). Because we do not have baseline MRI measures and the MZ discordant twin analysis was underpowered, direction of causality is suggestive but it cannot be confirmed in the present study. Other limitations may be our use of bilateral measures of brain structures since there is some evidence for brain laterality in PTSD (Kühn and Gallinat 2013; Morey et al. 2012; Morey et al. 2016). We also know little about mental health treatments these men might have sought or received that may have mitigated PTS or other symptoms across this time period. Some of the strengths of this study are that it is one of a very few to examine long-term associations between PTS symptoms and brain--including a 24-year follow-up of PTS symptoms, trauma exposures were heterogeneous, and we had good coverage of important covariates. The fact that PTS symptoms below the threshold for diagnosis were still associated with brain structure in late middle age is also an important finding.

Often overlooked in studies of aging, veterans comprise significant subsections of the population in the United States (Spiro III et al. 2018). The first wave of the 7.5 million Vietnam era veterans has just reached retirement age, and the projected veteran population as of September 2012 is 22.3 million adults (Department of Veterans Affairs Office of the Actuary 2013). Although only 6.1% met criteria for PTSD in the present study, veterans overall appear to be at higher risk for PTSD than the general population; previous research reported prevalence rates for Vietnam veterans as high as 31% lifetime and 15% in the past year (Kulka et al. 1990). One of the chief roles of military service vis-à-vis PTS may be to increase the likelihood of exposure to trauma compared to civilian life (Kulka et al. 1990; Lyons et al. 2011); however, at least half of the present sample reported a non-combat experience as their most serious traumatic event. PTS symptoms are known to persist and are costly in terms of high levels of co-morbidity, mortality and protracted suffering (Elder et al. 2009; Spiro III et al. 2018).

In sum, our findings demonstrate that PTS symptoms, even in the absence of a PTSD diagnosis, are present even decades after trauma exposure and associated with brain regions that are implicated in increased risk for neurodegeneration. The fact that even subclinical symptoms are associated with smaller regional brain volumes suggests that persistent PTS symptoms warrant clinical attention even if threshold for diagnosis has not been fully met (Arnsten et al. 2015; Freedy and Brock 2010; Freedy et al. 2010). Discordant MZ twin analyses added a unique component, consistent with a larger literature, showing that smaller volumes in relevant brain regions may be a pre-existing risk factor for PTS symptoms and their persistence over time. However, the number of MZ discordant pairs was too small to allow for firm conclusions. Taken together, the study results help to identify possible

mechanisms underlying the persistence of PTS symptoms as well as their potential contribution to the aging process (Lohr et al. 2015; Wolf and Schnurr 2016; Wolf and Schnurr 2016; Yaffe et al. 2010).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Table 1.**

Sample demographics at time of structural magnetic resonance (MRI) brain imaging (Time 2)

Variable	Mean (SD) or N (%)
Age (Time 1)	38 years (SD=2.7); range 32–44
Age (Time 2)	61.8 years (SD 2.4); range 56–67
Lifetime education	13.8 (SD 2.1); Associates degree/some college
Lifetime main occupation	5.5 (SD 1.8); Small business owner
Own income	5.5 (SD 2.9); \$50,000-\$59,999 per year
Center for Epidemiologic Studies-Depression	6.5 (SD 7.4)
Ethnicity (non-Hispanic White)	361 (87.4%)
Childhood trauma (# events rated as severe)	
0 events	325 (78.9%)
1 event	57 (13.8%)
>1 event	30 (7.3%)
Time 1 PTS symptoms	22.7 (SD 7.8); range 13–57 out of 65
Time 2 PTS symptoms	25.0 (SD 9.6); range 17–74 out of 85
Combat exposures (# types of exposures)	
Not in South East Asia	266 (64.4%)
In South East Asia, no exposure	33 ( 7.9%)
1–5 exposures	77 (18.6%)
6–16 exposures	37 ( 8.9%)
Recent life events (past 2 years)	9.0 (SD 7.6); range 0–55
APOE e 4 carrier	100 (24.9%)
Alcohol drinks in past 2 weeks	
None	175 (36%)
<= 1 per day	213 (44%)
>1 but <2 per day	38 (8%)
2 to <3 per day	25 (5%)
>= 3 per day	38 (8%)
Ever smoked	249 (60.3%)
Chronic health conditions (# of conditions)	
0 conditions	105 (25.4%)
1 condition	176 (42.6%)
2 conditions	83 (20.1%)
>2 conditions	49 (11.9%)
Any traumatic head injury with loss of consciousness	105 (25%)

**Table 2.**

Time 1 and change in posttraumatic stress symptoms predicting bilateral cortical, anterior cingulate, and subcortical brain volumetrics in late midlife: Model 1/predictive model.

Brain ROI (Time 2)	Time 1 PTS symptoms		Change	
	$\beta$	P	$\beta$	p
<b>Subcortical Structures</b>				
Hippocampal Volume	<b>-0.21 (0.08)</b>	<b>0.01</b>	<b>0.15 (0.08)</b>	<b>0.04</b>
Hippocampal Occupancy	<b>-0.15 (0.11)</b>	<b>0.04</b>	0.09 (0.07)	0.21
Amygdala	<b>-0.19 (0.08)</b>	<b>0.01</b>	0.11 (0.07)	0.13
<b>Anterior Cingulate (ACC) Volumes</b>				
Rostral ACC	0.03 (0.08)	0.74	0.05 (0.07)	0.53
Caudal ACC	0.10 (0.07)	0.20	0.00 (0.07)	0.98
<b>Prefrontal Cortex (PFC) Volumes (Exploratory)</b>				
Caudal Middle Frontal Gyrus	-0.13 (0.07)	0.09	0.09 (0.07)	0.21
Rostral Middle Frontal Gyrus	<b>-0.24 (0.07)</b>	<b>0.001</b>	0.17 (0.06)	0.01
Superior Frontal Gyrus	-0.14 (0.07)	0.04	0.07 (0.09)	0.26
Pars Orbitalis	-0.07 (0.08)	0.36	0.09 (0.07)	0.23
Pars Opercularis	-0.06 (0.08)	0.42	0.00 (0.07)	0.98
Pars Triangularis	-0.13 (0.08)	0.11	0.04 (0.07)	0.60
Medial Orbitofrontal Cortex	<b>-0.18 (0.07)</b>	<b>0.01</b>	0.15 (0.06)	0.02
Lateral Orbitofrontal Cortex	-0.06 (0.08)	0.42	0.05 (0.07)	0.47
Frontal Pole	-0.15 (0.08)	0.06	0.16 (0.08)	0.04

ROI=Region of interest. (N=247; df 58–60) Numbers in the table are parameter estimates ( $\beta$  coefficients), standard errors (SE), and p values from mixed models that account for non-independence of observations within twin pairs. Model includes time 1 PTS symptoms (intercept) and change (time 1 PTSD – time 2 PTS symptoms), age, ethnicity (non-Hispanic white vs other), early adult cognitive ability, apolipoprotein epsilon 4 (APOE) status (presence of any  $\epsilon 4$  allele [ $\epsilon 4+$ ] versus none [ $\epsilon 4-$ ]), head injury (yes, no), combat exposure, childhood trauma and life events. ROI measures are residuals of brain structure volume adjusted for scanner/site and intracranial volume. PTSD symptom measures were standardized with a mean of 0 and standard deviation of 1. Bolded exploratory PFC values are those significant following correction for multiple comparisons (Li, 2005 #132).

**Table 3.**

Posttraumatic stress symptoms averaged across 24 years and bilateral cortical, anterior cingulate, and subcortical brain volumetrics in late midlife.

Brain ROI (Time 2)	Model 1 (predictive)		Model 2 (Full)	
	$\beta$	P	$\beta$	p
<b>Subcortical Structures</b>				
Hippocampal Volume	<b>-0.17 (.07)</b>	<b>0.01</b>	<b>-0.21 (.09)</b>	<b>0.02</b>
Hippocampal Occupancy	<b>-0.13 (.06)</b>	<b>0.05</b>	-0.15 (.09)	0.08
Amygdala	<b>-0.17 (.06)</b>	<b>0.01</b>	-0.12 (.09)	0.17
<b>Anterior Cingulate (ACC) Volumes</b>				
Rostral ACC	0.02 (.07)	0.72	0.01 (.09)	0.87
Caudal ACC	0.08 (.06)	0.19	0.10 (.08)	0.24
<b>Prefrontal Cortex (PFC) Volumes (Exploratory)</b>				
Caudal Middle Frontal Gyrus	-0.11 (.06)	0.09	-0.12 (.08)	0.13
Rostral Middle Frontal Gyrus	<b>-0.20 (.06)</b>	<b>&lt;0.001</b>	<b>-0.23 (.08)</b>	<b>0.01</b>
Superior Frontal Gyrus	-0.12 (.06)	0.05	-0.16 (.08)	0.05
Pars Orbitalis	-0.06 (.07)	0.37	-0.05 (.09)	0.55
Pars Opercularis	-0.05 (.07)	0.41	-0.08 (.09)	0.35
Pars Triangularis	-0.11 (.07)	0.11	<b>-0.23 (.09)</b>	<b>0.01</b>
Medial Orbitofrontal Cortex	<b>-0.15 (.06)</b>	<b>0.01</b>	-0.16 (.08)	0.05
Lateral Orbitofrontal Cortex	-0.05 (.07)	0.42	-0.02 (.09)	0.84
Frontal Pole	-0.13 (.07)	0.07	-0.15 (.09)	0.10

ROI=Region of interest. Numbers in the table are parameter estimates ( $\beta$  coefficients), standard errors (SE), and p-values from mixed models that account for non-independence of observations within twin pairs. Model 1 (predictive; N=247 df=60) uses the average of time 1 and time 2 PTS symptoms as the independent variable as well as ethnicity (non-Hispanic white vs other), early adult cognitive ability, apolipoprotein epsilon 4 (APOE) status (presence of any  $\epsilon 4$  allele [ $\epsilon 4+$ ] versus none [ $\epsilon 4-$ ]), head injury (yes, no), number of combat exposures, number of severe childhood traumas and age as covariates; Model 2 additionally includes time 2 covariates for smoking (yes, no), alcohol consumption, chronic health conditions, Centers for Disease Control Depression Scale (CES-D), and life events. ROI measures are residuals of brain structure volume adjusted for scanner/site and intracranial volume. Bolded exploratory PFC values are those significant following correction for multiple comparisons.

**Table 4.**

Results for significant brain regions after omitting participants with presumptive PTSD

Time 2 brain regions	Averaged PTS symptoms		Change over time	
	$\beta$ ; p-value	PTS symptoms intercept (Time 1)	$\beta$ ; p-value	Change in PTS symptoms Time 1 to Time 2
Hippocampal Volume	<b>-0.18; p= 0.03</b>	<b>-0.20; p= 0.04</b>	<b>0.21; p= 0.01</b>	
Hippocampal Occupancy	-0.09; p= 0.24	-0.10; p= 0.09	0.12; p= 0.11	
Amygdala	-0.13; p= 0.10	-0.15; p= 0.11	0.12; p= 0.13	
Rostral MFG	-0.18; p= 0.02	<b>-0.21; p= 0.02</b>	0.09; p= 0.20	
Medial OFC	<b>-0.17; p= 0.03</b>	<b>-0.19; p= 0.03</b>	<b>0.14; p= 0.05</b>	
Pars Triangularis	-0.15; p= 0.09	-0.18; p= 0.01	0.18; p= 0.11	

N=220; df=46; MFG= middle frontal gyrus; OFC=orbitofrontal cortex