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Serum brain-derived neurotrophic factor remains elevated after long term follow-up of combat veterans with chronic post-traumatic stress disorder

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ABSTRACT

Attempts to correlate blood levels of brain-derived neurotrophic factor (BDNF) with post-traumatic stress disorder (PTSD) have provided conflicting results. Some studies found a positive association between BDNF and PTSD diagnosis and symptom severity, while others found the association to be negative. The present study investigated whether serum levels of BDNF are different cross-sectionally between combat trauma-exposed veterans with and without PTSD, as well as whether longitudinal changes in serum BDNF differ as a function of PTSD diagnosis over time. We analyzed data of 270 combat trauma-exposed veterans (230 males, 40 females, average age: 33.29 ± 8.28 years) and found that, at the initial cross-sectional assessment (T0), which averaged 6 years after the initial exposure to combat trauma (SD = 2.83 years), the PTSD positive group had significantly higher serum BDNF levels than the PTSD negative controls [31.03 vs. 26.95 ng/mL, $t(268) = 3.921$, $p < 0.001$]. This difference remained significant after excluding individuals with comorbid major depressive disorder, antidepressant users and controlling for age, gender, race, BMI, and time since trauma. Fifty-nine of the male veterans who participated at the first timepoint (T0) were re-assessed at follow-up evaluation (T1), approximately 3 years (SD = 0.88 years) after T0. A one-way ANOVA comparing PTSD positive, "subthreshold PTSD" and control groups revealed that serum BDNF remained significantly higher in the PTSD positive group than the control group at T1 [30.05 vs 24.66 ng/mL, $F(2, 56) = 3.420$, $p = 0.040$]. Serum BDNF levels did not correlate with PTSD symptom severity at either time point within the PTSD group [$r(128) = 0.062$, $p = 0.481$ and $r(28) = 0.157$, $p = 0.407$]. Serum BDNF did not significantly change over time within subjects [$t(56) = 1.269$, $p = 0.210$]

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nor did the change of serum BDNF from T0 to T1 correlate with change in PTSD symptom severity within those who were diagnosed with PTSD at T0 [$r(27) = -0.250$, $p = 0.192$]. Our longitudinal data are the first to be reported in combat PTSD and suggest that higher serum BDNF levels may be a stable biological characteristic of chronic combat PTSD independent of symptom severity.

1. Introduction

Post-traumatic stress disorder (PTSD) can occur among individuals who experience or witness, or are threatened with death, injury or violence, and includes symptoms such as: re-experiencing, avoidance, negative alterations in cognitions and moods associated with the traumatic event and hyperarousal (American Psychiatric Association, 2013). The life prevalence rate of PTSD is 8.3% among the general population in the United States (Kilpatrick et al., 2013) and 21.8% among combat veterans (Seal et al., 2009). In recent years, there have been increasing efforts to identify biological characteristics that are associated with PTSD for better diagnosis, personalized treatment options, prevention mechanisms, and an overall more in-depth understanding of PTSD (Blessing et al., 2017; Suliman et al., 2013; Zhang et al., 2014).

Brain-derived neurotrophic factor (BDNF) regulates neuronal survival, growth differentiation, and synapse formation, and as well as fear and memory formation in addition to other functions. It may have a central role in PTSD; however, the nature and direction of the relationship and its mechanisms are uncertain (Angelucci et al., 2014; Dell'Osso et al., 2009; Diniz et al., 2018; Mojtabavi et al., 2020). Animal models for chronic stress and PTSD have found greater concentrations of BDNF in the plasma and hippocampus of stressed animals when compared to non-stressed controls (Faure et al., 2007; Zhang et al., 2014). In accordance with these findings, a recent meta-analysis of 20 studies found that PTSD patients had increased serum BDNF levels compared with controls (Mojtabavi et al., 2020). These findings raise the possibility that elevated peripheral BDNF concentration may reflect a physiologic attempt to compensate for other neurobiological alterations that occur in PTSD (Hauck et al., 2009; Zhang et al., 2014). Alternatively, increased BDNF in PTSD could be a pathophysiological mechanism itself, due to the over-consolidation of traumatic memories around the time of the trauma (Diniz et al., 2018; Matsuoka et al., 2013). On the other hand, other studies that found significantly lower serum or plasma BDNF levels in individuals with PTSD argued for hippocampal volume reduction, as well as impaired memory and learning (Angelucci et al., 2014; Dell'Osso et al., 2009; Zhang et al., 2006).

The majority of published studies on BDNF in PTSD to date only present cross-sectional data and do not address changes in PTSD diagnoses, symptoms, and BDNF concentrations over time (Molendijk et al., 2011). Assessing psychopathology and biological markers at a single point in time, as opposed to longitudinal assessments, poses several limitations including variability in development, symptomatology and biology of PTSD over time (Schaie, 2005; Waller et al., 2016). One study found that BDNF levels were elevated in the acute trauma group when compared to controls, but the remote trauma group, those whose trauma occurred 10 years ago, was not significantly different from the controls (Hauck et al., 2010). Further, cross-sectional analyses only allow inter-individual assessments, which are not ideal, since PTSD symptomatology and the developmental stage vary between individuals (Waller et al., 2016). Longitudinal studies, however, assess individuals at two or more separate time points, allowing observation and analysis of intra-individual changes (Schaie, 2005). For example, a 6-month longitudinal study by Matsuoka et al. (2013) evaluated a small number of civilians traumatized by motor vehicle accidents and concluded that higher serum BDNF concentrations were associated with PTSD diagnoses at baseline and at 6 months, as well as with worsening PTSD symptom severity.

The present study extends our previous study (Blessing et al., 2017) and assessed serum BDNF in combat- exposed veterans with and without PTSD at two time points, T0 (baseline) and T1 (follow-up). We studied a relatively large sample of combat trauma exposed individuals, whose time since the index trauma was 6 years on average at T0 and who had a longitudinal follow-up interval of approximately 3 years later, T1. The aims of this study were to investigate: (1) serum BDNF levels in PTSD positive vs. PTSD negative controls at both the initial and follow-up assessments; (2) if serum BDNF concentrations change over time within individuals; (3) if serum BDNF levels correlated with PTSD symptom severity at each time point; and (4) if changes in BDNF levels were associated with changes in PTSD symptom severity between the two assessment time points.

2. Method

2.1. Participants

The current study included 230 male and 40 female American veterans from Operation Enduring Freedom (OEF) and/or Operation Iraqi Freedom (OIF) conflicts from a large Systems Biology of PTSD Consortium study (Dean et al., 2020) that investigates biomarkers for PTSD diagnosis. A detailed description of participant recruitment, study design, inclusion/exclusion criteria and blood sample collection have been described elsewhere (Dean et al., 2020). Briefly, participants were recruited by New York University (NYU), the Icahn School of Medicine at Mount Sinai (ISMMS), and the James J. Peters Veterans Administration Medical Center (JJPVAMC) and given written informed consent. All participants were exposed to DSM-IV PTSD Criterion A trauma during deployment (Kilpatrick et al., 2013). DSM-IV was the version of DSM in use at the time of this study. Of the total 270 participants, 130 were diagnosed with current PTSD and 140 participants had no history of PTSD. Participant characteristics are displayed in Table 1. Exclusion criteria included history of open head injury or closed head injury with loss of consciousness of more than 10 min, current drug abuse or dependence within the past year, current alcohol dependence or history of dependence within the past 8 months, lifetime history of any psychiatric disorder with psychotic features, bipolar disorder, or obsessive-compulsive disorder, currently exposed to recurrent trauma or exposed to a traumatic event within the past 3 months, participants with prominent suicidal or homicidal ideation, neurologic disorder or systemic illness affecting central nervous system function, anemia, recent blood donation in the past 2 months, and participants who were not stable for 2+ months on psychiatric medication, anticonvulsants, antihypertensive medication or sympathomimetic medication.

2.2. Assessment of clinical characteristics

All clinicians who conducted the clinical interviews for this study were post-doctorate level psychologists who had several years of experience working with veterans and trauma. All interviews were calibrated weekly with the PTSD program clinical team across sites, including a senior clinician, which ensured a similar use of diagnostic measures and consistent application of inclusion and exclusion criteria. Diagnoses were established with the Structured Clinical Interview for DSM-IV disorders (SCID) (Wisco et al., 2014) (which was the SCID manual in use at the time of the study) and Clinician Administered PTSD Scale (CAPS) criteria (Blake et al., 1995). PTSD subjects were positive for current war-zone-related PTSD of at least 3-month duration, as indexed by the DSM-IV with a CAPS score ≥ 40. PTSD negative controls were exposed to DSM-IV PTSD Criterion A trauma during deployment but

tested negative for lifetime combat or civilian PTSD and had current CAPS scores of ≤ 20. At T1, some participants no longer met the original PTSD criteria for the study but had symptoms intermediate between the PTSD positive and control groups and were characterized as "Subthreshold PTSD." More specifically, a participant was classified as subthreshold PTSD if he did not meet one or more of criteria B, C or D for PTSD defined by the DSM-IV and had a CAPS score between 21 and 39. Subthreshold PTSD is a classification defined by our study, not by the DSM-IV.

2.3. Blood sampling and BDNF assays

Blood at each time point was drawn between 8 am and 9 am in the morning after a night of fasting. Blood for BDNF analysis was collected into serum separator tubes (Vacutainer; BD, Franklin Lakes, NJ). After sitting at room temperature for at least 1 h to allow for clotting, blood was centrifuged at 2000*g* for 20 min at room temperature, and serum was separated and stored at −80 °C. Serum was assayed for BDNF in duplicate using a commercial BDNF ELISA assay kit (R&D Systems, Minneapolis, MN, USA). Sera were diluted 1:60 with diluent supplied by the kit manufacturer, to obtain BDNF concentrations within the linear range of the standard curve. To evaluate inter-assay variability, an internal control consisting of serum obtained from a single individual that had been frozen in multiple aliquots, was run on each plate processed, and was similar to that found by the manufacturer (between 2% and 12%); intra-assay variability was between 1% and 8%. The R&D Systems Human BDNF Quantikine ELISA Kit was found to have an acceptable 8–14% inter-assay variability of the internal control sample when measured on each plate run with human PTSD positive and negative subject samples. Samples were re-assayed if intra-assay coefficients of variance were > 10%. PTSD positive and negative subject samples were ran in the same assay in various batches. There was no inter-batch variability across the batches.

2.4. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 27.0 (SPSS Inc., Chicago, IL, USA). A two-tailed alpha of 0.05 was used as the cutoff for significance. Descriptive statistics, independent *t*-test, chi-square test, and ANOVA were performed to show sample characteristics. Normality of BDNF distribution was assessed using skewness, kurtosis and Q-Q plot. BDNF levels followed a normal distribution. Independent *t*-test was performed to examine group differences. Then ANCOVA was performed to examine group difference by PTSD status while controlling for age, gender, race, BMI, and time since trauma. Male and female subjects were then analyzed separately. We performed a paired *t*-test to examine BDNF changes overtime within subjects. Lastly correlation analyses were used to identify associations between serum BDNF and PTSD symptom severity cross-sectionally at each time point, as well as longitudinal changes in BDNF and symptom severity over time (T1 minus T0).

Table 2
PTSD status change of the recalled participants (n = 59).

	PTSD status at T0			
		PTSD+	PTSD-	Total
PTSD status at T1	PTSD+	16	1	17
	Subthreshold PTSD	10	1	11
	PTSD-	4	27	31
	Total	30	29	59

3. Results

Sample demographic and clinical characteristics are presented in Table 1. At T0, our sample included 130 PTSD positive and 140 PTSD negative control participants. Twenty-nine PTSD negative and 30 PTSD positive participants from T0, who were all male, returned for follow-up evaluation (T1). Of these 59 recalled participants, 17 were diagnosed with PTSD, 11 were sub-threshold PTSD and 31 did not have PTSD at T1. PTSD status changes of the 59 recalled participants can be found in Table 2.

At T0, there was no significant difference in age between groups [$t(268) = -0.521$, $p = 0.603$]. However, the two groups differed in education years [$t(268) = 4.113$, $p < 0.001$], body mass index (BMI) [$t(260) = -2.615$, $p = 0.009$], and time since trauma [$t(268) = -2.936$, $p = 0.004$] (Table 1). There was a higher proportion of Hispanic

Table 1
Summary of sample demographics and clinical characteristics.

	T0			T1			
	PTSD- (n = 140)	PTSD+ (n = 130)	*t or* χ^2 *(p value)*	PTSD- (n = 31)	Subthreshold PTSD (n = 11)	PTSD+ (n = 17)	*F or* χ^2 *(p value)*
Age, years [mean (sd)]	33.04 (8.45)	33.56 (8.12)	-0.521 (0.603)	35.90 (8.50)	35.64 (7.97)	33.59 (7.76)	0.458 (0.635)
Gender, male [n (%)]	119 (85%)	111 (85%)	0.008 (0.929)	31 (100%)	11 (100%)	17 (100%)	N/A
Race/ethnicity, [n (%)]			14.766 (0.011)				8.034 (0.430)
Hispanic	39 (28%)	56 (43%)		11 (37%)	3 (27%)	11 (65%)	
Non-Hispanic Asian	11 (8%)	4 (3%)		1 (3%)	0 (0%)	0 (0%)	
Non-Hispanic black	30 (21%)	36 (28%)		6 (20%)	3 (27%)	4 (24%)	
Non-Hispanic white	53 (38%)	31 (24%)		11 (37%)	4 (36%)	2 (12%)	
Non-Hispanic other	7 (5.0%)	3 (2%)		1 (3%)	1 (9%)	0 (0%)	
Education, years [mean (sd)]	15.01 (2.14)	13.98 (1.96)	4.113 (< 0.001)	15.42 (1.65)	15.27 (1.85)	14.53 (1.81)	1.492 (0.234)
Body mass index [mean (sd)]	27.86 (4.54)	29.49 (5.50)	-2.615 (0.009)	28.23 (4.25)	31.06 (8.05)	32.10 (4.97)	3.098 (0.053)
Time since trauma, year [mean (sd)]	5.51 (2.89)	6.48 (2.50)	-2.936 (0.004)	8.65 (2.28)	11.72 (7.08)	8.55 (2.67)	3.133 (0.051)
Follow up interval, year [mean (sd)]	N/A	N/A	N/A	3.51 (0.70)	3.12 (0.74)	2.92 (1.14)	2.836 (0.067)
Antidepressant use [n (%)]	5 (4%)	34 (27%)	29.376 (< 0.001)	2 (7%)	1 (9%)	6 (35%)	7.463 (0.024)
Major depressive disorder [n (%)]	4 (3%)	68 (52%)	83.732 (< 0.001)	0 (0%)	2 (18%)	9 (56%)	21.732 (< 0.001)
PTSD severity, Total CAPS [mean (sd)]	3.68 (4.93)	68.65 (18.08)	-39.630 (< 0.001)	4.87 (7.13)	37.64 (8.99)	71.00 (18.17)	179.436 (< 0.001)
BDNF, ng/mL [mean (sd)]	26.95 (8.91)	31.03 (8.11)	-3.921 (< 0.001)	24.66 (6.89)	27.42 (7.88)	30.05 (6.22)	3.420 (0.040)

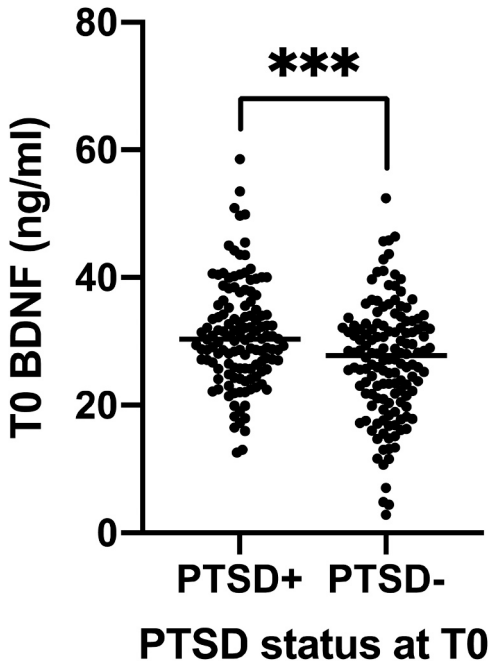

Fig. 1. Serum BDNF of PTSD positive vs PTSD negative participants at T0 (n = 270). Results from independent sample *t*-test showed that the PTSD positive group (n = 130; 31.03 ± 8.11 ng/mL) had significantly higher serum BDNF levels than the PTSD negative control group [n = 140; 26.95 ± 8.91 ng/mL; $t(268) = 3.921$, $p < 0.001$].

participants present in the PTSD positive group than in the control group [$\chi^2(5, n = 270) = 14.766$, $p = 0.011$]. The time since the index trauma at T0 was 6 years on average, with the PTSD positive group having a significantly longer time since the index trauma than the control group [6.48 ± 2.50 vs. 5.51 ± 2.89 years; $t(268) = 2.936$, $p = 0.004$]. As expected, the PTSD positive group had significantly higher proportions of individuals taking antidepressants [$\chi^2(1, n = 265) = 29.376$, $p < 0.001$] and having comorbid major depressive disorder [$\chi^2(1, n = 269) = 83.732$, $p < 0.001$]. At T1, PTSD negative, PTSD positive, and subthreshold PTSD participants did not differ in age, education years, BMI, time since trauma, or follow-up interval (time between T0 and T1) (Table 1). There was no significant difference in the distribution of race among the three groups studied at T1 [$\chi^2(8, n = 58) = 8.034$, $p = 0.430$]. In terms of antidepressant usage and comorbid major depressive disorder (MDD), the PTSD positive group had significantly higher percentages of antidepressant users and MDD comorbidities than subthreshold PTSD and control participants at T1 [$\chi^2(2, 59) = 7.463$, $p = 0.024$ and $\chi^2(2, 58) = 21.732$, $p < 0.001$].

3.1. Baseline analyses

At T0, serum BDNF was significantly greater in the PTSD positive group than in the PTSD negative group [31.03 ng/mL vs. 26.95 ng/mL, $t(268) = 3.921$, $p < 0.001$] (Fig. 1). We performed ANCOVA to examine serum BDNF levels between the two groups, covarying for potential confounder variables: age, gender, race, BMI, and time since trauma. The PTSD positive group continued to show significantly higher serum BDNF levels than controls [$F(1, 255) = 12.584$, $p < 0.001$]. When male and female participants were analyzed separately, male and female PTSD positive groups had significantly elevated serum BDNF compared to male and female controls [male: 30.98 ng/mL vs. 27.20 ng/mL, $t(228) = 3.348$, $p = 0.001$; female: 31.30 ng/mL vs. 25.55 ng/mL, $t(38) = 2.128$, $p = 0.040$]. There was no difference in BDNF levels between male vs. female participants in the entire sample [$t(268) = 0.498$, $p = 0.619$], within the control group [$t(138) = 0.784$, $p = 0.435$] or within the PTSD positive group [$t(128) = -0.156$, $p = 0.876$].

To address the potential effect of underlying MDD as a confounder variable, we performed a sensitivity analysis excluding participants who were diagnosed with MDD. Results were similar to those found earlier. PTSD positive participants who did not have comorbid MDD (n = 62) exhibited higher serum BDNF concentrations than did PTSD negative controls (n = 135) [31.66 ng/mL vs. 26.66 ng/mL, $t(195) = 3.780$, $p < 0.001$]. After applying age, gender, race, BMI and time since trauma as covariates to the non-MDD sample, the PTSD positive group continued to have significantly higher serum BDNF than the PTSD negative control group [$F(1, 183) = 13.651$, $p < 0.001$]. Past studies have showed an increase in BDNF concentrations in those taking antidepressants (Molendijk et al., 2011; Sen et al., 2008). To address such confounding effects, we performed a sensitivity analysis excluding those who were taking antidepressants. PTSD positive participants (n = 91) had greater serum BDNF levels than the PTSD negative controls (n = 135), both before (31.18 ng/mL vs. 26.55 ng/mL, $t(224) =$

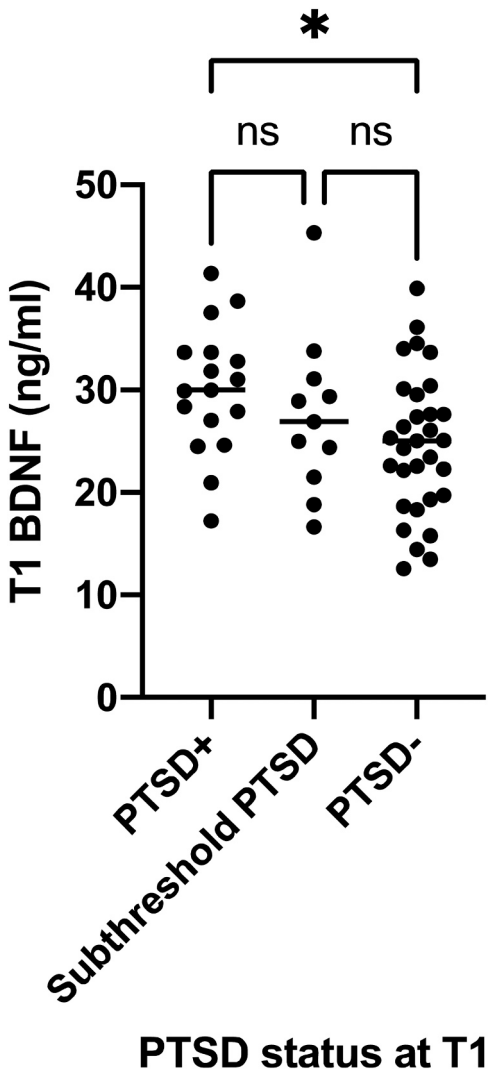

Fig. 2. Serum BDNF of PTSD positive, subthreshold PTSD and PTSD negative groups at T1 (n = 59). Serum BDNF significantly differed across PTSD positive (n = 17), subthreshold PTSD (n = 11) and PTSD negative (n = 31) participants at T1 [$F(2, 56) = 3.420$, $p = 0.040$], with post-hoc analyses finding that serum BDNF was significantly higher in the T1 PTSD positive group than in the T1 PTSD negative group (30.05 ± 6.22 ng/mL vs. 24.66 ± 6.89 ng/mL, $p = 0.012$). Subthreshold PTSD participants (27.42 ± 7.88 ng/mL) were not significantly different from either PTSD positive ($p = 0.328$) or PTSD negative groups ($p = 0.260$).

3.951, $p < 0.001$) and after applying the same covariates, ($F(1, 213) = 12.854$, $p < 0.001$).

We then performed two correlation analyses to assess relationships between serum BDNF and (a) time since the index trauma, and (b) PTSD symptom severity, measured by CAPS IV. Serum BDNF did not significantly correlate with time since the trauma [$r(268) = 0.050$, $p = 0.413$] across all participants, or with CAPS at T0 in the participants with PTSD [$r(128) = 0.062$, $p = 0.481$].

3.2. Longitudinal analyses

Next we analyzed our longitudinal dataset of 59 male veterans who returned for follow-up evaluation (T1). Serum BDNF did not change over time within all participants together [$t(56) = 1.269$, $p = 0.210$] or within the PTSD+, PTSD-, or subthreshold PTSD participants alone [$t(15) = 0.395$, $p = 0.698$; $t(29) = 0.792$, $p = 0.435$; $t(10) = 1.622$, $p = 0.136$ respectively]. Consistent with our T0 findings, serum BDNF significantly differed across PTSD negative, PTSD positive and subthreshold PTSD participants at T1 [$F(2, 56) = 3.420$, $p = 0.040$], with post-hoc analyses finding that serum BDNF was significantly higher in the T1 PTSD positive group than in the T1 PTSD negative group (30.05 vs 24.66 ng/mL, $p = 0.012$) (Fig. 2). Serum BDNF of the subthreshold PTSD participants did not differ from those of either PTSD positive ($p = 0.328$) or PTSD negative ($p = 0.260$) (Fig. 2). Then we performed the same sensitivity analyses as T0 using correspondingly smaller sample sizes. In the non-MDD sample (n = 47), serum BDNF missed statistical significance between the three groups [$F(2, 44) = 2.836$, $p = 0.069$]. Similarly, among those who were not taking antidepressant at T1 (n = 50), serum BDNF missed statistical significance between the three groups [$F(2, 47) = 2.696$, $p = 0.078$]. Nonetheless, the effect sizes for these latter comparisons were in the moderate range in the expected direction (Cohen's d = 0.37; Cohen's d = 0.32, respectively).

Among participants who were PTSD positive or subthreshold PTSD at T1, serum BDNF did not significantly correlate with symptom severity at T1 [$r(28) = 0.157$, $p = 0.407$]. Change in serum BDNF (calculated by T1 serum BDNF minus T0 serum BDNF) did not correlate with symptom severity change (calculated by T1 CAPS score minus T0 CAPS score) in either the entire recalled sample [$r(55) = -0.020$, $p = 0.883$] or in the subset that included only PTSD positive or subthreshold PTSD participants [$r(27) = -0.250$, $p = 0.192$].

4. Discussion

The present study examined serum BDNF in a sample of 270 male and female combat exposed veterans of which 59 male veterans have longitudinal follow-up data. We found significantly higher serum BDNF levels in the PTSD positive group than in PTSD negative group at both time points. Notable, both groups had experienced combat trauma sufficient to satisfy criterion A of the DSM-IV diagnosis of PTSD, suggesting that PTSD itself, rather than trauma exposure alone, is associated with the elevated BDNF levels. These differences in BDNF levels persisted over time, as far as approximately 9 years after the index trauma, and these levels did not change significantly within individuals over the follow-up period. Lastly, we found that serum BDNF levels were not significantly related to PTSD severity at either time point, and there was no significant relationship between changes in BDNF and changes in PTSD symptom severity over the follow-up period.

Our main finding of higher serum BDNF levels in PTSD is consistent with several (Matsuoka et al., 2013; Zhang et al., 2014), but not all (Aksu et al., 2018; Angelucci et al., 2014; Dell'Osso et al., 2009), reports in the literature (Mojtabavi et al., 2020). In a recent systematic review and meta-analysis, Mojtabavi et al. (2020) discussed inconsistency in the literature in regards to several confounder variables. These include antidepressant usage, MDD comorbidity, and time passed from trauma in the assessment of PTSD. Other confounder variables often mentioned in the BDNF literature include age, BMI, sex, and race in the measurement of BDNF (Mojtabavi et al., 2020; Nettiksimmons et al., 2014). The present study performed a thorough analysis taking these confounding effects into consideration, including performing ANCOVA models controlling for these covariables, and sensitivity analyses, excluding participants who had comorbid MDD and who used antidepressants. The confound of time since the index trauma may be especially important, since our study only assessed PTSD symptoms several years after the index trauma, and we cannot make any inferences about earlier differences in BDNF levels between PTSD positive and negative participants. In theory, time post trauma could affect the BDNF levels significantly (Mojtabavi et al., 2020). The literature has shown mixed results regarding BDNF levels following trauma in the acute phase vs. the chronic phase. One study concluded that plasma BDNF levels were higher in PTSD compared with controls in early years and then decreased as time passed from trauma (Hauck et al., 2010). Results from Matsuoka et al. (2013) suggest higher serum BDNF after a 6-month follow-up, compared to their baseline values. The consistency of our findings over the period of 6 years (T0) to 9 years (T1) after the index trauma, however, raises the possibility of either long-term changes in BDNF following combat trauma or else a pre-existing, stable, "trait"-like difference in serum BDNF levels in those who will develop PTSD following combat trauma. Our findings are unable to distinguish these possibilities.

Despite finding significant differences in BDNF in PTSD positive vs PTSD negative participants, we did not see a significant correlation between BDNF and PTSD symptom severity within the PTSD positive group, nor did we see significant within-subject changes in BDNF over time. The mechanisms underlying the present findings are unknown, and causal relationships between BDNF concentrations and PTSD status cannot be directly inferred. In their meta-analysis, Mojtabavi et al. (2020) refer to PTSD as a "neuroplastic disorder". To the extent a causal relationship does exist, however, at least two competing theories could help explain this relationship. The first considers that in individuals diagnosed with PTSD after combat trauma, increased serum BDNF concentrations could reflect a compensatory attempt, albeit largely ineffective, to lessen PTSD symptoms, perhaps by attempting to restore neuroplasticity to increase the potential for neural repair (Hauck et al., 2009), or to facilitate consolidation of fear memories, extinction learning, and reconsolidation (Andero and Ressler, 2012; Notaras and van den Buuse, 2020; Quirk and Mueller, 2008). Secondly, higher BDNF concentrations could have an intrinsic role in the pathophysiology of PTSD. Past animal models found that rats with upregulated BDNF in both blood and hippocampus had increased startle response, a core symptom of PTSD-hyperarousal, although the specific mechanism is still yet to be explored (Zhang et al., 2014, 2016).

There is only one other study, to our knowledge, that investigated serum BDNF and PTSD longitudinally. Matsuoka et al. (2013) recruited a sample of male and female participants who were survivors of motor vehicle accidents, of which 8 were determined (by CAPS score) to have full PTSD, 10 partial PTSD and 85 without PTSD. The participants were not treated with anti-depressants, and the two time points they analyzed were only 6 months apart. Despite some differences in study designs and trauma populations, our results are somewhat similar. Specifically, they found that, at follow up, serum BDNF concentrations were higher among individuals with full PTSD than those with partial PTSD or without PTSD. However, they found that participants with full PTSD exhibited significantly higher serum BDNF concentrations at the follow-up time point than at baseline. Such divergence of results could be due to the fact that their participants had experienced more recent trauma, whereas ours reflect more chronic stress or distant trauma.

Overall our study has several strengths. We recruited a sample of well-phenotyped combat veterans who were exposed to significant combat trauma averaging 6 years prior to participation in the study. This limits the aforementioned methodological variability in trauma types and disease development stage. Additionally, we analyzed our data both including and excluding individuals using antidepressant medication,

showing similar findings in both cases. Further, our control group (those without a diagnosis of PTSD) had been exposed to combat-related trauma, yet failed to develop PTSD. This could represent a strength, as it highlights the role of PTSD rather than that of the combat trauma exposure itself. Yet, it could also represent a weakness, in that these non-PTSD subjects could represent an especially resilient group. Finally, most prior studies utilized cross-sectional designs that lack information on how BDNF may change over time. This is the first non-interventional study to examine the longitudinal course of combat PTSD severity as a function of serum BDNF concentrations over time and at an index time point.

Limitations of our study include the fact that this was a study of chronic combat-related PTSD, limiting generalizability to other populations. BDNF levels were not measured either before or immediately after the trauma so we cannot conclude whether this increase in BDNF resulted from PTSD or if it was a pre-existing condition. Additionally, we analyzed one biological sample, serum, at only two discrete points in time separated by approximately 3 years. We also did not have detailed data regarding interventions before T0 or between T0 and T1. Since we did not have non-combat exposed controls, we cannot assess whether the relatively elevated BDNF seen in PTSD positive subjects is within the normal range or is physiologically elevated. The sample sizes of our three groups at T1 were relatively small; therefore, these findings should be replicated in larger and more diverse samples. Moreover, we saw a significantly different distribution of Hispanic ethnicity across the groups. Literature in the past has reported that BDNF can vary by ethnicity (Verhagen et al., 2010). Although differences in Hispanic vs other ethnicities per se have not been reported, future studies should examine that further as a possible contributing factor. Lastly, it is still unknown how well peripheral BDNF levels correlate with the CNS levels of BDNF in humans, though there have been animal models showing the blood levels of BDNF reflect brain tissue BDNF levels across species (Klein et al., 2011; Zhang et al., 2014).

In conclusion, our data showed that serum BDNF was elevated in combat veterans with chronic PTSD and remained stable over time, including within subject validation. Our findings are consistent with a role of BDNF in PTSD (Matsuoka et al., 2013; Mojtabavi et al., 2020; Zhang et al., 2016), although causality cannot be inferred from our data. The mechanisms underlying this association and effects of elevated serum BDNF concentrations in PTSD remain to be determined but may yield clues as to the involvement of neurotrophic factors in the pathophysiology and course of combat-related PTSD. This may contribute to biotyping subgroups of individuals with PTSD and to the consequent improvement of prediction of PTSD course and more personalized treatments. Further studies should explore the possible mechanisms of BDNF changes throughout the course of PTSD in larger and more diverse samples.

Disclaimer

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70-25.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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