Evidence for altered brain reactivity to norepinephrine in Veterans with a history of traumatic stress

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ABSTRACT

Background: Increases in the quantity or impact of noradrenergic signaling have been implicated in the pathophysiology of posttraumatic stress disorder (PTSD). This increased signaling may result from increased norepinephrine (NE) release, from altered brain responses to NE, or from a combination of both factors. Here, we tested the hypothesis that Veterans reporting a history of trauma exposure would show an increased association between brain NE and mental health symptoms commonly observed after trauma, as compared to Veterans who did not report a history of trauma exposure, consistent with the possibility of increased brain reactivity to NE after traumatic stress.

Methods: Using a convenience sample of 69 male Veterans with a history of combat-theater deployment, we examined the relationship between trauma-related mental health symptoms and the concentration of NE in cerebrospinal fluid (CSF). CSF NE levels were measured by HPLC in CSF from morning lumbar puncture. Behavioral symptoms associated with diagnoses of PTSD, depression, insomnia, or post-concussive syndrome (PCS), which together cover a wide variety of symptoms associated with alterations in arousal systems, such as sleep, mood, concentration, and anxiety, were assessed via self-report (PTSD Checklist [PCL] for PTSD, Patient Health Questionnaire 9 [PHQ9] for depression, Pittsburgh Sleep Quality Index [PSQI] for sleep problems including insomnia, and Neurobehavioral Symptom Inventory [NSI] for PCS) and structured clinical interview (Clinician-Administered PSTD Scale [CAPS]). Individuals meeting criterion A of the DSM-IV diagnostic criteria for PTSD were considered trauma-exposed. Linear regression models were used to quantify the association between CSF NE and symptom intensity in participants with and without a history of trauma exposure, as well as in participants with a history of trauma exposure who were currently taking the noradrenergic receptor antagonist prazosin.

Results: Fifty-two Veterans met criteria for a history of trauma exposure; of these, 36 met criteria for PTSD. CSF NE levels were not significantly different in Veterans with a history of trauma compared to those without, nor in Veterans with PTSD as compared to those without. Veterans with a history of trauma and who were not using the medication prazosin demonstrated a significantly more positive correlation between CSF NE and behavioral symptom expression than Veterans who had not experienced traumatic stress. No relationship between CSF NE and behavioral symptom expression was found in Veterans who had experienced traumatic stress and were taking prazosin at the time of the assessments.

Conclusions: These results are consistent with increased central nervous system responsiveness to noradrenergic signaling in individuals with a history of traumatic exposure, raising the possibility that there may be long-lasting physiologic effects of trauma-exposure that exist independently of whether an individual meets criteria for PTSD at any given point in time. Exploration of the mechanism by which brain responsiveness to NE is modulated following trauma holds the possibility of finding new strategies for both preventing and treating PTSD.

1. Introduction

Posttraumatic stress disorder (PTSD) is a condition that is frequently chronic, and develops following some, but not all, exposures to a severe trauma – such as when one's life or the life of another is in immediate danger (Breslau, 2002). Increased noradrenergic signaling has been found to contribute to the pathophysiology of PTSD, and may reflect long term upregulation of the brain noradrenergic system following trauma exposure (Hendrickson and Raskind, 2016).

Studies evaluating brain noradrenergic activity in PTSD have provided support for both increased presynaptic norepinephrine (NE) outflow, and, indirectly, for increased postsynaptic responsiveness or overall reactivity to NE: physiologic studies estimating brain noradrenergic signaling have demonstrated increased presynaptic NE outflow directly by measuring NE in cerebrospinal fluid (CSF) (Geracioti et al., 2001) or indirectly by measuring the purportedly brain-derived NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) in plasma (Southwick et al., 1993) or urine (Mellman et al., 1995). The study of Southwick *et al.* also provides inferential support for increased reactivity to NE in PTSD, as individuals with PTSD demonstrated significantly greater behavioral symptom exacerbation with pharmacologically provoked NE release than those without PTSD, out of proportion to the difference in NE release itself (Southwick et al., 1993; see also further discussion in Hendrickson and Raskind, 2016). The possibility of increased brain responsiveness to NE via the postsynaptic α_1 adrenoceptor (AR) in particular is supported by pharmacologic treatment studies demonstrating efficacy of the α_1 AR antagonist prazosin for the treatment of PTSD symptoms, particularly trauma nightmares and hyperarousal symptoms (Ahmadpanah et al., 2014; Germain et al., 2012; Raskind et al., 2013, 2007).

There is also an emerging literature suggesting that among individuals without PTSD, those without a history of trauma exposure (non-trauma-exposed controls) and those with a history of trauma exposure (trauma-exposed controls) do not have equivalent phenomenology; instead, trauma exposure may have a direct impact on stress-reactivity (Heim et al., 2002; van Nierop et al., 2018). In studies addressing aspects of PTSD pathophysiology ranging from hypothalamic pituitary adrenal (HPA) axis functioning (van Liempt et al., 2013) to sympathetic activation (Buckley and Kaloupek, 2001) to functional magnetic resonance imaging (fMRI) activation (Stark et al., 2015), a pattern of non-trauma-exposed controls showing an intermediate phenotype between the trauma-exposed controls (without PTSD) and trauma-exposed participants with PTSD has emerged as an unexpected but repeated finding. One way such an effect could be produced is if trauma exposure serves to "unmask" some sort of preexisting variation within the population. Here, we explore whether trauma exposure may increase brain reactivity to NE, allowing preexisting or subsequent variation in NE release to contribute to the variation in PTSD symptom expression among persons who have experienced a traumatic event.

In a sample of Veterans with and without PTSD following combat deployment(s) to Afghanistan and/or Iraq, we estimated presynaptic NE outflow in the central nervous system (CNS) by measuring NE concentrations in CSF ($[NE]_{CSF}$) obtained via morning lumbar puncture, and, in the same participants, we quantified the relationship between $[NE]_{CSF}$ and behavioral symptom expression. We hypothesized that Veterans who had experienced a major trauma, sufficient to meet criterion A of the DSM-IV diagnostic criteria for PTSD but independent of current PSTD status, would demonstrate a stronger positive correlation between $[NE]_{CSF}$ and behavioral symptoms than Veterans who had not experienced a major trauma – consistent with the possibility of increased brain reactivity to NE. We also hypothesized that in addition, and consistent with previous research (Geracioti et al., 2001), deployed Veterans meeting criteria for PTSD would have higher $[NE]_{CSF}$.

2. Materials and methods

2.1. Participants

Participants in this study formed a convenience sample, drawn from an ongoing longitudinal multimodal assessment study of Veterans with and without repetitive mild traumatic brain injury (mTBI) that has been previously described (Petrie et al., 2014). In brief, combat Veterans from the conflicts in Iraq and Afghanistan were recruited from the local VA medical center and community population. Medical and psychiatric history, including current medications, was obtained both via clinician interview and review of the medical chart. To meet inclusion criteria for the original study, Veterans had to have either no history of TBI, or at least one war-zone blast or combined blast/impact exposure that resulted in acute symptoms consistent with the American Congress of Rehabilitation Medicine (ACRM) criteria for mTBI (Kay, 1993). Behavioral health diagnostic assessments for diagnoses other than PTSD, but including substance use disorders, were based on the Structured Clinical Interview for *DSM-IV* (SCID) (First et al., 1996). None of the participants had a reported history of moderate or severe TBI, penetrating head wound, seizure disorder, or insulin-dependent diabetes. None of the participants were determined on assessment to have a current or past diagnosis of primary psychotic or neurodegenerative disorder, a current or past diagnosis of bipolar disorder, or a diagnosis of active substance abuse or dependence within the past 6 months. This study was approved by the VA Puget Sound Health Care System Human Subjects Committee. Prior to enrollment, all participants provided written informed consent.

2.2. Behavioral/symptom assessments

The Clinician-Administered PTSD Scale (CAPS) (Blake et al., 1995) was used both to determine whether or not a participant met diagnostic criteria for PTSD and to determine whether a participant had a history of a trauma exposure that met criterion A of the DSM-IV criteria for PTSD (henceforth referred to as "trauma-exposed").

The presence and intensity of behavioral symptoms commonly associated with disorders involving alterations of stress and arousal, including PTSD, depression, and insomnia, were assessed using standard instruments. Although these instruments are most often used to screen for and/or rate the intensity of symptoms associated with a specific mental health diagnosis, the individual items on these scales assess the frequency or intensity with which a participant reports symptoms such as "feeling tired or having little energy", "feeling irritable or having angry outbursts", or "trouble falling or staying asleep" – symptoms that are often present in the general population without PTSD or a history of trauma and that are often believed to be affected by NE signaling in these contexts as well (Hendrickson and Raskind, 2016). Specifically, self-reported symptom presence and intensity was assessed with: for PTSD symptoms, the PTSD Checklist for DSM-IV, military version (PCL) (Forbes et al., 2001); for symptoms commonly associated with a history of mTBI, a modified 28-item Neurobehavioral Symptom Inventory (NSI) (Cicerone and Kalmar, 1995); for sleep problems, the Pittsburgh Sleep Quality Index (PSQI) (Krakow et al., 2001); and for depression symptoms, the Patient Health Questionnaire 9 (PHQ-9) (Kroenke et al., 2001).

2.3. Quantification of catecholamines in CSF

CSF was obtained at a separate study visit via lumbar puncture carried out in the fasting state between 09:00 and 11:00 h, using a protocol previously described that has been verified as causing minimal distress or discomfort in research participants (Peskind et al., 2005). CSF samples used for catecholamine quantification consisted of a 0.5 ml aliquot of CSF from the 13th ml of CSF collected. These samples were added to 20 μL of 0.2M reduced glutathione and 0.24M EGTA and

immediately frozen in a polypropylene cryotube on dry ice at the bedside, then maintained in a −70 °C freezer until analysis.

Catecholamine concentrations in CSF were quantitated by high-pressure liquid chromatography (HPLC) with electrochemical detection. CSF was extracted using the alumina extraction method developed by Goldstein et al. (1981) and optimized by Holmes et al. (1994). Extracted samples were separated by HPLC using a Phenomenex reverse phase c18 Gemini column (150 mm × 4.6, 3 m, 110 Å; Phenomenex, Torrance, CA, USA). Detection was performed with an ESA Coulochem II electrochemical detector (ESA, Chelmsford, MA, USA) with the conditioning cell set at +350 mV, electrode 1 of the analytical cell set at +90 mV, and electrode 2 of the analytical cell set at −300 mV, with 3,4-dihydroxybenzylamine (DHBA) used as an internal standard (Wang et al., 2013). The intra-assay coefficient of variation (CV) for NE is 7.1% (based on measurements of the internal reference standard DHBA) and the inter-assay CV for NE is 10.8% (based on measurement of pooled CSF samples repeated in each assay).

Behavioral symptom assessment and lumbar puncture for catecholamine quantification were not carried out on the same day, as responding to trauma-related questionnaires may itself cause increased catecholamine release. The mean time between behavioral symptom assessment and lumbar puncture was approximately 3 months (SD 104 days).

2.4. Statistical analysis

Participants were divided into different groupings, depending on the analysis being conducted. To assess whether $[NE]_{CSF}$ differed as a function of trauma exposure and PTSD diagnosis, we compared $[NE]_{CSF}$ between (1) those who met criteria for PTSD versus those who did not, (2) those who had experienced a traumatic event versus those who had not, and (3) those who met criteria for mTBI versus those who did not. Comparison of symptoms and $[NE]_{CSF}$ between groups was based on linear regression, with age, which has been previously shown to have a strong association with $[NE]_{CSF}$ (Wang et al., 2013), included as a covariate.

As use of the α_1-AR antagonist prazosin is expected to disrupt the relationship between $[NE]_{CSF}$ and behavioral symptom expression (Hendrickson and Raskind, 2016), in analyses addressing the relationship of $[NE]_{CSF}$ to behavioral symptoms, participants were divided into a different set of three groups: (1) those with a history of trauma exposure (independent of PTSD diagnosis) but who were not on prazosin, (2) those without a history of trauma exposure (none of whom were on prazosin), and (3) those with a history of trauma exposure who were also taking prazosin. The relationship between symptom expression and $[NE]_{CSF}$ was investigated by fitting linear models using generalized least squares and allowing the variance to differ between groups. The first set of models excluded participants on prazosin (i.e., group 3 above), used raw symptom score as the response (dependent) variable, and included the predictor (independent) variables age, group, $[NE]_{CSF}$

(scaled by 100 so that the slope represents the change in the dependent variable per 100 pg/ml increase in $[NE]_{CSF}$), and the interaction term $[NE]_{CSF}$ by group. A significant interaction term indicates that the relationship between symptom severity and $[NE]_{CSF}$ depends on group membership. The second set of models included all three groups listed above and, in order to facilitate comparison of the relationship between symptom severity and $[NE]_{CSF}$ by group across all of the symptom assessments, used symptom z-score (based on normalizing across the entire study sample) as the response variable.

We also performed two sensitivity analyses for our results. The first set of analyses looked at the effect of use of SNRI or NRI medications (medications with a significant action on NE reuptake) on CSF NE levels by performing a two-tailed *t*-test to assess the difference in $[NE]_{CSF}$ between participants using these medications and those who were not. The second set of analyses looked at the effects of CSF concentrations of three other catecholamines besides NE: dopamine (DA), DOPA, and DHPG. First, to assess the relationship between the CSF concentration of the other major stress-relevant catecholamine, dopamine, and the impact of trauma exposure and PTSD diagnosis on behavioral symptom expression, we repeated the analyses described above for $[NE]_{CSF}$ with $[DA]_{CSF}$ in place of $[NE]_{CSF}$. To determine whether differences in synthetic capacity or degradation rate of NE between diagnostic groups might explain results of the relationship between $[NE]_{CSF}$ and behavioral symptom expression, we compared the ratios of NE to DOPA and NE to DHPG between our three primary trauma exposure and PTSD diagnosis analysis groups using one-way analysis of variance.

All statistical calculations were performed using R version 3.4.0 (R Development Core Team, 2017), RStudio version 1.0.136 (R Studio Team, 2016), and the *nlme* package (Pinheiro and Bates, 2000); tables and figures were generated using the *stargazer* (Hlavac, 2015) and *ggplot2* (Wickham, 2009) packages.

3. Results

3.1. Participant characteristics

Sixty-nine combat Veterans participated. Of these, 52 reported a history of a traumatic event meeting Criterion A of the DSM-IV diagnostic criteria for PTSD, and 36 met criteria for PTSD (Table 1). Forty-eight participants reported a history of mTBI; of those with a history of both mTBI and trauma exposure, the traumatic event meeting criterion A of the diagnostic criteria was sometimes, but not always, the same as the events in which the mTBI was sustained. Sixteen of the 69 Veterans (23%) were on prazosin at the time of the behavioral symptom evaluation, and all of these participants had experienced a trauma. The next most prevalent psychoactive medications reported were selective serotonin reuptake inhibitors (SSRIs, 35%), serotonin/noradrenaline or noradrenaline reuptake inhibitors (SNRI or NRIs, 16%), and beta blockers (9%). All medications were more common in the group that had experienced a trauma than in the group that had not, and all were

Table 1

Demographics for the total sample, broken down by history of trauma exposure and PTSD diagnostic status. Education and age given in years. SD = standard deviation.

	All Subjects (n = 69)	+ Trauma, +PTSD (n = 36)	+ Trauma, -PTSD (n = 16)	-Trauma (n = 17)
Race: N (%)				
White	52 (75)	27 (75)	12 (75)	13 (76)
African American	4 (6)	1 (3)	2 (12)	1 (6)
Other	13 (19)	8 (22)	2 (12)	3 (18)
Sex: % Male	100	100	100	100
Education: Mean (SD)	14.3 (1.9)	14.5 (2.1)	14.0 (1.5)	14.3 (1.8)
Age: Mean (SD)	34.3 (9.4)	35.8 (9.4)	33.7 (11.5)	31.6 (6.6)
mTBI: N (%)	48 (70)	33 (92)	14 (88)	1 (6)
Prazosin: N (%)	16 (23)	14 (39)	2 (12)	0 (0)

Table 2

Symptom intensity ratings for the total sample, broken down by history of trauma exposure and PTSD diagnostic status. PCL = PTSD Symptom Checklist, NSI = Neurobehavioral Symptom Inventory (symptoms associated with mTBI), PHQ-9 = Patient Health Questionnaire 9 (symptoms associated with major depressive disorder), PSQI = Pittsburgh Sleep Quality Index. All entries are mean (SD).

	All Subjects (n = 69)	+Trauma, +PTSD (n = 36)	+Trauma, -PTSD (n = 16)	-Trauma (n = 17)
PCL Total	43.6 (18.5)	57.0 (13.0)	35.2 (11.1)	23.1 (7.1)
NSI Total	25.5 (19.6)	37.1 (17.1)	19.5 (14.3)	6.5 (8.2)
PHQ-9 Total	9.1 (7.8)	13.6 (7.3)	5.8 (5.5)	2.8 (4.0)
PSQI Total	8.9 (5.0)	11.6 (4.2)	7.3 (4.9)	4.7 (3.1)

more common in those who met criteria for PTSD than in those who did not. Although female Veterans were eligible for the study, all participants were male.

3.2. Symptoms and NE concentrations by exposure or diagnostic group

Individuals with PTSD had significantly elevated ratings on each of the PCL, NSI, PHQ-9, and PSQI behavioral symptom scales compared to individuals without PTSD (Table 2, all p < 0.001), but there was no difference in $[NE]_{CSF}$ between these two groups (Table 3, p = 0.32). The same pattern was present when those with a history of trauma exposure, independent of PTSD status, were compared to the non-trauma-exposed control group (all p < 0.001 for behavioral symptoms, p = 0.80 for $[NE]_{CSF}$) and also when those with a history of mTBI were compared to those without (all p < 0.001 for behavioral symptoms, p = 0.24 for $[NE]_{CSF}$). In addition, because of the theoretical possibility that a medication with a significant action on NE reuptake could affect NE levels, we tested directly the effect of use of an SNRI or NRI medication on CSF NE levels, and found no significant effect (p = 0.80; data not shown).

3.3. Relationship between symptoms and NE concentration

Next, we looked for a relationship between the overall magnitude of PTSD symptoms, as quantified by the total PCL score, and $[NE]_{CSF}$. When excluding participants who reported the use of prazosin at the time of symptom assessment, and consistent with our primary hypothesis, we found a significantly more positive correlation between $[NE]_{CSF}$ and total PTSD symptom expression among participants with a history of trauma exposure (those with PTSD and trauma-exposed controls) than in those without such a history of trauma exposure (Fig. 1A; difference between the slopes relating $[NE]_{CSF}$ and total PCL score in the two groups = 18.0, p = 0.001; 95% CI for difference = [7.9, 28.0]). Among those with a history of trauma exposure, there was a trend toward a positive correlation between total PCL score and $[NE]_{CSF}$ (change in PCL per 100 pg/ml increase in $[NE]_{CSF}$ = 6.3, p = 0.11; 95% CI for slope = [-1.4, 14.0]). In contrast, among non-trauma-exposed controls, we found a significant

inverse relationship between total PCL score and $[NE]_{CSF}$ (change in PCL per 100 pg/ml increase in $[NE]_{CSF}$ = −11.7, p = 0.001; 95% CI for slope [-18.4,-5.0]).

When we examined the relationship between $[NE]_{CSF}$ and symptoms that are commonly associated with persistent post-concussive effects, as measured by the NSI, we again saw a significant inverse relationship between total NSI and $[NE]_{CSF}$ in non-trauma-exposed controls (Fig. 1B; change in NSI per 100 pg/ml increase in $[NE]_{CSF}$ = −15.7, p < 0.001; 95% CI for slope = [-21.4, −10.0]) and a significantly more positive relationship in those with trauma exposure (difference between slopes = 19.0, p = 0.001; 95% CI for difference between slopes [8.2, 29.7]).

To better characterize the specificity of this pattern to particular symptom scales or clusters, we quantified the slope relating $[NE]_{CSF}$ concentration to the normalized total symptom ratings (i.e., z-score based on the mean and standard deviation across all participants) on each of the individual symptom scales used, as well as for the three clusters of PTSD symptoms described in the DSM-IV. In this analysis, non-trauma-exposed controls, those with a history of trauma exposure but who were not on prazosin, and those with a history of trauma exposure who were taking prazosin at the time of symptom assessment were included as independent groups (Fig. 1C).

In addition to the above described effects for PCL and NSI totals, a similar, significantly greater positive relationship between symptom intensities and $[NE]_{CSF}$ for those with a history of trauma exposure (and not on prazosin) than for those without trauma exposure was also found for depressive symptoms (PHQ-9 total) and sleep symptoms (PQSI total), as well as each of the individual clusters of the PCL (Fig. 1C). For participants who were taking the α_1 AR antagonist prazosin at the time of symptom assessment (Fig. 1C, black bars), the increased positive relationship between $[NE]_{CSF}$ and symptom totals for those with a history of trauma exposure was lost for PCL cluster C, PCL cluster D, and PHQ-9, and the estimated effect was diminished for all assessment instruments. No such modification was seen for SSRI, SNRI/NRI, or beta blocker use (data not shown).

To characterize whether this pattern was specific to or driven by particular symptoms, we repeated this analysis using the normalized symptom rating for each of the individual items on each of the PCL, NSI, PHQ-9, and PSQI (results for the non-trauma-exposed group and the group that was trauma exposed but not using prazosin are shown for PCL and PSQI in Fig. 2, as well as for NSI and PHQ-9 in Supplemental Fig. 1). The basic pattern of a greater positive relationship between symptom intensities and $[NE]_{CSF}$ for those with a history of trauma exposure than for those without was maintained at the level of individual symptoms, regardless of the assessment scale or symptom category.

3.4. Relationships of other CSF catecholamine concentrations to behavioral symptom expression and NE concentrations

In an additional exploratory data analysis, two further questions were asked: First, we asked whether the main effect observed above was specific to NE, or whether a similar effect would be observed for

Table 3

Catecholamine concentrations in CSF for the total sample, broken down by history of trauma exposure and PTSD diagnostic status. NE = Norepinephrine, DA = Dopamine, DOPA = 3,4-dihydroxyphenylalanine (DA precursor), DHPG = 3,4-dihydroxyphenylglycol (NE metabolite). All concentrations are pg/ml and reported as mean (SD).

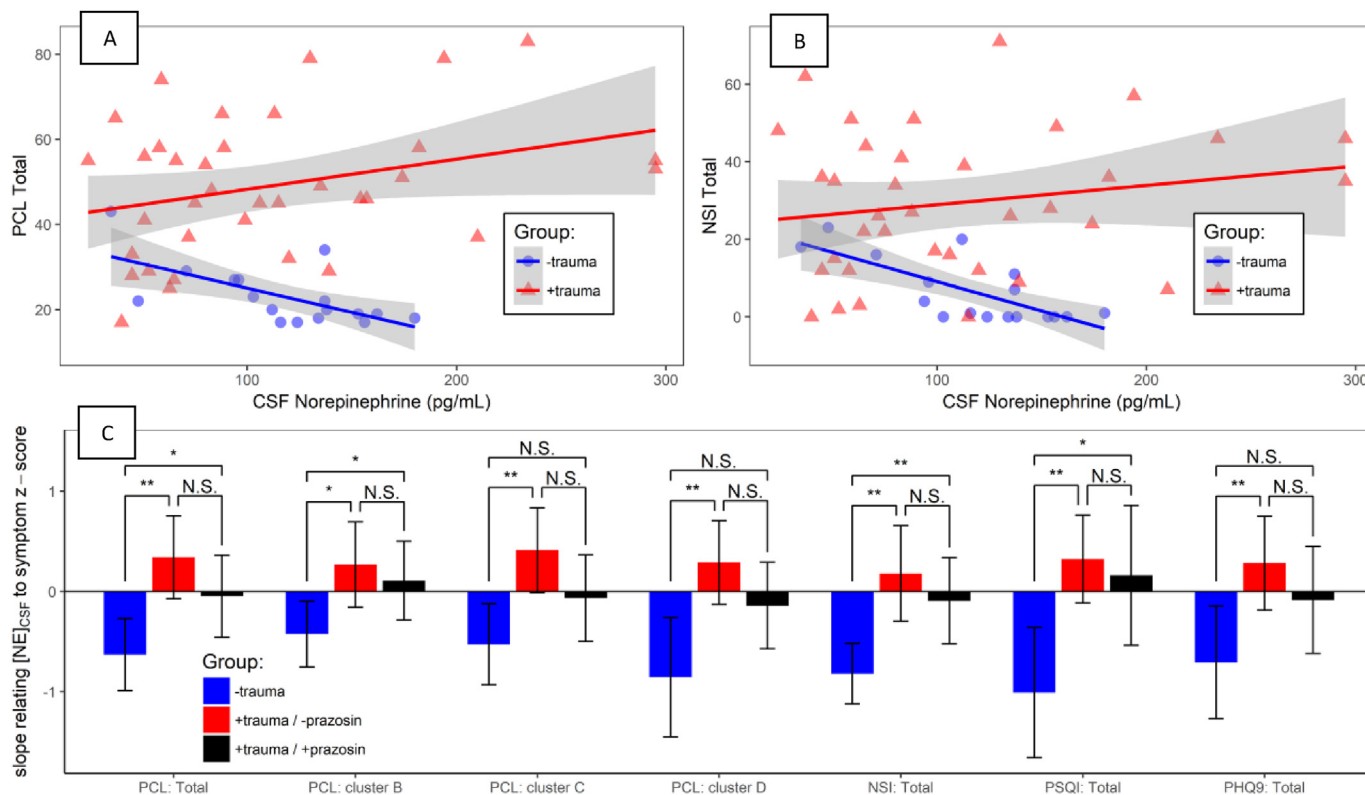|	All (n = 69)	+Trauma, +PTSD (n = 36)	+Trauma, -PTSD (n = 16)	-Trauma (n = 17)
NE (SD)	132 (82)	146 (94)	115 (84)	117 (40)
DA (SD)	18 (26)	18 (24)	19 (31)	15 (26)
DOPA (SD)	656 (208)	713 (224)	580 (160)	605 (187)
DHPG (SD)	2115 (645)	2159 (688)	1832 (445)	2291 (658)
DOPA/NE (SD)	6.3 (3.1)	6.5 (3.5)	6.8 (3.2)	5.5 (1.8)
DHPG/NE (SD)	20.8 (10)	19.8 (10.5)	22.8 (11.9)	21.1 (6.9)

Fig. 1. Relationship of $[NE]_{CSF}$ to mental health symptoms in individuals with and without a history of trauma exposure. (A) $[NE]_{CSF}$ is inversely related to total PCL score in non-trauma-exposed controls (blue circles) but is significantly more positively associated with total PCL in individuals with a history of trauma exposure (red triangles; shaded area represents 95% CI); individuals using prazosin at the time of the assessment are not shown in this plot. (B) The same pattern is present for total NSI score. (C) The relationship between $[NE]_{CSF}$ and mental health symptoms is significantly more positive in those with a history of trauma exposure (blue) than those without (red) for all clusters of PCL, as well as for the total PSQI and PHQ-9 scores (error bars depict 95% CIs). Furthermore, in participants who reported taking the α_1 antagonist prazosin (black), the effect is lost for PCL C, PCL D, and PHQ-9, and the estimated effect is attenuated for all assessments. [* = p < 0.05, ** = p < 0.01]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
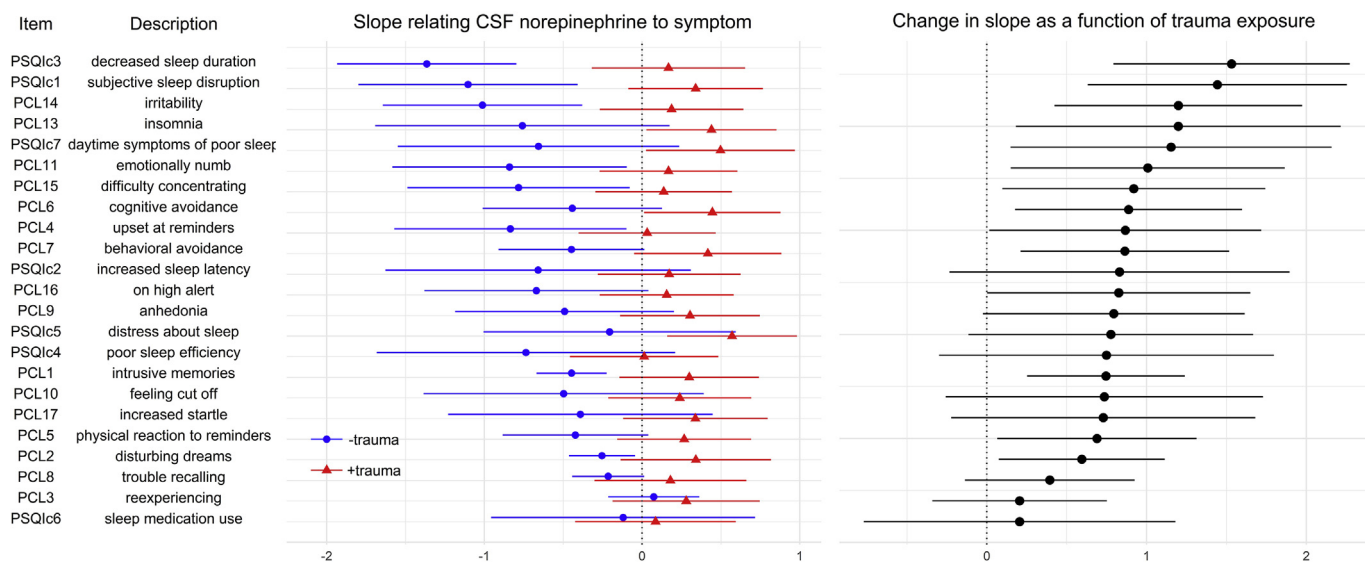

Fig. 2. Effect of criterion A trauma history on the relationship between $[NE]_{CSF}$ and normalized values (z-scores) for individual PTSD symptoms (PCL items) and sleep complaints (PSQI items). Items are ordered by the magnitude of the change in slope as a function of trauma exposure, shown in the far-right column. The slope relating $[NE]_{CSF}$ and normalized values for individual items are shown in the center-right column for non-trauma-exposed controls (blue circles) and those with a history of trauma exposure but who were not using prazosin (red triangles). Bars represent 95% CIs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
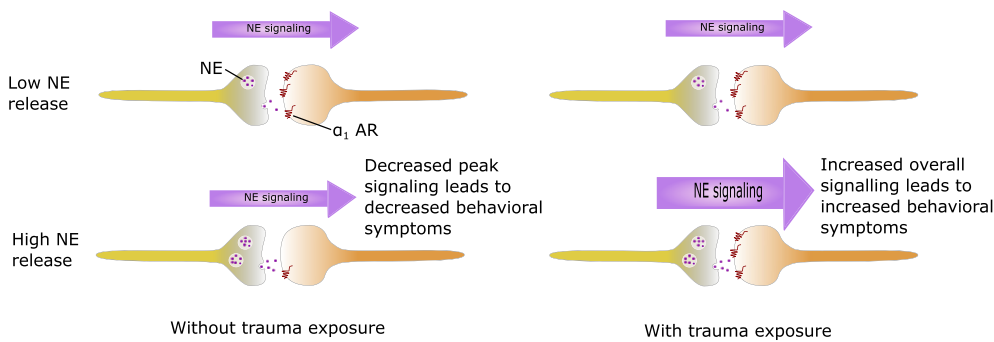
Fig. 3. Hypothesized postsynaptic mechanism for the change in the relationship between NE and behavioral symptoms in individuals with a history of trauma exposure. In the first column, individuals without a history of trauma exposure and who have lower average levels of NE release have normal overall levels of NE signaling and low levels of behavioral symptoms commonly associated with α_1 AR activation. When individuals in this group have higher average levels of NE release, as in the lower left panel, there is hypothesized to be a homeostatic mechanism by which the post-synaptic receptor density or gain is decreased so that the overall average levels of NE signaling are maintained, and behavioral symptoms associated with α_1 AR activation are, if anything, decreased due to decreases in peak signaling capacity. In the second column, in individuals with a history of trauma exposure, those with low average levels of NE release have similar overall levels of NE signaling as those without a history of trauma exposure and thus do not have elevated behavioral symptoms. However, as shown in the lower right panel, when those individuals have increased levels of average NE release, we hypothesize that they do not have the same degree of homeostatic postsynaptic downregulation. This leads to increased overall NE signaling – including increased activation of α_1 ARs – and thus increased expression of associated behavioral symptoms. Alternative mechanisms to explain the observed effect include changes in downstream reactivity to NE or changes in regions that modulate the response to NE, such as the medial prefrontal cortex (mPFC). This hypothesized change in homeostatic regulation may also occur in combination with increases in presynaptic NE release following chronic stress or trauma (not shown).

dopamine, the other major stress-relevant catecholamine. When we repeated the analyses described in sections 3.2 and 3.3 using $[DA]_{CSF}$ rather than $[NE]_{CSF}$, we found no significant difference in dopamine levels as a function of PTSD diagnosis (p = 0.71) or history of trauma exposure (p = 0.88, Table 3). In addition, we found no significant relationship between $[DA]_{CSF}$ and behavioral symptom intensities, nor any change in the relationship between $[DA]_{CSF}$ and symptom intensities as a function of diagnostic group or trauma exposure (all estimated slopes relating $[DA]_{CSF}$ and normalized total scores on the PCL, PSQI, NSI, PHQ-9, and PCL symptom clusters between −0.3 and 0.3 with 90% CI including zero; all comparisons between groups as defined in section 3.3 above non-significant at $\alpha = 0.05$).

Second, we asked whether examination of the primary NE metabolite, 3,4-dihydroxyphenylglycol (DHPG) (Eisenhofer, 2004), or the dopamine precursor 3,4-dihydroxyphenylalanine (DOPA), provided any evidence that differences in synthetic capacity or degradation rate of NE can provide an alternative explanation for the altered relationship between CSF NE and behavioral symptom expression in those with versus without a history of traumatic stress. Although the gold-standard strategy for assessing noradrenergic synthetic capacity requires pharmacologic provocation studies that were outside the scope of this study (Raskind et al., 1999), differences in the ratio of NE to DHPG between groups would provide a very rough indication of differences in turnover and clearance of NE, while differences in the ratio of NE to DOPA would provide a very rough indication of differences in synthetic capacity. However, there were no significant differences in these ratios between any of our primary analysis groups (Table 3).

4. Discussion

Consistent with our primary hypothesis, Veterans with a history of trauma exposure meeting criterion A of the diagnostic criteria for PTSD demonstrated a significantly more positive correlation between $[NE]_{CSF}$ and behavioral symptom expression compared to non-trauma exposed Veterans. These results are consistent with exposure to a traumatic event resulting in an alteration in the brain's response to central NE release, such that higher NE levels now lead to increased symptoms, even when the same NE levels are not associated with increased symptoms in those without a history of trauma exposure. This finding is potentially consistent with a number of possible mechanisms, including increased postsynaptic gain of NE receptors, such as the α_1 AR; increased downstream intrinsic reactivity to NE signaling; or decreased modulation of NE reactivity by other brain areas.

There is some pre-clinical support for the presence of mechanisms that increase the responsiveness of CNS stress response systems in general, and α_1 ARs in particular, following exposure to a trauma. In 2015, Rajbhandari and colleagues found that rodents exposed to a predator stress demonstrated long-lasting increases in behavioral manifestations of anxiety, and that these behavioral effects were dependent on a corticotropin-releasing factor (CRF)-dependent sensitization of α_1 ARs to NE (Rajbhandari et al., 2015). An alternative mechanism that could lead to similar behavioral-level changes in the response to increased NE release is supported by the work of Gresack and Risbrough, who found that the ability of NE to modulate startle reactivity in a mouse model is dependent on concurrent CRF signaling (Gresack and Risbrough, 2011).

A model in which trauma exposure changes, in particular, the sensitivity of postsynaptic α_1 ARs to NE is also consistent with our recent finding that an individual's standing systolic blood pressure at baseline is associated with the responsiveness of that individual's PTSD symptoms to prazosin (Raskind et al., 2016). As the support of blood pressure during the several minutes following standing is in non-elderly populations predominantly mediated by peripheral NE acting through α_1 ARs on arteriolar smooth muscle cells, this finding was interpreted as resulting from a concomitant upregulation of both central and peripheral α_1 ARs, such that an increased gain in the reflexive loop following standing was predictive of a similar upregulation of α_1 ARs' responsivity to central NE in individuals with symptoms of PTSD.

The finding of an inverse relationship between $[NE]_{CSF}$ and symptom ratings in non-trauma-exposed controls was unexpected and initially appears counterintuitive. It is potentially consistent, however, with previous suggestions of an inverse relationship between plasma NE levels and the response profile of NE receptors. For example, the degree of pupillary dilation in response to agonism of pupillary α_1 ARs when phenylephrine is applied to the iris has been found to be inversely related to plasma NE concentration (Sitaram et al., 1984b). This has been presumed to result from a homeostatic process that regulates α_1 AR density and/or gain in response to circulating NE levels, evidence of which can also be seen in the well-documented development of α_1 AR supersensitivity when noradrenergic innervation is interrupted, such as occurs in Horner's syndrome (Freedman and Brown, 2005). Previous assessments correlating pupillary dilation in response to phenylephrine and anxiety symptoms have found an inverse relationship between the magnitude of pupillary dilation and harm avoidance in a sample of healthy individuals (White and Depuea, 1999), as well as an inverse relationship between the magnitude of pupillary dilation and both generalized anxiety and panic symptoms in a clinical sample selected for depression and anxiety symptoms (Sitaram et al., 1984a). These findings have been interpreted to suggest that these symptoms may be driven by the amount of NE released, such that they are then inversely

related to the magnitude of pharmacologic pupillary dilation, an indication of α_1 AR sensitivity (White and Depuea, 1999).

One potential explanation for our result, then, is that the symptoms identified as being inversely related to $[NE]_{CSF}$ in non-trauma-exposed controls are positively associated with NE postsynaptic receptor activation, with postsynaptic receptor sensitivity (either density or gain) being the limiting factor for symptom generation – but they appear inversely related to $[NE]_{CSF}$ because $[NE]_{CSF}$ is most accurately considered an indication of the average amount of norepinephrine release over a period of time, and in individuals without a history of trauma, the average amount of norepinephrine release over time is inversely related to the receptor sensitivity (Fig. 3). This type of a positive association between NE postsynaptic receptor activation and symptom expression would also mean that if a homeostatic mechanism that decreases receptor sensitivity when neurotransmitter levels increase were to be disrupted by trauma, such that higher average levels of NE release were less compensated for by decreased postsynaptic reactivity, then higher levels of $[NE]_{CSF}$ would become more positively associated with higher levels of NE receptor signaling, as seen in our data.

Alternatively, a similar pattern could be produced by analogous changes downstream from NE signaling or in systems that modulate the reactivity to NE signaling – for example, if the medial prefrontal cortex (mPFC) can regulate the impact of increasing levels of NE release in individuals without a history of trauma, but following trauma this regulatory activity is decreased. This type of a mechanism would be consistent with what is known about the effects of prolonged stress on the relative strengths of amygdala activation and mPFC regulation of the amygdala and limbic response to stressful stimuli (Arnsten et al., 2015), although it would not provide an explanation for the association between increased systolic blood pressure after standing and prazosin responsiveness of PTSD symptoms noted above (Raskind et al., 2016).

Finally, the inverse relationship seen between $[NE]_{CSF}$ and behavioral symptom intensity in non-trauma-exposed controls could also be produced if NE release in these individuals were accompanied by release or activation of a modulator of NE's effects that more than compensated for the increased NE release. For example, neuropeptide Y (NPY) is an anxiolytic compound that is often co-released by NE neurons in the central and peripheral nervous system and that can attenuate release of NE and/or its stimulating effects at postsynaptic receptors (Schmeltzer et al., 2016). NPY has been found to be lower in the CSF of Veterans with PTSD than both civilian controls (Sah et al., 2009) and combat-exposed controls (Sah et al., 2014). Future studies that examine both $[NE]_{CSF}$ and $[NPY]_{CSF}$ in the same population will be informative.

The lack of obvious specificity of the observed effect for symptoms of PTSD as opposed to symptoms categorized as relating to depression or post-concussive syndrome has a number of potential explanations. First, even beyond the obvious overlap in the symptoms identified as part of the diagnostic criteria for each condition (e.g., changes in sleep, concentration, and mood are part of all three categories), the majority of the items in each assessment tool are known to be present at a high rate in individuals with each of the three diagnoses (e.g., headaches are common in depression and PTSD despite not being part of the diagnostic criteria). This may be because there is substantial overlap in the pathophysiologic mechanisms by which symptom expression occurs in these disorders (Stein and McAllister, 2009), which could also help to explain the observed modification by trauma of the relationship between NE and the majority of symptoms assessed. In addition, there may be dependencies between items that are assessed as independent symptoms. For example, because there is particularly strong evidence of a role for NE in the generation of hyperarousal symptoms, we expected that the connection to these symptoms would be the strongest; and, indeed, hyperarousal and especially sleep symptoms tended to be among those with the strongest measured effect. However, impairment in sleep can directly worsen problems with many other domains, such as mood, concentration, or pain (Kyung Lee and Douglass, 2010; Palmer

and Alfano, 2016). Thus, a change in the relationship of NE to mood or pain could potentially be observed secondary to a change in the relationship of NE to sleep.

When examining the effect of concurrent medication use on our results, we found that the relationship between $[NE]_{CSF}$ and behavioral symptom expression was diminished in those with a history of trauma exposure who reported taking prazosin at the time of symptom assessment. Prazosin is a CNS-penetrant noradrenergic modulator that has been found in other studies to decrease both daytime and nighttime symptoms of PTSD (Raskind et al., 2013), an effect that has been postulated to be via either antagonism or inverse agonism of α_1 AR in the CNS (Hendrickson and Raskind, 2016; Oganesian et al., 2011; Zhu et al., 2000). Although the present data set cannot be used to test hypotheses addressing the efficacy of prazosin for PTSD, given the absence of a control condition in a setting with a high likelihood of confounding-by-indication, the findings present a new, if indirect, line of evidence about the relationship between prazosin use and the interruption of PTSD symptom generation that is consistent with this mechanism of action.

In contrast with previous studies, we did not find a statistically significant increase in $[NE]_{CSF}$ in Veterans with a diagnosis of PTSD compared with those without a diagnosis of PTSD. This is also in contrast to our expectations based on pre-clinical research, where, for example, chronic stress has been found to lead to significant increases in the fraction of rat prefrontal cortex (PFC) noradrenergic axons coexpressing both the NE synthetic enzyme tyrosine hydroxylase and the NE transporter (NET) (Miner et al., 2006). Mean $[NE]_{CSF}$ and $[DHPG]_{CSF}$ were both numerically higher in Veterans with a diagnosis of PTSD, however, and given the limited sample size, a negative result does not preclude an effect that simply was not captured here. Furthermore, the sample without PTSD in this study was significantly different than in previous work: in our study, all participants were deployed Veterans, the vast majority of whom had experienced combat deployments; it is likely that many of the individuals who did not report a history of exposure to a traumatic event meeting criterion A of the diagnostic criteria for PTSD may nonetheless have had a greater and often prolonged period of exposure to more moderately traumatic events or periods of time requiring sustained vigilance than are common in the civilian population. In the previous study by Geriacioti et al. comparing $[NE]_{CSF}$ in combat Veterans with PTSD to individuals without PTSD, the control group consisted of healthy adult males who were not necessarily Veterans (Geracioti et al., 2001). Thus our results remain consistent with a potential combined effect of trauma on noradrenergic signaling, with some effect of traumatic exposure on $[NE]_{CSF}$ outflow, and an additional effect of traumatic exposure on brain reactivity to $[NE]_{CSF}$. The difference between our results and previous work also raises the question of whether prolonged stress, such as experienced in a war-zone deployment, may lead to alterations in noradrenergic signaling even in the absence of a traumatic experience meeting criterion A of the diagnostic criteria for PTSD, as well as whether these alterations may differ from those that result from a criterion A qualifying event.

This work has a number of important limitations. As an observational study, there is always the risk of unknown sources of confounding. Furthermore, although the findings are consistent with our hypothesis that trauma exposure causes a change in brain responsivity to NE, causality cannot be directly inferred from an observational study of this type. In addition, this work was done entirely in male combat Veterans who were young to middle aged, and whether these findings would generalize to civilians, women, children, or older men is unknown. The separation in time between the quantification of behavioral symptoms and $[NE]_{CSF}$ was also significant, at a mean of 3 months (SD 104 days). Although it is not expected that this separation in time would produce the effect that was observed, it may have artificially obscured its magnitude, and it is possible that the strength of the relationship between $[NE]_{CSF}$ and symptom intensity would be significantly stronger if the measurements were carried out closer

together.

Finally, the overwhelming majority of individuals in this study with a history of trauma exposure also had a history of mTBI (47 out of 52), whether or not that was related to their documented criterion A qualifying traumatic event. Although the five individuals with a history of trauma exposure but no history of mTBI generally displayed a pattern of symptom intensities and $[NE]_{CSF}$ values similar to the trauma-exposed participants with a history of mTBI, consistent with this being an effect of trauma exposure, the degree of overlap between mTBI and trauma exposure makes a true disambiguation statistically impossible in this data set. Furthermore, this may not turn out to be pathophysiologically meaningful, either: evidence from animal models suggests that mTBI may result in increases in both NE synthesis (Kobori et al., 2006) and α_1 AR transcription (Kobori et al., 2011), while impairments in working memory associated with increased catecholamine signaling following mTBI show improvement after administration of prazosin (Kobori et al., 2011). Thus, increased α_1 AR-mediated signaling may represent a final common pathway following both psychological trauma and mTBI, potentially explaining some of both the very high rates of comorbidity as well as the significant overlap in observed symptoms, which have previously been suggested as indicating the possibility of a common pathophysiological process (Stein and McAllister, 2009). Larger studies that allow determination of the separate contributions of psychological trauma and mTBI to the effects observed in this work will be an important area for future research.

This study also has a number of strengths. This is the largest study published to date characterizing both CSF NE levels and trauma-associated mental health symptoms, with the only previous study having been possibly too small for a pattern such as presented here to have been detected. Furthermore, selection of participants in a way that was unbiased with respect to the presence or absence of a PTSD diagnosis facilitated the observation of the distribution of such symptoms across the spectrum seen in a sample of combat Veterans.

The implications of these results are significant. First, they raise the possibility that there may be a long-lasting physiologic response to trauma exposure that can exist independently of whether an individual meets criteria for PTSD at any given point in time. This would provide a potential explanation for the repeatedly observed but so far unexplained pattern of individuals sometimes showing PTSD symptoms only after a substantial delay following their trauma exposure, or intermittently over time (Mota et al., 2016; Utzon-Frank et al., 2014; Waller et al., 2015). The existence of a generalized mechanism whereby reactivity to NE is persistently increased following trauma exposure could also help to explain some of the increase in stress reactivity seen in PTSD as well as other disorders, such as the finding that individuals with both a primary psychotic disorder and a history of childhood trauma show increases in mood symptoms and in psychotic symptoms in response to stress (Lardinois et al., 2011). Second, these results provide further independent support for the role of CNS NE in the pathophysiology of PTSD in general and, indirectly, for the role of prazosin in treating PTSD by blocking this pathway in particular. Third, these results suggest that when searching for novel targets for clinical intervention, an exploration of the mechanism by which the response to increased NE is modulated following trauma holds the possibility for finding ways to more effectively or even permanently modulate the changes that occur. Finally, this work suggests that immediate targets for further research would include both testing for a specific increase in α_1 AR density following trauma exposure and seeking to identify additional non-invasive biomarkers of central noradrenergic tone in human clinical populations.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ynstr.2018.03.001.

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