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Posttraumatic stress disorder influences the nociceptive and intrathecal cytokine response to a painful stimulus in combat veterans

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ABSTRACT

Objective: Although posttraumatic stress disorder (PTSD) and chronic pain frequently occur in tandem, the pathophysiological mechanisms mediating this comorbidity are poorly understood. Because excessive inflammation occurs in both conditions, we examined the cerebrospinal fluid (CSF) concentrations of inflammatory response mediators interleukin 1-beta (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor-alpha (TNFα) and interleukin 10 (IL-10) after prolonged suprathreshold pain stimulus in 21 male combat veterans; 10 with PTSD and 11 combat controls (CC).

Methods: After completing baseline quantitative sensory testing (QST) and psychological profiling, all patients received an injection of capsaicin into the quadriceps muscle. Spontaneously reported pain was measured for 30 min after the capsaicin injection. The evoked pain measure of temporal summation was tested between 70 and 110 min post capsaicin injection. Inflammatory (IL-1β, IL-6, IL-8 TNFα) and anti-inflammatory (IL-10) CSF cytokines were measured before (baseline) and after capsaicin injection over a time frame of 110 min.

Results: Following intramuscular capsaicin injection, pro-inflammatory cytokines [TNFα, IL-6, IL-8] significantly increased (percent rise from baseline) in both groups, whereas IL-1β significantly increased in the PTSD group only. The anti-inflammatory cytokine IL-10 showed an immediate (within 10 min) increase in the CC group; however, the IL-10 increase in the PTSD group was delayed and not consistently elevated until 70 min post injection.

Conclusion: These findings show significant central nervous system (CNS) differences in the inflammatory response to a deep pain stimulus in combat veterans with and without PTSD. They support the concept that abnormally elevated neuroinflammatory response to pain stimuli may be one CNS mechanism accounting for the high co-occurrence of PTSD and pain.

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1. Introduction

1.1. Cytokines, pain and PTSD

PTSD and chronic pain co-occur frequently and for both there is accumulating evidence of peripheral and CNS hyperinflammation. Since 1995, mounting evidence has implicated excessive release of pro-inflammatory cytokines in the periphery and CNS in the generation of pathologic pain states (Clatworthy et al., 1995; Sommer et al., 1999; Woolf, 2011). These important inflammatory mediators regulate the amplitude and duration of inflammation, likely potentiating the painful experience, a concept bolstered by numerous studies in non-human animal models demonstrating that pro-inflammatory cytokines have an important role in nociceptive pain signaling (Covey et al., 2000; Myers et al., 2006). During a peripheral nerve injury, the release of local cytokines promotes recruitment of macrophages to the injury site (Liefner et al., 2000). Activated macrophages secrete components of the complement cascade, coagulation factors, proteases, hydrolases, interferons, and

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other cytokines that ultimately facilitate degradation and phagocytosis of the injured tissue. In addition to local cytokine release, resident macrophages and glia cells found in the CNS and meningeal covering become activated and react to nerve injury by releasing cytokines that promote recruitment of macrophages to the CNS (Ji and Suter, 2007; Scholz and Woolf, 2007; Zhuang et al., 2006). Activated glial cells and astrocytes are believed to release cytokines that can trigger inflammatory responses in the dorsal horn of the spinal cord (Kawasaki et al., 2008; Zhang et al., 2011) and the dorsal root ganglion (Ji and Suter, 2007; Scholz and Woolf, 2007; Tsuda et al., 2005) which facilitate neuropathic-mediated pain. In addition to inflammatory cytokines released from the spinal cord, meningeal-derived fibroblasts, macrophages, mast cells, and dendritic cells also release cytokines, including TNFα, IL-6, and IL-1β, into the CSF during the pain-mediated inflammatory response. Thus, pro-inflammatory cytokines derived from CNS cells (microglia, astrocytes, meningeal fibroblasts, macrophages, mast cells, and dendritic cells) react to a painful stimulus and can promote persistent neuropathic pain.

1.2. Inflammation and PTSD

Through these same pathways, central and peripheral hyperinflammation linked to PTSD may contribute to pain amplification. The current literature provides accruing evidence for excessive peripheral inflammation in PTSD. Evaluation of basal or stimulated peripheral cytokines (Maes et al., 1999; Spivak et al., 1997; Wong et al., 2000; Bob et al., 2010; Gill et al., 2010; Hoge et al., 2009; Symes et al., 2010; Tucker et al., 2010; von Känel et al., 2010; Lindqvist et al., 2014; Tursich et al., 2014; Plantinga et al., 2013) indicates there are abnormally increased concentrations of pro-inflammatory cytokines in patients with PTSD in most, but not all studies (de Kloet et al., 2007; Miller et al., 2001; Song et al., 1999). Few studies have examined cytokine levels in the CSF of individuals with PTSD. Of those, one reported higher concentrations of CSF IL-6 in combat veterans with PTSD (Baker et al., 2001), whereas the other reported no difference in CSF IL-6 concentrations in civilians with PTSD (Bonne et al., 2011). Cytokines may contribute to the pathophysiology of PTSD through a number of mechanisms that are independent of co-morbid depression and physical illnesses (Lindqvist et al., 2014; Tursich et al., 2014; Plantinga et al., 2013). Recently published gene expression studies show accumulating evidence of innate immune dysregulation in PTSD, and that this malfunctioning may be a risk factor for development of the disorder (Breen et al., 2015; Glatt et al., 2013; Tylee et al., 2015; Guardado et al., 2016). Taken together, there is growing evidence that PTSD is associated with inflammation in the periphery and in the CNS, which may be associated with the comorbid chronic pain often found in this population.

1.3. Cytokines and capsaicin

Capsaicin activates the vanilloid receptor subtype 1 (TRPV1) expressed on peptide-containing C fibers, elicits a sensation of burning pain, and causes neurogenic inflammation. Activation of TRPV1 stimulates the exocytosis of substance P (SP) from nociceptors, leading to mast cell degranulation of histamine and serotonin release from platelets. Pro-inflammatory cytokines, such as TNFα, enhance the capsaicin sensitivity of neurons by increasing the depolarization potential (Nicol et al., 1997) in peripheral C fibers as well as C and Aβ fibers within the dorsal root ganglion. TNFα, known to enhance capsaicin sensitivity in non-human animal models, increases nocifensive behavior (e.g., foot withdrawal to painful stimuli), and enhances release of calcitonin gene-related peptide (known to heighten pain sensitivity) (Khan et al., 2008). At the level of the spinal cord dorsal horn, intradermal capsaicin elicits the release of the neurotransmitters glutamate, aspartate, and glycine which then results in spinal cord-mediated release of inflammatory cytokines (Malmberg et al., 1995). Taken together, capsaicin induces cytokine release in the CNS, and this action on CNS cytokine release can be amplified by the surrounding inflammatory milieu. Our project assessed whether excessive capsaicin-mediated pro-inflammatory cytokine release and its intensification of painful experience are biomarkers for the abnormal peripheral and CNS inflammation occurring in individuals with PTSD.

1.4. Study objectives

Prior work by our group showed that intramuscular capsaicin injection is rated as higher in spontaneously reported pain intensity and unpleasantness by individuals who have PTSD, as compared to healthy combat controls (CC) (Moeller-Bertram et al., 2014). We also showed that the same PTSD subjects have signs of central sensitization (CS), as evidenced by the enhanced evoked pain measure of temporal summation after capsaicin injection. The aim of the current study was to test the hypothesis that PTSD participants have an abnormal CNS inflammatory response if subjected to a prolonged painful stimulus (i.e., an intramuscular capsaicin injection) when compared to healthy CC. To investigate this hypothesis: 1) intramuscular capsaicin was injected into a group of 10 PTSD and 11 CC, 2) participants were queried about spontaneously reported pain measures for 30 min after capsaicin injection, 3) patients were serially queried about evoked pain measures of central sensitization (temporal summation) from 70 to 110 min after capsaicin injection and 4) CSF cytokines were measured at baseline (before) and serially after capsaicin injection for a 110 min period. The goal was to better characterize the relationships of CSF concentrations of pro-inflammatory cytokines (IL-1β, IL-6, IL-8 TNFα) and the anti-inflammatory cytokine (IL-10) concurrently measured after a capsaicin pain challenge.

2. Materials and methods

The local Institutional Review Board (University of California San Diego, San Diego, CA and Research and Development Committee, San Diego VA Healthcare Services) approved all study procedures, and all participants provided written, informed consent. All participants were tested in the Clinical Research Unit (CRU) at the VA San Diego Healthcare Center (VASDHS) in a quiet room at ambient temperature after they were familiarized with the testing environment. The study assessments were divided into two separate experimental days. On experimental day 1, each study participant received a comprehensive medical examination, a structured clinical interview, baseline quantitative sensory testing (QST) testing and filled out several paper-and-pencil questionnaires. PTSD was diagnosed by Structured Clinical Interview for DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*) Axis I Disorders (SCID) and the Clinician Administered PTSD Scale (CAPS), which was used as a continuous measure reflecting the severity of PTSD. Exclusionary psychiatric disorders and current substance or alcohol use/dependence were identified by the SCID interview and substance/alcohol use was confirmed with a urine toxicology screen. On experimental day 2, which occurred within 30 days of experimental day 1, all subjects received an intramuscular capsaicin injection (see Section 2.4) at 13:00 h. CSF samples were taken 10 min after the injection and subsequently at 20-min intervals up to 110 min later (see Section 2.8).

2.1. Subjects

A total of 21 male Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) veterans from the greater San Diego area

were enrolled. Ten had a diagnosis of PTSD and 11 were enrolled in the CC group; groups had similar levels of combat exposure and were closely matched on age, race, and health habits. Inclusion criteria for all subjects were: 1) history of deployment to a combat zone, 2) physical examination results within normal range, 3) no chronic or acute pain complaint, and 4) no medication for at least 5 half lives, prior to CRU admission (including non-steroidal anti-inflammatory medications and over-the-counter pain medications). Further inclusion criteria for study subjects were: prior combat deployment and diagnosis of PTSD (PTSD group), and for the CC group members, no PTSD diagnosis as determined by SCID and CAPS. Exclusion criteria for the PTSD and CC group were: 1) Age younger than 18 years or older than 65 years; 2) Inability to give informed consent; 3) History of substance abuse within 6 months of study participation; 4) Significant head trauma, as judged by loss of consciousness or a post concussive syndrome by history; and 5) In the case of CC, any current DSM-IV Axis I disorder, or for PTSD subjects, any other current DSM-IV Axis I comorbid disorder except for dysthymia or major depression, if secondary to (chronologically after) the PTSD diagnosis.

2.2. Questionnaires

2.2.1. CAPS

CAPS, considered a gold standard PTSD symptom scale, is a structured interview scaled on a 136-point numerical scale that provides both a categorical diagnosis and a measure of the severity of PTSD symptoms. The CAPS has good psychometric properties across a wide variety of clinical populations and research settings (Weathers et al., 2001; Bernstein et al., 1994).

2.2.2. Combat experiences scale

Combat exposure was measured using a standardized seven-item questionnaire, the Combat Experiences Scale (CES); (http://www.ptsd.va.gov/) (Keane et al., 1989).

2.2.3. Childhood trauma questionnaire (CTQ)

The CTQ is a 28-item self-report inventory that provides a brief, reliable, and valid screening for histories of childhood abuse and neglect. It evaluates five different types of childhood traumatic experiences, including emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect (Bernstein et al., 1994). Rating is performed using a Likert scale ranging from 1 (never) to 5 (very often), with final scores ranging from 5 to 25.

2.2.4. Pain anxiety symptom scale (PASS)

The PASS is a 40-item self-report measure of fear and anxiety symptoms associated with pain (McCracken et al., 1992). It is designed to assess the following four components of pain-related anxiety: cognitive, fear, escape/avoidance, and physiologic. It is scored using a 6-point Likert scale, ranging from never (0) to always (5).

2.2.5. Pain catastrophizing scale (PCS)

Catastrophizing is an irrational thought or belief that something is far worse than it actually is (Sullivan et al., 1995). The PCS is a 13-item self-report measure of pain catastrophizing based on past painful experiences (Osman et al., 2000). The experiences are rated on a 5-point Likert scale. The reliability and validity of the PCS has been demonstrated in studies with undergraduates (Osman et al., 2000) and samples of outpatients with pain (Gescheider, 1997).

2.2.6. Beck depression inventory-II (BDI-II)

Depression severity was assessed using the Beck Depression Inventory (BDI) (Beck et al., 1998). The BDI-II is a 21-question multiple-choice self-report measure with components related to symptoms of depression, including cognition and physical symptoms. The BDI does not directly yield diagnoses of depression but does provide a numerical score of the depression symptom severity.

2.3. QST

Cool and warm detection thresholds, as well as cold and heat pain thresholds were determined using a Thermal Sensory Analyzer (Medoc Advanced Medical System, Ramat Yashai, Israel). Four trials of cool/warm detection thresholds and three trials for heat/pain thresholds were applied and averaged together to determine the thresholds.

Briefly, a standard thermode (3×3-cm surface) was placed on the mid-portion of either left or right thigh (randomly assigned) to heat or cool the skin (ramping at $1\,^{\circ}C/s$). The standard program using the "method of limits" (Pitman et al., 1990) was applied and subjects were instructed to push a button when they detected change in temperature (detection thresholds) and when the temperature became painful (pain thresholds). Mechanical detection thresholds were obtained using standardized Von Frey hairs delivering increasing force when pushed onto the skin at the same location on the thigh (range: 0.008–300 g). Pressure was applied to bend the filament and held for 1 s. The up/down method was used, with the last stimulus detected after the third change used as the recorded threshold value. Pressure pain thresholds were determined using a manual pressure algometer that repeatedly applied pressure to the skin with a standardized contact of $1\,cm^2$ (Wagner FPK manual pressure algometer). Averaging the three trials derived the pressure pain threshold.

2.4. Intramuscular pain stimulus

Each subject received a capsaicin injection (100 μg in 10 μL) using a microsyringe and a 1.5-in., 30-gauge needle into the center portion of the quadriceps muscle. The injection site was identical to that of the previous QST testing. A dose-response curve for intradermal capsaicin was previously performed in a separate small set of healthy volunteers. The data showed a dose-dependent pain response, with 10 μg of capsaicin eliciting pain and 100 μg of capsaicin required to elicit a reliable production of moderate spontaneous pain response for up to 30 min. In contrast, doses of 0.1 μg and 1.0 μg, respectively, did not reliably induce pain. Based on these observations, we used a 100-μg dose of capsaicin in 10-μL volume for intramuscular injection into both the CC and PTSD group.

Spontaneous pain discomfort, intensity and unpleasantness was quantified with the visual analog scale (VAS) ranging from "no pain" to "worst pain imaginable" at 5-min intervals for 30 min. Only 30 min of spontaneous pain measures were obtained because most subjects reported resolution of their spontaneous pain after 30 min. Subjective pain rating data were analyzed with the repeated measures ANOVA by group (PTSD, CC) as a between-subjects factor and as a within-subjects repeated measure. Both primary and interaction effects were examined. Intensity and unpleasantness ratings were run in two separate models.

2.5. Temporal summation to pressure stimuli

Evoked pain measured with temporal summation was assessed every 30 min starting 70 min after the capsaicin injection (when the spontaneous pain had subsided) and continued to the 110-min point. Ten repetitive pressure stimuli at 0.3-Hz frequency using a pressure algometer [with a standardized contact of $1\,cm^2$ (Wagner FPK manual pressure algometer)] were applied to both PTSD and CC groups. To reach each participant's pressure threshold, the applied force for each stimulus was increased manually (by 1 lb) over 1 s to attain that individual's thresholds. The resulting maxi-

mal force ranged between 0.1 and 0.5 lb above the threshold and was then repeatedly applied for a total of ten times. Pain ratings of C-fiber-mediated secondary pain occurring 1–1.5 s after the stimulus application were rated on a VAS after the first, fifth, and tenth stimulus. Data were analyzed using a linear mixed effects model in which group (PTSD/CC) and repeated measures (first/fifth/tenth stimulus) were entered as fixed factors and subject was entered as a random factor.

2.6. Cerebrospinal fluid (CSF) collection

The day of the capsaicin injection, subjects had an intravenous catheter secured. A 24-gauge catheter was placed in the lumbar subarachnoid space at the L3/4 or L4/5 level under fluoroscopic guidance. Participants were allowed to rest for 90 min after catheter placement and assessed for any immediate complications. Prior to (within one min before) intramuscular capsaicin injection (time 0 min), CSF (0.9 mL and separated into four 0.225 mL aliquots) was withdrawn. To control for the potential influence of diurnal rhythms, the CSF collections (time 0 min) were initiated at 13:00 h (+/−20 min) for all subjects. Ten min after injection (time 0 min), the first CSF was withdrawn and subsequently collected thereafter at 20 min intervals for the next 110 min. The last cerebrospinal fluid draw (110 min after time 0 min) occurred at 15:00 h (+/−20 min).

2.7. Cerebrospinal fluid cytokine analyses

Five cytokines were determined by multi-cytokine array using an electrochemiluminescence platform and multiplexing SECTOR Imager 2400 for analyte detection (Meso Scale Discovery, Gaithersburg, MD). Each specimen was referenced to a standard curve generated from 5 calibrators with known cytokine concentrations. The panel included IL-1ß, IL-6, IL-8 IL-10 and TNFα. The lower limit of detection was 0.1 pg/mL. This assay has a large dynamic range up to 2000 pg/mL, and no values were above the upper limit of detection. Each assay also included a pool to monitor for assay drift. The intra-assay coefficients of variation were: IL-1β = 28%, IL-6 = 7%, IL-8 = 5%, IL-10 = 26%, and TNFα=29%. The inter-assay coefficients of variation for each cytokine were IL-1β = 28%, IL-6 = 8%, IL-8 = 6%, IL-10 = 33%, and TNFα = 21%. The MSD multiplex was able to consistently detect very low concentrations of IL-1β in CSF, but with better CVs as values rose to 1 pg/mL. To minimize an influence of drift between kits, all specimens collected serially for an individual were run in duplicate on the same kit. Further, we compared the serial response after the capsaicin pain challenge to baseline levels, employing a within subjects analytic design to strengthen the reliability of detecting sutained increments or decrements in cytokine levels.

2.8. Statistical analysis

To model CSF cytokine differences between PTSD and CC subjects, and to assess within-subject changes over time, we used the Mann Whitney *U* test and Wilcoxon signed-rank test to compare the percent changes in each cytokine. To correct for multiple comparisons we applied the Benjamini-Hochberg procedure maintaining a 5% False Discovery Rate (FDR). For analysis of pain measures over time, we used a likelihood-based approach to select either an exchangeable or autoregressive correlation structure and a likelihood ratio test to select either homogenous or heterogeneous variance by group, and assessed the significance of covariates with repeated measures ANOVA. To correlate pain measures with the change in CSF cytokines, the Pearson's correlation coefficient (and *P* values) between pain measures and the percent change in CSF cytokines from baseline to subsequent time points was carried out. Comparison of the correlations between the group cytokine concentrations was carried out using Fischer r-to-z transformation at each time point and the significance determined with a two-tailed *t*-test.

3. Results

3.1. Subject demographics

All 21 subjects served during the OEF/OIF conflicts with three military services.

- PTSD group: Marine Corps (3), Navy (6), Army (1)
- CC group: Marine Corps (6), Navy (1), Army (4)

All were male subjects; the groups did not significantly differ in average age, race, or length of service (Table 1).

3.2. Clinical measures

As expected, the two groups differed significantly on PTSD severity (i.e., CAPS), childhood trauma (i.e., CTQ), and depressive symptom severity (BDI-II) (Table 1). The ratings for combat exposure using the combat experience scale (CES) were comparable between the two groups. Despite the presence of PTSD, the pain anxiety and pain catastrophizing ratings during baseline assessments did not differ significantly between the groups.

3.3. QST

PTSD and CC groups did not differ significantly in baseline thermal and/or mechanical sensitivity in any of the evaluated measures (Table 2).

3.4. Spontaneously reported pain after intramuscular pain stimulus

Spontaneously reported pain to the intramuscular capsaicin injection was measured for 30 min after injection. The mean VAS ratings during and immediately following the injection are portrayed in Fig. 1A and B, for pain intensity and pain unpleasantness, respectively. There was a significant group difference in the pain ratings, resulting in a significant interaction term, because the PTSD group had higher pain ratings for both pain intensity ($F(1,18) = 6.5$, $P = 0.02$) and pain unpleasantness ($F(1,18) = 6.5$, $P = 0.019$). These results substantiated that participants with PTSD experienced enhanced pain after the capsaicin injection. There was not a group effect on the subjective pain ratings for pain discomfort ($P > .05$). However, over time, both the pain intensity and unpleasantness ratings decreased in the CC group, but not for the participants with PTSD (Fig. 1A and B).

Because both depression and childhood trauma can affect pain sensitivity, we examined whether the observed between-group differences in subjective pain ratings over time were related to these measures. Similarly, to more specifically determine if these psychiatric comorbidities, including depression, anxiety or prior childhood trauma, contributed to the sustained painful experience (i.e., pain discomfort, pain unpleasantness and pain intensity) in the PTSD group, we also ran repeated measure ANOVA tests for each measure separately. The influence of PTSD on pain intensity and unpleasantness remained significant even when taking into account the potential influence of depression or childhood trauma: 1) including a depression covariate as measured by BDI-II alone (intensity: $F(1,18) = 10.9$, $P = 0.004$; unpleasantness: $F(1,18) = 12.3$, $P = 0.003$), 2) including a childhood trauma covariate as measured with CTQ alone (intensity: $F(1,18) = 10.7$, $P = 0.004$; unpleasantness: $F(1,18) = 5$, $P = 0.039$), or after considering both BDI-II and CTQ

Table 1
Baseline Subject characteristics and clinical measures for both groups.

	PTSD		Combat Control		Statistical Values	
	Mean	SD	Mean	SD	t	*P*
Demographic Variables						
Gender	10 male		11 male			
Age (yrs)	28.9	8.8	28.5	7.0	0.13	0.89
Race[a]						
Caucasian	8		9		0.01	0.92
Other	2		2			
Injured During Service	3		0		3.9	0.05
Service Duration (years)	6.5		7.1		0.3	0.8
Clinical Variables						
CAPS Score	68.7	12.7	15.6	16.7	8.1	0.00
Combat Exposure	22.5	9.4	18.3	4.4	1.3	0.20
BDI-2	18.3	9.8	3.2	4.5	4.6	0.00
Childhood Trauma	81.1	8.1	72.3	5.7	2.9	0.01
Pain Anxiety Symptom Scale	53.5	26.1	52.0	18.6	0.16	0.88
Pain Catastrophizing Scale	11.3	8.4	10.4	6.2	0.3	0.77

PTSD: posttraumatic stress disorder, CAPS: Clinician Administered PTSD Scale, BDI-2: Beck depression inventory-2, SF-36 MHC: quality of life assessment.
[a] This measure was compared using a χ^2 test rather than a *t*-test.

Table 2
Baseline Quantitative Sensory Testing (QST).[a] Using a Medoc Analyzer, baseline thresholds for somatosensory stimuli were obtained. Shown are mean values and standard deviation; lbs.: pound-force per square inch; Pressure pain thresholds were assessed on the subject's thumb nail bed of the dominant hand and the thigh muscle quadriceps muscle. Thermal detection and other pain thresholds were assessed on subjects' quadriceps. gr: gram; lbs.: pound-force per square inch.

	PTSD		Combat Control		Statistical Values	
	Mean	SD	Mean	SD	t	DF
Thermal Testing						
Warm Detection Threshold (°C)	35.46	2.11	36.58	2.32	1.15	19
Cool Detection Threshold (°C)	28.99	2.21	27.85	2.51	1.10	19
Heat Pain Threshold (°C)	46.45	3.21	46.68	1.78	0.19	13.8
Cold Pain Threshold (°C)	8.41	11.99	1.93	5.05	1.59	11.9
Mechanical Testing						
Touch Detection Threshold (gr)	3.48	0.50	3.54	0.46	0.27	19
Pressure Pain Threshold (lbs.)	13.12	5.4	11.39	5.8	0.71	19

Pressure pain thresholds were assessed on the subjects' quadriceps muscle. Thermal detection and other pain thresholds were assessed on subjects' quadriceps.
Pressure pain thresholds were assessed on the subject's thumb nail bed of the dominant hand and the thigh muscle.
PTSD: posttraumatic stress disorder, SD: standard deviation, gr: gram, lbf: pound-force per square inch.
[a] Baseline thresholds for somatosensory stimuli were obtained using a Medoc Analyzer.

(intensity: $F(1,18) = 14.2$, $P = 0.002$; unpleasantness: $F(1,18) = 8.4$, $p = 0.01$). These analyses supported the conclusion that PTSD accounted for the exacerbation of spontaneous pain as reflected by the measures of pain intensity and unpleasantness after capsaicin injection.

3.5. Evoked pain measure of temporal summation to pressure stimuli

Temporal summation was used to measure evoked pain responses after spontaneous pain had abated (70 min after capsaicin injection). Assessment of the temporal summation to pressure stimuli indicated that there was a significant increase in pain intensity ratings over time in both groups (Fig. 1C):

- VAS rating for PTSD:
  - 1 st stimulus: 13.5 ± 18.8
  - 5th stimulus: 23.6 ± 21.6
  - 10th stimulus: 31.9 ± 26.8
- VAS rating for CC:
  - 1 st stimulus: 5.5 ± 6.4
  - 5th stimulus: 9.9 ± 9.4
  - 10th stimulus: 14.2 ± 12.8

However, the increase in pain intensity ratings following repeated application of pressure stimuli was significantly greater in the participants with PTSD when compared to the CC group ($F(1,399) = 16.97$, $P < 0.001$) (Fig. 1C). This significant increase in the temporal summation of pressure pain did not change when BDI-II and/or CTQ covariates ($P < 0.001$) were added to the statistical models, again indicative of a robust association with PTSD.

3.6. CSF cytokine analysis

The concentrations of CSF cytokines, including IL-6, IL-8, and TNFα, measured as a percent increase from baseline (prior to capsaicin injection), increased significantly in both the PTSD and CC group at 10 min and remained elevated up to 110 min post injection ($p < .05$). In the CC group, IL-1β remained low and did not increase significantly throughout the 110-min assessment ($P > 0.05$) (Fig. 2A). In contrast, for participants with PTSD, CSF IL-1β showed an immediate (up to 30 min) ($P < 0.05$) and a sustained increase (lasting up to 110 min) ($P < 0.06$) (Fig. 2A). The anti-inflammatory and regulatory cytokine, IL-10, showed an immediate increment from baseline in the CC group at the 10-min time point and remained elevated throughout the assessment ($p \leq .05$) (Fig. 2B). In contrast, in individuals with PTSD, the CSF IL-10 did not increase significantly until 30 min post injection and then did not remain consistently elevated until after 70 min post-capsaicin (Fig. 2B). Multiple comparison analysis with the

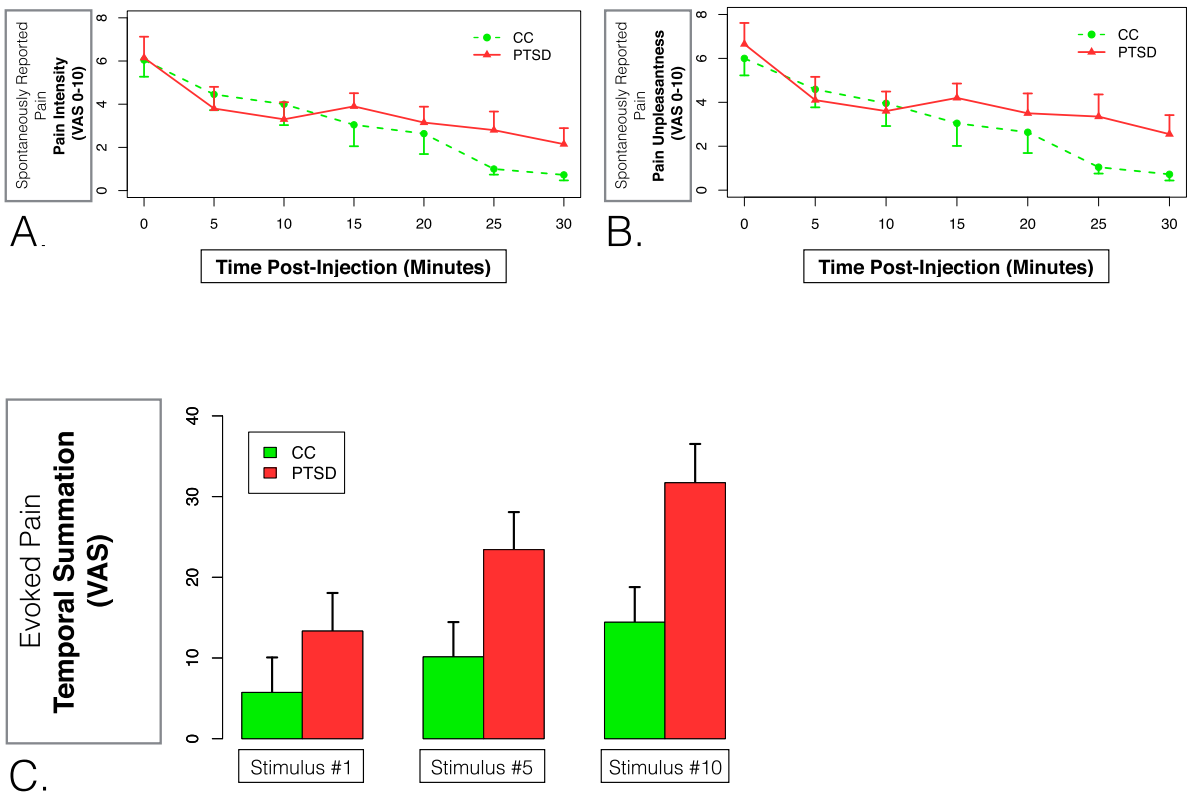

Fig. 1. Spontaneous Pain Reported as measured with the visual analog scale (VAS) after capsaicin injection for Pain Intensity 1A and for Pain Unpleasantness 1B. Temporal summation measures of repeated mechanical stimuli referred to as an "Evoked Pain" measure 1C. Compared to the Combat control group (CC), the PTSD group showed continued elevated levels of reported spontaneous pain unpleasantness ($F(1,18) = 6.5$, $P = 0.019$) and intensity ($F(1,18) = 6.5$, $P = 0.02$). Shown are pain intensity responses (VAS scores) for first, 5th and 10th stimulus just above pressure pain threshold. The increase in the evoked pain intensity rating following repeated application of pressure stimuli was significantly greater in the PTSD compared to the CC group ($F(1,399) = 16.97$, $P < 0.001$). PTSD: post traumatic stress disorder group; CC: combat control group; VAS: visual analog scale; data are shown as Mean ± SEM.

Benjamini-Hochberg False Discovery Rate (FDR) correction supported the conclusion of the significant differences in the cytokine responses between groups obtained with the Wilcoxon signed-rank test (Fig. 2A aand B).

3.7. CSF cytokine correlation to spontaneously reported pain measures (intensity, discomfort and unpleasantness)

The increase in CSF TNFα in the PTSD group was significantly correlated with the initial measure of spontaneous pain intensity ($r = 0.836$, $n = 10$, $P = 0.019$) as well as the spontaneous pain unpleasantness ($r = 0.866$, $n = 10$, $P = 0.012$) at the 30-min time point. The correlation with unpleasantness approached significance at the 10-min measurement ($r = 0.805$, $n = 10$, $P = 0.053$). When compared to the controls, PTSD participants spontaneously reported significantly higher levels of pain discomfort and unpleasantness. These pain indices showed significantly larger correlations with CSF TNFα levels at both 10 and 30 min and with IL-8 levels at 30 min after capsaicin injection in the PTSD group, as compared with the CC group (tested by Fischer r-to-z transformation). None of the proinflammatory cytokines in CSF correlated with pain discomfort, intensity, or unpleasantness in the CC group.

Interestingly, in the CC group, in contrast to the PTSD group, spontaneously reported pain measures of discomfort, intensity and unpleasantness showed significantly larger correlations with the levels of IL-10, the anti-inflammatory cytokine (tested by Fischer r-to-z transformation). In the CC group, CSF IL-10 levels were negatively correlated with discomfort at 10 min ($r = -0.755$, $n = 11$, $P = 0.012$) and approached significance at 30 min ($r = -0.625$, $n = 11$, $P = 0.053$). Further, at 30 min after capsaicin injection, IL-10 and self-reported pain intensity were significantly correlated in the CC participants ($r = -0.737$, $n = 11$, $P = 0.015$) (Table 3).

3.8. CSF correlation to evoked pain measures (temporal summation)

In the PTSD group, TNFα levels in CSF correlated with signs of central sensitization (evoked pain mediated temporal summation) at 90-min ($r = 0.793$, $n = 10$, $P = 0.019$) and 110-min ($r = 0.833$, $n = 10$, $P = 0.010$) post-capsaicin. Also, in the PTSD group, IL-6 correlated with evoked pain mediated temporal summation at 110 min ($r = 0.746$, $n = 10$, $P = 0.034$) and approached significance at 70 min ($r = 0.692$, $n = 10$, $P = 0.057$) and 90 min ($R = 0.659$, $n = 10$, $P = 0.076$). The correlation with IL-1β also approached significance at 110 min ($r = 0.705$, $n = 10$, $P = 0.051$). There were no significant correlations between the CSF cytokines and temporal summation in the CC group (Table 4).

4. Discussion

PTSD and pain have a well-documented high comorbidity, although the pathophysiological mechanisms linking these conditions are currently poorly understood. We examined changes in CSF concentrations of IL-1β, IL-6, IL-8, IL-10, and TNFα after painful intramuscular capsaicin injection. Capsaicin-stimulated pain elicited cytokine changes in the CSF of both PTSD and CC par-

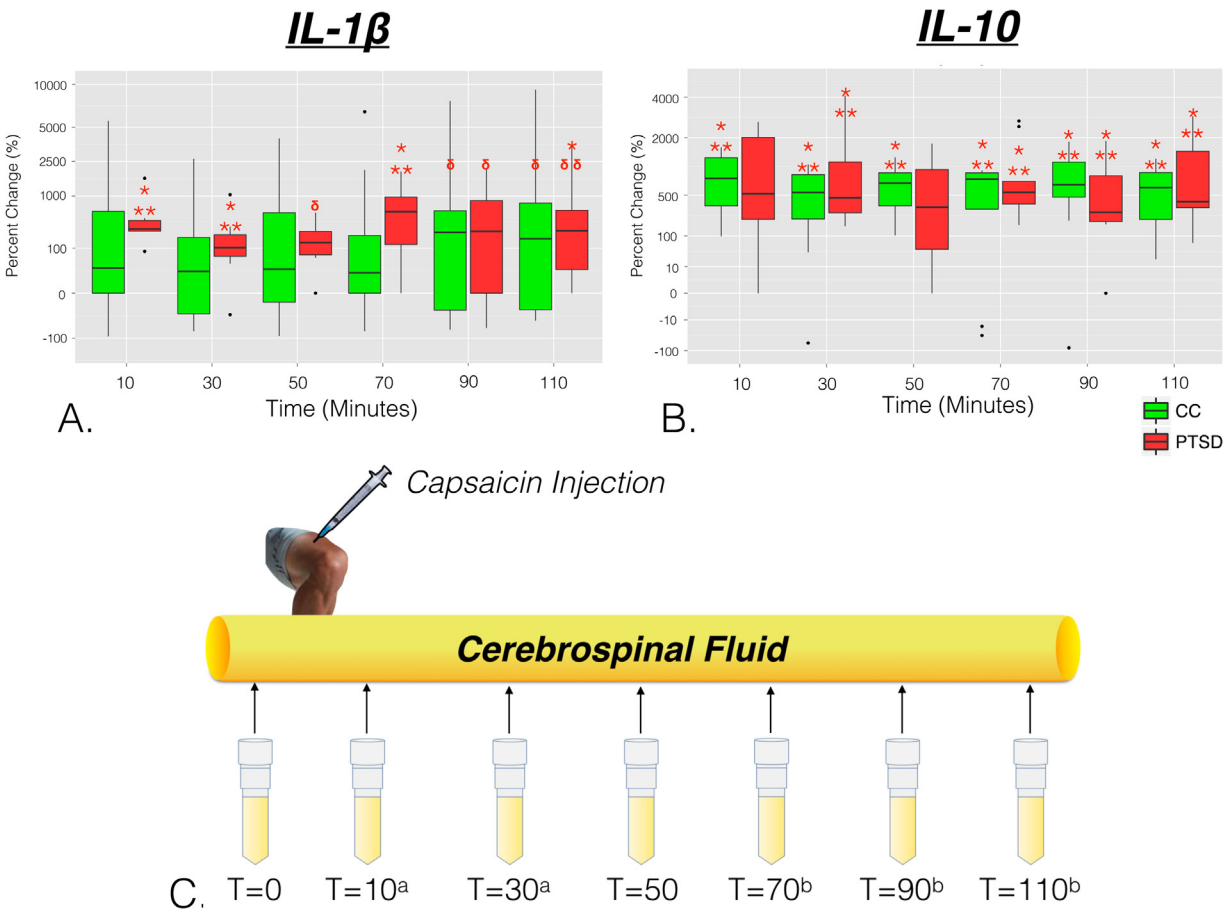

Fig. 2. CSF IL-1β, measured as percent increase, was significantly greater in the PTSD group at 10 min and remained elevated up to 110 min after capsaicin injection (Fig. 2A). In the CC group, IL-1β remained low and showed no significant increase throughout the 110-min measurement ($P < 0.05$) (Fig. 2A). CSF IL-10 percent increase was significantly greater in the CC group at the 10-min measurement and remained elevated throughout all subsequent measurements (Fig. 2B). In contrast, the PTSD group percent increase in IL-10 was not significantly greater until the 30-min measurement and remained consistently elevated only from the 70- to 110-min measurements after capsaicin injection (Fig. 2B). Baseline cytokine levels T = 0 min were not different between groups: CC; (IL-1β) 0.12 (+/−.07) pg/mL, (IL-10) 0.41 (+/−0.28) pg/mL versus PTSD; 0.10 (+/−.01) pg/mL, (IL-10) 0.38 (+/−.35) pg/ml. Panel C. Overview of the baseline CSF draw at T = 0 min followed by serial CSF draws at T = 10 min and then subsequent 20 min intervals i.e., T = 30 min etc. a. T = 10 and T = 30 min spontaneous pain was recorded demarcated. b. At T = 70–110 min Evoked pain measures were recorded. Percent change was obtained by comparing post-capsaicin to pre-capsaicin CSF cytokine levels. Measured with Mann Whitney *U* test and Wilcoxon signed-rank test $^{*}p \leq .05$, $^{\delta}p < .06$. Adjustment for multiple comparisons with the Benjamini–Hochberg False Discovery Rate (FDR) correction $^{**}p \leq .05$, $^{\delta\delta}p < .06$.

Table 3
CSF Cytokine Correlations to Pain Discomfort, Intensity, and Unpleasantness. Pearson's correlation coefficient (and p-values) between each pain measurement and percentage change of various cytokines in cerebrospinal fluid from baseline at each available time point.

CSF Cytokines	Pain Discomfort		Pain Intensity		Unpleasantness	
	PTSD	CC	PTSD	CC	PTSD	CC
TNF-α						
10 min	0.771 (0.073)	0.360 (0.307)	0.754 (0.083**)	−0.254 (0.478)	0.805 (0.053**)	−0.292 (0.412)
30 min	0.692 (0.085)	0.320 (0.367)	0.836 (0.019*,**)	0.132 (0.715)	0.866 (0.012*,**)	−0.014 (0.968)
IL-6						
10 min	0.192 (0.715)	0.237 (0.510)	0.335 (0.516)	−0.421 (0.226)	0.375 (0.463)	−0.437 (0.207)
30 min	−0.268 (0.561)	0.212 (0.557)	0.131 (0.780)	0.193 (0.594)	0.101 (0.830)	−0.337 (0.341)
IL-8						
10 min	0.389 (0.446)	−0.030 (0.934)	0.603 (0.205**)	−0.374 (0.287)	0.508 (0.304)	−0.378 (0.281)
30 min	−0.122 (0.794)	−0.109 (0.765)	0.280 (0.543)	−0.139 (0.701)	0.183 (0.694)	−0.275 (0.442)
IL-1β						
10 min	0.013 (0.980)	0.101 (0.782)	0.207 (0.694)	−0.505 (0.137)	0.148 (0.780)	−0.467 (0.174)
30 min	−0.400 (0.374)	−0.537 (0.110)	−0.018 (0.970)	−0.518 (0.125)	−0.057 (0.904)	−0.486 (0.154)
IL-10						
10 min	0.172 (0.745)	−0.755 (0.012*,**)	0.237 (0.651)	−0.438 (0.205)	0.380 (0.457)	−0.375 (0.285)
30 min	0.313 (0.494)	−0.625 (0.053**)	0.556 (0.195)	−0.737 (0.015*,**)	0.611 (0.145)	−0.357 (0.311**)

PTSD: Post-traumatic stress disorder. CC: Combat controls.
* 0.05 within group correlation to measure.
** <.05 between group Fischer r-to-z transformation.

Table 4
Temporal summation of CSF Cytokines. Pearson's correlation coefficient (and p-values) between each pain measurement and percent change of various cytokines in CSF from baseline at each available time point.

CSF Cytokines	Temporal Summation	
	PTSD	CC
TNF-α		
70 min	0.587 (0.126)	0.540 (0.133)
90 min	0.793 (0.019*)	0.251 (0.484)
110 min	0.833 (0.010$^{*,\delta\delta}$)	0.210 (0.561)
IL-6		
70 min	0.692 (0.057)	−0.072 (0.855)
90 min	0.659 (0.076)	−0.119 (0.743)
110 min	0.746 (0.034*,**)	−0.108 (0.766)
IL-8		
70 min	0.118 (0.780)	0.183 (0.638)
90 min	0.106 (0.803	0.131 (0.718)
110 min	0.311 (0.453)	0.097 (0.790)
IL-1β		
70 min	0.560 (0.148)	−0.142 (0.716)
90 min	0.553 (0.155)	−0.317 (0.372)
110 min	0.705 (0.051$^{\delta,**}$)	−0.320 (0.368)
IL-10		
70 min	−0.179 (0.671)	−0.420 (0.260)
90 min	−0.127 (0.764)	−0.307 (0.389)
110 min	0.239 (0.568)	−0.447 (0.195)

PTSD: post-traumatic stress disorder. CC: combat controls.
* 0.05 within group correlation to measure.
** <.05 between group Fischer r-to-z transformation.
$^{\delta}$ <.06 within group correlation measure.
$^{\delta\delta}$ <.06 between group Fischer r-to-z transformation.

ticipants, although the associated subjective and objective indices of pain differed. Both groups showed a similar increase in TNFα and IL-6 concentrations, but only the PTSD group evinced significantly positive correlations between inflammatory cytokine levels (TNFα) and spontaneously reported pain and the evoked pain measure of temporal summation. In contrast, for the CC group, there was an inverse relationship between levels of the anti-inflammatory cytokine IL-10 and spontaneously reported pain discomfort and intensity, suggesting that the release of higher amounts of IL-10 in the CNS was associated with experiencing less pain. The dynamic changes in the CSF levels of IL-1β and IL-10 also differed significantly over time between the groups, suggesting that there was a dysregulation of pain-associated inflammatory and anti-inflammatory responses in the individuals with PTSD as compared CC group. Specifically, the participants with PTSD showed an immediate increase in the release of the pro-inflammatory cytokine IL-1β and greater variability in the anti-inflammatory cytokine IL-10 levels, which contributed to the observed delay in the IL-10 increment over time. In contrast, the CC group showed an early and sustained increase in IL-10 (Fig. 2B).

To our knowledge, this study is the first to show:

1) An increase in cytokines in CSF in response to intramuscular capsaicin injection in humans.
2) Differences in the temporal dynamics of CSF cytokine responses to pain in individuals with PTSD.
3) A positive correlation in the PTSD group, but not in the CC group, between pro-inflammatory cytokines in CSF and spontaneously reported pain and the evoked pain measure of temporal summation.

Elevated CSF pro-inflammatory cytokine levels indicative of excessive neuroinflammation are thought to be associated with chronic pain states, and have been reported in patients with chronic nociceptive (Lundborg et al., 2010; Backonja et al., 2008; Bjurstrom et al., 2016) as well as neuropathic pain (Kotani et al., 2004). Besides signs of neuroinflammation as reflected by CSF cytokine indices, brain positron emission tomography (PET) scans of patients with chronic back pain show microglial cell specific activation (greater than healthy controls) in: 1) medial thalamic, 2) post central gyrus, and 3) paracentral lobule, suggesting that chronic pain mediated neuroinflammation and central sensitization likely co-occur in both the brain and spinal cord (Loggia et al., 2015). The mechanisms of central sensitization are known to involve neuronal interaction with activated glia cells and astrocytes (Milligan and Watkins, 2009; Watkins and Maier, 2005). Activated microglia and astrocytes release pro-inflammatory cytokines/chemokines such as TNFα, IL-1β, IL-6 and IL-8, in addition to glutamate and substance P, which collectively are known to amplify pain (Milligan and Watkins, 2009; Watkins and Maier, 2005).

In this study, both the PTSD and CC groups showed immediate and sustained elevation of CSF levels of TNFα, IL-6 and IL-8. TNFα and IL-6 were significantly correlated with evoked pain measures in the PTSD group, whereas there was no correlation between pro-inflammatory cytokine concentrations and any pain measure in the CC group. CNS release of TNFα can lower pain thresholds by modulating the number of neuronal membrane ion channels and their functional activity (Choi et al., 2010), which can result in hyper-excitable pain circuits within dorsal horn (Reeve et al., 2000; Park et al., 2011; Kawasaki et al., 2008). TNFα binding to the microglial TNFα receptor (TNFα-R) stimulates p38 mitogen-activated protein kinase (P38 MAPK) phosphorylation and activation. The activated P38 MAPK cascade activates nuclear factor kappa B (NFκB) that, in turn, promotes the secretion of multiple cytokines (IL-6, IL-8, TNFα, and IL-1β) that mediate pain facilitation (Ji and Suter, 2007). In our study, the release of TNFα, IL-6, and IL-8 into CSF increased immediately after the intramuscular painful stimulus, and both TNFα and IL-6 responses were significantly correlated with spontaneously reported pain and evoked temporal summation measures in the PTSD, but not in the CC group.

Of particular note was the significantly increased immediate and sustained release of IL-1β into the CSF after intramuscular capsaicin injection in the PTSD, but not in the CC group. Similar to TNFα, both inflammatory and neuropathic pain models have shown that IL-1β is synthesized and secreted from astrocytes, neurons, and microglia. IL-1β enhances activity in the lamina II of the dorsal horn by multiple mechanisms (Reeve et al., 2000; Park et al., 2011; Kawasaki et al., 2008) and is known to amplify pain signaling. Increased IL-1β secretion in the PTSD-group may enhance pain nociception, which is a conclusion supported in our study by the following: 1) increases in spontaneously reported pain intensity and unpleasantness and 2) the increased evoked pain measure of temporal summation. Moreover, the CSF IL-1β elevation in the PTSD group was more highly correlated with temporal summation at 110 min after capsaicin as compared to the control group and the correlation approached a significant within-group value at that time point (.705, $P = 0.06$). While CSF TNFα levels were increased in both groups, we hypothesize that downstream TNFα signaling in glial cells that regulates the immediate activation of IL-1β and the NFκB-mediated secretion of multiple cytokines (Ji and Suter, 2007) may be differentially regulated in the PTSD and CC groups. We further postulate that the TNFα signal-mediated IL-1β translation and secretion in the CNS may by dysregulated in PTSD, which would contribute to the observed pain facilitation.

Interestingly, the PTSD group showed a delayed increment in the release of the anti-inflammatory cytokine IL-10, as compared to the immediate and sustained increase of IL-10 in the controls. Upregulation of TNFα is known to elicit a compensatory release of anti-inflammatory cytokines such as IL-10. IL-10 then activates STAT3 signaling by IL-10 receptors 1 and 2, resulting in decreased synthesis of multiple cytokines, including TNFα, IL-1β, and IL-6 (de

Waal Malefyt et al., 1991; Murray, 2005; Sabat et al., 2010). Supporting the anti-nociceptive and anti-inflammatory properties of IL-10, the administration of exogenous IL-10: 1) decreases TNFα induced hyperalgesia and 2) produces prolonged reversal of neuropathic pain when administered in the intrathecal space (Milligan et al., 2006a; Milligan et al., 2006b; Wagner et al., 1998). Clinical research has documented further that there are significantly lower levels of IL-10 in the CSF of patients with chronic pain as compared to age-matched healthy controls (Backonja et al., 2008). It was only in the CC group that there was an inverse correlation between the CSF IL-10 concentrations and spontaneously reported pain intensity and discomfort (Table 3). Based on our current data, we hypothesize that IL-10 may directly modulate the painful experience by modulating the inflammatory response or by a recently identified immunoproteostasis mechanism (Chakrabarty et al., 2015).

5. Study limitations

A clear limitation of this study was the multiple measures and small sample size, and thus the study should be viewed as a pilot in need of replication. However, we applied the Benjamini-Hochberg False Discovery Rate correction to further verify original analyses. Further we believe the strict inclusion and exclusion criteria help to substantiate the relevance of these observations to combat veterans with PTSD, but larger clinical studies are needed to confirm the robustness and ability to generalize more broadly to other pain-related syndromes. This project was not intended to be a clinical study with a treatment arm, and thus it should also be acknowledged that the lack of long-term patient follow-up clearly limits our ability to link these acute findings to the more sustained chronic pain experienced by individuals with PTSD. Longitudinal research is now needed to investigate whether central neuroinflammation and pain sensitization are predictive of the vulnerability to enhanced chronic pain in individuals with PTSD.

6. Conclusion

For the first time, to our knowledge, we show that adults with PTSD secrete abnormally high levels of the pro-inflammatory cytokine IL-1β into their CSF directly after intramuscular capsaicin injection, and have a delayed increase in release of the anti-inflammatory cytokine IL-10. The central neuro-inflammatory response and amplified central sensitization suggest that mechanisms described in the basic science literature (e.g., microglial and astrocyte dysregulation) may play a significant role in chronic pain syndromes known to be comorbid with PTSD. Future investigations of inflammatory activity in the CNS may potentially lead to new therapeutic options for treating these co-occurring chronic pain symptoms.

Conflicts of interest

None.

Contributors

Dr. Imanuel Lerman wrote the manuscript and devised the analysis. Drs. Dewleen Baker, Richard Hauger, Piyush Patel, Christopher Coe and Bryan Davis edited the manuscript. Dr. Dewleen Baker, Piyush Patel and Tobias Moeller Bertram carried out the study design. Dr. Tobias Moeller Bertram carried out the study. James Proudfoot carried out the statistical analysis. All authors had access to the manuscript and reviewed it.

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