




Baroreflex dysfunction and augmented sympathetic nerve responses during mental stress in veterans with post-traumatic stress disorder

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Key points

- Patients with post-traumatic stress disorder (PTSD) are at a significantly higher risk of developing hypertension and cardiovascular disease. The mechanisms underlying this increased risk are not known.
- Studies have suggested that PTSD patients have an overactive sympathetic nervous system (SNS) that could contribute to cardiovascular risk; however, sympathetic function has not previously been rigorously evaluated in PTSD patients.
- Using direct measurements of sympathetic nerve activity and pharmacological manipulation of blood pressure, we show that veterans with PTSD have augmented SNS and haemodynamic reactivity during both combat-related and non-combat related mental stress, impaired sympathetic and cardiovascular baroreflex sensitivity, and increased inflammation.
- Identifying the mechanisms contributing to increased cardiovascular (CV) risk in PTSD will pave the way for developing interventions to improve sympathetic function and reduce CV risk in these patients.

Abstract Post-traumatic stress disorder (PTSD) is associated with increased cardiovascular (CV) risk. We tested the hypothesis that PTSD patients have augmented sympathetic nervous system (SNS) and haemodynamic reactivity during mental stress, as well as impaired arterial baroreflex sensitivity (BRS). Fourteen otherwise healthy Veterans with combat-related PTSD were compared with 14 matched Controls without PTSD. Muscle sympathetic nerve activity (MSNA), continuous blood pressure (BP) and electrocardiography were measured at baseline, as well as during two types of mental stress: combat-related mental stress using virtual reality combat exposure (VRCE) and non-combat related stress using mental arithmetic (MA). A cold pressor test (CPT) was administered for comparison. BRS was tested using pharmacological manipulation of BP via the Modified Oxford technique at rest and during VRCE. Blood samples were analysed for inflammatory biomarkers. Baseline characteristics, MSNA and haemodynamics were similar between the groups. In PTSD vs. Controls, MSNA ($+8.2 \pm 1.0$ vs. $+1.2 \pm 1.3$ bursts min^{-1} , $P < 0.001$) and heart rate responses ($+3.2 \pm 1.1$ vs. -2.3 ± 1.0 beats min^{-1} , $P = 0.003$) were significantly augmented during VRCE. Similarly, in PTSD vs. Controls, MSNA ($+21.0 \pm 2.6$ vs. $+6.7 \pm 1.5$ bursts min^{-1} , $P < 0.001$) and diastolic BP responses ($+6.3 \pm 1.0$ vs. $+3.5 \pm 1.0$ mmHg, $P = 0.011$) were significantly augmented during MA but not during CPT ($P =$ not significant). In the PTSD group, sympathetic BRS (-1.2 ± 0.2 vs. -2.0 ± 0.3 burst incidence mmHg^{-1} , $P = 0.026$)

and cardiovagal BRS (9.5 ± 1.4 vs. 23.6 ± 4.3 ms mmHg⁻¹, $P = 0.008$) were significantly blunted at rest. PTSD patients had significantly higher highly sensitive-C-reactive protein levels compared to Controls (2.1 ± 0.4 vs. 1.0 ± 0.3 mg L⁻¹, $P = 0.047$). Augmented SNS and haemodynamic responses to mental stress, blunted BRS and inflammation may contribute to an increased CV risk in PTSD.

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Abbreviations AU, arbitrary unit; BP, blood pressure; BRS, baroreflex sensitivity; CAPS, Clinician Administered PTSD Scale; CPT, cold pressor test; CV, cardiovascular; DBP, diastolic blood pressure; hsCRP, highly sensitive C-reactive protein; IL, interleukin; MA, mental arithmetic; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; OEF, Operation Enduring Freedom; OIF, Operation Iraqi Freedom; OND, Operation New Dawn; PCL-M, PTSD Checklist – Military Version; PTSD, post-traumatic stress disorder; SBP, systolic blood pressure; SNA, sympathetic nerve activity; SNS, sympathetic nervous system; VRCE, virtual reality combat exposure.

Introduction

Post-traumatic stress disorder (PTSD) is a highly prevalent mental health condition amongst both military Veterans and the general population in those who have been exposed to psychologically traumatic events. This disorder is associated with a significantly increased risk for the development of cardiovascular (CV) disease and hypertension (Kang *et al.* 2006; Bedi & Arora, 2007; Kubzansky *et al.* 2007; Boscarino, 2008; Cohen *et al.* 2009; Kubzansky *et al.* 2009; Edmondson *et al.* 2013; Vaccarino *et al.* 2013; Beristianos *et al.* 2016). Multiple large epidemiological studies have demonstrated an independent link between PTSD and the risk of CV disease and hypertension (Kang *et al.* 2006; Bedi & Arora, 2007; Kubzansky *et al.* 2007; Boscarino, 2008; Cohen *et al.* 2009; Kubzansky *et al.* 2009; Edmondson *et al.* 2013; Vaccarino *et al.* 2013; Beristianos *et al.* 2016); however, the physiological mechanisms underlying this association have not been determined. Prior studies have demonstrated a decrease in low frequency heart rate (HR) variability (Cohen *et al.* 1998; Chang *et al.* 2013; Shah *et al.* 2013) and increased urinary noradrenaline levels (Wingenfeld *et al.* 2015) in PTSD patients, suggestive of heightened sympathetic nervous system (SNS) activity, which could be a potential mechanism contributing to an increased risk of CV disease and hypertension. Despite these findings, direct assessments of sympathetic nerve activity (SNA) have never previously been measured in this population or used to assess SNS and haemodynamic reactivity to mental stress, nor have they been used to investigate aberrancies in SNS regulation such as blunted arterial baroreflex sensitivity that could contribute to chronic SNS overactivity and subsequently to an increased CV risk.

The present study aimed to investigate haemodynamic and SNS reactivity and regulation at rest and during mental stress in young PTSD patients, prior to the development of hypertension and CV disease. The study

population included young, otherwise healthy military Veterans with and without PTSD from the wars in Iraq and Afghanistan (Operation Enduring Freedom, Operation Iraqi Freedom and Operation New Dawn; OEF/OIF/OND, respectively). We tested the hypothesis that Veterans with PTSD have significantly greater SNS and haemodynamic responses during mental stress associated with their PTSD symptoms (i.e. combat-related mental stress). We further hypothesized that these augmented sympathetic and haemodynamic responses extend to other forms of mental stress (i.e. non-combat-related mental stress) and are mediated by blunted arterial baroreflex sensitivity (BRS) and increased inflammation. Characterizing SNS reactivity and regulation in PTSD could uncover key physiological alterations in autonomic function in PTSD patients, which could, in turn, expedite the development of therapies to mitigate SNS overactivation and thereby improve long-term CV risk in these patients.

Methods

Ethical approval

This study conformed to the standards set by the *Declaration of Helsinki* and was approved by the Emory University Institutional Review Board (IRB #54697) and the Atlanta VA Medical Centre Research and Development Committee. All participants provided signed informed consent for study participation via forms approved by the aforementioned regulatory committees.

Study population

The study population consisted of 28 participants: 14 patients with PTSD and 14 age-matched Controls without PTSD. All study participants (both PTSD and Controls) were physically healthy OEF/OIF/OND Veterans, without hypertension or other medical comorbidities. Participants

were recruited from mental health clinics serving OEF/OIF/OND Veterans at the Atlanta VA Medical Center and surrounding clinics. Exclusion criteria for all participants included hypertension, smoking, diabetes, heart or vascular disease, illicit drug use, excessive alcohol use (> 2 drinks per day), hyperlipidaemia, autonomic dysfunction, medications known to affect SNS (clonidine, β -blockers, angiotensin-converting enzyme inhibitors), treatment with monoamine oxidase inhibitors and any serious systemic disease. Participants with a prior documented diagnosis of PTSD from medical records were recruited to the PTSD group and those without a prior diagnosis were recruited to the Control group. The diagnosis of PTSD was confirmed in PTSD patients and excluded in Controls using the Clinician Administered PTSD Scale for DSM (CAPS)-IV (Blake *et al.* 1995) or the PTSD Checklist – Military Version (PCL-M) (Blanchard *et al.* 1996). PTSD patients required a CAPS severity score of ≥ 45 to confirm the presence of PTSD (Weathers *et al.* 2001).

Measurements and procedures

Blood pressure. Beat-to-beat arterial BP was measured continuously and non-invasively using digital pulse photoplethysmography (CNAP; CNSystems, Graz, Austria). Absolute values of BP were calibrated with upper arm BP readings at the start and every 15 min throughout the study. This device has been validated to reflect accurate absolute and beat-to-beat fluctuations in BP when compared to invasive intra-arterial catheter readings (Jelezov *et al.* 2010; Ilies *et al.* 2012).

Muscle sympathetic nerve activity. Multiunit post-ganglionic sympathetic nerve activity directed to muscle (MSNA) was recorded from the peroneal nerve using microneurography, as described previously (Vallbo *et al.* 1979; Wallin & Fagius, 1988). A tungsten microelectrode (tip diameter 5–15 μm ; Bioengineering, University of Iowa, Iowa City, IA, USA) was inserted into the nerve and a reference microelectrode was inserted in close proximity. The signals were amplified (total gain 50 000–100 000), filtered (700–2000 Hz), rectified and integrated (time constant 0.1 s) to obtain a mean voltage display of sympathetic nerve activity (Nerve Traffic Analyzer, Model 662C-4; Bioengineering, University of Iowa). The neurogram was recorded using LabChart 7 (PowerLab 16sp; ADInstruments, Sydney, Australia) along with continuous ECG recordings using a bioamp. All MSNA recordings met previously established criteria (Delius *et al.* 1972a, b; Mano *et al.* 2006) and were analysed by a single investigator who was blinded to the participant's status as a Control or patient. MSNA was expressed as burst frequency (bursts per minute) and total activity (arbitrary units per minute).

Virtual reality combat exposure (VRCE). Participants wore head-mounted goggles with peripheral display and stereo earphones for auditory input (SamSung Gear VR, Ridgefield Park, NJ, USA). These goggles displayed computer-generated first-person battle scenarios from Iraq (Virtual Iraq for OIF/OND) and Afghanistan (Virtual Afghanistan for OEF) (Rizzo *et al.* 2010, 2011; Rothbaum *et al.* 2010, 2014; McLay *et al.* 2011; Roy *et al.* 2013) for a duration of 3 min, followed by an additional 4 min during concomitant arterial baroreflex testing. These VRCE clips have been shown to increase reactivity of psychophysiological measures, such as acoustic startle, fear-enhanced startle and galvanic skin response (Roy *et al.* 2013), are associated with elevations in startle reactivity and salivary cortisol levels (Rothbaum *et al.* 2014; Norrholm *et al.* 2016) and have been reported by patients as immersive and anxiogenic.

Mental stress via mental arithmetic (MA). Participants were asked to serially subtract a one or two digit number from a three or four digit number and were urged to do so as quickly and accurately as possible, for a duration of 3 min (Carter *et al.* 2005; Carter & Ray, 2009). An investigator used flash cards with a three or four digit number and were urged by two additional study team members in white coats to answer 'faster' and 'get it right'. This test has been shown to induce mental stress and increase MSNA (by ~ 5 –15 bursts min^{-1}) and BP (by ~ 10 –16 mmHg), as well as HR (by ~ 16 –24 beats min^{-1}), in both healthy humans and patient groups (Matsukawa *et al.* 1991b; Noll *et al.* 1996; Dishman *et al.* 2003; Kuniyoshi *et al.* 2003; Carter *et al.* 2013).

Arterial BRS testing using the Modified Oxford technique. The gold standard method for evaluation of arterial BRS is performed by measuring changes in MSNA and R-R interval during arterial BP changes induced by nitroprusside and phenylephrine (Rudas *et al.* 1999). Sodium nitroprusside (100 μg in 10 mL of normal saline (NS)) was bolused through an antecubital i.v. catheter, followed 60 s later by a bolus of phenylephrine (150 μg in 10 mL of NS) during continuous MSNA, ECG and haemodynamic monitoring. Medications were room temperature at the time of administration. These medications induce a decrease of ~ 15 mmHg, followed by an increase of ~ 15 mmHg in arterial BP.

Cold pressor test (CPT). CPT is a standard human physiology research technique with a powerful sympathoexcitatory and pressor effect that was performed by submerging the participant's hand up to the wrist in an ice-water slurry (~ 0 – 1°C) for 1 min (Wood *et al.* 1984; Victor *et al.* 1987; Fagius *et al.* 1989; Cui *et al.* 2002; Dishman *et al.* 2003; Park *et al.* 2012). SNS activation during CPT is not regulated by baroreflexes

(Cui *et al.* 2002) and allows for discernment between baroreflex-mediated *vs.* generalized or non-baroreflex mediated increases in SNS activation.

Inflammatory biomarkers. Blood was drawn in all subjects at baseline for the following inflammatory biomarkers: highly sensitive C-reactive protein (hsCRP), interleukin (IL)-2 and IL-6. hsCRP was determined by immunoturbidometric method on the Beckman AU460 (Beckman Diagnostics, LaBrea, CA, USA) using reagent kits from Sekisui Diagnostics (Lexington, MA, USA). IL-2 and IL-6 measurements were determined using enzyme-linked immunosorbent assay kits from R&D Systems (Minneapolis, MN, USA).

CAPS-IV and the PCL-M. The diagnosis of PTSD was confirmed in PTSD patients and excluded in Controls using CAPS-IV or PCL-M. CAPS-IV was administered and interpreted by a single trained investigator for all participants. A severity score of ≥ 45 on either survey was required to confirm the presence of PTSD (Weathers & Ruscio, 1999; Weathers *et al.* 2001).

Experimental protocol

Screening visits. All participants presented for one or two screening visits prior to experimental procedures. During the screening visit(s), BP was taken a total of three times (separated by 5 min) and averaged to ensure the absence of hypertension and obtain baseline values. CAPS-IV or the PCL-M was performed during the screening visit to confirm the diagnosis of PTSD in patients and to exclude the diagnosis of PTSD in Controls.

Experimental study visit. All participants were studied in the morning, after abstaining from food, caffeine and alcohol for at least 12 h, as well as smoking and exercise for at least 24 h. The study room was quiet, semidark and temperate ($\sim 21^\circ\text{C}$). Participants were placed in a supine position on a comfortable stretcher. A 20 gauge i.v. catheter was placed into the antecubital vein of the arm and blood was obtained for inflammatory biomarkers. Finger cuffs were fitted and placed on the fingers of the dominant arm for continuous beat-to-beat arterial BP measurements, and an upper arm cuff was placed for intermittent automatic calibrations with the finger cuffs. ECG patch electrodes were placed for continuous ECG recordings and a belt-type sensor was placed for monitoring continuous respiratory rates. The leg was positioned for microneurography, and the tungsten microelectrode was inserted and manipulated to obtain a satisfactory nerve recording. After 10 min of rest, baseline BP, HR and MSNA were recorded continuously for 15 min. After baseline measurements, BRS testing was performed by infusing i.v. boluses of 100 μg of nitroprusside, followed

60 s later by 150 μg of phenylephrine, as described above. After 30 min of rest to ensure sufficient washout of drugs and return to baseline conditions, participants underwent mental arithmetic for 3 min and VRCE for 3 min. The order of these manoeuvres was randomized and separated by 30 min of rest. In a subset of participants, VRCE was extended for an additional 4 min, during which time BRS testing was repeated by infusing nitroprusside and phenylephrine during concomitant VRCE. After 30 min of rest, the cold pressor test was performed for 1 min.

Data analysis

MSNA. MSNA, BP and ECG data from the study visit were exported from Labchart 7 into WinCPRS (Absolute Aliens, Turku, Finland) for analysis. The software automatically marks the R-waves from the continuous ECG recording that was then reviewed and edited manually if needed. MSNA bursts were detected and marked by the software using the criteria: 3:1 burst to noise ratio within a 0.5 s search window, with an average latency of 1.2–1.4 s from the previous R-wave. The MSNA neurograms were also inspected for accuracy of detection by a single investigator (JP) and edited if needed. For total activity measurements, burst amplitudes were normalized by assigning the largest burst amplitude during at least 5 min of baseline recordings to be equated to a measurement of 1000 arbitrary units (AU). MSNA was expressed as burst frequency (bursts min^{-1}), burst incidence [bursts (100 heart beats) $^{-1}$], and total activity (AU min^{-1}), which is a sum of the amplitudes of normalized bursts min^{-1} .

Assessment of BRS. Sympathetic BRS was defined as the slope of the linear regression between values of MSNA (burst incidence and total activity) on the y -axis and the diastolic BP on the x -axis derived during the 2 min of drug boluses and following 2 min of data. Linear regression was performed using the WinCPRS program that grouped diastolic BP into 3 mmHg bins and then averaged the MSNA burst incidence and normalized total activity values within each bin. Cardiovascular BRS was assessed as the slope of the linear regression between R-R intervals measured from the continuous ECG recording and the systolic BP during drug infusions. Only regressions with a coefficient of determination (R^2) > 0.70 were included in the analysis. The operating point around which the arterial baroreflex operates was derived from the mean resting systolic and diastolic blood pressure, with the corresponding mean R-R interval, MSNA burst incidence and normalized MSNA total activity during at least 5 min of baseline recordings.

Statistical analysis. Statistical analysis was performed using SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA). Baseline characteristics and baroreflex slopes were

Table 1. Baseline characteristics and measurements

	PTSD (n = 14)	Controls (n = 14)	P value
Age	34.4 ± 1.7	32.2 ± 1.5	0.331
Sex (male/female)	12/2	13/1	1.0
Race (black/white)	10/4	12/2	0.645
Weight (kg)	93.8 ± 6.2	88.1 ± 4.8	0.416
BMI (kg m ⁻²)	30.1 ± 1.6	28.4 ± 1.2	0.393
Depression (yes/no)	2/12	2/12	1.0
SSRI (yes/no)	4/10	2/12	0.645
SBP (mmHg)	133 ± 3	128 ± 4	0.351
DBP (mmHg)	81 ± 2	77 ± 4	0.389
MAP (mmHg)	99 ± 3	95 ± 4	0.451
HR (beats min ⁻¹)	68 ± 4	62 ± 2	0.267
MSNA (bursts min ⁻¹)	19.8 ± 1.3	19.9 ± 2.5	0.952
MSNA [bursts (100 heart beats) ⁻¹]	32.2 ± 3.1	32.7 ± 4.2	0.929
MSNA (AU min ⁻¹)	9934 ± 1022	10950 ± 1648	0.605
CAPS-IV score	81 ± 9	39 ± 3	0.001

Baseline measurements of body mass index (BMI), SBP, DBP, HR and MSNA calculated as burst frequency (bursts min⁻¹) and burst incidence were compared between PTSD and Controls without PTSD. SSRI, serotonin reuptake inhibitor. CAPS-IV was used to confirm PTSD diagnosis in subjects and exclude PTSD in Controls. Values are the mean ± SE. **P* < 0.05 was considered statistically significant.

compared between the two groups (PTSD vs. Controls) using two-tailed two-sample *t* tests for continuous variables and Fisher's exact test for categorical variables. Repeated measures ANOVA was performed using PROC GLM to determine differences between the groups with respect to change in MSNA, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and HR with time during each intervention: MA, VRCE and CPT. When the overall *F* test was significant, the contrast option for *post hoc* analysis was used to compare the difference from baseline to each time point between the groups. For correlation analyses, the *t* statistic for correlation coefficient was used to evaluate whether or not a linear relationship existed between hsCRP levels and MSNA responses, as well as cardiovagal and sympathetic baroreflex sensitivity. Results are expressed as the mean ± SEM. *P* < 0.05 was considered statistically significant.

Results

Baseline characteristics

There was no significant difference in age, sex, race, weight or body mass index between groups (Table 1). Groups were well-matched for age with a mean ± SEM age of 34.4 ± 1.7 years in PTSD patients and 32.2 ± 1.5 years in

Controls. The majority of participants in each group were African-American and male. There were no significant differences in the presence of comorbid depression or use of serotonin reuptake inhibitors between the groups. Mean baseline blood pressure and HR were similar between the groups. There was no significant difference in resting MSNA quantified as burst frequency, burst incidence or total activity between the groups. As expected, the mean CAPS-IV score was significantly higher amongst the PTSD patients compared to Controls without PTSD.

VRCE

These experiments aimed to determine whether the haemodynamic and MSNA responses during combat-related mental stress (i.e. mental stress related to PTSD symptoms) were augmented amongst young Veterans with PTSD. During 3 min of VRCE, there was no statistically significant difference in the SBP, DBP and MAP, responses during VRCE between the groups (Fig. 1A–C)(Table 2). However, there was a small but significantly greater increase in HR during VRCE amongst PTSD patients compared to Controls (ANOVA, *F* test, *P* = 0.003) (Fig. 1D). There was also a significantly greater increase in MSNA quantified as change in burst frequency (ANOVA, *F* test, *P* < 0.001) and total activity (ANOVA, *F* test, *P* < 0.001) (Fig. 1E and F) amongst PTSD patients compared to Controls.

MA

These experiments aimed to determine whether the haemodynamic and MSNA responses during non-combat-related mental stress (i.e. mental stress that was not related to PTSD symptoms) were augmented amongst young Veterans with PTSD. During 3 min of MA, there was no significant difference in the SBP response amongst PTSD patients compared to Controls (Fig. 2A) (Table 2). However, there was a significantly greater increase in the DBP response amongst PTSD patients (ANOVA, *F* test, *P* = 0.011) (Fig. 2B), although there was no statistically significant difference in the MAP and HR responses during MA between the groups (Fig. 2C and D). There was a significantly greater increase in MSNA quantified as change in burst frequency (ANOVA, *F* test, *P* < 0.001) amongst PTSD patients compared to Controls (Fig. 2E) and a trend towards a significant difference when quantified as change in MSNA total activity (ANOVA, *F* test, *P* = 0.056) (Fig. 2F).

CPT

These experiments aimed to determine whether there was a difference in the haemodynamic and MSNA responses between PTSD patients and Controls during a non-mental

stress type of sympathoexcitatory stimulus that is not mediated by arterial baroreflexes (Cui *et al.* 2002) for comparison. During 1 min of CPT, there was no significant difference in the MSNA response quantified as the change in burst frequency or change in total activity between the PTSD and Control groups (Fig. 3A and B) (Table 2). There was also no significant difference in BP and HR responses during CPT between the groups (Fig. 3C and D).

Arterial BRS

Sympathetic BRS was quantified as the slope of the linear relationship between MSNA burst incidence and diastolic BP during pharmacological manipulation of BP. This standard quantification method controls for potential differences in HR responses during baroreflex testing. At baseline, the mean slope of the individual linear regressions between MSNA burst incidence and diastolic BP was significantly less steep in PTSD patients *vs.* Controls, demonstrating decreased sympathetic BRS in PTSD patients ($P = 0.026$) (Fig. 4A and B). Similarly, under baseline conditions, the mean slope of the individual linear regressions between RRI and systolic BP was significantly less steep in PTSD patients *vs.* Controls, demonstrating decreased cardiovagal BRS in the PTSD group ($P = 0.008$) (Fig. 4C and D).

Arterial baroreflex sensitivity was repeated in a subset of PTSD ($N = 9$) and Controls ($N = 11$) during concomitant VRCE. Several of the participants in each group did not have repeat baroreflex testing as a result of side effects during IV nitroprusside administration, including headache, palpitations and hypotension. There was no difference in baseline BRS in the subset of Controls and PTSD subjects that had repeat baroreflex testing. During VRCE, there was no difference in the mean slope of the individual linear regressions between MSNA burst incidence and diastolic BP between PTSD patients and Controls, demonstrating no significant difference in sympathetic BRS during VRCE between the groups ($P = 0.135$) (Fig. 5A and B). However, the mean slope of the individual linear regressions between the RRI and systolic BP was significantly less steep in PTSD patients *vs.* Controls, demonstrating decreased cardiovagal BRS in PTSD patients during VRCE ($P = 0.014$) (Fig. 5C and D).

Inflammatory biomarkers

PTSD patients had significantly elevated hsCRP levels (PTSD *vs.* Controls, 2.1 ± 0.4 *vs.* 1.0 ± 0.3 mg L⁻¹, $P = 0.047$) (Fig. 6A). There was no significant difference in IL-2 or IL-6 levels between the groups (Fig. 6B and C). There was a significant linear relationship between

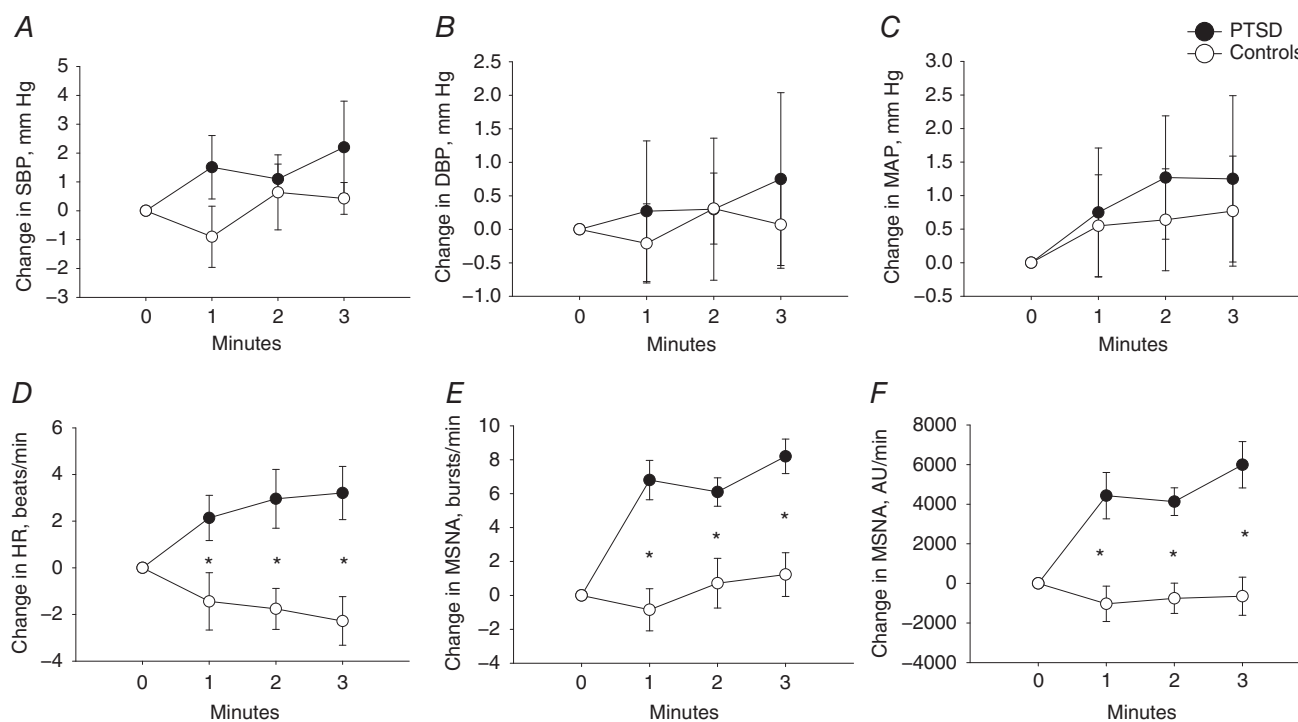


Figure 1. Hemodynamic and sympathetic reactivity during virtual reality combat exposure

Change in SBP (A), DBP (B), mean arterial pressure (MAP) (C), heart rate (HR) (D), muscle sympathetic nerve activity (MSNA) quantified as burst frequency (bursts/min) (E) and total activity (AU/min) (F) during 3 min of virtual reality combat exposure in PTSD patients (filled circles) and Controls (open circles). * $P < 0.05$ between PTSD and Control groups.

Table 2. Haemodynamic and sympathetic nerve responses during sympathoexcitation in PTSD and Controls

	VRCE		MA		CPT	
	M0	M3	M0	M3	M0	M1
SBP (mmHg)						
PTSD	129 ± 4	131 ± 4	127 ± 4	134 ± 4	123 ± 4	128 ± 6
Controls	127 ± 6	128 ± 6	129 ± 5	132 ± 5	120 ± 5	120 ± 6
DBP (mmHg)						
PTSD	74 ± 2	75 ± 2	73 ± 2	79 ± 3	77 ± 3	83 ± 3
Controls	77 ± 4	77 ± 5	79 ± 4	82 ± 5	73 ± 4	78 ± 4
MAP (mmHg)						
PTSD	93 ± 2	94 ± 2	93 ± 2	99 ± 3	94 ± 2	101 ± 3
Controls	95 ± 5	96 ± 5	97 ± 5	101 ± 5	91 ± 4	96 ± 5
HR (beats min ⁻¹)						
PTSD	65 ± 3	68 ± 3*	64 ± 4	71 ± 4	68 ± 4	75 ± 3
Controls	66 ± 5	64 ± 5	67 ± 4	70 ± 5	65 ± 4	73 ± 5
MSNA (bursts min ⁻¹)						
PTSD	19.3 ± 1.6	27.6 ± 1.9*	17.4 ± 1.4	38.0 ± 3.2*	24.5 ± 1.7	31.8 ± 2.0
Controls	22.3 ± 5.0	23.5 ± 4.9	24.5 ± 3.9	30.5 ± 3.2	22.5 ± 3.9	34.2 ± 5.9
MSNA (AU min ⁻¹)						
PTSD	12960 ± 1082	17873 ± 2448*	8814 ± 1305	25629 ± 4206	15993 ± 1898	26814 ± 2636
Controls	11855 ± 2599	11207 ± 3611	13464 ± 3151	20015 ± 3082	13517 ± 2950	21478 ± 4445

SBP, DBP, MAP, HR and MSNA quantified as burst frequency (bursts min⁻¹) and total activity (AU min⁻¹) at rest (minute 0; M0) and during minute 3 (M3) of VRCE and MA, as well as during minute 1 (M1) of CPT in PTSD vs. Controls. Values are the mean ± SE. *Significant difference in response between PTSD and Control groups ($P < 0.05$).

hsCRP levels and MSNA total activity response to VRCE ($P = 0.011$, $R = 0.556$). There were no significant correlations between hsCRP and MSNA responses during MA, nor to cardiovagal or sympathetic BRS.

Discussion

The present study focused on a relatively young population of Veterans from the current combat operations in Iraq and Afghanistan without hypertension or CV disease. The aim was to identify pre-existing biological mechanisms that are present in young individuals who are currently healthy but at increased risk of CV disease and hypertension by virtue of having PTSD (Cohen *et al.* 2009). More than 2 000 000 OEF/OIF/OND service members have been deployed to Iraq and Afghanistan over the past 15 years and are returning with high rates of PTSD, estimated at up to 20% post-deployment (Hoge *et al.* 2004). PTSD is also common in the civilian population (Bedi & Arora, 2007) and is associated with significant impairment, making PTSD a major public health problem (Kessler, 2000). One less well-recognized but highly significant consequence of PTSD is an increased risk of hypertension and CV disease (Brudey *et al.* 2015). In a prospective study that included 562 twins discordant for PTSD, those with PTSD had more than two-fold greater risk of developing coronary heart disease than their counterparts without PTSD, and this association

remained significant even after controlling for lifestyle factors, comorbidities and major depression (Vaccarino *et al.* 2013). Data from the US National Comorbidity Survey showed that PTSD patients had a 2.9-fold greater risk for developing hypertension (Kibler *et al.* 2009) and multivariate analyses that included over 300 000 OEF/OIF Veterans showed that those with PTSD had a 59% higher chance of developing hypertension (Cohen *et al.* 2009). In a prospective study of over 4000 Vietnam Veterans without CV disease at baseline, those with PTSD had a 2.2-fold greater risk of CV mortality determined using a multivariate model that adjusted for comorbid conditions, lifestyle and combat exposure (Boscarino, 2008). In older Veterans without CV disease at baseline, PTSD was associated with a significantly greater risk of developing CV disease, chronic heart failure, myocardial infarction and peripheral vascular disease (Beristianos *et al.* 2016). Thus, multiple large epidemiological studies demonstrate that PTSD is significantly and independently associated with an increased risk of hypertension, CV disease and mortality.

The mechanisms underlying the association between PTSD and CV risk have not been fully determined. PTSD patients have chronic ongoing mental stress that may lead to biological changes as a result of allostasis. Allostasis (Bedi & Arora, 2007) refers to physiological adjustments made to meet demands from external challenges. Such allostatic responses are engaged during times of stress

and include activation of the SNS, which is initially adaptive. However, we hypothesized that exposure to extreme stress with subsequent development of PTSD results in chronic physiological changes as a result of high 'allostatic load', including chronic elevations of SNS activity, which is maladaptive. High allostatic load also leads to inflammation and oxidative stress, which in turn could lead to SNS overactivity directly via central effects, and indirectly via arterial baroreflex dysfunction. SNS overactivity has a major role in causing and sustaining hypertension (Grassi, 2009), which is a major risk factor for CV disease, and also leads to a number of deleterious effects on end organs that are independent of blood pressure, including the development of heart failure (Julius, 1993), arrhythmias (Grassi *et al.* 2004), atherogenesis (Erami *et al.* 2002), insulin resistance (Jamerson *et al.* 1993) and renal sclerosis (Amann *et al.* 2000).

Prior studies have provided indirect evidence that PTSD may be characterized by SNS overactivity. PTSD patients have a higher resting HR and BP, as well as decreased HR variability, suggesting a state of chronic SNS overactivity (Buckley & Kaloupek, 2001; Bedi & Arora, 2007). Studies have shown that PTSD patients have decreased low-frequency and high-frequency HR variability (Cohen *et al.* 1998; Chang *et al.* 2013; Shah *et al.*

2013), suggestive of increased sympathetic and decreased parasympathetic tone to the heart. In addition, urinary noradrenaline levels have been shown to be significantly elevated in PTSD patients compared to Controls without PTSD (Wingenfeld *et al.* 2015). In the present study, we present the first direct assessments of sympathetic nerve activity using microneurography in PTSD patients compared to age-matched Controls. Although we did not observe baseline differences between the groups in MSNA or haemodynamics at rest, PTSD patients had significantly augmented MSNA responses during both combat-related and non-combat-related mental stress. These data suggest that SNS drive in these patients is exacerbated by mental stress associated with PTSD symptoms, such as that experienced during flashbacks, as well as non-related mental stressors.

Previous studies assessing sympathetic and haemodynamic reactivity during mental stress in PTSD have shown mixed results. Although some have reported that PTSD patients had greater increases in HR, BP and noradrenaline levels during mental stress (Bedi & Arora, 2007), others found that PTSD was characterized by blunted haemodynamic responses during mental stress induced by recall of traumatic events (Cohen *et al.* 1998). A unique feature of the present studies is the use of direct sympathetic nerve measurements and

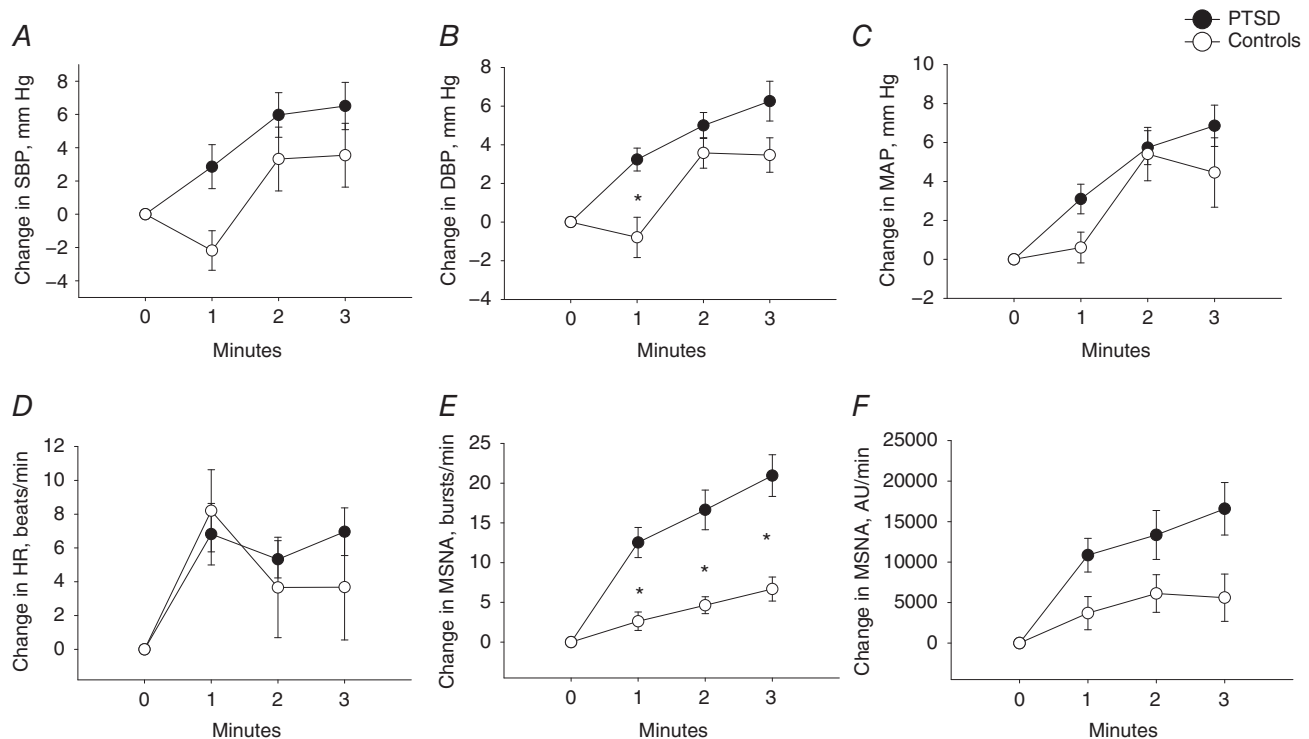


Figure 2. Hemodynamic and sympathetic reactivity during mental arithmetic

Change in SBP (A), DBP (B), MAP (C), HR (D), MSNA quantified as burst frequency (bursts min^{-1}) (E) and total activity (AU min^{-1}) (F) during 3 min of mental arithmetic in PTSD patients (filled circles) and Controls (open circles).

* $P < 0.05$ between PTSD and Control groups.

continuous beat to beat haemodynamics during two different types of mental stress: (i) combat-related mental stress (i.e. mental stress that is associated with PTSD symptoms) and (ii) non-combat-related mental stress that is not related to PTSD symptoms. We showed that young Veterans with PTSD have significantly greater increases in MSNA and HR during VRCE compared to Controls, suggesting that PTSD patients have heightened sympathetic and haemodynamic responses during combat-related mental stress. PTSD patients also had augmented MSNA and diastolic BP responses during mental arithmetic, suggesting that these exaggerated haemodynamic and sympathetic responses also extend to non-combat related mental stress in PTSD. However, there was no difference in the haemodynamic and MSNA responses during the CPT, suggesting that augmented haemodynamic and sympathetic responses are not generalized to all sympathoexcitatory stimuli, nor to non-baroreflex-mediated stimuli. Thus, the augmented SNS and haemodynamic responses in PTSD are specific to mental stress, both related and unrelated to PTSD symptoms. Exaggerated increases in SNS activity during mental stress could contribute to an increased risk of hypertension and CV disease by increasing the burden of SNS activity. Moreover, exaggerated SNS responses

during mental stress have been shown to be associated with an increased risk of hypertension (Matthews *et al.* 1993) and CV disease (Proietti *et al.* 2011).

VRCE is considered a neutral stimulus in those without combat-related PTSD; therefore, it is not surprising that there was minimal MSNA and BP reactivity observed during VRCE amongst the Control group. However, in PTSD patients, the MSNA response was significantly heightened during VRCE but, despite the heightened MSNA response, the BP responses were not augmented. By contrast, MSNA responses during MA were also significantly augmented in PTSD, although they were accompanied by concomitant augmentation of diastolic BP responses. These observations suggest that there may be differences in the degree of vasoconstriction and therefore increase in blood pressure in response to sympathetic nerve activation (i.e. neurovascular transduction) during combat-related *vs.* non-combat-related mental stress in PTSD. Although not tested in the present study, the differences in neurovascular transduction observed between stress protocols could be a result of differences in the release of noradrenaline at sympathetic nerve terminals, the generation of vasodilators and alternative vasoconstrictors, and adrenergic receptor density and responsiveness. In particular, chronic

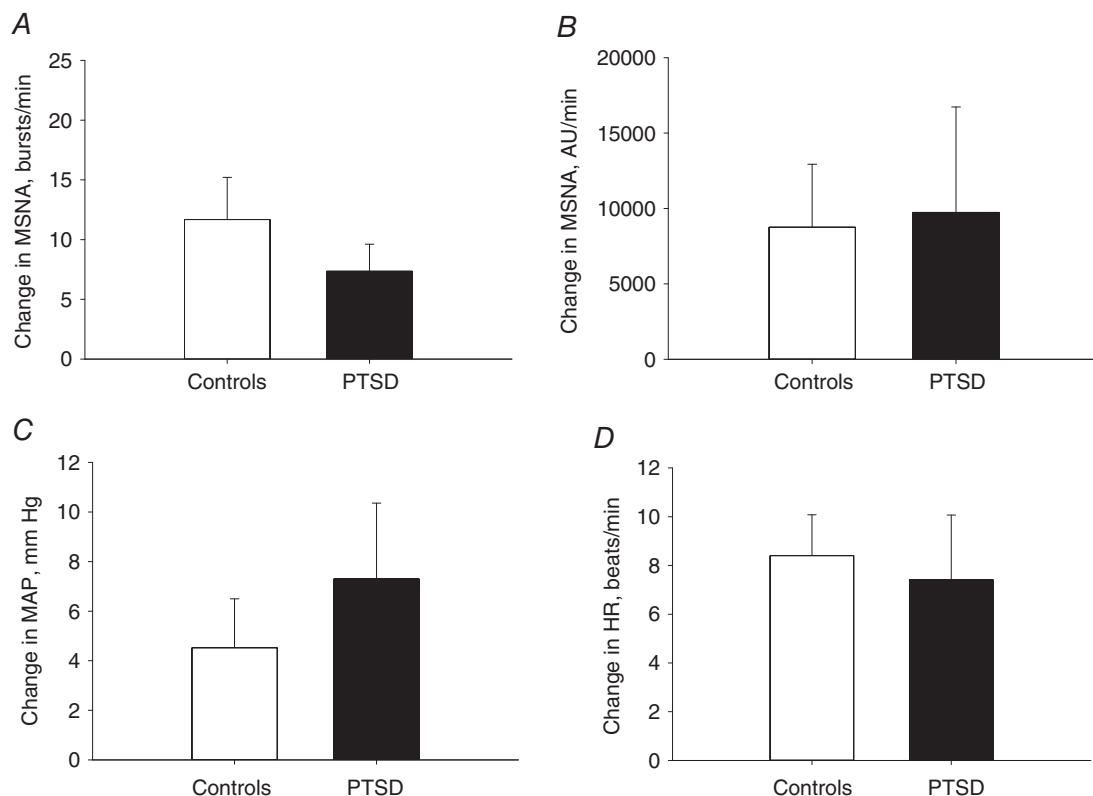


Figure 3. Hemodynamic and sympathetic reactivity during the cold pressor test

Change in MSNA quantified as burst frequency (bursts min^{-1}) (A) and total activity (AU min^{-1}) (B), MAP (C) and HR (D) during 1 min of CPT in PTSD patients vs. Controls.

elevations in sympathetic activation during stress related to PTSD symptoms, could lead to downregulation of α -1 adrenergic receptors, as has been suggested in other states characterized by high sympathetic activity (Feng *et al.* 1999; Dinunno *et al.* 2002), and this may lead to blunted BP responses relative to the degree of sympathetic activation. These and other mechanisms underlying neurovascular transduction during mental stress in PTSD should be evaluated in future studies.

One potential mechanism underlying SNS over-activation in PTSD is decreased arterial BRS. The arterial baroreceptors are important in the moment-to-moment control of BP by buffering acute fluctuations in arterial BP during postural, physiological or mental stress. In

addition, arterial baroreceptors tonically inhibit SNS activity to maintain BP around a set point. BRS is diminished or blunted if increases and decreases in BP via the baroreflex arc fail to elicit the appropriate changes in SNS activity (sympathetic BRS) and parasympathetic activity (cardiovascular BRS) to maintain appropriate levels of arterial BP, or are chronically reset to higher BP levels as in chronic hypertension. Using the Modified Oxford technique to assess arterial baroreflex sensitivity, we showed that baseline sympathetic and cardiovascular BRS was indeed blunted in PTSD patients compared to Controls, and also that cardiovascular BRS was blunted during concomitant combat-related mental stress, suggesting that impaired BRS may contribute to abnormal SNS responses

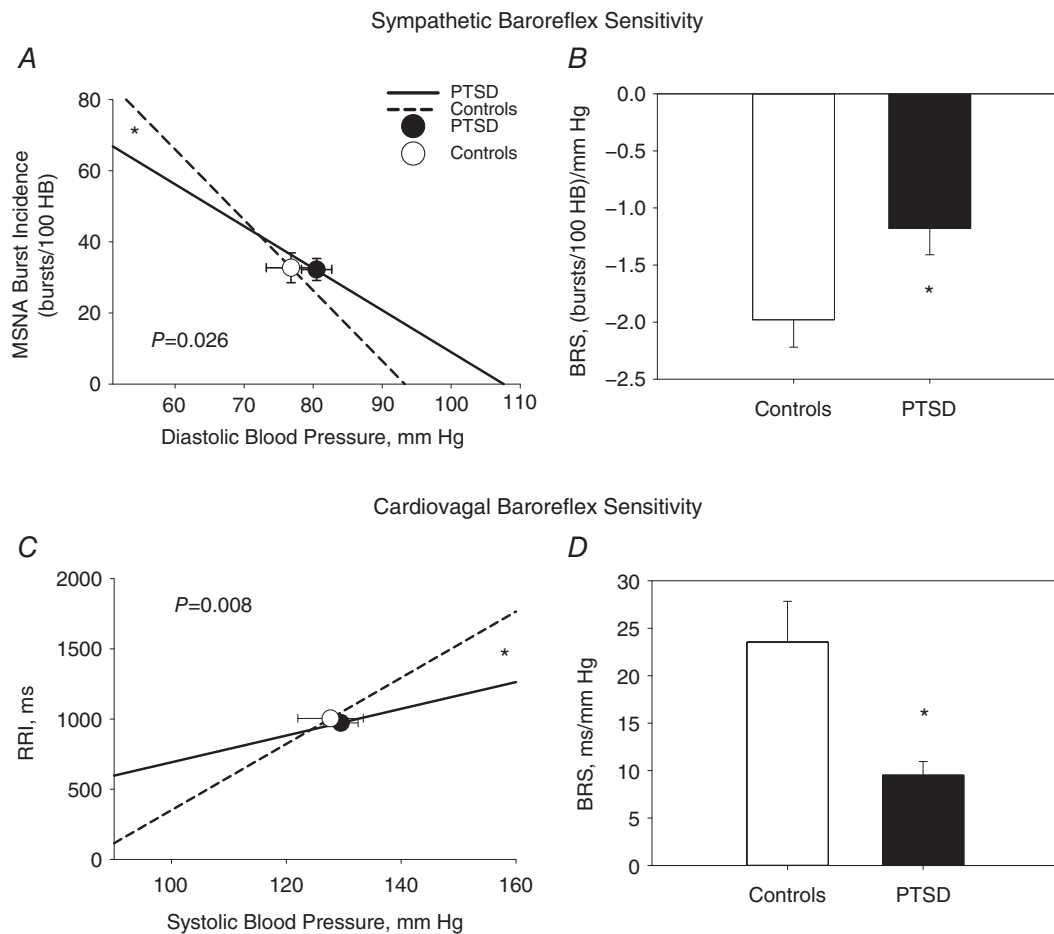


Figure 4. Sympathetic and cardiovascular baroreflex sensitivity at rest

The linear relationships between diastolic blood pressure and MSNA burst incidence (A) and between SBP and R-R interval (RRI) (C) are shown at rest and were derived from the mean slope values from individual linear regressions performed during baroreflex testing in PTSD (continuous line) vs. Controls (dashed line). The mean slope of the individual linear regressions between DBP and MSNA represents mean sympathetic BRS and was significantly less steep in PTSD [-1.2 ± 0.2 bursts (100 heart beats) $^{-1}$ mmHg $^{-1}$; $R^2 = 0.77 \pm 0.02$] vs. Controls [-2.0 ± 0.3 bursts (100 heart beats) $^{-1}$ mmHg $^{-1}$; $R^2 = 0.85 \pm 0.02$; $P = 0.026$] (A and B). The mean slope of the individual linear regressions between systolic blood pressure and RRI represents mean cardiovascular BRS and was significantly less steep in PTSD [9.5 ± 1.4 ms mmHg $^{-1}$; $R^2 = 0.74 \pm 0.02$] vs. Controls [23.6 ± 4.3 ms mmHg $^{-1}$; $R^2 = 0.75 \pm 0.02$; $P = 0.008$] (C and D). The operating point around which the baroreflex operates is derived from baseline measures and signified by the closed circles (PTSD) and open circles (Controls). * $P < 0.05$ between PTSD and Control groups.

during mental stress in PTSD. Decreased BRS contributes to the pathogenesis of many conditions characterized by SNS overactivity, including chronic heart failure (Grassi *et al.* 1995), obesity (Grassi *et al.* 1998) and hypertension (Matsukawa *et al.* 1991a), and the data obtained in the present study demonstrate that impaired BRS is present in Veterans with PTSD. Decreased BRS could be one contributing mechanisms underlying the link between PTSD and cardiovascular disease risk.

Chronic inflammation may contribute to blunted BRS in PTSD because several studies have demonstrated that immune dysfunction is present in this population (von Kanel *et al.* 2007; Spitzer *et al.* 2010; Plantinga *et al.* 2013) and, in experimental animal models of

CV disease, anti-inflammatory treatments have been shown to affect autonomic and baroreceptor function (Marvar *et al.* 2016). PTSD is associated with higher circulating levels of inflammatory markers (von Kanel *et al.* 2007; Spitzer *et al.* 2010; Plantinga *et al.* 2013) including CRP, IL-4, IL-6 and intercellular adhesion molecule-1, higher brain angiotensin II levels (Matsumura *et al.* 1998; Saavedra *et al.* 2011), and greater arterial stiffness (Walczevska *et al.* 2011). In the population in the present study, we found that young OEF/OIF/OND patients with PTSD had significantly elevated hsCRP levels compared to age-matched Controls, suggesting that chronic inflammation may play a contributory role in impaired BRS and heightened SNS responses

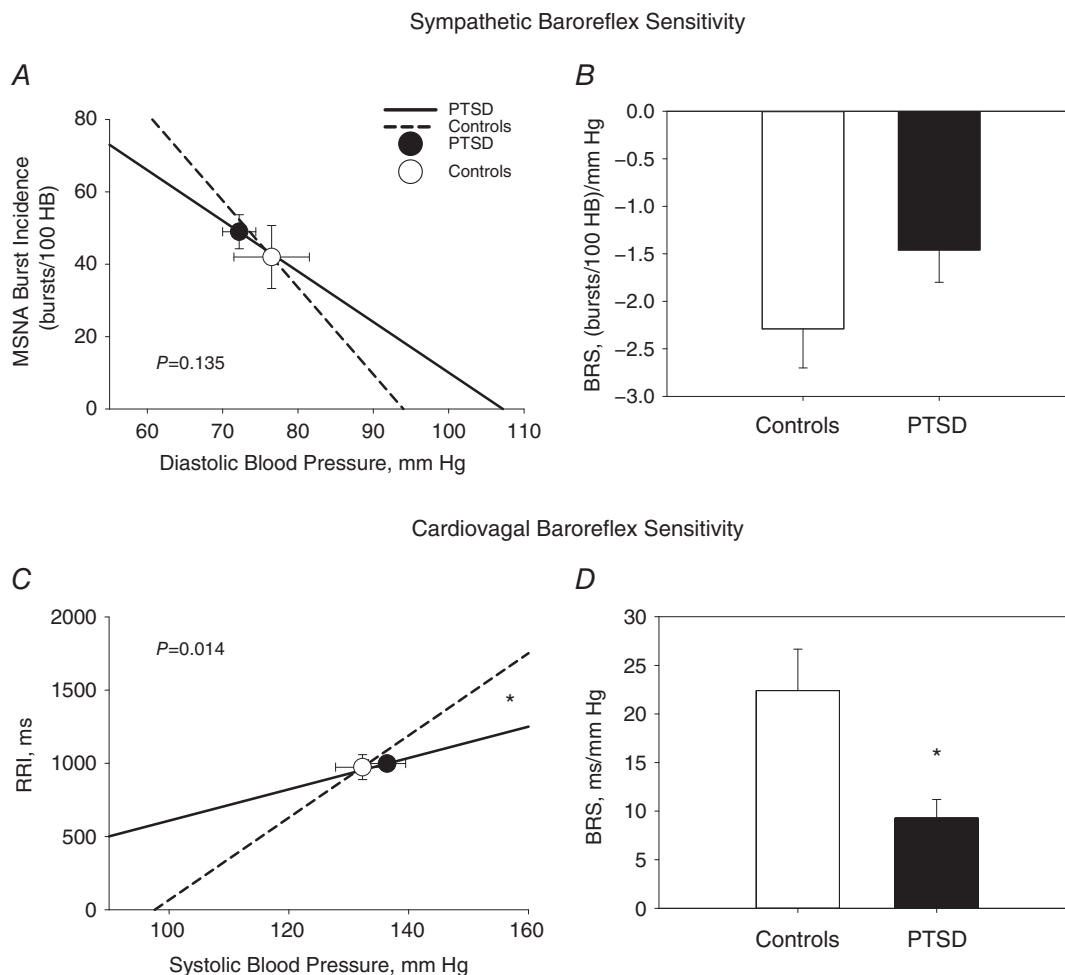


Figure 5. Sympathetic and cardiovagal baroreflex sensitivity during virtual reality combat exposure
 The linear relationship between diastolic blood pressure (DSB) and MSNA burst incidence (A) and between systolic blood pressure and R-R interval (RRI) (C) are shown during concomitant VRCE, and were derived from the mean slope values from individual linear regressions performed during baroreflex testing in PTSD (continuous line) vs. Controls (dashed line). There was no significant difference between the groups in the mean slope of the individual linear regressions between DBP and MSNA that represents mean sympathetic BRS (A and B). The mean slope of the individual linear regressions between systolic blood pressure and RRI represents cardiovagal BRS and was significantly less steep in PTSD ($9.3 \pm 1.9 \text{ ms mmHg}^{-1}$; $R^2 = 0.75 \pm 0.02$) vs. Controls ($22.4 \pm 4.3 \text{ ms mmHg}^{-1}$; $R^2 = 0.75 \pm 0.03$; $P = 0.014$) (C and D). The operating point around which the baroreflex operates is signified by the closed circles (PTSD) and open circles (Controls). * $P < 0.05$ between PTSD and Control groups.

in PTSD. Our finding that hsCRP levels correlate with heightened MSNA responses during VRCE suggest that heightened basal levels of inflammation may be linked to enhanced sympathetic reactivity in PTSD. CRP levels are correlated with increased risk of cardiovascular disease in multiple large epidemiological studies (Ridker *et al.* 1998; Corrado & Novo, 2007; Greenland *et al.* 2010; Kaptoge *et al.* 2010) and the results of the present study provide additional support indicating that basal levels of inflammation are heightened in PTSD and may be linked to enhanced sympathetic activity and baroreflex dysfunction. In addition, there is evidence that, prior to the development of overt CV disease, chronic inflammation could lead to BRS dysfunction at vascular nerve endings or along its tract and within the brainstem (Chapleau *et al.* 2001; Zubcevic *et al.* 2011; McBryde *et al.* 2013). In the present study, although we observed elevated basal hsCRP levels in PTSD, we did not observe a significant correlation between hsCRP and baroreflex sensitivity. However, these analyses may have been limited by a small sample size and the relatively small range of hsCRP values.

We recognize several other limitations to these studies. First, our study population primarily comprised

African-American males with combat-related PTSD. Whether these findings can be generalized to females, other races and ethnicities or PTSD related to non-military trauma is unclear. Second, we used a CAPS-IV score ≥ 45 to confirm the diagnosis of PTSD in patients, and a score of < 45 to exclude the presence of PTSD in Controls (Weathers & Ruscio, 1999; Weathers *et al.* 2001). The Control group comprised OEF/OIF/OND Veterans without PTSD and had a mean CAPS-IV score of 38, which is below the threshold for PTSD diagnosis but was within the range for subthreshold PTSD (CAPS-IV score range 20–39). Indeed, all Control participants had a CAPS-IV score within the subthreshold PTSD range. Recent investigations of service members with subthreshold PTSD have also revealed physiological alterations (Costanzo *et al.* 2014). A more robust difference in physiological measures may have been demonstrated if the Controls included only those without any symptoms of PTSD (score range 0–19) or non-Veterans without combat exposure. Third, although we observed a significantly lower cardiovagal and sympathetic BRS at baseline, and significantly lower cardiovagal BRS in the PTSD group during VRCE, there was a non-significant trend towards

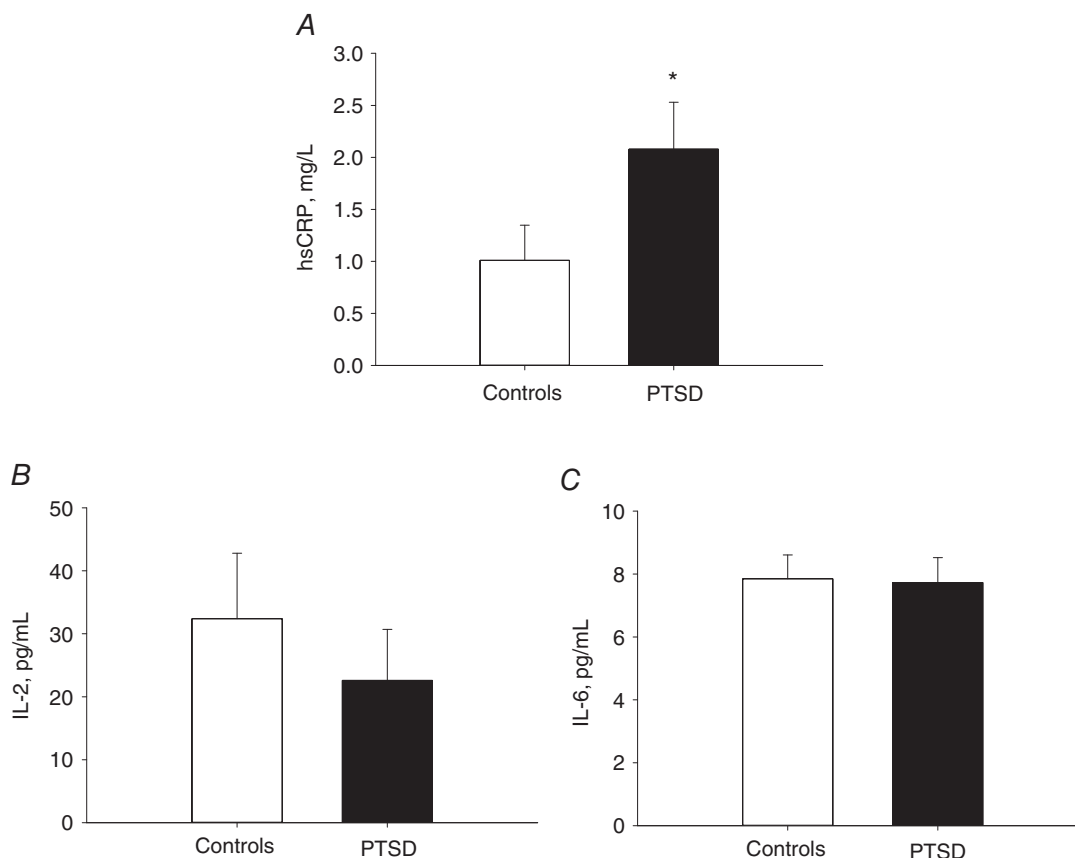


Figure 6. Inflammatory biomarkers

Highly sensitivity hsCRP (A), interleukin 2 (IL-2) (B) and interleukin 6 (IL-6) and (C) levels in PTSD patients vs. Controls. * $P < 0.05$ between PTSD and Control groups.

reduced sympathetic BRS in the PTSD group during VRCE. The study may have been underpowered to detect differences in sympathetic BRS during concomitant VRCE. Fourth, although we observed significantly higher levels of basal hsCRP levels in PTSD, IL-2 and IL-6 levels were not elevated in this cohort. Although we did not observe a difference in basal levels of IL-2 and IL-6, this does not exclude the possibility that these cytokines may be enhanced in PTSD during periods of acute stress or exposure to fear stimuli, and should be tested in future studies. Lastly, it is unclear from this study whether augmented sympathetic responses to mental stress and decreased BRS, are a result of having PTSD, or are present prior to the development of PTSD and associated with a higher propensity for developing PTSD. Because SNS responses to mental stress are variable in the general population, those with higher SNS responses to stress and blunted BRS may be at greater risk of developing PTSD in the setting of high allostatic load. Studies assessing physiological responses to mental stress before and after the development of PTSD, or long-term prospective studies evaluating the incidence of PTSD amongst those with high vs. low SNS reactivity to mental stress, represent potential strategies for determining whether increased SNS reactivity is associated with an increased risk of developing PTSD.

In conclusion, PTSD is a highly prevalent and debilitating illness that is associated with an increased risk of CV disease and hypertension. Augmented sympathetic and haemodynamic responses to mental stress not only associated with PTSD symptoms, but also generalized to other types of mental stress, may lead to an overall increased burden of sympathetic activity that leads to a greater risk of developing hypertension and CV disease. Blunted sympathetic and cardiovagal BRS may contribute to increased sympathetic and decreased parasympathetic activation in PTSD, and chronic inflammation may play a causative role in decreasing BRS and increasing central sympathetic output. Identifying physiological mechanisms that contribute to an increased CV risk in PTSD patients will allow for the development of interventions aiming to modify CV risk in these patients. Future studies testing therapies to improve SNS reactivity, BRS and inflammation should be conducted to evaluate potential long-term benefits on CV risk in PTSD patients.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

JP, PM and BR contributed to the conception or design of the study. JP, PL, MK, SN, RD, SA, NL and BR contributed to the acquisition, analysis or interpretation of data. JP, PM, PL, MK, SN, RD, SM, NL and BR contributed to drafting the manuscript or revising it critically for important intellectual content. All authors approve of the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, and all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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