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Dual-hormone stress reactivity predicts downstream war-zone stress-evoked PTSD

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ABSTRACT

Background: The crucial role of the hypothalamic-pituitary-adrenal axis (HPA) in stress-related homeostasis suggests dysregulated HPA involvement in the pathogenesis of post-traumatic stress disorder (PTSD), yet most studies examining linkages between HPA axis measures and PTSD have yielded null findings. One untested explanation for this inconsistency is a failure to account for simultaneous adrenal and gonadal influence. Here we tested the singular and interactive effects of cortisol (C_R) and testosterone (T_R) reactivity as moderators of war-zone stress evoked PTSD emergence in the war-zone.
Methods: U.S. soldiers (N = 120) scheduled for deployment to Iraq completed pre-deployment measures of C_R and T_R stress reactivity to a CO_2 inhalation challenge. Once deployed, monthly assessments of exposure to traumatic war-zone stressors and PTSD symptoms were collected via a web-based assessment system.
Results: Cortisol hypo-reactivity potentiated the pathogenic impact of war-zone stressors only in soldiers for whom the CO_2 challenge did not elevate testosterone, suggesting that the dual hormone stress reactivity profile of blunted cortisol and testosterone may confer increased risk for PTSD emergence by potentiating the pathogenic effects of war-zone stressors.
Conclusions: Findings underscore the utility of assessing both HPA and HPG stress reactivity when assessing PTSD vulnerability and may help inform efforts for enhanced soldier screening and inoculation to war-zone stressors.

1. Introduction

The HPA axis' central role in the maintenance of stress-related homeostasis (see Charmandari et al., 2005) implies HPA dysregulation should be involved in the pathogenesis of stress-related disorders, including PTSD. Seminal studies supported this view in demonstrating strong associations between hypo-corticolism and PTSD (McFarlane et al., 1997; Resnick et al., 1995; Yehuda et al., 1995). Subsequent studies have replicated these findings (De Kloet et al., 2007; Rohleder et al., 2004; Steudte-Schmiedgen et al., 2015), but many studies have not. In fact, existing meta-analyses summarizing three decades of research have shown mixed, and overall null findings for HPA dysregulation in PTSD etiology (Klaassens et al., 2012; Meewisse et al., 2007).

One potential explanation for the mixed findings across studies is a failure to account for gonadal influence on HPA-axis function. Testosterone and other androgens exhibit potent anti-glucocorticoid effects (Agarwal et al., 1979; Danhaive and Rousseau, 1988; Sasson and Mayer, 2013), mediated by androgen-sensitive afferents to structures central to HPA-modulated stress regulation, including the medial pre-optic area, central and medial amygdala, and bed nuclei of the stria terminalis (Viau et al., 1999; Viau and Meaney, 1996). Further, testosterone has direct anxiolytic effects (Hermans et al., 2006), likely due to inhibitory activation of gamma-amino-butyric-acid (GABA) receptors (Bitran et al., 1993).

PTSD is generally regarded as a disorder of dysregulated threat reactivity. In line with this view, the peritraumatic period is marked by elevated cortisol and stress-evoked cortisol hyper-reactivity, followed by a temporally-graded reduction in HPA-axis

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activity, culminating in cortisol hypo-reactivity (Meewisse et al., 2007; Resnick et al., 1995; Rubinow et al., 2005; Valtysdóttir et al., 2001; Weems and Carrion, 2007; Yehuda et al., 2007). By inhibiting trauma-evoked HPA-axis hyper-reactivity during the *peri*-traumatic period (Handa et al., 1994b), testosterone might short-circuit the transition to blunted HPA-axis stress-reactivity in the post-trauma period. Moreover, testosterone's anti-glucocorticoid effects may protect against structural damage in the hippocampus (Gouras et al., 2000), producing long-term protection against the stress-evoked glucocorticoid-mediated neural degeneration thought to characterize PTSD (Kitayama et al., 2005; Sapolsky et al., 1990). Thus, evidence for androgen modulation of HPA-axis hyper-reactivity suggests that peritraumatic elevations in testosterone might protect against the subsequent emergence of PTSD.

1.1. Study overview

Capitalizing on the established reputation of CO_2 inhalation as a laboratory stressor (Harrington et al., 1996; Perna et al., 1995; Schmidt and Zvolensky, 2007; Telch et al., 2011, 2010), we examined whether differences in hormonal reactivity to a single 35% CO_2/65% O_2 inhalation stress challenge accounts for variability in war-zone stress-evoked PTSD symptoms. Prior to their first-ever military deployment, U.S. soldiers bound for Iraq (N = 120) provided salivary hormone samples immediately prior to, and 30 min after CO_2 inhalation. Then, during their 16-month deployment, soldiers completed monthly web-based assessments of war-zone stressors and PTSD symptoms. This prospective design allowed us to examine the singular and joint effects of pre-deployment basal cortisol (C) and testosterone (T) and CO_2 challenge-evoked cortisol reactivity (C_R) and testosterone reactivity (T_R) as moderators of traumatic war-zone stressor effects on the subsequent emergence of PTSD symptoms in theater. The strength of PTSD predictors should be proportional to the degree to which they tap threat reactivity. Therefore, hormonal stress reactivity indices may exert a more potent influence in the pathogenesis of PTSD, relative to basal concentrations.

2. Methods and materials

2.1. Participants and procedures

Participants (N = 120) were recruited for the Texas Combat PTSD Risk Project, which aimed to prospectively identify biological, cognitive, and psychosocial risk factors for war-zone psychopathology. They were from 9 Army units (4 combat service support units, 4 combat units, and 1 combat support unit) planning to deploy from Ft. Hood to Iraq between August 2007 and August 2009. To reduce the possibility of perceived coercion to participate, unit leaders did not attend recruitment sessions. Soldiers were briefed about the study by the project director and principal investigator in the presence of an Army ombudsman. Prior to deployment, participants visited the University of Texas at Austin to complete an extensive assessment battery that included measures in neuroimaging, cognitive, genetic, hormonal, and psychosocial domains. During deployment, soldiers received monthly email reminders to complete a de-identified, web-based assessment called the Combat Experiences Log (CEL; Lee et al., 2011; see Measures for details).

Soldiers were required to meet the following criteria to enroll in the study: (1) age 18 or older, (2) no prior deployment to a war-zone, and (3) planned deployment to Iraq within 3 months of consent. Of the 223 soldiers attending the briefings, 184 (82.51%) consented to participate, 6 did not deploy, 1 withdrew consent, and 16 completed no assessments in theater. Of the remaining 161 soldiers, 24 were excluded from hormonal analysis due to endocrine disorders and/or current use of antidepressants, stimulants, or steroid hormones, 8 were excluded due to invalid hormone measures (i.e., coefficients of variance ≥0.15), and an additional 9 did not complete the in-theater measures included in this analysis. The final sample therefore included 120 soldiers (see Table 1 for demographics and descriptive statistics). Post-hoc power analyses using Optimal Design software (Spybrook et al., 2006) revealed sufficient power to detect a medium effect size (δ = 0.40–0.50, β = 0.82–0.95).

Table 1
Descriptive Statistics.

Demographic Variables	N	%	
Lifetime Axis I Diagnosis[a]	64	53.33	
Male	104	86.67	
Female	16	13.33	
Prior Trauma[b]	65	54.17	
Pre-Deployment and Deployment Variables	N	M	SD
Number of Monthly CEL Entries – PCL-Short[c]	120	4.44	3.37
Monthly Average Number of PTEs[d]	120	1.83	1.85
PTSD Symptoms (PCL-Short)[c]	537	5.28	2.13
C (pg/ml) Pre-CO_2[e]	120	0.12	0.06
C (pg/ml) Post-CO_2[e]	120	0.15	0.13
C_R (pg/ml) Post-Pre CO_2[e]	120	0.03	0.12
T (pg/ml) Pre-CO_2[e]	120	83.07	26.74
T (pg/ml) Post-CO_2[e]	120	84.91	33.72
T_R (pg/ml) Post-Pre CO_2[e]	120	1.83	23.05

Descriptive statistics for all modeled variables.
[a] Indicates presence of lifetime DSM-IV-TR defined Axis I disorders.
[b] History of trauma based on DSM-IV PTSD Criterion A defining exposure to a traumatic event prior to pre-deployment.
[c] PCL-Short = Post-traumatic Stress Checklist – 4 Item Version (N = 537 repeated in theater observations).
[d] PTEs = Potentially traumatic war-zone stressors.
[e] Basal salivary cortisol (C) and testosterone (T) were measured before and 30 min after a single inhalation of 35% CO_2/65% O_2 gas at pre-deployment, and reactivity measures (C_R, T_R) were derived by subtracting pre-CO_2 from post-CO_2 hormone levels.

2.2. Institutional review board approval

All study procedures were approved by the Institutional Review Board of the Office of Research Support at The University of Texas at Austin and the Brooks Army Medical Center Scientific and Human Use Review Committee. All participants provided informed consent.

2.3. Pre-deployment measures

2.3.1. Demographics, lifetime history of mental disorders, and prior trauma exposure

Soldiers completed a demographics questionnaire, and the SCID – Structured Clinical Interview for Axis I DSM-IV Diagnoses. Doctoral-level psychology students, with >1 year of assessment experience administered the SCID under supervision of the Principal Investigator (M.J. Telch).

2.3.2. Hormone assessments

Two, 3 mL saliva samples were taken for each participant to measure basal cortisol (C), basal testosterone (T), cortisol reactivity (C_R), and testosterone reactivity (T_R). Samples were collected between 1400 and 1700 h to minimize the impact of diurnal variation. Participants first completed a 30-min rest period before providing a passive drool sample used to assess C and T. To assess C_R and T_R evoked by a potentially stressful task, participants then completed a CO_2 challenge. A second saliva sample was taken 30 min after the CO_2 challenge, and the difference in hormone concentration from the first to second sample (post-CO_2 level minus pre-CO_2) was used to calculate C_R and T_R. After the collection of the second

sample, both samples were frozen. Later they were packed in dry ice and sent to Salimetrics (State College, PA, USA), where cortisol and testosterone concentrations were assayed in duplicate. The intra-assay and inter-assay coefficients of variance were in the acceptable range (4.6% and 9.9% for testosterone, 3.5% and 5.1% for cortisol).

2.3.3. 35% CO_2 stress reactivity challenge

The CO_2 inhalation challenge occurred between 2 PM and 4 PM and followed procedures similar to those described in other CO_2 challenge studies conducted in our laboratory (Telch et al., 2011, 2012). Participants were seated individually in a soundproof room and fitted with an ambulatory heart rate monitor. Following a 5-min resting baseline period, participants viewed a 3-min video containing the rationale, procedural instructions, and a demonstration of the CO_2 inhalation procedure. They were then instructed to take a full vital capacity breath of the gas mixture containing 35% CO_2/65% O_2 through a plastic mask and to hold it in their lungs for 5 s. Participants were then instructed to breath normally until the effects of the gas subsided (approximately 30 s), at which point they completed the Acute Panic Inventory (Dillon et al., 1987) – a widely used self-report instrument for assessing emotional responding to CO_2 challenge (see Telch et al., 2012 for additional information on the CO_2 challenge procedures).

2.4. Measures of war-zone stressors and PTSD symptoms

During deployment, soldiers completed the Combat Experiences Log (CEL) – a web-based longitudinal tracking system for collecting repeated self-report in-theater assessments of soldiers' exposure to war-zone stressors, and their psychological adjustment to the war-zone environment (Lee et al., 2011). The date for each soldier's monthly entry was automatically recorded by the system and used to calculate the number of days deployed at the time of each survey entry. The CEL was designed to be completed within 10–15 min and utilized brief versions of empirically established measures of deployment-related stressors and psychological symptoms (see Lee et al., 2011 for more detailed information on the CEL).

2.4.1. Assessment of war-zone stressors

Exposure to war-zone stressors in the past 30 days were measured with dichotomous (present vs. absent) items from the CEL which were adapted from the Deployment Risk and Resilience Inventory[8]. Given our interest in exposure to potentially traumatic events likely to evoke trauma-related reactions, two advanced clinical doctoral students (AC and CL) and the PI (MJT) independently selected subsets of all stressors from the original checklist that met PTSD DSM-5 criteria for a traumatic event ("exposure to actual or threatened death, serious injury or sexual violence", p. 271) (American Psychiatric Association, 2013). Additionally, two free-response items that allowed reporting of stressors not included in the checklist were independently hand-coded. There was perfect inter-rater agreement (see Supplementary Table S1 for selected items and their respective frequencies).

2.4.2. Assessment of post-traumatic stress symptoms

The Post-Traumatic Stress Disorder Checklist (PCL-Short) (Bliese et al., 2008) was used to assess the three core symptom domains of PTSD (re-experiencing, avoidance, and hyper-arousal symptoms) on a scale of intensity from 1, not at all, to 5, extremely. The PCL-Short has demonstrated diagnostic accuracy comparable to the full 17-item version of the PCL (Bliese et al., 2008).

2.5. Statistical analyses

Two-level mixed effects growth models of monthly in-theater PTSS (PCL-Short) were used to estimate the main, incremental and interactive moderation effects of pre-deployment basal cortisol (C), basal testosterone (T), and the absolute value of cortisol (C_R) and testosterone reactivity (T_R) to the 35% CO_2 challenge on subsequent symptomatic reactions to monthly potentially traumatic war-zone stressors. All analyses were conducted using the lme4 (version 1.1.8) and associated packages in R [33] (code available upon request). Standard modeling procedures began with determining the best-fitting linear and polynomial change trajectories, followed by the addition of predictors, and higher-order interaction terms[32].

Full information maximum likelihood estimation was used to determine functional forms of in-theater symptoms, and to compare deviance statistics of nested models, whereas restricted maximum likelihood was used to produce the final estimates (Kenward and Roger, 1997). Unstructured variance-covariance matrices were specified across models, as other structural restrictions produced poorer model fit. Degrees of freedom for all fixed effect significance tests were derived using Satterthwaite approximations[34]. Effect sizes for each parameter estimate were derived from t-statistics with the following formula: $r = \sqrt{[t2/(df + t^2)]}$, where t equals the value of the t-statistic, and df equals the degrees of freedom. All statistical tests were two-tailed, with the alpha criterion set at 0.05. No *p*-value adjustments for multiple comparisons were made, as our modeling approach more efficiently addresses error inflation through precision-weighted estimation (Gelman et al., 2012). Diagnostic analyses of final models revealed no violations of statistical assumptions, including no systematic pattern in missingness (Raudenbush and Bryk, 2002).

The number of days since deployment was centered at 8 months, and included in its raw metric. Dichotomous predictors including gender (male = 0, female = 1) and past or current DSM-IV Axis I psychopathology (absent = 0, present = 1) were significant in preliminary models, and were therefore retained as control variables in all final models. All continuous covariates were z-transformed for ease of interpretation. To allow inclusion of both males and females in the analyses, basal hormone assays (C and T) were z-transformed separately within genders. Further, both indices of reactivity (T_R and C_R) were centered at zero, reflecting no change from pre-to-post CO_2 challenge, and scaled within genders. Negative values indicate decreases, and positive values indicate increases in hormone levels from pre-to-post CO_2. Transforming within genders effectively removed gender differences from the absolute levels and range of the hormone indices. As an additional check for the suitability of including both genders, effects were examined separately for males and females, revealing no significant sex differences in the magnitude or pattern of hormone effects.

War-zone stress was modeled by including each individual's average monthly number of potentially traumatic combat stressors (PTE_{BP}). The effect of PTE_{BP} can be interpreted as a total between-soldier effect of having a higher monthly average level of stressor exposure. We also simultaneously modeled within-soldier monthly deviations from their own average number of stressors (PTE_{WP}), which reflects the within-soldier effect of monthly deviations in stressor exposure. The PTE_{WP} analyses can be found in Supplementary Tables S2–S4. Modeling both PTE_{BP} and PTE_{WP} is useful because it avoids imposing the problematic assumption that their effects are equal (Hoffman and Stawski, 2009). To evaluate significant stress-moderation effects, a standard approach was used (Aiken et al., 1991) in which hormone indices in interaction with PTEs were centered 1 SD above and below their respective zero-points to estimate the conditional main effects of PTEs given a particular hormone profile (i.e., high or low basal levels, or CO_2-evoked increases or decreases in hormone levels).

Table 2
Incremental Main Effects on In-Theater PTSD Symptoms.

Parameter	*b*	*se*	*df*	*t*	*p*	Effect Size
Time	0.02	0.03	54.60	0.66	0.511	0.09
Time x Time	−0.02	0.00	213.20	−4.89	**0.000**	0.32
Sex[a]	0.59	0.51	77.70	1.16	0.249	0.13
Lifetime Axis I Diagnosis[a]	0.50	0.35	89.40	1.45	0.151	0.15
C[b]	−0.07	0.18	90.30	−0.37	0.711	0.04
T[b]	−0.21	0.18	89.20	−1.19	0.237	0.13
C_R[b, c]	−0.10	0.17	70.00	−0.56	0.577	0.07
T_R[b, c]	−0.08	0.18	89.70	−0.44	0.660	0.05
PTE_{BP}[d]	0.46	0.14	113.50	3.38	**0.001**	0.30
PTE_{WP}[d]	0.29	0.06	458.50	4.41	**0.000**	0.20

All modeled covariates were z-transformed with the exception of time, time^2, sex, and DSM-IV lifetime Axis I diagnosis.
[a] Sex (male = 0; female = 1) and lifetime Axis I diagnosis (absent = 0; present = 1) were dichotomously coded.
[b] Basal salivary testosterone (T), basal salivary cortisol (C), testosterone reactivity (T_R), and cortisol reactivity (C_R) were z-transformed separately within males and females.
[c] C_R and T_R were calculated by subtracting post-CO_2 salivary levels from pre-CO_2 challenge levels, and were centered at zero reflecting no change.
[d] PTE = potentially traumatic event. PTE_{BP}: Reflects the total between-soldier effect of having a higher monthly average exposure to PTEs, across deployment months. PTE_{WP}: Indicates the effect of having 1 additional PTE relative to the individual soldier's monthly average number of stressors, in any single deployment month. *P*-values ≤ 0.05 are in bold type.

Finally, post-hoc analyses were performed to test the robustness of the observed hormone effects. Given that prior trauma exposure may have sensitized hormonal responding to stressors, we controlled for the main effects of prior trauma, defined as events prior to deployment meeting DSM-IV PTSD criterion A (absent = 0, present = 1). We also tested whether emotional reactivity to the 35% CO_2 stressor challenge might account for the observed effects. This involved controlling for the stress-moderation effects of the highest level of fear (0 = "no fear"; 100 = "extreme fear") expressed during the stressor challenge. These effects were entered simultaneously, with the peak fear x PTEs interaction term included for both the between (PTE_{BP}) and within (PTE_{WP}) stressor effects. Results of these analyses are presented in Supplementary Table S4.

3. Results

Incremental main effects for all modeled variables are presented in Table 2. Controlling for all covariates, soldiers' pre-deployment cortisol reactivity to the CO_2 challenge modulated the effects of war-zone stressors on PTSD symptom emergence during deployment. As presented in Fig. 1 and Table 3, relative to soldiers who exhibited an increase in cortisol in response to the CO_2 challenge (b = 0.25, se = 0.17, t = 1.48, p = 0.142, r = 0.15), soldiers showing a decrease in cortisol showed greater PTSD vulnerability to increasing war-zone stressors (b = 0.72, se = 0.19, t = 3.75, p < 0.000, r = 0.32).

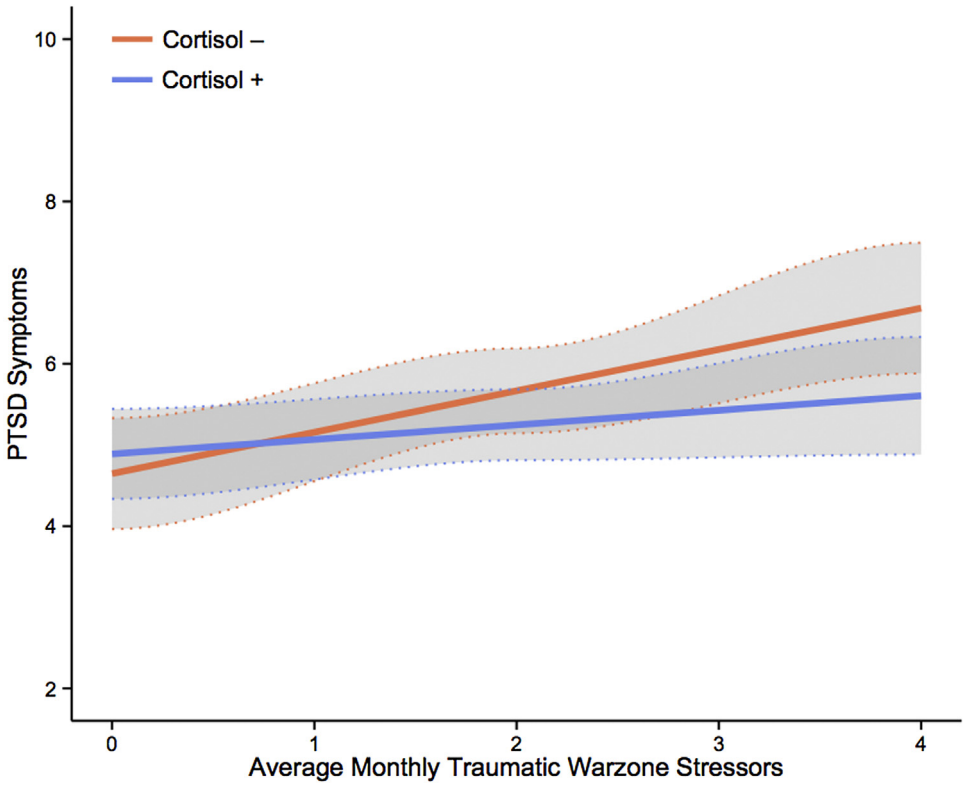

Fig. 1. Pre-Deployment Cortisol Reactivity × Between-Soldier Effects of Traumatic Warzone Stressors on In-Theater PTSD Symptoms. Effects of between-soldier differences in average monthly exposure to potentially traumatic war-zone stressors (PTEBP) for soldiers exhibiting increases (+1 SD) or reductions (−1 SD) in salivary cortisol following a single inhalation of 35% CO2/65% O2 gas at pre-deployment. Shaded regions indicate asymmetric bootstrap-derived 95% confidence limits. Undulations reflect variation in data density across the length of each regression line.

Table 3
Single Hormone Stress-Moderation Models of In-Theater PTSD Symptoms.

Parameter	*b*	*se*	*df*	*t*	*p*	Effect Size
$C \times PTE_{BP}$	0.09	0.11	129.50	0.86	0.393	0.08
PTE_{BP} | Low C	0.35	0.19	116.50	1.87	0.064	0.17
PTE_{BP} | High C	0.54	0.16	126.60	3.37	**0.001**	0.29
$T \times PTE_{BP}$	0.16	0.14	106.10	1.15	0.255	0.11
PTE_{BP} | Low T	0.33	0.18	100.60	1.83	0.071	0.18
PTE_{BP} | High T	0.65	0.21	106.30	3.15	**0.002**	0.29
$C_R \times PTE_{BP}$	−0.23	0.12	92.00	−1.95	0.054	0.20
PTE_{BP} | C_R-	0.72	0.19	124.70	3.75	**0.000**	0.32
PTE_{BP} | C_R+	0.25	0.17	97.50	1.48	0.142	0.15
$T_R \times PTE_{BP}$	−0.11	0.16	93.10	−0.73	0.469	0.08
PTE_{BP} | T_R-	0.55	0.18	109.00	3.05	**0.003**	0.28
PTE_{BP} | T_R+	0.33	0.24	95.90	1.38	0.171	0.14

Results from multi-level growth models including only one of the pre-deployment hormone moderator variables in interaction with the between-soldier variance component of stressors, which reflects average monthly stressor exposure (PTE_{BP}). Hormone reactivity stress-moderation effects (i.e., $C_R \times PTE_{BP}$, $T_R \times PTE_{BP}$) were estimated controlling for basal hormone levels (i.e., C, T) as main effects. Omnibus effects ($C \times PTE_{BP}$; $C_R \times PTE_{BP}$, etc.) are followed by conditional effects (PTE_{BP} | Low C; PTE_{BP} | C_R-, etc.), which indicate the effects of PTE_{BP} given each combination of high (+1 SD from the mean) or low (−1 SD from the mean) basal hormone levels (i.e., C, T) measured prior to the pre-deployment CO_2 challenge, *or* increases (+1 SD from 0) or reductions (−1 SD from 0) in salivary hormone levels (i.e., $C_R-/+, T_R-/+$) from pre-to-post CO_2 challenge. *P*-values ≤ 0.05 are in bold type.

Table 4
Dual-Hormone Stress-Moderation Models of In-Theater PTSD Symptoms.

Parameter	*b*	*se*	*df*	*t*	*p*	Effect Size
$C \times T \times PTE_{BP}$	0.05	0.14	108.90	0.36	0.723	0.03
PTE_{BP} | Low C, Low T	0.34	0.25	102.20	1.38	0.172	0.13
PTE_{BP} | High C, Low T	0.36	0.26	115.20	1.37	0.174	0.13
PTE_{BP} | Low C, High T	0.51	0.32	98.00	1.59	0.116	0.16
PTE_{BP} | High C, High T	0.73	0.24	107.30	2.99	**0.003**	0.28
$C_R \times T_R \times PTE_{BP}$	0.60	0.13	103.00	4.62	**0.000**	0.41
PTE_{BP} | C_R-, T_R-	1.39	0.24	124.10	5.78	**0.000**	0.46
PTE_{BP} | C_R+, T_R-	−0.61	0.30	115.80	−2.05	**0.043**	0.19
PTE_{BP} | C_R-, T_R+	−0.04	0.36	101.50	−0.10	0.919	0.01
PTE_{BP} | C_R+, T_R+	0.37	0.22	93.10	1.65	0.103	0.17

Results from multi-level growth models that included either both basal hormone variables, or both hormone reactivity variables in interaction with the between-soldier variance component of stressors (PTE_{BP}). Hormone reactivity stress-moderation effects (i.e., $C_R \times T_R \times PTE_{BP}$) were estimated controlling for basal hormone levels (i.e., C, T) as main effects. Omnibus effects ($C \times T \times PTE_{BP}$; $C_R \times T_R \times PTE_{BP}$, etc.) are followed by conditional effects (PTE_{BP} | Low C, Low T; PTE_{BP} | C_R-, T_R-, etc.), which indicate the effects of PTE_{BP} given each combination of low (+1 SD above the mean) or high basal hormone levels (i.e., C, T), or increases (+1 SD from 0) or reductions (−1 SD from 0) in salivary hormone levels (i.e., C_R-, C_R+, T_R-, T_R+). *P*-values ≤ 0.05 are in bold type.

Next, to test the joint effect of dual-hormone reactivity, we examined whether the cortisol-reactivity effects depicted in Fig. 1 were contingent on soldiers' testosterone reactivity to the pre-deployment CO_2 challenge. As presented in Fig. 2 and Table 4, there was a large and statistically significant three-way interaction between cortisol reactivity, testosterone reactivity and war-zone stressor exposure (b = 0.60, se = 0.13, t = 4.62, p < 0.000, r = 0.41). Probing revealed that soldiers displaying a pre-deployment hormone profile of blunted cortisol reactivity and blunted testosterone reactivity to the CO_2 stress challenge were significantly more vulnerable to the effects of war-zone stressors with respect to PTSD symptom emergence (b = 1.39, se = 0.24, t = 5.78, p < 0.000, r = 0.46). This is in sharp contrast to those soldiers exhibiting blunted cortisol reactivity but robust testosterone reactivity (b = −0.04, se = 0.36, t = −0.10, p = 0.919, r = 0.01). In light of these dual-hormone reactivity effects, the main effect of cortisol reactivity (presented in the previous paragraph and displayed in Fig. 1 and Table 1) is misleading; cortisol reactivity was *only* associated with vulnerability to the effects of war-zone stress among soldiers exhibiting blunted testosterone reactivity.

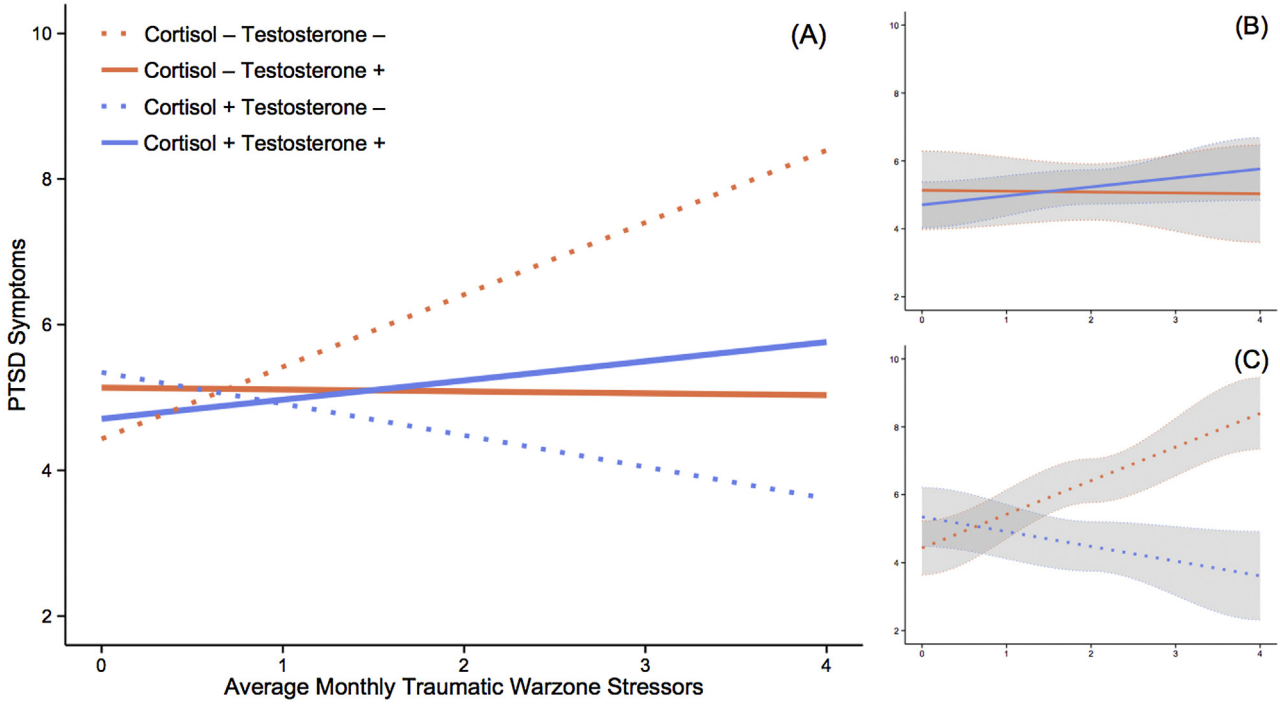

Fig. 2. Pre-Deployment Cortisol Reactivity × Testosterone Reactivity × Between-Soldier Effects of Traumatic Warzone Stressors on In-Theater PTSD Symptoms. (A) represents the effects of between-soldier differences in average monthly exposure to potentially traumatic war-zone stressors for soldiers exhibiting increases (+1 SD from no change) or decreases (−1 SD from no change) in salivary cortisol and/or testosterone following a single inhalation of 35% CO_2/65% O_2 gas at pre-deployment. Subplots on the right contrast the effects of stressors on symptoms as a function of increases and decreases in salivary cortisol for soldiers exhibiting increases (B) or decreases (C) in salivary testosterone. Shaded regions indicate asymmetric bootstrap-derived 95% confidence limits. Undulations reflect variation in data density across the length of each regression line.

Finally, to test the robustness of the stress-moderation findings, post-hoc analyses controlled for the main effects of trauma exposure prior to deployment, and incorporated interaction terms including peak fear expressed during the CO_2 stressor challenge in interaction with PTE_{BP} and PTE_{WP}. Results revealed only negligible changes to the parameter estimates (see supplemental online text, Table S4), indicating the hormone reactivity effects were independent of prior traumatization, and subjective distress in response to the CO_2 challenge.

4. Discussion

Our findings identified a specific pre-deployment dual-hormone profile of PTSD risk vulnerability among healthy first-time deployed soldiers. After controlling for relevant covariates including basal levels of cortisol and testosterone, prior trauma, and emotional stress reactivity, soldiers displaying a hormone profile of blunted cortisol and blunted testosterone reactivity to a CO_2 stressor challenge showed heightened PTSD symptom emergence at high levels of war-zone stressor exposure (see Fig. 2). Heightened cortisol reactivity was not pathogenic in soldiers displaying elevations in testosterone in response to the CO_2 stress reactivity challenge.

At first glance, our data showing no appreciable effects of pre-deployment cortisol in predicting PTSD symptom emergence (see Fig. 1) are consistent with the many published reports showing no reliable influence of the HPA-axis on PTSD (Klaassens et al., 2012; Meewisse et al., 2007). Yet, consistent with evidence for the protective role of cortisol release (de Quervain and Margraf, 2008; Soravia et al., 2009), and with recent findings from a study of German soldiers that lower cortisol stress reactivity predicted increased PTSD symptomatology at 12 months post-deployment (Steudte-Schmiedgen et al., 2015), our data show that heightened cortisol reactivity protects against the in-theater pathogenic effects of war-zone stressors. However, this protection was only observed for the subset of soldiers who show blunted testosterone reactivity (see Fig. 2). Similarly, our data suggest that testosterone reactivity may too provide a protective function for PTSD by reducing the pathogenic impact of war-zone stressors; however, this protective effect was observed only for soldiers displaying blunted HPA reactivity. These findings converge in highlighting the importance of the interaction between the HPA and HPG axes in PTSD.

Another remarkable aspect of our findings is that we observed significant moderation of between-soldier differences in overall stressor exposure (PTE_{BP}), but not more acute within-soldier changes in stressor exposure (PTE_{WP}). This pattern is consistent with a dose-response model of the relation between stress-evoked hormone elevations, and trauma-related psychopathology. Specifically, whereas acute deployment of glucocorticoids in response to stressors is adaptive, unencumbered chronic elevations may promote neurotoxic effects (Kitayama et al., 2005; Sapolsky et al., 1990), ultimately contributing to the emergence of psychopathology. Thus, in addition to emphasizing the need for more complete and functional neuroendocrine models, the present findings demonstrate the importance of simultaneously modeling the impact of chronic and acute stressor exposure, instead of imposing the untenable assumption that their effects are equal (Hoffman and Stawski, 2009).

4.1. Potential mechanisms of hormonal stress moderation effects

Several putative causal mechanisms warrant discussion. Glucocorticoids and androgens exhibit both slow genomic and rapid non-genomic effects subject to a number of biological, genetic, and contextual influences beyond those considered here (Joëls et al., 2011; Johnson et al., 2015), and the interplay of their effects are thought to account for robust sex differences in the prevalence of mood, anxiety and stress-related disorders (McHenry et al., 2014). Moreover, these hormonal effects occur in brain regions that have been implicated in emotion regulation as well as the formation, consolidation, and retrieval of fear and extinction memory (Kindt and Kindt, 2014; Shin and Liberzon, 2010). For instance, glucocorticoid involvement in fear acquisition and extinction is very well-established (Rodrigues and LeDoux, 2009; Roozendaal et al., 2009). Specifically, stress-induced glucocorticoid release exerts bi-directional effects on emotional memory, serving to enhance memory consolidation, but to impair subsequent retrieval (Wolf, 2009), possibly by blunting activation in the medial temporal lobe[47].

Consistent with cortisol's impairment effects on fear memory retrieval, cortisol administration has been shown to facilitate fear extinction during exposure to phobic stimuli of public speaking (Soravia et al., 2006), spiders (Soravia et al., 2014), and heights (de Quervain et al., 2011). Cortisol has also been shown to reduce trauma symptoms in PTSD patients (de Quervain and Margraf, 2008), and chronic stress symptoms following critical illness and surgery (Schelling et al., 2004). Taken together, these data suggest the observed protective effects of heightened stress-evoked cortisol reactivity may operate by disrupting retrieval of traumatic memories in the war-zone.

Like glucocorticoids, the literature also suggests sex hormones are regulators of fear acquisition and extinction (Hermans et al., 2006; Milad et al., 2010; van Wingen et al., 2011). For instance, testosterone has been demonstrated to attenuate perceived fear and facilitate fear extinction in animals (Aikey et al., 2002; Bitran et al., 1993) and humans (Hermans et al., 2006). The testosterone metabolites androstanediol and androsterone are potent GABA-a agonists, which in turn have been implicated in fear reduction via regulation of amygdalar norepinephrine (NE) (Aikey et al., 2002), consistent with evidence that GABA-ergic agonist drugs reduce anxiety primarily by regulating NE within the amygdala (Hatfield et al., 1999; McGaugh, 1989). Further, some evidence directly links GABA-a to PTSD, including one report of 41% lower pre-frontal GABA binding affinity in veterans with PTSD versus controls (Bremner et al., 2000). Thus, along with existing evidence, the present results suggest that stress-evoked elevations in testosterone may serve as an endogenous safety signal through its GABA-ergic effects, thereby attenuating the emotional impact of traumatic war-zone stressors.

In addition to direct stress-moderating effects, the observed interaction effects deserve emphasis, given that the direction of the effects of both hormone reactivity indices were contingent on the other. Reports showing cortisol-mediated suppression of HPG function far outnumber reports of testosterone-mediated suppression of HPA function. However, in support of the present evidence that testosterone may interfere with the stress-regulatory capacity of the HPA-axis, several anatomical loci for the HPA-suppressive action of testosterone have been identified, including within the periphery (Rubinow et al., 2005) and the CNS (Handa et al., 1994a). It should also be noted that HPG suppression of the HPA-axis may not be limited to testosterone, as other sex hormones have been reported to inhibit HPA function (Saltzman et al., 1998), suggesting the present findings may generalize to other forms of hormonal cross-talk.

The possible mechanisms governing the effects of CO_2 inhalation on HPA and HPG activation also deserve mention. CO_2 challenge is known to induce panic-like reactions in both clinical and healthy humans (Harrington et al., 1996; Perna et al., 1995; Schmidt and Zvolensky, 2007; Telch et al., 2011, 2010), and freezing and anxiety-like behavior in animals, by signaling both amygdalar and extra-amygdalar fear circuitries, which are responsive to hypercarbia and acidosis induced by CO_2 inhalation (Feinstein

et al., 2013; Taugher et al., 2014; Ziemann et al., 2009). Accompanying the subjective response is a physiological stress response, with clear evidence for CO2-induced activation of both the HPA and HPG axes, and for the functional involvement of these hormones in hypoxia chemo-sensitivity (Semple et al., 1981; Tatsumi et al., 1994; van Duinen et al., 2005).

4.2. Study limitations

Several study limitations should be noted. First, sole reliance on salivary measures precluded inferences pertaining to central vs. peripheral involvement in the observed hormonal effects. Second, we were not able to collect hormonal measures in the war-zone. Reijnen et al. (2015) found that although testosterone levels steadily increased from pre- to post-deployment, the trajectory of change did not differ between those with low versus high posttraumatic stress symptoms at 1 and 2 years post-deployment. Third, inferences from the present findings are limited to the emergence of symptoms during deployment, and cannot speak to the emergence of disorder, or longer-term post-deployment outcomes. Fourth, efforts to collect monthly web-based assessments of soldiers' war-zone stressors and psychological symptoms during deployment were not without its challenges. Although we were successful in obtaining in theater data from most soldiers (91%), missing data were common (see Lee et al., 2011 for details). Thus, although selection bias may have influenced study findings, there was no pattern to missingness, and our analytic approach is well suited for handling missing observations (Raudenbush and Bryk, 2002). Finally, woman in the sample were too few in number to allow for reliable testing of gender-specific hormonal stress-moderation effects.

5. Conclusion

Despite a strong theoretical basis, the sum of prior work investigating relations between HPA markers and trauma-related psychopathology has revealed overall null effects across studies (Klaassens et al., 2012; Meewisse et al., 2007). Consistent with our approach, efforts to resolve this conflicting literature have assumed ubiquitous model misspecification is responsible, and have appropriately called for more integrative and comprehensive neuroendocrine models, including identifying moderators of the HPA-PTSD association (e.g., major depression; see Morris et al., 2012). The present study also emphasizes the need to develop functional models, both with respect to hormonal measures of stress reactivity (cf. Steudte-Schmiedgen et al., 2015), as well as implementing diathesis-stress frameworks to determine how contextual factors govern their expression.

Guided by evidence for HPA-HPG cross talk, we identified a pre-deployment hormonal risk profile – namely, blunted cortisol and testosterone stress reactivity, that prospectively predicted PTSD symptom emergence in the war-zone. Within a diathesis-stress framework, we found preliminary evidence suggesting that this hormonal reactivity profile confers increased risk for PTSD by potentiating the pathogenic effects of war-zone stressors. These findings underscore the utility of simultaneously examining both HPA and HPG markers, and stress reactivity, and may help resolve inconsistencies in the literature suggesting overall negligible effects of HPA functioning in PTSD. They may also aid in informing the development of enhanced soldier screening, and inoculation to war-zone stressors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.01.013.

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