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Sex Differences in Peritraumatic Inflammatory Cytokines and Steroid Hormones Contribute to Prospective Risk for Nonremitting Posttraumatic Stress Disorder

Chloe S. Lalonde[1], Yara Mekawi[2], Kelly F. Ethun[3,4], Eleonore Beurel[5,6], Felicia Gould[5], Firdaus S. Dhabhar[5,7,8], Katharina Schultebraucks[9], Isaac Galatzer-Levy[10], Jessica L. Maples-Keller[2], Barbara O. Rothbaum[2], Kerry J. Ressler[2,11], Charles B. Nemeroff[12], Jennifer S. Stevens[2] and Vasiliki Michopoulos[2,3]

Abstract

Women are at higher risk for developing posttraumatic stress disorder (PTSD) compared to men, yet little is known about the biological contributors to this sex difference. One possible mechanism is differential immunological and neuroendocrine responses to traumatic stress exposure. In the current prospective study, we aimed to identify whether sex is indirectly associated with the probability of developing nonremitting PTSD through pro-inflammatory markers and whether steroid hormone concentrations influence this effect. Female ($n = 179$) and male ($n = 197$) trauma survivors were recruited from an emergency department and completed clinical assessment within 24 h and blood samples within ~three hours of trauma exposure. Pro-inflammatory cytokines (IL-6, IL-1β, TNF, IFNγ), and steroid hormone (estradiol, testosterone, progesterone, cortisol) concentrations were quantified in plasma. Compared to men, women had a higher probability of developing nonremitting PTSD after trauma ($p = 0.04$), had lower pro-inflammatory cytokines and testosterone (p's<0.001), and had higher cortisol and progesterone (p's<0.001) concentrations. Estradiol concentrations were not different between the sexes ($p = 0.24$). Pro-inflammatory cytokines were a significant mediator in the relationship between sex and probability of developing nonremitting PTSD ($p < 0.05$), such that men had higher concentrations of pro-inflammatory cytokines which were associated with lower risk of nonremitting PTSD development. This effect was significantly moderated by estradiol ($p < 0.05$), as higher estradiol levels in men were associated with higher pro-inflammatory cytokine concentrations and lower risk for developing nonremitting PTSD. The current results suggest that sex differences in the pro-inflammatory cytokine response to trauma

[1]Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA
[2]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia, USA
[3]Yerkes National Primate Research Center, Atlanta, Georgia, USA
[4]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia, USA
[5]Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, Miami, Florida, USA
[6]Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, Florida, USA
[7]Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, Florida, USA
[8]Department of Psychology, University of Miami, Coral Gables, Florida, USA
[9]Department of Emergency Medicine, Columbia University Irving Medical Center, New York, New York, USA
[10]Department of Psychiatry, New York University School of Medicine, New York, New York, USA
[11]Mclean Hospital, Harvard Medical School, Belmont, Massachusetts, USA
[12]Department of Psychiatry and Behavioral Sciences, University of Texas at Austin, Dell Medical School, Austin, Texas, USA

Corresponding author:
Vasiliki Michopoulos, Department of Psychiatry and Behavioral Sciences, Yerkes National Primate Research Center, Emory University, 954 Gatewood Rd, Atlanta, GA 30329, USA.
Email: vmichop@emory.edu

exposure partially mediate the probability of developing nonremitting PTSD, and that the protective ability to mount an pro-inflammatory cytokine response in men may depend on higher estradiol levels in the aftermath of trauma exposure.

Keywords
sex differences, PTSD, inflammation, cytokines, hormones, steroids

Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric condition that develops after experiencing a traumatic event, characterized by alterations in physiology (e.g., heightened startle responses) and affect (e.g., anhedonia, negative cognition).[1] Risk of developing PTSD is derived from predisposing genetic and psychosocial factors, peritraumatic physiological and psychological responses to index trauma exposure, and subsequent posttrauma response and environment which may protect against or potentiate the development of the disorder.[2] Trauma-exposed women are twice as likely to suffer from PTSD than men.[3] However, the mechanisms by which sex impacts the development and chronicity of PTSD symptoms to confer this increased risk in women remains unclear. The peri-traumatic physiological response to traumatic stressxposure was found to be more predictive of PTSD development than pretrauma risk factors in a large meta-analysis.[4] Sexually dimorphic physiologic response to traumatic stress exposure may confer differential risk for PTSD development in women and men.

Among the multiple physiologic systems that respond to traumatic stress exposure, the immune system response differs quantitatively by sex [5] and is initiated to prepare the body for injury, infection, and wound repair that may result from stress exposure.[6] Broadly, women have lower rates of multi-organ failure and sepsis after trauma exposure [7,8] than men, which has been attributed in part to steroid hormone influence of immune response in each affected organ system.[8,9] Androgens suppress immunological response to trauma through multiple mechanisms, including decreased inflammatory cytokine release.[9,10] Castrated male mice produced higher levels of inflammatory cytokines after trauma-hemorrhage compared to sham-surgery male mice, with no difference in posttrauma corticosterone levels.[11] Androgen administration depresses posttrauma cytokine response in castrated male mice,[12] while 17β-estradiol administration increases interleukin 2 (IL-2) production.[10,13] This preclinical data has prompted clinical study of estradiol administration or androgen-suppressive therapy after trauma exposure.[9].

The quantitative sex difference in immune response to trauma may confer additional susceptibility or resilience to development of PTSD. Recent work has implicated the role of the immune system and inflammation in the development of PTSD. Neutrophil, lymphocyte, and monocyte levels have been shown to have high impact on a predictive algorithm of PTSD course following a traumatic stressor.[14] Furthermore, lower TNF and interferon-gamma (IFNγ) concentrations in the acute posttrauma period are associated with prospective risk for developing chronic PTSD.[15] These results corroborate other data showing that a decreased inflammatory response to stress exposure is associated with greater psychophysiological hyperarousal (e.g. startle) [16] and posttraumatic anxiety.[17] However, it remains unclear whether an attenuated pro-inflammatory response to a traumatic event contributes to the sex difference in PTSD symptom development.

Multiple physiological systems modulate activity of the immune system in the context of stress exposure, which may contribute to differential responses to trauma exposure in females versus males. Stress-induced release of cortisol in response to activation of the hypothalamic-pituitary-adrenal (HPA) axis acts via glucocorticoid receptors to impact immune cell distribution and activity.[18] Cortisol release also inhibits pro-inflammatory gene expression by decreasing nuclear factor-$_K$B (NF-$_K$B) activity.[12,13] Dysregulation of the HPA axis is characteristic of PTSD [19] and low basal cortisol levels and blunted glucocorticoid response to stressors are associated with the prospective development of PTSD in soldiers [20] and police officers.[21] Importantly, sex differences in HPA axis function may impact the immune contribution to traumatic stress exposure. For example, men have higher basal salivary cortisol concentrations than women, whereas women have a larger salivary cortisol response after experimental stress.[18] Furthermore, sex differences in other steroid hormones, including progesterone and testosterone, have previously been implicated in PTSD risk [16,22] and can modulate immune system activity [5] and the HPA axis.[23] Similar to cortisol, these other steroid hormones, predominantly, but not exclusively, made in the gonads, may also impact risk for PTSD by influencing the expression of inflammatory cytokines in response to trauma exposure. Indeed, recent evidence shows that stress exposure decreases testosterone and increases estradiol levels in both women and men in a manner that is not dependent on cortisol responsivity to stress exposure.[23]

The goal of the current study was to extend our previous investigation of the role of peri-traumatic inflammation in conferring increased risk for prospective PTSD development [15] by identifying whether sex differences in the inflammatory response to traumatic stress exposure contribute to prospective sex differences in the probability of developing nonremitting PTSD. We hypothesized that women would have increased prospective risk for developing nonremitting PTSD and lower levels of pro-inflammatory cytokines following traumatic stress exposure compared to men. Further, we

hypothesized that women would have lower concentrations of testosterone and higher concentrations of cortisol, estradiol, and progesterone compared to men in the peri-traumatic period. Finally, we conducted exploratory analyses to test the hypothesis that sex differences in the inflammatory response after trauma may be partially attributed to sex differences in concentrations of cortisol, estradiol, progesterone, and testosterone levels following trauma exposure.

Methods

Participants and Procedure

Participants ($N = 505$) were enrolled in the emergency department (ED) of a Level 1 trauma center in Atlanta, Georgia from 2016−2018 within 24 h after the experience of a Criterion A trauma, per Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) guidelines.[24] Inclusion criteria included ability to provide informed consent, age between 18 and 65 years old, ability to speak and read English, provision of a blood sample, and access to a telephone to schedule follow-up contact. Exclusion criteria included active psychoses, hemodynamic instability, respiratory distress, intoxication or severe pain limiting interview, plans for immediate surgery or admission to the intensive care unit, or anticipation of admission to the hospital for >72 h.[15] Follow up visits at 1, 3, 6, and 12 months were completed to assess for the development of PTSD symptoms. Study procedures were reviewed and approved by the Emory University Institutional Review Board and Grady Hospital Research Oversight Committee. All study data were recorded, de-identified, and managed electronically using REDCap.[25].

Measures

A standardized trauma interview (STI) was conducted in the ED to collect sociodemographic and medication data (Supplemental Table 1), and characteristics of the presenting trauma. The STI included clinician-rated severity of trauma, which assessed physical severity of injuries and trauma on a 5-point scale (high score reflecting more severe trauma), distinct from patient experience of index trauma.[15] The PTSD Symptom Scale (PSS) was collected at 1, 3, 6, and 12-month follow-up time points.[26] Interrater reliability was 97%.

PTSD Outcome Trajectories

Latent growth mixture modeling (LGMM) was applied using Mplus Version 7 to determine the number of distinct symptom class trajectories that existed among the data set, based on the PSS symptoms collected throughout the 12-month course of the study for each patient as previously described.[7,8,21] LGMM analyses were run only on the sample of participants who returned for at least one follow-up assessment. Participants were assigned a probability of class membership to three identified trajectories (nonremitting, recovery, or resilient) based on their symptom course as previously described.[7,8,21] For the present study, probability of nonremitting PTSD symptoms was used as the outcome variable of interest as this score accounts for the loadings on other classes for all individuals and captures variability across trajectories of symptom over time.[27,28] Furthermore, this approach characterizes an individual's response to a traumatic stressor over time, which is more illustrative and predictive of future resilience or pathologic adaptations than a single time point.[28]

Blood Sample and Biomarker Assessment

Venous blood samples were collected in EDTA tubes in the ED by medical staff using standard techniques. The mean time elapsed between trauma exposure and blood sampling was 201 min (standard error of the mean: 12.8) as previously described.[15] Within six hours of collection, EDTA tubes were centrifuged at 4°C and the plasma was aliquoted into 500 uL samples and frozen at −80°C until time of assay. As previously described,[15] interleukin 1 beta (IL-1β), interleukin 6 (IL-6), tumor necrosis factor (TNF), and interferon gamma (IFNγ) were analyzed using commercially available human multiplex assay (M500KCAF0Y, Bio-Rad, Hercules, Calif.) and a MAGPIX instrument (Luminex, Austin, TX).

Plasma concentrations of cortisol, progesterone, and testosterone were quantified at the Yerkes National Primate Research Center Biomarkers Core Laboratory using liquid chromatography -triple quadrupole tandem mass spectrometry (LC-MS/MS) multiplex steroid assays. Prior to LC-MS/MS analysis, supported liquid extraction (SLE+) was used to extract each steroid from 100 µL of plasma. Separation was achieved by gradient elution according to the following LC conditions using a Shimadzu Nexera X2 UHPLC system: Phenomenex Kinetex® 2.6 µm, C18, 100 Å, 50 × 2.1 mm LC column at 50°C with mobile phases composed of 0.1% formic acid in H20 and 0.1% formic acid in acetonitrile. A time gradient was created using 2 LC-30AD pumps running in series at a flow rate of 0.8 mL/min. d4-cortisol, 13C3-progesterone, d3-testosterone were used as internal standards. Thirteen calibrator levels covering a range of 0.01 to 100 µ/dL were processed together with each set of samples, quality control samples, and reagent blank. Three fortified quality control samples were analyzed in duplicate in each run. Mass spectrometric detection for each steroid was performed with a QTRAP®6500 system (AB SCIEX, Framingham, MA, USA). The system for these steroids were operated in positive electrospray ionization and multiple reaction monitoring mode. All data was acquired and processed using Analyst®1.5.2 software with hotfixes (AB SCIEX). Samples were assayed in duplicates and hormone levels were determined from a standard curve using linear regression analysis. The lower limit of quantitation (LLOQ)

for cortisol was 0.01 ng/mL, while the LLOQ for testosterone and progesterone was 0.05 ng/mL. Intra-run and inter-run precision for all steroids were < 10%. Plasma concentrations of 17β -estradiol were quantified using a commercially available ELISA kit (R&D Systems, Minneapolis, MN).

Samples were run by analysts who were blind to participant PTSD symptoms, and samples whose coefficient of variation (CV) between the replicates exceeded 15% were repeated and the values were averaged. Steroid hormones and cytokine levels were nonnormally distributed and were transformed to normality using a natural log transformation. To obtain a more comprehensive assessment of each participant's overall inflammatory response, and to avoid error due to over-reliance on any single representative cytokine when cytokines were intercorrelated with each other ($r = 0.33$ -0.52), a total pro-inflammatory cytokine score was calculated for each participant.[29] This was obtained by summing the natural log of IL-6, IL-1β, TNF, and IFNγ concentration for each participant.

Data Analytic Plan

The data were analyzed using SPSS v26 [30] and summarized as mean values + /- 95% confidence intervals. Figures were created in R [31] with ggplot2.[32] The alpha level was set at <0.05 for statistical significance. Analyses included participants with at least one follow-up assessments of PTSD symptoms allowing for LGMM estimation of their PTSD symptom trajectory ($N = 376$; 197 men and 179 women). Steroid hormone values that were <LLOQ upon assay were set at the LLOQ for analyses. Natural log of steroid hormone concentrations (estradiol, testosterone, progesterone, cortisol) and pro-inflammatory cytokine sum values >3 interquartile ranges for each sex were excluded as outliers for each respective analysis. Five of 10 pregnant or breastfeeding women met these criteria and were excluded from the analyses as a consequence. Welch's t-tests and chi-square tests were used to assess baseline sociodemographic differences between men and women, as well as differences in PTSD symptom trajectory and biomarker concentrations. Pearson's correlations were used to assess relationships between biomarker concentrations and probability of nonremitting PTSD in the overall sample, and within males and females. Mediation and moderated mediation analyses were conducted using PROCESS package which uses bootstrap sampling to create confidence intervals of effect sizes.[33] Moderated mediation analyses using each steroid hormone were exploratory. The moderation effect of each steroid hormone on the indirect effect of sex on probability of nonremitting PTSD outcome via inflammation was stratified by 1 SD above and below each sex-specific mean per Hayes.[34] Body mass index (BMI), time of day that trauma occurred (day/night), time elapsed between trauma event and blood draw, and whether the traumatic event involved interpersonal violence were included as covariates in all models.[15]

Results

Sex Differences in Demographics and Nonremitting PTSD Development

In this study population, men were significantly older than women ($t_{370.64} = 2.89$, $p < 0.001$; Table 1). There were no significant differences between the two sexes regarding race, BMI, clinician-rated severity of trauma, time of day that trauma occurred, elapsed time between trauma event and blood sampling, and whether the traumatic event involved interpersonal violence (all *p's*>0.05; Table 1). Women had a significantly higher probability of being in the nonremitting PTSD symptom trajectory than men ($t_{338.65} = -2.04$, $p = .04$; Figure 1A).

Sex Differences in Pro-inflammatory Cytokines and Steroid Hormone Concentrations

In posttrauma blood sample, pro-inflammatory cytokine scores were significantly greater in men than women ($t_{269.91} = 4.35$, $p < 0.001$; Table 1, Figure 1B). Estradiol concentrations did not differ between men and women ($t_{149.48} = -1.19$, $p = .24$; Table 1, Figure 2A). Men had significantly higher testosterone than women ($t_{133} = 12.68$, $p < 0.001$; Table 1, Figure 2B), and women had significantly higher progesterone concentrations than men ($t_{143} = -5.14$, $p < 0.001$; Table 1, Figure 2C). Finally, cortisol concentrations were significantly greater in women than men ($t_{234.31} = -5.24$, $p < 0.001$; Figure 2D).

Relationships Between Proinflammatory Cytokines, Steroid Hormone Concentrations, and PTSD Risk

In the overall sample, pro-inflammatory cytokine scores were significantly associated with probability of developing nonremitting PTSD ($r = -0.18$, $p < 0.01$) and cortisol concentrations ($r = -0.14$, $p = 0.02$), but not with concentrations of estradiol, testosterone, and progesterone (*p*'s>0.05; Table 2). Pro-inflammatory cytokine scores were significantly associated with estradiol concentrations in men ($r = 0.31$, $p < 0.001$), but not women ($r = 0.00$, $p = 0.98$; Table 2). Estradiol, testosterone, a cortisol concentration did not predict nonremitting PTSD risk in the overall sample, or in either sex ($p > 0.05$, Table 2). Progesterone was found to predict probability of developing nonremitting PTSD in men ($r = 0.23$, $p < 0.01$, Table 2) but not women ($p = 0.25$).

Indirect Effect of Sex on PTSD Risk Through Inflammation

To assess whether sex differences in the inflammatory response to traumatic stress exposure contribute to prospective sex differences in the development of nonremitting

Table 1. Sociodemographic, Biomarker Concentrations, and Trauma Characteristics of Participants Broken Down by Sex.

	Male		**Female**		**Analysis**		
	Mean	SD	Mean	SD	t	df	*p*
Age (years)	37.86	12.75	34.05	12.74	2.89	370.64	<0.001
Body mass index	27.40	5.86	28.96	8.81	−2.00	305.47	.05
Clinician-rated severity of trauma (0–5)	2.73	0.97	2.64	1.04	0.80	363.48	.42
Time elapsed between trauma and blood draw (hours)	3.37	3.47	4.01	4.22	−1.55	333.69	.12
	N	%	N	%	$\chi 2$		*p*
Race					8.27	5.00	.14
Black	141	72	141	79			
White	39	20	22	12			
Other	17	8	16	9			
Interpersonal trauma					3.18	1.00	.08
Yes	28	14	39	22			
No	169	86	140	78			
Time of day trauma occurred					4.46	2.00	.11
Day	161	82	136	76			
Night	36	18	40	22			
Unknown	0		3	2			
	Mean	SD	Mean	SD	t	df	*p*
Estradiol (pg/mL)	56.09	8.62	62.77	67.2	−1.19	149.48	.24
Testosterone (ng/mL)	4.37	3.37	0.13	0.15	14.59	133.51	<0.001
Progesterone (ng/mL)	0.05	0.02	2.93	5.74	−6.03	143	<0.001
Cortisol (ng/mL)	186.07	100.00	275.95	180.00	−5.24	234.31	<0.001
Pro-inflammatory cytokine sum (ln ng/mL)	7.65	1.55	6.86	1.46	4.35	269.91	<0.001

Reference range for steroid hormones: estradiol: male 20−50 pg/mL, premenopausal female 10−300 pg/mL; testosterone: male 2.91−11 ng/mL, female 0.18 −0.54 ng/mL; progesterone: male 0.12−0.3 ng/mL, luteal female 2−30 ng/mL; AM cortisol: 50−250 ng/mL.[35]

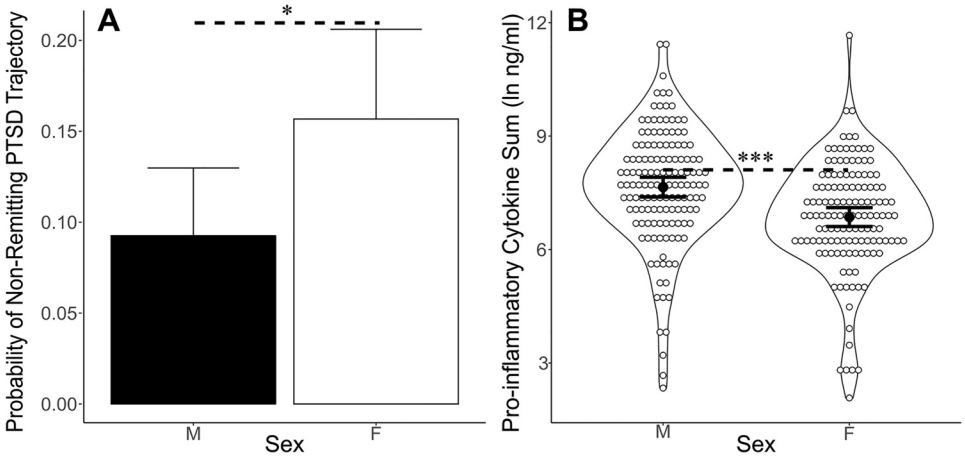

Figure 1. (A) Females (F; $n = 179$) had a significantly higher probability of belonging to the nonremitting posttraumatic stress disorder (PTSD) symptom trajectory than males (M; $n = 197$). (B) Sex differences in pro-inflammatory cytokines collected posttrauma (sum of natural log transforms of interleukin [IL]-6, IL-1β, tumor necrosis factor [TNF], and interferon-gamma [IFNγ]). Bold points represent means, empty points represent individual values. In both figures, error bars denote 95% confidence intervals. *$p < 0.05$, ***$p < 0.001$.

PTSD symptoms, we examined the indirect effect of sex on the probability of belonging to the nonremitting PTSD trajectory through its effect on the pro-inflammatory cytokine sum score using mediation analysis (Figure 3). Sex was significantly associated with pro-inflammatory cytokine sum score ($B = -0.82$, $\beta = -0.45$, $p < 0.001$), as men had higher levels of inflammation than women. Lower pro-inflammatory cytokine levels in the ED significantly predicted probability of developing nonremitting PTSD symptoms ($B = -0.03$, $\beta = -0.15$, $p < 0.01$). The direct effect of sex on PTSD outcome was $B = 0.03$. The total effect of sex on PTSD outcome via pro-inflammatory cytokine levels was $B = 0.06$.

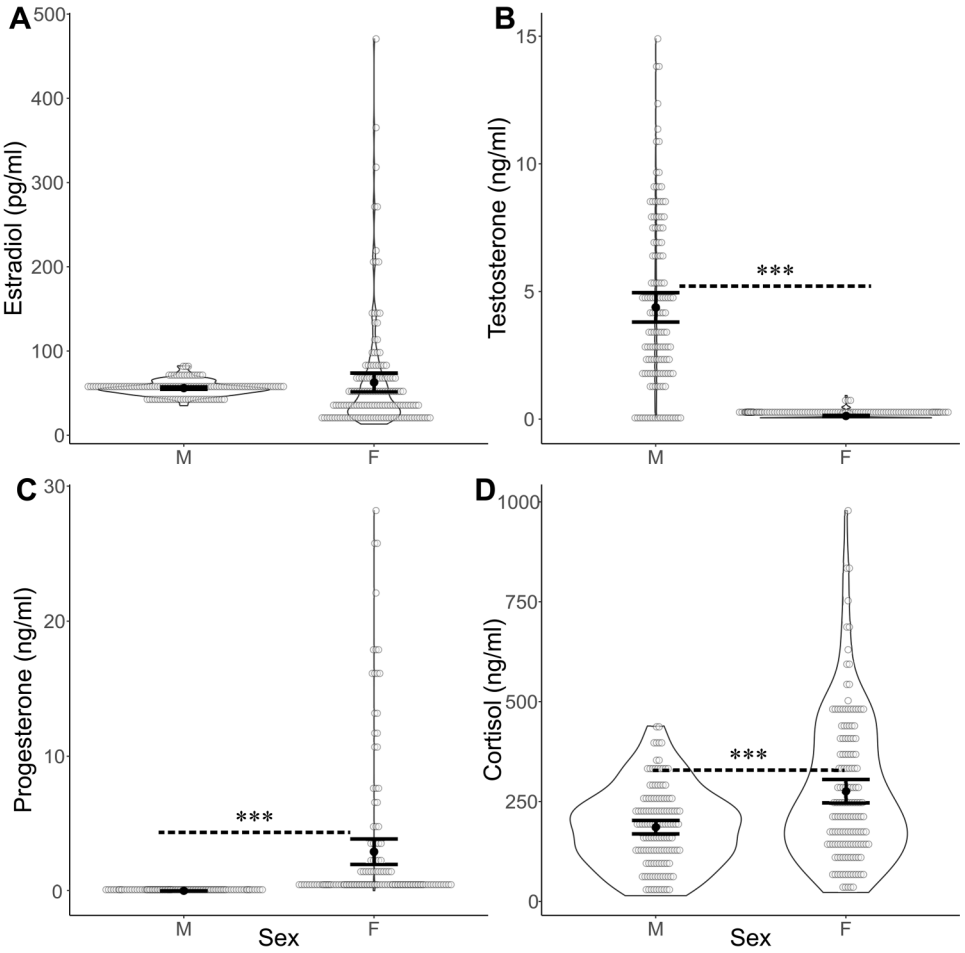

Figure 2. Distribution of estradiol (A), testosterone (B), progesterone (C), and cortisol (D) concentrations collected in a posttrauma blood sample by sex. In all plots, bolded point represents the mean, error bars demonstrate 95% confidence limits. Empty points represent individual values. ***$p < 0.001$.

Table 2. Correlations Between Pro-inflammatory Scores, Steroid Hormone Concentrations, and PTSD risk in the Overall Sample, and Within Each Sex.

	Pro-inflammatory sum score			**Nonremitting PTSD**		
	r(*p*-value) Overall	Male	Female	r(*p*-value) Overall	Male	Female
Nonremitting PTSD	**−0.18(<0.01)**	**−0.20(0.02)**	−0.12(0.16)			
Estradiol	0.01(0.93)	**0.31(<0.001)**	0.00(0.98)	0.09(0.13)	−0.14(0.13)	0.11(0.17)
Progesterone	−0.06(0.36)	−0.04(0.70)	0.03(0.72)	0.03(0.61)	**0.23(<0.01)**	−0.10(0.25)
Testosterone	0.13(0.05)	−0.06(0.55)	−0.16(0.08)	−0.06(0.29)	0.05(0.53)	−0.02(0.83)
Cortisol	**−0.14(0.02)**	−0.09(0.32)	−0.09(0.31)	0.04(0.50)	0.02(0.78)	0.00(0.96)
Pro-inflammatory sum score				**−0.18(<0.01)**	**−0.2(0.02)**	−0.12(0.16)

Abbreviation: PTSD, posttraumatic stress disorder.

The indirect effect of sex on probability of developing nonremitting PTSD symptoms through pro-inflammatory cytokine levels was significant (ind. = 0.03, β = 0.07, $p < 0.05$), such that men had greater inflammation which was associated with lower risk of PTSD symptom development. In addition to this indirect effect via pro-inflammatory cytokine sum score, post hoc exploratory analyses of individual cytokines showed the indirect effect of sex on probability of developing nonremitting PTSD via log IFNγ alone was significant (ind. = 0.02, β = 0.07, $p < 0.05$; Supplemental Table 2).

Conditional Indirect Effect of Sex Based on Steroid Hormone Concentrations

We then used moderated mediation analyses to examine whether the indirect effect of sex on probability of developing nonremitting PTSD via pro-inflammatory cytokines was contingent upon sex steroids known to impact pro-inflammatory cytokines. These exploratory analyses showed that the indirect effect of sex on PTSD outcome via inflammation was significantly moderated by estradiol ($B = -3.15$, $p < 0.01$, Figure 4). Specifically, in men, higher concentrations of estradiol were protective against probability of developing nonremitting PTSD. At low concentrations of estradiol (1 SD below the mean), the indirect effect of sex on nonremitting PTSD trajectory was $B = -0.01$. At the mean and high ($+1$ SD) concentrations of estradiol, the indirect effect of sex was $B = 0.02$ ($p < 0.05$) and $B = 0.08$ ($p < 0.05$), respectively. Cortisol, testosterone, and progesterone were not found to significantly moderate the indirect effect of sex on probability of developing nonremitting PTSD (all *p's*>0.05).

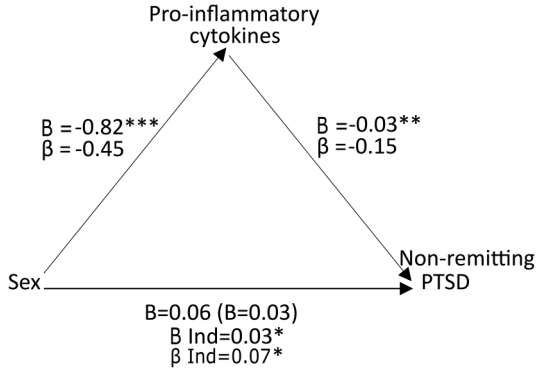

Figure 3. Results of mediation model examining sex, pro-inflammatory cytokines collected posttrauma (sum of natural log transforms of interleukin [IL]-6, IL-1β, tumor necrosis factor [TNF], and interferon-gamma [IFNγ]), and probability of being on a nonremitting posttraumatic stress disorder (PTSD) symptom trajectory. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Discussion

The purpose of the present study was to characterize how the inflammatory and steroid hormone response to trauma exposure prospectively contributes to differential risk for developing nonremitting PTSD between men and women. The results show that in a civilian sample presenting to the emergency department of a large, urban hospital after trauma exposure, women prospectively had a higher probability of developing nonremitting PTSD than men, corroborating previous cross-sectional and epidemiological findings.[3] There was no difference in clinician-rated severity of trauma between men and women in the study samples, and no difference between the sexes in proportion of index traumas involving interpersonal violence. Our assessment of inflammatory markers and steroid hormones in the aftermath of traumatic stress exposure revealed significant sex differences such that women exhibited lower pro-inflammatory cytokines and testosterone, and higher cortisol and progesterone concentrations than men. Estradiol concentrations, however, were not different between the sexes. Lastly, we found that higher inflammatory cytokine levels after trauma in men were protective against probability of developing nonremitting PTSD in a manner that was dependent on estradiol concentrations. Taken together, these results underscore

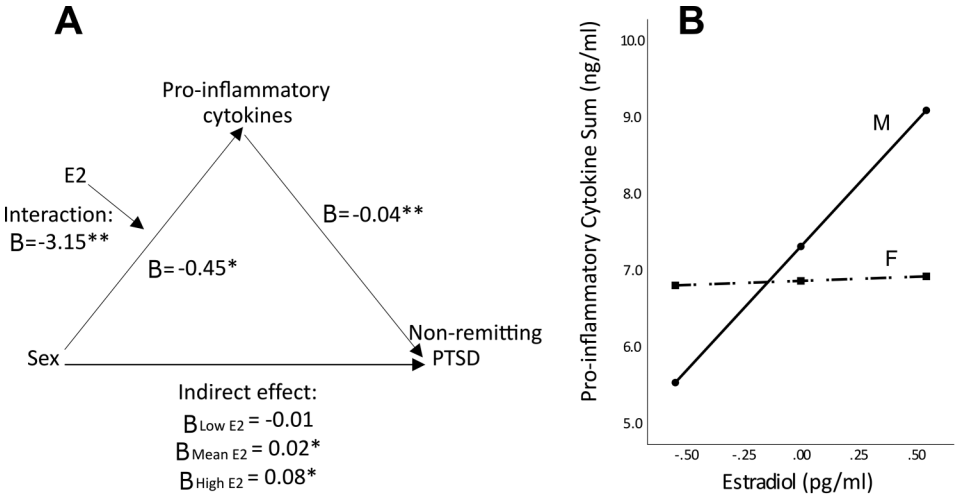

Figure 4. (A) Results of moderated mediation model examining sex, estradiol, pro-inflammatory cytokines collected posttrauma (sum of natural log transforms of interleukin [IL]-6, IL-1β, tumor necrosis factor [TNF] and interferon-gamma [IFNγ]), and probability of being on a nonremitting posttraumatic stress disorder (PTSD) symptom trajectory. (B) Interpolation lines showing the interaction effect between estradiol level and pro-inflammatory cytokine sum in males (M; solid line) and females (F; dashed line). *$p < 0.05$, **$p < 0.01$.

the role of inflammatory signaling and steroid hormones in the aftermath of trauma exposure in conferring sex-specific risk for the prospective development of PTSD, and highlight the importance of studying sex differences within and across biological systems that may historically only have been assessed in one sex versus the other (e.g. assessing estradiol in men when historically it has been labeled as a female sex hormone).

The current results extend our previous finding that lower TNF and IFNγ concentrations in the aftermath of traumatic stress exposure are prospectively associated with increased risk of nonremitting PTSD trajectory [15] by demonstrating that the pro-inflammatory response to traumatic exposure mediates differential prospective risk in the development of nonremitting PTSD between men and women. Women showed lower pro-inflammatory cytokine levels compared to men in the aftermath of trauma exposure and these lower levels of pro-inflammatory cytokines were associated with increased risk of developing nonremitting PTSD. Importantly, pro-inflammatory markers influence memory consolidation and fear conditioning, as low doses of IL-1 increase acquisition of hippocampal-dependent passive avoidance learning in rats [36] and TNF and its receptors enhance synaptic plasticity via increasing surface expression of AMPA receptors [37] and can impact contextual fear conditioning.[38] Our results corroborate data showing that a decreased inflammatory response to stress exposure is associated with greater psychophysiological hyperarousal (e.g. startle) [16] and posttraumatic anxiety,[17] and suggest that increased inflammation in men following traumatic stressor exposure exerts a protective effect on future PTSD development.[39]

We used exploratory analyses to assess whether the sex difference in inflammatory response to trauma was related to cortisol, estradiol, progesterone, and testosterone concentrations in the acute aftermath of trauma,[6,37] as cortisol, testosterone and estradiol levels are dynamically altered in response to trauma [40] and stress exposure,[23] and can modulate activity of the immune system.[9,10,18] Although levels of testosterone, progesterone and estradiol were not related to the inflammatory response to traumatic stress exposure in the overall sample, higher cortisol concentrations were associated with lower pro-inflammatory score. While this relationship is consistent with the notion that HPA axis-induced secretion of cortisol inhibits the pro-inflammatory immune response via glucocorticoid negative feedback,[12,13] previous studies have shown that the inability to mount a cortisol response to traumatic stress exposure is predictive of greater PTSD risk.[41,42] The current data suggest that mounting an inflammatory response to traumatic stress is protective against PTSD development,[39] and indicates that individual-specific factors that influence this response, including steroid hormones, should be considered when determining the efficacy of pharmacological early interventions, such as hydrocortisone,[22] in attenuating prospective risk for PTSD development. This notion is further supported by the current data describing sex differences in steroid hormone levels and their relationships to pro-inflammatory markers in the acute aftermath of traumatic stress exposure.

Women in the current study exhibited lower testosterone levels, and higher progesterone concentrations than men, corroborating previous data showing quantitative sex differences among these steroid hormones.[43] Women in the current study had significantly greater cortisol concentrations posttrauma than men, consistent with studies of healthy college-aged individuals subjected to social stress demonstrating increased cortisol reactivity to psychosocial stress exposure.[18] Interestingly, peripheral estradiol concentrations were not different between the sexes at this posttraumatic timepoint, as posttrauma estradiol concentrations of men were elevated above the normal serum range, while posttrauma estradiol concentrations in women were more variable as expected due to menstrual cycling and menopausal status and remained within normal limits.[35] Our data suggest that men may have increased estradiol levels posttrauma, although an absolute increase cannot be determined without a pretrauma baseline sample. However, increased estradiol concentrations have been noted in both men and women after trauma [40] and stress exposure,[23] and may be due to stress-induced upregulation of aromatase.[44] It is important to note that a potential bias may have been introduced by setting steroid hormone values below the LLOQ to the LLOQ for analyses. This decision was made to include these participants and maintain power in our analyses, and primarily resulted in upward correction of progesterone levels in men, testosterone levels in women, estradiol levels in both sexes, and minimal adjustment in cortisol levels in both sexes.

Although most often associated with gonadal and adrenal production, steroidogenesis also occurs in the brain (neurosteroidogenesis) and can be induced by immune system activation in response to injury and stress exposure. More specifically, estradiol is synthesized from testosterone by aromatase in neurons within the hypothalamus, amygdala, hippocampus, and cerebral cortex.[45] Translational rodent studies have shown that inflammatory cytokines induced by mechanical injury increase central aromatase expression in reactive astroglia [46] in a manner that is dependent on TNF.[47] In songbirds, acute restraint stress exposure increases central aromatase activity,[48] and inflammatory cytokines IL-6 and IL-1β induced by the irritant phytohemagluttin (PHA) in the absence of injury are sufficient to upregulate central aromatase.[49] Increased neurosteroidogenesis of estradiol is neuroprotective [45] and decreases neurotoxin-induced neurodegeneration in male rats.[46] Administration of fadrozole, an aromatase inhibitor, increases reactive gliosis and neurodegeneration after mechanical injury in male birds,[50] further suggesting that endogenous aromatase activity and increased estradiol is critically important in recovery from traumatic stressor exposure.

Greater estradiol concentrations were associated with higher inflammatory cytokine levels in men but not

women, and higher inflammatory cytokines were inversely associated with prospective risk for nonremitting PTSD. This result suggests that the steroid hormone modulation of inflammatory response to traumatic stress confers risk for PTSD via its ability to modulate the immune system and cytokine expression. This notion is supported by evidence showing that estradiol administration in humans increases neutrophil numbers in blood [51] that have previously been shown to be important for prospective PTSD development,[14] and *in vitro* treatment of natural killer cells with estradiol enhances the production of IFNγ in mice.[52] Other *in vitro* studies indicate that estradiol's ability to modulate cytokine production in human monocytes and macrophages is dose-dependent, as low estradiol concentrations enhance the production of pro-inflammatory cytokines and high concentrations attenuate their concentration.[41,42] Taken together, these findings suggest the sex difference in the effect of estradiol concentrations on prospective nonremitting PTSD risk may be explained by differential sensitivity of the inflammatory response in men and women to estradiol fluctuations.

While our results showed that estradiol moderates the relationship between sex and inflammation, estradiol did not directly mediate the effect of sex on probability of developing nonremitting PTSD. We did not find any impact of cortisol, progesterone and testosterone on conferring risk of an attenuated inflammatory response to traumatic stress and concomitant development of PTSD. These results were unexpected, as cortisol, progesterone, and testosterone have all been shown to modulate the immune system activity and inflammation [6,11–13] and have been implicated in the etiology and maintenance of PTSD.[13–16,22] An important caveat however is that most research on these steroid hormones to date have focused on their contribution to symptoms of the PTSD disease state rather than their contribution to prospective risk at the time of trauma.[53] Additionally, this research has historically been conducted in a sex-specific manner where steroids typically categorized as sex steroids for females (estradiol and progesterone) have not been extensively studied in males, and *vice versa* regarding testosterone levels in females.

A limitation of the present study is that while we show that sex differences in differential risk for PTSD may be related to differential sensitivity of the inflammatory response to fluctuations in estradiol concentrations between men and women, we were unable to account for natural and induced hormonal variation. Hypogonadal women (menopausal, surgically/pharmacologically suppressed) would be expected to have attenuated estradiol concentrations and inflammatory response after trauma and increased risk of developing nonremitting PTSD. In contrast, naturally cycling women with greater variance in estradiol concentrations may have decreased sensitivity to estradiol that may result in smaller inflammatory response to trauma exposure. As overall reported use of contraceptives and synthetic hormones in our study sample was low (Supplemental Table 1), future studies are necessary to better understand how menopausal status and pharmaceuticals that alter endogenous steroid levels (e.g., contraceptives, hormone replacement therapies, aromatase inhibitors) impact endogenous steroid levels and contribute to PTSD development.

The current findings are also limited by our analyses of multiple biomarkers from a single blood sample collected within 24 h of index trauma and follow-up assessments up to 12-months posttrauma exposure. As has been shown in studies of healthy participants and individuals with PTSD, in such dynamic systems as the HPA axis and immune response, characterizing an individual's response to a traumatic stressor over time is more illustrative and predictive of future resilience or pathologic adaptations than a single time point.[21] Future studies following individuals for >12 months following trauma exposure and incorporating serial blood draws in the immediate posttrauma period would enable a more comprehensive assessment of biomarkers of risk for developing nonremitting PTSD. Future studies also are necessary to assess the role of the immune system and steroid hormones in the development of subcluster symptoms of PTSD following trauma exposure, as well as recovery from and resilience to PTSD after trauma exposure. Finally, while medication usage that may impact the immune response to traumatic stress exposure was relatively low in our study sample (Supplemental Table 1), we were not able to account for chronic comorbidities that also may impact inflammation beyond adjusting for BMI.

Conclusion

In summary, we show that women prospectively have a higher probability of developing nonremitting PTSD than men in the aftermath of civilian trauma exposure, and that the peripheral pro-inflammatory cytokine response to traumatic stress exposure mediates this sex difference in the prospective development of PTSD. Overall, the current data suggest that mounting an inflammatory response to traumatic stress is protective against PTSD development,[39] and indicates that individual-level and contextual factors that influence this response, including steroid hormones, should be considered when identifying at-risk individuals and determining the efficacy of psychological and pharmacological early interventions for preventing PTSD development. With heightened interest in the clinical relevance of the inflammatory response as a result of the COVID-19 pandemic,[54] IL-6 assays are now available in many health systems as an in-house assay. TNF and IFNγ may also become routine orders that could be evaluated in patients presenting to trauma centers, allowing for the establishment of follow-up protocols that connect trauma survivors at risk for developing nonremitting PTSD to evidenced based treatment.

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Declaration of Conflicting Interests

Dr. Galatzer-Levy receives a salary from Facebook. Dr. Rothbaum has received funding from the Wounded Warrior Project, the Department of Defense (clinical trial grant W81XWH-10-1-1045), and the McCormick Foundation; she has served on advisory boards for Aptinyx, Genentech, Jazz Pharmaceuticals, Neuronetics, Nobilis Therapeutics, and Sandoz; and she receives royalties from Oxford University Press, Guilford Publications, American Psychiatric As- sociation Publishing, and Emory University. Dr. Ressler has received research funding from the Burroughs Wellcome Foundation, the Howard Hughes Medical Institute, and NARSAD; he has served as a consultant for Alkermes, Biogen, and Resilience Therapeutics; and he holds patents for a number of targets related to improving extinction of fear (he has received no equity or income within the past 3 years related to these). In the last year, Dr. Nemeroff has received research support from NIH; he has served a consultant for SK Pharma, Silo Pharma, Signant Health, Intra-Cellular Therapies, Navitor Pharmaceuticals, Prismic Pharmaceuticals, Sunovion Pharmaceuticals, Taisho Pharmaceutical, Total Pain Solutions, ANeuroteck, Neuritek and Xhale; he is a stockholder in Antares, BI Gen Holdings, Celgene, Seattle Genetics, and Xhale; he has served on scientific advisory boards for the Anxiety Disorders Association of America (ADAA), the Brain and Behavior Research Foundation, the Laureate Institute for Brain Research, Skyland Trail, and Xhale; he has served on boards of directors for ADAA and Gratitude America; he has income sources or equity of $10,000 or more from American Psychiatric Publishing, Signant Health, Intra-Cellular Therapies, and Silo Pharma, and he has patents on a method and devices for transdermal delivery of lithium (US 6,375,990B1) and on a method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by ex vivo assay (US 7,148,027B2). Dr. Maples-Keller has received funding from COMPASS Pathways and has received a speaking fee from COMPASS Pathways.

All remaining authors have nothing to disclose.

ORCID iDs

Chloe S. Lalonde https://orcid.org/0000-0002-6775-3299
Charles B. Nemeroff https://orcid.org/0000-0001-7867-1160
Vasiliki Michopoulos https://orcid.org/0000-0003-2531-923X

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