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Altered overnight levels of pro-inflammatory cytokines in men and women with posttraumatic stress disorder

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ABSTRACT

Background: Posttraumatic stress disorder (PTSD) is associated with disturbed sleep and elevated levels of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Studies in animals and healthy humans have also shown that disrupted sleep elevates pro-inflammatory cytokines, including IL-6 and TNF-α. A better understanding of overnight cytokine levels and sleep might shed light on possible mechanisms for elevated inflammation in PTSD. Thus, we investigated overnight levels of IL-6 and TNF-α in individuals with and without PTSD while recording sleep polysomnography (PSG).

Method: Serum samples were collected from otherwise healthy, medication-free participants with chronic PTSD (n = 44; 50% female; M age = 30.34 ± 8.11) and matched controls (n = 49; 53% female; M age = 30.53 ± 6.57) during laboratory PSG. Levels of IL-6 and TNF-α were measured at hours 0, 2, 4, 6, and 8 after typical sleep onset time using serial serum samples. Plasma IL-6 and TNF-α levels were quantified using enzyme-linked immunosorbent assays.

Results: Growth model analysis indicated a significant*group* by *time* interaction for IL-6 (t[247] = -2.92, p = .005) and a significant *group* by *sex* by *time* interaction for TNF-α (t[275] = 2.02, p = .04). PTSD positive men and women initially had higher IL-6 and TNF-α at sleep onset, but not at the end of their sleep cycle. Men with PTSD showed a peak of TNF-α at the end of the sleep cycle, whereas male control subjects demonstrated an inverted U-shaped profile. There were no significant differences in TNF-α levels overnight between women with and without PTSD.

Conclusion: To our knowledge, this is the largest study to examine IL-6 overnight in a PTSD sample and the first study to examine overnight TNF-α in PTSD. Overnight IL-6 and TNF-α levels may be altered in individuals with PTSD compared to those without PTSD, and TNF-α trajectories also differed by sex. The current findings highlight the need to consider sex, sleep, time of day, and circadian variation when examining inflammation in PTSD. Additional research in broader study samples will be necessary to clarify associations between disrupted sleep, cytokines, and increased risk for disease in PTSD.

1. Introduction

Posttraumatic Stress Disorder (PTSD) is a debilitating condition with a lifetime prevalence of approximately 8% (Kessler et al., 1995). In addition to severely impairing psychological wellbeing, PTSD is associated with increased risk for cardiovascular, autoimmune and metabolic disorders, and premature mortality (Boscarino, 2006; Cohen et al., 2009; O'Donovan et al., 2015). Inflammation can drive the development of multiple physical diseases and has been proposed as a mechanism linking PTSD with ill health (O'Donovan et al., 2013). Though studies differ with respect to methodology (Hussein et al., 2017), numerous studies and a meta-analysis have confirmed that

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individuals with PTSD tend to exhibit elevated levels of inflammatory markers, including levels of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α; Gill et al., 2008; Lindqvist et al., 2014; Passos et al., 2015), as well as other inflammatory markers such as interferon-gamma (IFN-γ) and high-sensitivity C-reactive protein (Bruenig et al., 2018; Lindqvist et al., 2017). Additionally, certain treatment strategies, such as psychopharmacological agents (Kenis and Maes, 2002) as well as psychotherapy (Himmerich et al., 2016) can lead to changes in levels of inflammatory markers. However, people with PTSD are a highly heterogeneous group and not all individuals with PTSD display elevated inflammation (Söndergaard et al., 2004). Thus, identifying the specific symptoms of PTSD associated with inflammatory activity could be a first step towards designing interventions that reduce inflammation in PTSD.

Most individuals with PTSD exhibit substantial sleep disturbance, in the form of insomnia and frequent nightmares (Neylan et al., 1998). Objective studies of sleep in PTSD have shown decreased sleep continuity (Mellman et al., 1997) decreased slow-wave sleep (Kobayashi et al., 2007; Richards et al., 2013) and alterations in rapid eye movement (REM) sleep (Breslau et al., 2004). However, objective sleep disturbance in PTSD appears to differ by sex; women with PTSD show more REM sleep than controls (Otte et al., 2007; Richards et al., 2013). Disrupted sleep in the early aftermath of trauma is associated with worse subsequent clinical symptoms (Mellman et al., 2002), indicating a potential role of sleep as a mechanism of PTSD symptoms. Disrupted sleep is also associated with poor physical health outcomes in PTSD (Boyko et al., 2013), indicating a potential role for sleep in increasing physical disease risk in this population.

Research in healthy controls and other sleep-disordered groups indicates that sleep and inflammation are closely intertwined. Some studies show sleep disruption leads to increased concentrations of both IL-6 and TNF-α (Irwin et al., 2006), and a large meta-analytic study showed a significant association of short sleep with higher levels of IL-6 when sleep was measured objectively (Irwin et al., 2016). Circadian release of TNF-α has also been shown to be disrupted in sleep apnea patients, another group with considerable sleep disturbance (Entzian et al., 1996). PTSD-related sleep disturbance, therefore, could plausibly promote the production of pro-inflammatory cytokines during the night and thereby increase risk for disease. Given that elevated levels of pro-inflammatory cytokines may feed back to the nervous system to further interrupt sleep, particularly delta sleep (Redwine et al., 2000), the relationship between PTSD-related sleep disturbance and pro-inflammatory cytokines may be bidirectional.

Despite the evidence for an interrelationship between disrupted sleep and elevated cytokines in PTSD, few studies have examined this directly by studying overnight trajectories of pro-inflammatory cytokines in people with PTSD compared to controls. One study (Gill et al., 2010) examined nine participants with PTSD comorbid with major depressive disorder, nine participants with only PTSD, and 14 non-traumatized control subjects, comparing these three groups on IL-6 levels overnight. In this sample, participants with major depressive disorder and PTSD had significantly higher mean levels of IL-6 than participants in either of the other two groups. Furthermore, peak overnight IL-6 levels correlated significantly with PTSD symptomatology, indicating that people with more severe PTSD showed elevated IL-6 concentrations. These findings support the notion that PTSD is associated with elevated overnight cytokine levels. However, this study did not examine differences in overnight IL-6 by sex and it had a relatively small sample size in each group. Given sex differences in both objective sleep in PTSD (Richards et al., 2013) and associations between sleep and markers of inflammation (Suarez, 2008; Prather et al., 2013), the relationship between disrupted sleep and cytokine production may differ in men and women. To our knowledge, no studies have compared overnight concentrations of TNF-α in people with and without PTSD or examined trajectories of any cytokine overnight.

In the present study, we assessed overnight trajectories for IL-6 and TNF-α in sex- and age-matched adults with and without PTSD. We expected to observe elevated IL-6 and TNF-α concentrations across the night in people with PTSD compared to healthy controls. We also expected that overnight IL-6 and TNF-α would be related to total sleep time such that participants with the shortest sleep duration would show the highest levels of both cytokines.

2. Methods

2.1. Study participants

Our sample included 85 participants recruited as part of a larger study (N = 93) focused on sleep abnormalities in PTSD. We acknowledge that a mean of 54 on the Clinician Administered PTSD Scale (CAPS) is lower than many studies with treatment seeking patients, which may be due to our inclusion criteria of being medically healthy and on no medication (see below). As detailed in other studies using this sample (e.g., O'Donovan et al., 2011), participants were recruited through ads and flyers distributed in the community as well as in relevant local clinics for the PTSD sample. Eight participants were excluded from the present study due to difficulties in overnight blood collection. In our sample, 43 participants were positively diagnosed with current, chronic PTSD and 42 were sex- and age-matched medically healthy controls (see Table 1). PTSD diagnosis was established via the CAPS (Blake et al., 1995) conducted by trained clinical interviewers. Eligible participants met DSM-IV criteria for PTSD; see Table 2 for clinical characteristics of the sample. Exclusion criteria included a history of traumatic brain injury; presence of neurologic disorders or systemic illness; use of psychiatric, anticonvulsant, antihypertensive, sympathomimetic, steroidal, statin or other prescription medications; obesity as defined by a body mass index of BMI $>$ 30; alcohol abuse or dependence in the prior 2 years; substance abuse or dependence in the previous year; any psychiatric disorder with psychotic features; bipolar disorder or obsessive-compulsive disorder. Subjects with extreme morning tendencies (score $<$ 19) and extreme evening tendencies (score $>$ 47) on the Smith Morningness Scale (Smith et al., 1989) were excluded. For female participants, pregnancy was an exclusion criterion and all were premenopausal (indicated by having at least one menstrual period in the past 12 months) and scheduled during a follicular phase of the menstrual cycle. Further exclusion criteria for control participants included a history of current or lifetime PTSD and a lifetime history of major depressive disorder (MDD) or panic disorder. All participants provided written informed consent before participating in any study procedures and the project was approved by the Committee on Human Research at the University of California, San Francisco.

2.2. Procedure

Participants stayed three consecutive nights at the sleep laboratory in a General Clinical Research Center (GCRC; see Richards et al., 2013 for more details). The current study involved data from the second night, after one habituation night in the sleep laboratory. To account for individual variability of sleep onset, participants were asked to report habitual sleep onset (HSO) via 1-week sleep diary prior to the study. HSO was then used as an individual onset for data recording and acted as the starting point of the experiments, as well as a point of reference for this study. Two hours before HSO, a catheter was inserted into an antecubital vein for repeated blood sampling. Whole blood samples were drawn every 15 min starting with HSO until 8 h after HSO. For this study, blood samples from 0, 2, 4, and 6 h after HSO were used. Furthermore, a last blood draw was added between 7 A M and 10 A M after habitual waking time, marking the last timepoint in our analyses.

Table 1
Demographics of controls and PTSD positive participants.

	Control (n = 42)	PTSD (n = 43)	d/ϕ_c	DF	t/X^2	p
Age (*M* (*SD*))	30.48 (8.26)	30.63 (6.63)	−0.02	78.48	−0.09	.93
Years of Education (*M* (*SD*))	15.38 (2.02)	14.86 (2.23)	0.24	82.55	1.13	.26
BMI (*M* (*SD*))	24.45 (3.74)	26.93 (4.69)	−0.59	79.80	−2.71	.01
Total sleep time (M (SD))	774.84 (29.16)	748.63 (36.20)	0.20	73.17	0.88	.38
Clock time of HSO (M (SD)	00:07 h (69 min)	23:48 h (76 min)	−0.26	82.61	1.21	.23
Average IL-6 (*M ln pg/ml* (*SD*))	0.10 (0.80)	0.13 (0.97)	−0.02	80.78	−0.11	.86
Average TNF-α (*M ln pg/ml* (*SD*))	0.03 (0.35)	0.00 (0.33)	0.07	82.33	0.34	.74
Sex (Male %)	21 (50.00)	22 (51.16)	0.00	1	0.00	> .99
Smoking Status (%)	8 (19.05)	11 (25.58)	0.05	1	0.21	.64
Veteran Status (%)	0 (0)	10 (23.26)				
Ethnicity (%)			0.24	4	14.62	.01
African American	1 (2.38)	5 (11.63)				
Asian	7 (16.67)	2 (4.65)				
Caucasian	31 (73.81)	23 (53.49)				
Hispanic	3 (7.14)	6 (13.95)				
Others	0 (0.00)	7 (16.28)				
Marital Status (%)			0.21	2	7. 49	.02
Divorced/Separated	1 (2.38)	8 (18.60)				
Married	6 (14.29)	2 (4.65)				
Single	35 (83.33)	33 (76.74)				

Note: Welch two-sample *t*-test were applied to compare the groups on continuous measures and X^2-tests were applied to discrete measures. For statistical testing, cytokine measures were log-transformed before entering the analyses to approximate normal distributions. BMI = body mass index; HSO = habitual sleep onset.

Table 2
Clinical Characteristics.

	Control (n = 42)	PTSD (n = 43)
Trauma-Exposed (n (%))	11 (26.19)	43 (100.00)
Clinician Administered PTSD Scale Total Score (M (SD))	0 (0.00)	54.00 (14.91)
Time Since Trauma (Years (SD))	11.53 (10.20)	9.01 (9.36)
Trauma Type (n (%))		
Combat	0 (0.00)	8 (18.60)
Motor Vehicle Accident	4 (9.52)	0 (0.00)
Physical Violence/Abuse	4 (9.52)	18 (41.86)
Sexual Assault/Abuse	0 (0.00)	16 (37.21)
Sudden/Violent death	2 (4.76)	2 (4.65)
Other	1 (2.38)	5 (11.63)

Note: For trauma type, numbers and percentages overlap because some Criterion A events fit multiple categories. "Other" category included almost drowning (n = 1, PTSD group), stalking (n = 2, PTSD group), and complications from a medical procedure (n = 1, Control group).

2.3. Measures

2.3.1. PTSD diagnosis

Lifetime and current PTSD were assessed with the Clinician Administered PTSD Scale (CAPS), a structured interview that corresponds to DSM-IV criteria for PTSD (Blake et al., 1995). The CAPS is a 30-item scale that assesses the frequency and intensity of re-experiencing, avoidance, and hyperarousal symptoms of PTSD. Diagnosis of PTSD was based on symptoms experienced in the previous month associated with the subject's self-identified worst traumatic event. Other psychiatric disorders were assessed by administration of the Structured Clinical Interview for DSM-IV (First et al., 1995).

2.3.2. Cytokines

The human IL-6 Quantikine high sensitivity enzyme-linked immunosorbent assay (hsELISA) and human TNF-α Quantikine hsELISA were used to measure IL- 6 and TNF-α respectively (R&D Systems, USA). The lower limits of detection were 0.17 pg/ml for IL-6 and 0.62 pg/ml for TNF-α. Where duplicates differed more than 20%, samples were repeated in duplicate. Intra-assay coefficients of variation were < 10% for both IL-6 and TNF-α. One sample had an IL-6 level below the lowest detectable limit of the assays, and 19 had TNF-α levels below the detectable limit; these samples were all recoded as being one unit below the lowest detectable limit.

2.3.3. Total sleep time

As previously described in Richards et al. (2013), polysomnography recordings were obtained with ambulatory polysomnography (Nihon Kohden Trackit Ambulatory Recording System) in accordance with standardized guidelines (Rechtschaffen, 1968). Pass Plus was utilized for both visual scoring and quantitative EEG analysis of the digitized polysomnography data. Visual scoring was conducted by a highly experienced registered polysomnography technician, who classified all 30-second epochs in every sleep record as wake; stages 1, 2, 3; REM; or movement using current AASM criteria (AASM, 2007). Sleep onset was defined as the first minute of eight consecutive minutes of stage 2 sleep with no more than 2 intervening minutes of stage 1 sleep or minutes awake. Total sleep time was defined by time spent in epochs scored as NREM stages 1 through 3 and stage REM over a time window of ten hours after HSO.

2.3.4. Covariates

Over and beyond the described variables of interest, biological sex ("*female*" vs "*male*") and age, as well as total hours of sleep, entered the analyses of this study as covariates. Given that there was a strong association between PTSD status and body mass index (BMI), BMI was also added as a covariate.

2.4. Statistical analyses

Multilevel modeling was used to assess growth curve analyses on the trajectories of the inflammatory responses for each participant, with two-hour cytokine measurements nested within participants. Growth curve modeling was conducted as implemented in the 'nlme' library of R (Pinheiro et al., 2014). In all analyses, time was treated as a within-subject continuous variable, group, sex and the covariates as between-subject variables, and the two inflammatory marker responses of IL-6 and TNF-α served as the outcome variables. For all analyses, we modeled time with a quadratic effect in order to account for the non-linear trajectories of nocturnal cytokine levels previously reported in other studies (Cuesta et al., 2016; Nilsonne et al., 2016). Since time was not centered, the intercept represented HSO for each participant and the beginning of the growth curve.

Backward model selection was performed by contrasting the deviance of a complex model with that of a simple model using a log-likelihood test (Bliese and Ployhart, 2002). Model parameters were estimated using restricted maximum likelihood. If the complex model fitted the data significantly better than the simple model, we retained the complex model. When indicated by the model-selection procedure, a random participant intercept and/or slope were included in the model. Secondly, we determined whether a linear, quadratic or cubic time effect fitted the data best. As suggested by Bliese and Ployhart (2002), we then added a more complex error structure (i.e. accounting for autocorrelation or heteroscedasticity) to the models, if model comparison indicated significant differences. Post-hoc contrasts were analyzed with the 'LSMEANS' library of R and multiple comparisons were corrected with false discovery rate (FDR; Benjamini and Hochberg, 1995) with a p-value of 0.05.

In order to achieve normal distribution for IL-6 and TNF-α, natural log transformations were conducted on both biological measures (see Table 1 for IL-6 and TNF-α levels for both the PTSD and control groups, expressed in log-transformed units). Visual inspection indicated that the two transformed variables were normally distributed. To control for multivariate outliers, we use Mahalanobis distance to identify influenceable individuals (Hadi, 1992). As a consequence, data of three participants were deemed influential and subsequently dismissed from the final analyses.

3. Results

Table 1 provides an overview of the demographic information of the N = 85 participants by group. There was a significant group difference in BMI (t[79.80] = -2.71; p = 0.01), driven by a lower mean BMI of controls compared to PTSD-positive participants. Groups also differed in ethnicity (X^2[4] = 14.62; p = 0.006), with the control group having a higher frequency of Caucasians compared to the PTSD group.

Bivariate Pearson correlations revealed no significant associations among the continuous covariates and mean overnight cytokine levels. However, there was an association between the participants age and BMI, indicating that older participants had significantly higher BMI ($r_{age,BMI}$ = .27; p = .01), and a marginal positive association between the two mean overnight cytokine levels ($r_{IL\text{-}6,TNF\text{-}\alpha}$ = .20; p = .07; Supplementary Table 1).

We conducted independent samples t-tests for each two-hour segment of the experiment where we compared the cytokine levels between the two groups. None of these t-tests indicated a significant group differences between these levels across all timepoints (see Fig. 1).

3.1. Nocturnal IL-6 trajectory

Our analyses showed a substantial dependency amongst IL-6 responses within subjects (ICC = .46). To account for within-person change of IL-6, we continued with specifying our growth model. Model specification indicated that the within-subject factor time predicted IL-6 responses best when squared. Furthermore, our model selection process identified a random intercept and slope model for IL-6 responses, while accounting for heteroscedasticity.

To test our first assumption, that nocturnal IL-6 levels would be different between controls and participants with PTSD, we added group as a fixed effect to our above specified model. In this model, *group* interacted significantly with both *time* (B = 0.16, SE = 0.07, p = .02) and *time*^{2} (B = -0.02, SE = 0.01, p = .01). To test our hypothesis that *group* and *sex* accounted for a significant amount of variability in *IL-6 levels* over the course of the sleep cycle, we added *sex* to our specified random intercept and slope model. In this model, none of the interactions involving *sex* were significant (all p > .21), leading us to treat *sex* as a covariate in subsequent models. Controlling for *age*, *sex* and *BMI,* the IL-6 model still indicated a significant 2-way interaction of *group* x *time* (B = 0.19, SE = 0.06, p = .003) and *group* x *time*^{2} (B = -0.02, SE = 0.01, p < .001). Fig. 2 shows that IL-6 levels increased after HSO for both groups and dropped again approximately after 4 h, following an inverted U-shaped trajectory of IL-6 levels for both groups. This trajectory was distinctively more pronounced in the PTSD group than in the control group, and the control group showed an almost flat slope (see Table 3).

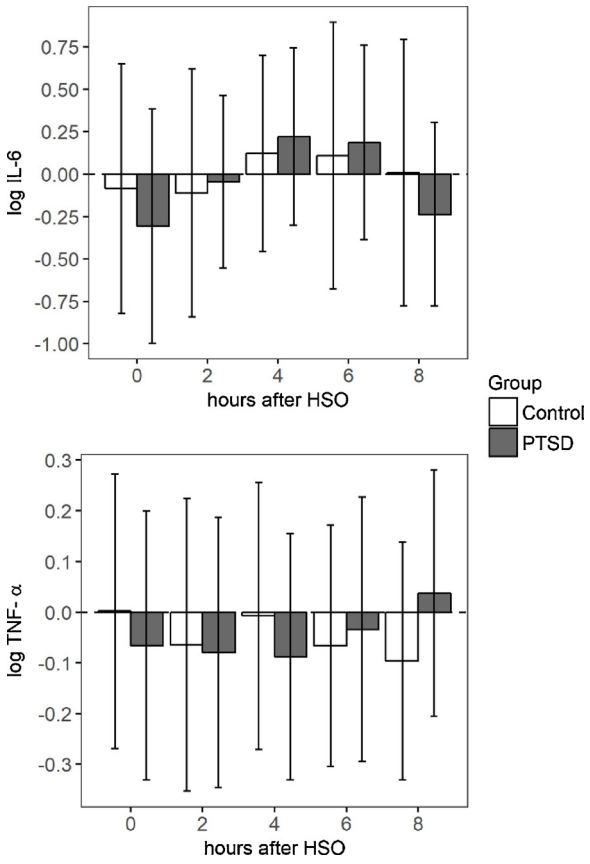

Fig. 1. Barplots of unadjusted, log-transformed cytokine levels over the 5 timepoints by group. Note. Error bars represent +/- 1 standard errors.

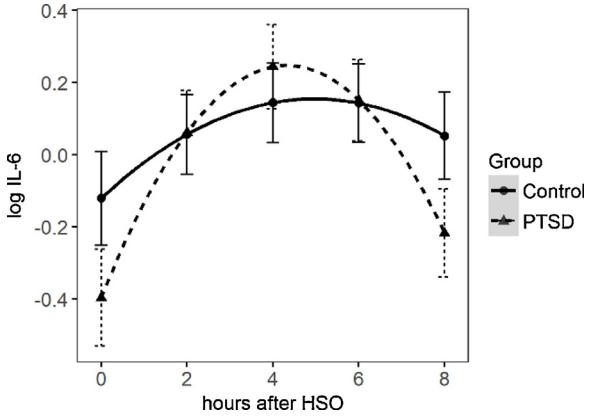

Fig. 2. Predicted IL-6 levels of PTSD positive subjects and healthy controls. Note. Error bars represent +/- 1 standard errors.

FDR adjusted follow-up analyses examined the differences in predicted IL-6 level at each time point compared to each other time point, based on the fully adjusted model. Within the PTSD group, all pairwise contrasts between time points were statistically significant, showing the robustness of changes over the night (see Supplementary Table 3). No pairwise comparisons between time points were significant within the control group, indicating the relative stability of IL-6 levels in this

Table 3
Fixed effects of fully adjusted overnight cytokine models by group.

Fully adjusted model for IL-6 overnight levels of control participants

Parameter	Coefficient (B)	SE	DF	*t*	*p*
Intercept	−0.05	0.72	135	−0.72	.47
Age	0.01	0.01	37	0.68	.50
BMI	0.01	0.03	37	0.20	.84
Sex (Men)	−0.09	0.20	37	−0.42	.68
Time	0.10	0.03	135	3.12	**.01**
Time^2	−0.01	0.004	135	−2.35	**.02**

Fully adjusted model for IL-6 overnight levels of PTSD participants

Parameter	Coefficient (B)	SE	DF	*t*	*p*
Intercept	1.01	0.50	119	−2.03	**.04**
Age	0.01	0.01	37	1.35	.19
BMI	0.002	0.02	37	0.11	.92
Sex (Men)	0.19	0.16	37	1.19	.24
Time	0.31	0.05	119	6.58	**.001**
Time^2	−0.04	0.01	119	−6.84	**.001**

Fully adjusted model for TNF-α overnight levels of control participants

Parameter	Coefficient (B)	SE	DF	*t*	*p*
Intercept	0.14	0.27	139	0.52	.60
Age	−0.001	0.01	35	−0.11	.92
BMI	−0.01	0.01	35	−0.68	.50
Sex (Men)	0.04	0.10	35	0.43	.67
Time	−0.02	0.02	139	−0.99	.32
Time^2	0.002	0.002	139	0.99	.32
Sex x Time	0.06	0.03	139	2.36	**.02**
Sex x Time^2	−0.01	0.00	139	−3.03	**.003**

Fully adjusted model for TNF-α overnight levels of PTSD participants

Parameter	Coefficient (B)	SE	DF	*t*	*p*
Intercept	−0.36	0.25	136	−1.43	.16
Age	−0.005	0.01	37	−0.87	.39
BMI	0.02	0.01	37	2.22	**.03**
Sex (Men)	−0.13	0.10	37	−1.37	.18
Time	−0.04	0.02	136	−1.86	.07
Time^2	0.005	0.002	136	2.22	**.03**
Sex x Time	0.01	0.03	136	0.50	.62
Sex x Time^2	0.000	0.003	136	−0.09	.93

Note: The coefficients for *Time* entered the models as hours. All cytokine measures were log-transformed before entering the analyses to approximate normal distributions.

group.

In order to assess if our findings were independent from *total sleep time*, we computed a final model that adjusted for the total amount of sleep within a ten-hour window. This model revealed the same *group* x *time* interactions (i.e. linear and quadratic), but no significant effect of *total sleep time* (p = .14).

3.2. Nocturnal TNF-α trajectory

Measurements of TNF-α were dependent within subjects (ICC = .66). Subsequent model comparison indicated that a random intercept and slope model fitted the TNF-α responses best. Furthermore, model specification indicated that we did not have to account for autocorrelation, but again for heteroscedasticity.

Following the same process as described for IL-6, we started by adding *group* to the model. In this model, we found a significant *group* x *time*^{2} (B = 0.007, SE = 0.002, p = .01) and a significant *group* x *time* interaction (B = -0.04, SE = 0.02, p = .04). We then proceeded by adding *sex* to our model. This analysis revealed a 3-way interaction between *group*, *sex* and *time*^{2} (B = 0.009, SE = 0.004, p = .05). The analysis also revealed significant *sex* x *time*^{2} interaction (B = -0.01, SE = 0.003, p = .02) and a *sex* x *time* interaction (B = -0.07, SE = 0.03, p = .01), while all the other effects were not significant (all p > .23). Adding the covariates *age* and *BMI* to the model did not change any of the above-mentioned results.

To better examine our marginal 3-way interaction between *group*, *sex* and *time*^{2}, we ran our final models stratified by group. The model with only *sex* and *time* of the control sample showed a significant interaction between *sex* and the linear effect of *time* (B = 0.06, SE = 0.03, p = .02), as well as with a quadratic effect of *time* (B = -0. 01, SE = 0.003, p = .01). For the PTSD group, the interaction effects were not significant (both p > .55). But there was a significant quadratic effect of *time* (B = 0.005, SE = 0.002, p = .02) and a trend towards a linear effect of *time* (B = -0.04, SE = 0.002, p = .06); the effect of *sex* was not significant (p = .64). Adding the covariates *age* and *BMI* to both models did not substantially change the estimates for either group, but BMI was significantly associated with TNF-α levels (B = 0.02, SE = 0.01, p = .03; see Table 3).

The 3-way interaction of *time*^{2}, *group* and *sex* is reflected in an inverted U-shaped trajectory for TNF-α levels of the male control participants over the course of the 8-hour period, peaking around 4 h after self-reported HSO, while the other three subsamples (control female participants and PTSD positive females and males) followed a U-shaped trajectory (see Fig. 3). But while the TNF-α levels of control males followed a pronounced slope and dropped below the initial level (measured at hour 0), TNF-α levels for female controls remained largely unchanged. Both men and women in the PTSD positive group had higher TNF-α levels after 8 h, while this effect was more pronounced in PTSD positive males and flat in PTSD positive females.

As illustrated by Fig. 3, FDR-adjusted follow-up analysis of the predicted means from the fully adjusted model within the control male subsample indicated that the increase of TNF-α levels from hour 0 to 2 was marginally significant, while the drops between hour 4 to 6 and 6

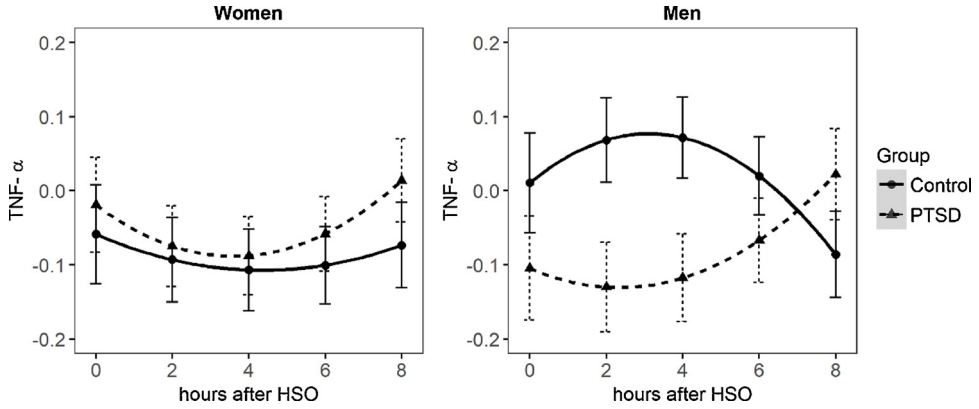

Fig. 3. Predicted TNF-α levels of women and men, by PTSD group. Note. Error bars represent +/- 1 standard errors.

to 8 were significant. The drop from hour 0 to 8 was marginally significant. Within the PTSD positive male subsample, the elevations of hour 4 to 6, the elevation of hour 6 to 8, and the overall increase from hour 0 to hour 8 were significant. All comparisons within the female subsamples were not significant, indicating no significant within-group changes.

3.2.1. Effects of sleep

We finally also assessed if the *TNF-α* was independent from *total sleep time* and computed a sleep-adjusted model. Adding the sleep measure resulted in non-significant 3-way interactions (both *ps* > .11), albeit without any impact on effect sizes derived from our original models. The *sex* x *time* (B = 0.07, SE = 0.03, *p* = .02) and *sex* x *time*^{2} (B = -0.01, SE = 0.003, *p* = .01) interactions were unchanged, while none of the previous main effects were significant (all *ps* > .16). *Total sleep time* had a significant positive effect (B = 0.001, SE = 0.0005, *p* = .02), indicating that more sleep was associated with higher peak *TNF-α* levels overnight.

4. Discussion

The current study examined levels of pro-inflammatory cytokines overnight in a sample of PTSD-positive adults in comparison to age- and sex-matched controls. Broadly, our results showed no significant differences between the PTSD-positive participants and controls at any single timepoint. However, the groups did differ on their overnight trajectories of cytokine levels. With regard to IL-6, the PTSD-positive participants showed more variation overnight, with an exaggerated inverted U-shaped curve consisting of lower IL-6 levels than the controls at the start and end of the night and higher midpoint IL-6 levels four hours after habitual sleep onset. Regarding TNF-α, PTSD-positive participants showed elevated cytokine levels at the end of the night in comparison to controls, though the trajectory of TNF-α levels overnight differed by sex. While the male control participants showed an inverted U-shaped trajectory of TNF-α levels overnight, all three other groups showed flatter U-shaped trajectories of TNF-α overnight.

Although the current study did not support our initial hypothesis that PTSD-positive participants would show elevated cytokines overnight, they did differ from healthy controls when considering the overall shape of the trajectories of cytokine levels over the course of the night. For example, the overnight peak in IL-6 was higher in the PTSD sample in comparison to controls, while TNF-α was elevated in comparison to the controls 8 h after habitual sleep onset. Additionally, the men without PTSD showed altered TNF-α trajectories overnight in comparison to all other groups. All of these differences suggest alterations in time course of pro-inflammatory activity overnight in PTSD, indicating the importance of considering time of day and trajectories of responses when trying to understand altered inflammatory activity in PTSD.

Based on previous studies showing elevated inflammatory activity in PTSD compared to controls (Passos et al., 2015), disrupted sleep in PTSD (Kobayashi et al., 2007), and elevated cytokines associated with sleep disruption (Irwin et al., 2016), we expected overall overnight elevations in both IL-6 and TNF-α in PTSD. There may be a number of reasons why results from the current study were not broadly consistent with this pattern. Importantly, there is a high degree of inter-individual variability in both sleep disruption (Straus et al., 2015) and inflammatory activity in PTSD (O'Donovan et al., 2017), with several studies suggesting that not all PTSD patients show sleep disruption or inflammation. Additionally, previous research has suggested that elevated cytokine levels overnight is associated with severity of PTSD symptoms (Gill et al., 2010). The current study selected for physically healthy, medication-free younger adults with PTSD. Interestingly, PTSD severity in our sample was much lower in comparison to the sample recruited by Gill and colleagues (mean CAPS score = 54 versus 82 in the comorbid PTSD + depression group from Gill et al.). Selection criteria for the current study may have constrained our sample to participants with relatively less severe symptoms.

There are several limitations of the current study worth noting. First, our small sample size limits the ability to draw strong conclusions. Importantly, as stated above, the current study consisted of physically healthy, non-obese, non-medicated younger adults with PTSD, which allowed us to examine associations of PTSD with inflammation independent of some of these potential confounds. However, given that PTSD is associated with increased risk for a number of physical diseases, including cardiovascular, autoimmune, and metabolic disorders (Boscarino, 2006; Cohen et al., 2009; O'Donovan et al., 2015), the current sample may not be generalizable to a more representative sample of PTSD patients. Though the samples were matched based on age and sex, there are a number of ways in which participants with PTSD differed from control participants (trauma exposure, type of trauma, veteran status, ethnicity, smoking), so future studies should attempt to control for these confounding factors. The current study only included analyses of TNF-α and IL-6, though other inflammatory markers such as IFN-γ and high-sensitivity C-reactive protein may also be elevated in PTSD (Breunig et al., 2018; Lindqvist et al., 2017). In addition, both TNF-α production and receptor levels may be modified by treatments such as psychotherapy (Himmerich et al., 2016). Though the current study excluded medication use, no information was collected about IL-6 or TNF-α receptors, and no information is available about prior history of psychotherapy use in the sample. Future studies should more closely examine these associations. Additionally, the current study was constrained to examining a single night of data collection. Finally, the current study sampled cytokines at only five timepoints overnight, only three of which occurred during the habitual sleep period, limiting the opportunity to examine trajectories at a more fine-grained level of detail, including examining detailed relationships between sleep architecture/sleep cycles and their relationship to cytokine levels during the sleep period. Future studies using more frequent sampling during the sleep period will be able to examine these relationships more thoroughly.

To our knowledge, this is the largest study to examine IL-6 overnight in a PTSD sample and the first study to examine overnight TNF-α in PTSD. The current findings demonstrate altered overnight levels of pro-inflammatory markers in men and women with PTSD in comparison to healthy control participants. Additional research in broader study samples will be necessary to continue to examine relationships between disrupted sleep, cytokines, and increased risk for disease in PTSD. The current findings highlight the need to consider sex, sleep, time of day, and circadian variation when examining inflammation in PTSD.

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Credit author statement

AK: Conceptualization, Formal Analysis, Writing – Original Draft. LDS: Writing – Original Draft. AAP, SSI, and AR: Writing- Reviewing and Editing. JS and EM: Data Curation. TJM: Methodology, Software, Writing – Reviewing and Editing. TCN: Funding Acquisition, Conceptualization, Supervision, Writing – Reviewing and Editing. AO: Conceptualization, Supervision, Writing – Reviewing and Editing.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.12.002.

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