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Signature:

Evelina Sterina

Date

Time of trauma prospectively affects PTSD symptom severity: the impact of circadian rhythms and cortisol

By
Evelina Sterina
Master of Science

Clinical Research

Jennifer Stevens, PhD
Advisor

Amita Manatunga, PhD
Committee Member

Jennifer Felger, PhD
Committee Member

Accepted:

Kimberly Jacob Arriola, Ph.D, MPH
Dean of the James T. Laney School of Graduate Studies

Date

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By

Evelina Sterina
B.A., University of Chicago, 2016

Advisor: Jennifer Stevens, PhD

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Abstract

Time of trauma prospectively affects PTSD symptom severity: the impact of circadian rhythms and cortisol

By Evelina Sterina

A key feature of posttraumatic stress disorder (PTSD) is a disruption of hypothalamic-pituitary-adrenal (HPA) axis feedback sensitivity and cortisol levels. Despite known diurnal rhythmicity of cortisol, there has been little exploration of the circadian timing of the index trauma and consequent cortisol release. Stress-related glucocorticoid pulses have been shown to shift clocks in peripheral organs but not the suprachiasmatic nucleus, uncoupling the central and peripheral clocks. A sample of 425 participants was recruited in the Emergency Department following a DSM-IV-TR Criterion A trauma. The Zeitgeber time of the trauma was indexed in minutes since sunrise, which was hypothesized to covary with circadian blood cortisol levels (high around sunrise and decreasing over the day). Blood samples were collected $M(SD)=4.0(4.0)$ hours post-trauma. PTSD symptoms six months post-trauma were found to be negatively correlated with trauma time since sunrise ($r(233)=-0.15$, $p=0.02$). The effect remained when adjusting for sex, age, BMI, race, clinician-rated severity, education, pre-trauma PTSD symptoms, and trauma type ($\beta=-0.21$, $p=0.00061$). Cortisol levels did not correlate with blood draw time, consistent with a masking effect of the acute stress response obscuring the underlying circadian rhythm. Interactions between trauma time and expression of *ARNTL* ($p_{\text{unadj}}=0.043$) and *TIMELESS* ($p_{\text{unadj}}=0.010$) predicted six-month PTSD symptoms. The interaction of trauma time and cortisol concentration was significantly correlated with the expression of *PER1* ($p_{\text{unadj}} = 1.4 \times 10^{-3}$). The differential effect of time of day on future symptom severity suggests a role of circadian effects in PTSD development, potentially through peripheral clock disruption

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is a common, often persistent and debilitating, mental health disorder in which patients suffer hyperarousal symptoms, intrusive re-experiencing, negative cognitions, and avoidance of related stimuli following exposure to a traumatic event. Though such events are estimated to occur in 50-60% of the population, only a subset of people who experience a traumatic event (5-50%) are diagnosed with the disorder (Kessler et al., 1995). Numerous psychosocial risk factors have been identified, including female sex, history of family and personal psychopathology and trauma, severity and duration of the trauma, and lack of family and social support (Tortella-Feliu et al., 2019; Yehuda et al., 2015). However, known factors do not adequately capture the probability of developing PTSD in prospective studies and experimental studies continue to explore the underlying neurobiology to identify the causal pathways leading to psychopathology (Tortella-Feliu et al., 2019). With a better understanding of the biological mechanisms connecting risk factors and symptom development trajectories, pharmacotherapeutic interventions can be designed to more precisely target key steps in the pathway at the appropriate time (Abdallah et al., 2019; Walton et al., 2021).

Among the key physiologic features of PTSD are low basal levels of the stress hormone cortisol and an exaggerated negative feedback sensitivity of the body's hormonal stress response system – the hypothalamic-pituitary-adrenal (HPA) axis (Yehuda et al., 2015). However, there remain inconsistencies in descriptions of this relationship, particularly its role in the early stages of PTSD development (Speer et al., 2019). Results are often mixed, and it remains unclear if disruptions of the HPA axis are a risk factor for PTSD (Yehuda, 1999), a mechanism in its development (Rauch et al., 2020), an indicator of prior trauma exposure (Resnick et al., 1995), or a component of the symptom profile (Neylan et al., 2005). Several prospective studies have

shown that lower cortisol concentrations in the period following trauma exposure were predictive of increased chronic PTSD symptoms (Delahanty et al., 2000; Yehuda et al., 1998). Furthermore, glucocorticoids could have a role in the treatment and/or prevention of PTSD, as more recent studies in animals (Cai, 2006) and humans (Michopoulos et al., 2017) show that exogenous glucocorticoid administration enhances fear extinction processes that are dysregulated in individuals with PTSD. Small-scale clinical trials with glucocorticoid-based interventions such as hydrocortisone target both treatment and prevention of PTSD with some promising results, but the timing and duration of treatment has not been investigated or optimized (de Quervain et al., 2017). Due to the practical limitations in collecting pre-traumatic cortisol levels, knowledge of peri-traumatic levels is limited to post-traumatic collections. It can be speculated that lower pre-traumatic cortisol would similarly lead to higher PTSD symptoms. However, to our knowledge, this hypothesis has not been previously explored and would better characterize the role of cortisol in the peritraumatic period and help distinguish its role in PTSD pathophysiology.

While pre-traumatic cortisol cannot be measured in a natural scenario, variability in timing of the trauma exposure may have substantial implications, as cortisol and other effectors of the HPA axis follow endogenous 24 hour circadian rhythms. Interactions between the expression of circadian “clock” genes, a system dubbed the transcription-translation feedback loop (TTFL), keep time across systems of the body, including the circadian cortisol rhythm (Son et al., 2008). Cortisol peaks shortly after wake in the morning hours and falls in a linear fashion over the day (Horrocks et al., 1990). Given the dramatic fluctuations in cortisol throughout the day due to the circadian system, the point or “phase” in the circadian rhythm at time of trauma may be an important determinant of both pre-trauma levels of cortisol and the amplitude of the cortisol response to the traumatic event. Light, through retinohypothalamic communication, is the

strongest “Zeitgeber” or “time giver” that entrains the circadian clock in the suprachiasmatic nucleus (SCN) of the hypothalamus (Golombek & Rosenstein, 2010). Even in a world increasingly filled with artificial light and variability in schedules of daily activities, the outside light-dark cycle and clock time remains a reliable estimate of circadian phase in the general population and an important biological variable to consider in biomedical research (Honma et al., 2003; Nelson et al., 2021).

Although existing data suggest that the effect of the endogenous rhythms of cortisol or other circadian-mediated functions may impact the development of PTSD, studies to date have not explored how timing of trauma exposure affects cortisol responsivity to traumatic stress to impact the pathogenesis and early course of PTSD development (Delahanty et al., 2000; Meewisse et al., 2007). For instance, stress-related glucocorticoid pulses are shown to shift clocks in peripheral organs but not the suprachiasmatic nucleus, uncoupling the central and peripheral clocks (Balsalobre et al., 2000; Kiessling et al., 2010). As such, cortisol levels may moderate the expression of peripheral clock genes at different times of the day and circadian phase.

We conducted a longitudinal prospective study of trauma exposure, recruiting participants in the Emergency Department (ED) of a large urban Level 1 trauma center within several hours of trauma exposure. We hypothesized that traumas experienced earlier in the day, closer to the circadian cortisol peak, would be associated with lower 6-month PTSD symptoms severity based on previously reported associations between higher peri-traumatic cortisol, which occurs earlier in the day due to the endogenous circadian rhythm of cortisol, and lower PTSD symptoms. However, it is unknown whether cortisol response to the stress of the trauma would be dampened or facilitated by an ongoing circadian background of low cortisol during the

afternoon hours. To explore potential mediating factors, we assessed the relationship between blood cortisol levels and circadian gene expression with time of trauma and PTSD symptom severity 6 months following trauma.

METHODS

Participants

The current study used a sample of participants enrolled in a cohort study of primarily African American participants recruited at the Emergency Department (ED) of Grady Memorial Hospital, a publicly funded hospital in Atlanta, Georgia. All study procedures were reviewed and approved by the Emory Institutional Review Board (IRB) and the Grady Hospital Research Oversight Committee. Participants were included if they spoke English, were between 18 and 64 years of age, and suffered a DSM-IV-TR Criterion A trauma in the past 24 hours. Trauma types included motor vehicle collisions, sexual assaults, non-sexual assaults, industrial or home accidents, and any other events involving actual or threatened death or serious injury to self or others. Participants were excluded if they had previous hospitalizations for mental health reasons; a history of mania, schizophrenia, or other psychosis; current suicidal ideation; attempted suicide in the last 3 months; current intoxication; loss of consciousness (>5 minutes) as a result of the trauma; or any impairment on the Glasgow Coma Scale (<15) (Teasdale & Jennett, 1974).

Assessment

Eligible patients were approached in the ED after initial medical evaluation, appropriate laboratory testing, and medical clearance occurred. Following patient agreement to participate

and signing of informed consent, a trained assessor administered a series of demographic and symptom evaluations. Demographic information and information about the index trauma was gathered using the Standardized Trauma Interview (STI), a 41-item clinician-administered interview gathering information on relevant aspects of the trauma at baseline, including clinician-rated severity of trauma, and demographic information (Foa & Rothbaum, 2001). As part of the STI, clinician-rated severity of trauma was assessed on a 1-5 Likert scale based on degree of injury sustained and, when applicable, degree of involvement of a weapon: 1 = mildly severe (minor injuries, not requiring medical attention), 2 = somewhat severe (requires medical attention, threat of unseen weapon), 3 = clearly severe (threat of weapon- weapon clearly present), 4 = very severe (serious injury, injured with weapon), and 5 = near death (severely injured, left for dead) (Rothbaum et al., 2012). PTSD symptoms were assessed at baseline using the Posttraumatic Stress Diagnostic Scale (PDS) (Foa et al., 1997), a 49-item self-report measure based on the DSM-IV, to assess previous trauma history and any PTSD symptoms in the month preceding the index trauma. Time of trauma, asked as “what time did the trauma start?,” was reported by the participants at the time of interview in the ED. A follow-up assessment using the 17-item PTSD Symptoms Scale (PSS), a validated, shorter assessment of PTSD severity (Foa & Tolin, 2000), was administered approximately 6 months after trauma exposure. The 6-month timepoint was chosen to evaluate chronic symptomatology rather than acute stress reaction.

Blood collection and assay of cortisol and mRNA

Whole blood (EDTA and Tempus) samples were collected on the day of trauma and collection time was recorded. Blood samples were collected $M(SD) = 4.03(3.99)$ hours after trauma exposure. Within six hours of collection, EDTA tubes were centrifuged at 4°C and the

plasma was aliquoted into 500 μ L samples and frozen at -80°C until time of assay.

Plasma concentrations of cortisol 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 cortisol from 100 μ L of plasma. Separation was achieved by gradient elution according to the following LC conditions using a Shimadzu Nexera X2 UHPLC system: WatersTM Acquity UPLC BEH C18 1.7 μ m, 50 x 2.1 mm LC column at 50°C with mobile phases composed of 0.1% formic acid in H₂O 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. Thirteen calibrator levels covering a range of 0.01 to 100 μ g/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 cortisol levels were determined from a standard curve using linear regression analysis. The lower limit of quantitation (LLOQ) for cortisol was 0.01 μ g/dL. Intra- and inter-assay precisions were < 10%.10%.

RNA was extracted from Tempus RNA tubes with the Versagene kit (Gentra Systems, Minneapolis, MN), quantified using a Nanophotometer, and checked for quality with the Agilent Bioanalyzer. RNA was reverse-transcribed and biotin-labeled using the Ambion kit (AMIL1791; Applied Biosystems, Foster City, CA), and the cRNA was hybridized to Illumina HT-12 v3.0

arrays (Illumina, San Diego, CA) or v4 BeadChip arrays and incubated overnight for 16 hours at 55 °C. Arrays were washed, stained with Cy3 labeled streptavidin, dried, and scanned on the Illumina BeadScan confocal laser scanner, following Lori et al. (Lori et al., 2021). Briefly, the gene expression was \log^2 transformed, normalized for sequencing depth, and transformed to improve heteroscedasticity following the variance stabilization transformation pipeline (Love et al., 2014). MRNA analyses focused on 20 genes associated with circadian function (*ARNTL*, *ARNTL2*, *CLOCK*, *CRY1*, *CRY2*, *CSNK1E*, *ESR1*, *ESR2*, *FKBP5*, *NPAS2*, *NR1D1*, *NR1D2*, *PER1-3*, *RORA*, *RORB*, *TEF*, *TIMELESS*, *VIP*, and *VIPR2*) and a rhythmically expressed key stress system gene (*FKBP5*) (Linnstaedt et al., 2018). Genes were selected if they are implicated in the core circadian feedback mechanism or are transcriptional regulators of clock-controlled genes involved in neurotransmission, immune processes, and endocrine signaling.

Data Analysis

Analyses were conducted in R version 4.0.3 (R Core Team, 2021). An alpha of 0.05 (5%) was used for statistical significance.

Time of Trauma

A linear regression model was used to evaluate the association between a continuous measure of time of trauma with 6-month PTSD symptoms, controlling for participant race, sex, body mass index (BMI), age, education, baseline PTSD symptoms, interpersonal versus non-interpersonal trauma type, and clinician-rated trauma severity (1-5). The time of trauma was indexed in minutes since sunrise, which was hypothesized to covary with baseline circadian cortisol levels (high around sunrise and decreasing over the day), reflecting a Zeitgeber time of

trauma. Sunrise time in Atlanta, Georgia was calculated via the US National Oceanic & Atmospheric Administration's Solar Calculator (US Department of Commerce), and minutes elapsed from the last sunrise were calculated. Henceforth, "time" refers to Zeitgeber time, indexed in minutes since sunrise, rather than clock time.

Cortisol

Log-transformed cortisol levels were assessed for association with time of blood draw, indexed in minutes after sunrise, controlling for participant race, sex, BMI, age, education, baseline PDS scores, and clinician-rated trauma severity in a linear regression model. To estimate the effect of time of trauma on cortisol levels, while accounting for variability in time between trauma occurrence and blood draw, a linear regression model was conducted with time of trauma as the independent variable and time of blood draw, also indexed as minutes since sunrise, as an additional covariate. Association of cortisol levels with 6-month PTSD symptoms was assessed with a linear regression model controlling for participant race, sex, BMI, age, education, baseline PDS scores, clinician-rated trauma severity, and time of blood draw. Of note, time of blood draw was only included in models that utilized a blood-based metric (i.e., cortisol, gene expression).

Gene Expression

Linear regression models were used to estimate the effects of time of trauma, gene expression (separately for each gene), and their interaction, in predicting 6-month PTSD symptom severity. These models included covariates for the first three ancestry principal components derived from genome-wide genotyping (PsychArray BeadChip, Illumina, San

Diego, CA) from autosomal independent genomic markers (pairwise $r^2 < 0.25$) to control for population structure (Lori et al., 2021), as well as batch for ancestry genotyping and RNA, race, sex, BMI, age, education, pre-trauma PTSD symptom severity (PDS scores), clinician-rated trauma severity, interpersonal vs. non-interpersonal trauma type, and blood draw time.

Additional analyses using the same model structure were conducted to examine whether time of trauma and post-trauma cortisol interacted to affect gene expression, as well as whether time of trauma and gene expression interacted to affect PTSD symptom severity. Due to multiple comparisons, p values of gene expression analyses were adjusted for a false discovery threshold of 5% (Benjamini & Hochberg, 1995). Adjusted p values are demarcated by subscript “FDR” and unadjusted p values are demarcated by subscript “unadj.”

RESULTS

Sample characteristics

A total of 425 participants were recruited at Grady Memorial Hospital, and 234 (55%) of the 425 returned for the 6-month follow up assessment. Of participants who returned for the 6-month assessment (n = 234), peritraumatic cortisol measures were collected from 182 (78%), and gene expression measures were collected from 131 (56%) (Figure 1). The total cohort was 46% female, ethnically diverse (74% Black, 18% White, 1% Asian, 3% multiracial, and 3% other race), included subjects with varied levels of education (12% BA/BS or graduate degree, 39% AA or some college, 29% high school graduate, 15% no high school degree), and had an average age of 35.7 years (standard deviation (SD) = 12.9, range = 18-64). Motor vehicle collisions constituted the majority of the represented traumas (67%), followed by non-sexual assaults

(16%), industrial or home accidents (9%), sexual assaults (6%), and other events that involved actual or threatened death or serious injury to self or others (1%). In the total cohort, clinician-rated trauma severity was found to be significantly higher in traumas that occurred greater minutes after sunrise ($\beta = 0.13$, $p = 0.006$). Of the 425 participants, 59% ($n = 251$) reported that they previously experienced a Criterion A trauma. The overall mean baseline PTSD symptom score was 9.7 ($SD = 10.4$) on the PDS (possible score range 0-51, with higher scores indicating higher perceived stress), and there was no significant association between baseline PTSD scores and time of trauma ($\beta = 0.04$, $p = 0.38$). There were no statistically significant differences in the distribution of sex, race, clinician-rated trauma severity, education, trauma type, age, or baseline PTSD scores between the total cohort, participants who returned for the 6-month follow up, participants with cortisol measures, and participants with gene expression measures (Table 1).

Association between time of trauma and 6-month PTSD symptom severity

A linear regression analysis was performed to assess PTSD symptoms as a function of time of trauma, indexed as minutes since sunrise to reflect decreasing circadian levels of baseline cortisol throughout the day. Higher trauma time since sunrise predicted less PTSD symptom severity at the 6-month timepoint ($\beta = -0.15$, $p = 0.02$, **Figure 2A**). The effect held when adjusting for sex, BMI, age, race, clinician-rated trauma severity, education, interpersonal vs. non-interpersonal trauma type, and baseline PTSD symptoms ($\beta = -0.21$, $p = 0.00061$); adjusted $R^2 = 0.32$ for full model. The relationship was present for participants without reported prior Criterion A traumas ($\beta = -0.29$, $p = 0.004$), as well as participants with reported prior Criterion A traumas ($\beta = -0.19$, $p = 0.014$).

Blood draw time did not predict cortisol levels ($\beta = -0.04$, $p = 0.54$), consistent with an acute stress cortisol response masking the underlying circadian rhythm (**Figure 2B**). However, time of trauma predicted cortisol levels when controlling for blood draw time ($\beta = -0.13$, $p = 0.045$), suggesting that the magnitude of the HPA stress response may be influenced by the circadian state. Of note, the relationship between blood draw time and time of trauma was not found to be significant ($\beta = 0.01$, $p = 0.79$); blood samples were collected $M(SD) = 4.03(3.99)$ hours after trauma, and analyses that included blood-based measures (e.g., cortisol and gene expression) were controlled for blood draw time.

Cortisol levels did not predict 6-month PTSD symptoms ($\beta = 0.005$, $p = 0.94$; **Figure 2C**). Mediation analysis was therefore not undertaken, as the mediator variable (cortisol), was not significantly associated with the outcome variable (PTSD symptoms).

Association between gene expression and time of trauma, cortisol, and 6-month PTSD symptom severity

As shown in **Table 2**, time of trauma predicted the expression of *CRY1* ($\beta = -0.14$, $p_{\text{unadj}} = 0.047$), *CSNK1E* ($\beta = -0.16$, $p_{\text{unadj}} = 0.026$), and *PER1* ($\beta = -0.22$, $p_{\text{unadj}} = 1.2 \times 10^{-3}$). Of those, *PER1* ($p_{\text{FDR}} = 0.025$) was significant after FDR correction. Log-adjusted cortisol concentration predicted the expression of ten genes: *ARNTL2* ($\beta = -0.18$, $p_{\text{unadj}} = 0.037$), *CLOCK* ($\beta = -0.30$, $p_{\text{unadj}} = 1.8 \times 10^{-4}$), *ESR1* ($\beta = -0.19$, $p_{\text{unadj}} = 0.030$), *FKBP5* ($\beta = 0.21$, $p_{\text{unadj}} = 0.0075$), *NPAS2* ($\beta = -0.18$, $p_{\text{unadj}} = 0.027$), *NR1D1* ($\beta = -0.18$, $p_{\text{unadj}} = 0.028$), *NR1D2* ($\beta = -0.29$, $p_{\text{unadj}} = 0.0020$), *PER1* ($\beta = 0.52$, $p_{\text{unadj}} = 6.1 \times 10^{-11}$), *RORA* ($\beta = -0.23$, $p_{\text{unadj}} = 0.0068$), and *TIMELESS* ($\beta = -0.17$, $p_{\text{unadj}} = 0.036$). Of those, five were significant after FDR correction: *CLOCK* ($p_{\text{FDR}} = 0.0019$),

FKBP5 ($p_{\text{FDR}} = 0.032$), *NR1D2* ($p_{\text{FDR}} = 0.014$), *RORA* ($p_{\text{FDR}} = 0.032$), and *PER1* ($p_{\text{FDR}} = 1.3 \times 10^{-9}$).

The expression of *VIP* predicted 6-month PTSD symptoms ($\beta = -0.26$, $p_{\text{unadj}} = 0.022$, $p_{\text{FDR}} = 0.46$), but this effect was not significant after FDR correction. As mediation analysis could not be conducted, moderation was instead conducted to evaluate the interaction between trauma timing and cortisol in predicting circadian gene expression. A complementary analysis of the interaction between trauma timing and gene expression predicting PTSD severity was conducted to further evaluate the hypothesis that trauma-induced shifts in the peripheral clock may moderate the effect of trauma timing on PTSD symptomatology.

Interaction between trauma timing and cortisol in predicting expression of circadian genes

As shown in Table 3, the interaction of trauma time and cortisol concentration predicted the expression of *PER1* prior to FDR correction ($\beta = -1.6$, $p_{\text{unadj}} = 1.4 \times 10^{-3}$, $p_{\text{FDR}} = 0.053$), where at earlier trauma times higher cortisol concentration predicted higher *PER1* expression, a relationship that was not present at later trauma times (**Figure 3**). *ESR2* ($\beta = -1.4$, $p_{\text{unadj}} = 0.029$, $p_{\text{FDR}} = 0.22$) and *TEF* ($\beta = 1.4$, $p_{\text{unadj}} = 0.031$, $p_{\text{FDR}} = 0.22$) had similar effects, but the relationship was less robust.

Interaction of trauma timing and expression of circadian genes in predicting PTSD severity

As shown in **Table 4**, interactions between trauma time and expression of *ARNTL* ($\beta = -6.7$, $p_{\text{unadj}} = 0.043$, $p_{\text{FDR}} = 0.33$) and *TIMELESS* ($\beta = 4.2$, $p_{\text{unadj}} = 0.010$, $p_{\text{FDR}} = 0.21$) predicted 6-month PTSD symptoms, though the effect was not significant after FDR correction. There was also a main effect for *VIP* expression post-trauma predicting fewer PTSD symptoms at 6 months ($\beta = -0.36$, $p_{\text{unadj}} = 0.0044$, $p_{\text{FDR}} = 0.046$), but no interaction with trauma time ($p > 0.05$). The

expression of *ARNTL* and *TIMELESS* did not appear to be impacted by the trauma timing as time of trauma did not predict expression ($p>0.05$). No other genes showed significant main effects or interactions with time of trauma in predicting 6-month PTSD symptoms. Notably, no significant main effects of *PER1* and *CLOCK* on PTSD symptom severity were found, therefore preventing mediation analysis to test the hypothesized model of gene expression mediating an association of time of trauma and PTSD symptoms severity.

DISCUSSION

The current study found that future PTSD symptoms are higher when the incident trauma occurred fewer minutes after sunrise, despite more severe traumas occurring greater minutes after sunrise. This significant relationship was strengthened when accounting for demographic factors, baseline PTSD symptoms, and trauma severity. Our results suggest that timing of trauma should be considered in PTSD risk prediction models, as patients who experience traumas early in the day may be at higher risk for more severe, chronic PTSD symptoms in the future. The mechanism for this effect remains to be explored, with potential explanation implicating the HPA axis, the circadian biological clock, or an interaction between the two systems.

As post-trauma cortisol levels were not found to predict 6-month PTSD symptoms, it remains unclear if this effect of time of trauma exposure is mediated through the HPA axis. However, baseline cortisol levels would be expected to be higher during the trauma times that resulted in higher PTSD symptoms at 6-months, which contradicts studies suggesting the protective nature of cortisol in the peritraumatic period. The reason for this discrepancy is unclear, but a possible explanation is that the degree of cortisol response to trauma may vary throughout the day or circadian phase, which combined with varying pre-trauma cortisol levels

leads to measured post-traumatic cortisol levels that appear non-rhythmic. Although it is not feasible to assess pre-trauma cortisol necessary for calculation of cortisol response to a stressor, a meta-analysis of time of day differences in a much more mild stressor, the Trier Social Stress Test (TSST), suggests higher cortisol response later in the day, although the results were not significant and the majority of the analyzed studies did not report the time of test (Goodman et al., 2017). A combined reanalysis of five studies conducted with the same TSST protocol by a single laboratory showed that though there were no significant differences in cortisol response by time of day (potentially explained by variability in the degree of synchronization to the external light/dark cycle between participants), higher basal cortisol significantly correlated with lower cortisol response to the TSST (Kudielka et al., 2004). This is consistent with our findings that cortisol levels in the ED acutely post-trauma were not predicted by blood draw time, reflecting that the acute stress cortisol response to the trauma likely masked the underlying circadian rhythm. As emerging findings suggest that stress-related increases in cortisol can reset peripheral clocks, including the adrenal gland clock, such a shift could result in subsequent peripheral rhythm disruption, which has been linked to negative health outcomes (Balsalobre et al., 2000; Kiessling et al., 2010).

The mechanism underlying the rhythmicity of glucocorticoids is multifactorial, involving the SCN, the pituitary gland, and the adrenal gland, but at each of these locations, the TTFL of cyclic genes forms the basis of timekeeping. This interaction is bidirectional, as the “clock” genes (e.g., *PER1*, *PER2*, *TIMELESS*, *NPAS2*) that generate circadian rhythms are shown to be regulated by stress hormones like cortisol (Segall et al., 2009; So et al., 2009). Studies report several of these genes to also be implicated in PTSD risk. The first genome-wide association study for posttraumatic stress symptoms identified genetic variants of the retinoid-related orphan

receptor alpha (*RORA*) gene, a gene that dampens environmental influences on the circadian rhythm, to be associated with increased risk of such symptoms (Logue et al., 2013). Another gene that has been of particular interest due to its role in a number of stress-related psychiatric disorders is *FKBP5* (Binder et al., 2008), a chaperone protein involved in glucocorticoid receptor transport, which is rhythmically expressed in most tissues.^{1,6} And more recently, a significant stress-dependent relationship was found between a genetic variant in the circadian rhythm-associated thyrotroph embryonic factor (*TEF*) gene and post-traumatic stress symptoms across multiple cohorts (Linnstaedt et al., 2018). While we found the expression of *RORA* and *FKBP5* to be associated with post-trauma cortisol levels, *TEF* was only predicted by the interaction of trauma timing and cortisol levels prior to FDR correction.

Our investigation revealed a significant correlation between the expression of *PER1*, a core TTFL gene (Albrecht et al., 1997), and cortisol concentration, as well as an interaction between trauma time and cortisol concentration in predicting *PER1* expression. This finding is consistent with experimental findings of *PER1* hypersensitivity to corticosteroids, an effect that then shifts other circadian genes (Reddy et al., 2012). Glucocorticoid-induced disruptions of peripheral rhythms are mediated via the glucocorticoid receptor (GR), which directly binds to DNA and regulates gene expression after binding to glucocorticoids. Its sensitivity to glucocorticoids is flexible, varying by tissue and at different points in time (Meijsing et al., 2009; Yamamoto et al., 2005). Due to *PER1*'s significant role in the TTFL, the time-dependent sensitivity of *PER1* to cortisol may play an early role in subsequent peripheral clock desynchronization.

Interestingly, the expression of two genes interacted with trauma time in predicting PTSD symptoms: *ARNTL* and *TIMELESS*. Neuronal PAS Domain Protein 2 (NPAS2) is a core

transcription factor in the TTFL found to be analogous to the canonical circadian CLOCK protein (Reick, 2001). NPAS2-deficient mice show deficiencies in complex emotional long-term memory and behavior, including decreased reactions to both cued and contextual fear tasks and decreased anxiety-like behaviors (Garcia, 2000; Ozburn et al., 2017). Moreover, NPAS2 is expressed in neurons within the hippocampus, dentate gyrus, and amygdala complex (Zhou et al., 1997). *TIMELESS* was identified as a core part of the TTFL in *Drosophila*, but the extent of its role in the humans is less clear, with mammalian studies implicating it in DNA replication in addition to circadian rhythm generation (Barnes, 2003). The involvement of these three genes in PTSD development has not been previously documented, warranting further elucidation of their specific roles in the disease processes.

Although our study is prospective and longitudinal, it is limited by its observational nature. As we are unable to control the time of trauma occurrence, there may be characteristics that participants share depending on the time of trauma. Though potential confounders were selected *a priori* to control for such differences, there may be unaccounted variables. Of the hypothesized confounders, clinician-rated trauma severity was found to be significantly different between the groups, with more severe traumas occurring during the night. However, PTSD symptoms were higher for daytime traumas, despite increased PTSD symptoms correlating with increased trauma severity. Other limitations include the variable timing of blood draw and the lack of pre-trauma samples. Cortisol concentrations reported in the current study reflect the sum of baseline cortisol levels and cortisol released in response to the stressor, and these components cannot be effectively separated. Although time of day in reference to sunrise was used to estimate baseline cortisol, the phase and robustness of participants' peritraumatic rhythms could not be evaluated. Participants' circadian rhythms may have been out of phase with the daylight cycle, on which we

base our estimates. This includes, but is not limited, to inability to control for variations in participants' sleep and sleep-wake cycles prior to data collection, which may shift or disrupt cortisol rhythms. The trauma time of day was collected via participants' self-reported estimates of the start of the trauma, which may be skewed by recall bias. Additionally, due to limitations in staffing, most of the patient recruitment and blood collection occurred during the day, so our sample includes primarily participants who experienced daytime traumas. Moreover, though the 6-month assessment time was chosen to reflect chronic symptoms, it is unknown how long these effects may persist.

Potential future directions include analyses using alternate methods of estimating circadian phase at time of trauma. As direct measures of the SCN are not possible in humans, the gold standard peripheral marker of circadian phase is dim-light melatonin onset (DLMO), which is obtained by measuring melatonin concentrations in dim lighting prior to sleep (Lewy et al., 1999). Therefore, DLMO is not a practical measure for indexing circadian timing in acutely traumatized patients seen in emergency departments. Other ways to estimate circadian phase are becoming increasingly possible through transcriptomic and/or metabolomic biomarkers such as clock and clock-controlled genes and fatty acids (Laing et al., 2017; von Schantz & Skene, 2015). Of note, it remains unclear whether peripheral estimates, including circadian gene expression, reflect the central clock in the SCN, clocks in peripheral organs, or a combination of inputs (Balsalobre et al., 2000). Sleep disruptions have been shown to have a bidirectional relationship with the central clock (Archer et al., 2014), so the organization of sleep-wake cycles would inform of the stability of the subject's circadian system and test the hypothesis that timing of the trauma contributes to PTSD development via clock disruption. Tracking changes in the sleep-wake cycle would provide insights into whether the subject's overall diurnal rhythmicity

may mediate the observed relationship between trauma time of day and PTSD development (Cakmak et al., 2021). Such an integration of measures of rhythmicity with studies of peritraumatic sleep are especially pertinent given recent findings that pre-trauma sleep disturbances are predictive of future PTSD symptoms, and they could potentially be further explored via sleep-wake tracking with wearable devices (Neylan et al., 2020). Future studies would also benefit from comparison of participants' trauma timing estimates against emergency medical service reports for increased accuracy. Additionally, psychological explanations such as shock of experiencing trauma at a seemingly safe morning time could guide future directions. Continued search for an explanatory mechanism of this relationship would help elucidate the processes explaining why only a fraction of people who experience trauma go on to experience chronic distressing symptoms. Moreover, longer-term is necessary to determine whether the effects persist beyond the 6-month period, as well as to explore measures that may mitigate this response.

In conclusion, the current study contributes further insight into prospective risk factors of PTSD, implicating time of trauma as a prospective factor. Analysis of potential variables mediating this effect did not yield clear candidates, contrary to our hypothesis that rhythmicity of the HPA axis could explain the relationship. Further characterization of the impact of circadian rhythms could have implications on ongoing studies exploring hydrocortisone as a treatment modality. This result further suggests that the role of the HPA axis in PTSD pathophysiology is nuanced and multi-dimensional, necessitating further characterization before it can be effectively leveraged for prevention and treatment.

AUTHOR CONTRIBUTIONS

K.J.R, B.O.R., and V.M. substantially contributed to the design of the study and developed the study concept. V.M., J.S.S., A.L., A.P.W., K.F.E were involved in the data collection process. E.S., J.S.S. developed the data analytical plan and E.S. performed data analysis. V.M., S.D.L., T.C.N., G.D.C., provided supervision. E.S. wrote the first draft of the manuscript and all co-authors reviewed and revised the manuscript critically for important intellectual content. All co-authors approved the version of the manuscript to be published.

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Table 1
Demographic Characteristics and Trauma Severity at Each Analysis Step

Characteristic	All patients (N=425)		6-Month Follow-up (N=234)		6-Month Follow-up with Cortisol (N=182)		6-Month Follow-up with Gene Expression (N=131)		Chi-Square Test		
	N	%	N	%	N	%	N	%	df	χ^2	# p
Sex											
Male	230	54%	130	56%	96	53%	68	52%	3	0.57	0.90
Female	195	46%	104	44%	86	47%	63	48%			
Race											
Black	315	74%	179	76%	133	73%	95	73%	12	4.94	0.96
White	78	18%	35	15%	31	17%	23	18%			
Asian	4	1%	4	2%	3	2%	1	1%			
Mixed	14	3%	8	3%	8	4%	5	4%			
Other	14	3%	10	4%	8	4%	8	6%			
Trauma severity (clinician-rated)											
1	37	9%	18	8%	18	10%	12	9%	12	5.52	0.94
2	138	32%	76	32%	61	34%	47	36%			
3	147	35%	86	37%	65	36%	49	37%			
4	89	21%	47	20%	35	19%	22	17%			
5	14	3%	7	3%	3	2%	1	1%			
Education											
B.A./B.S. or beyond	75	18%	48	21%	41	23%	30	23%	9	3.86	0.92
A.A./A.S. or some college	164	39%	92	39%	71	39%	46	35%			
High school graduate	123	29%	63	27%	47	26%	37	28%			
No high school degree	63	15%	31	13%	23	13%	18	14%			
Trauma type											
Motor vehicle collision	283	67%	172	74%	137	75%	102	78%			0.46
Non-sexual assault	70	16%	30	13%	19	10%	13	10%			
Sexual assault	27	6%	11	5%	8	4%	5	4%	12	11.8	
Industrial or home accident	39	9%	17	7%	15	8%	8	6%			
Other	6	1%	4	2%	3	2%	3	2%			
Trauma minutes since sunrise											
0 – 359	236	56%	134	57%	117	64%	87	66%	9	9.61	0.38
360 – 719	83	20%	41	18%	30	16%	23	18%			
720 – 1079	29	7%	17	7%	9	5%	4	3%			
1080 – 1440	77	18%	42	18%	26	14%	17	13%			
	All patients		6-Month		6-Month		6-Month			One-Way	

	(N=425)		Follow-up (N=234)		Follow-up with Cortisol (N=182)		Follow-up with Gene Expression (N=131)		ANOVA		
	M	SD	M	SD	M	SD	M	SD	df	F	p
Age	35.7	12.9	36.7	12.9	37.7	13.2	36	12.8	3	1.07	0.36
Total baseline symptoms, PDS	9.7	10.4	9.1	9.6	8.8	9.6	9.2	9.6	3	0.43	0.73
BMI	28.0	6.7	28.6	7.5	29.1	7.7	29.0	7.4	3	1.33	0.26

Table 2
Association Between Gene Expression and Time of Trauma, Cortisol, and 6- Month PTSD Symptom Severity

	Trauma Minutes After Sunrise ¹			Log-adjusted Cortisol ¹			6-Month PTSD Symptoms ²		
	β	p _{unadj}	p _{FDR}	β	p _{unadj}	p _{FDR}	β	p _{unadj}	p _{FDR}
<i>ARNTL</i>	0.0078	0.92	0.97	0.083	0.40	0.52	0.11	0.25	0.89
<i>ARNTL2</i>	-0.047	0.52	0.77	-0.18	0.037*	0.078	0.10	0.31	0.89
<i>CLOCK</i>	0.012	0.87	0.96	-0.30	0.00018**	0.0019**	-0.054	0.58	0.89
<i>CRY1</i>	-0.14	0.047*	0.33	-0.042	0.63	0.76	0.070	0.49	0.89
<i>CRY2</i>	-0.038	0.59	0.77	-	0.96	0.96	0.054	0.59	0.89
<i>CSNK1E</i>	-0.16	0.026*	0.27	0.032	0.70	0.76	-0.0031	0.98	0.99
<i>ESR1</i>	-0.065	0.34	0.68	-0.19	0.030*	0.078	0.040	0.67	0.92
<i>ESR2</i>	-0.077	0.26	0.68	0.0066	0.94	0.96	-0.074	0.46	0.89
<i>FKBP5</i>	0.013	0.86	0.96	0.21	0.0075**	0.032*	-0.020	0.85	0.99
<i>NPAS2</i>	-0.063	0.39	0.68	-0.18	0.027*	0.078	-0.18	0.088	0.84
<i>NR1D1</i>	-0.017	0.81	0.96	-0.18	0.028*	0.078	-0.075	0.46	0.89
<i>NR1D2</i>	-0.065	0.29	0.68	-0.29	0.0020**	0.014*	-0.068	0.46	0.89
<i>PER1</i>	-0.22	0.0012**	0.025*	0.52	6.1E-11**	1.3E-9**	-0.064	0.51	0.89
<i>PER2</i>	0.038	0.59	0.77	-0.12	0.16	0.26	-0.040	0.70	0.92
<i>PER3</i>	0.0028	0.97	0.97	-0.16	0.062	0.18	0.091	0.38	0.89
<i>RORA</i>	-0.092	0.17	0.60	-0.23	0.0068**	0.032*	-0.0055	0.95	0.99
<i>RORB</i>	-0.10	0.14	0.59	0.038	0.65	0.76	-0.0045	0.96	0.99
<i>TEF</i>	-0.053	0.47	0.76	-0.14	0.086	0.15	0.00073	0.99	0.99
<i>TIMELESS</i>	-0.066	0.37	0.68	-0.17	0.036*	0.078	-0.17	0.12	0.84
<i>VIP</i>	-0.084	0.26	0.68	0.072	0.34	0.48	-0.26	0.022*	0.46
<i>VIPR2</i>	-0.12	0.12	0.59	-0.088	0.27	0.41	0.11	0.29	0.89

*p<0.05; **p<0.01

FDR: False discovery rate (5% threshold)

All models adjust for three ancestry principal components, batch for genotyping and RNA, race, sex, age, BMI, education, pre-trauma PTSD symptom severity (PDS scores), clinician-rated trauma severity, interpersonal vs non-interpersonal trauma type, and blood draw time

¹Models with gene expression as outcome variable

²Model with gene expression as predictor variable and symptoms as outcome variable

Table 3
Main Effects and Interactions Between Time of Trauma and Cortisol on Circadian Gene Expression

Gene	Trauma Minutes After Sunrise ¹			Log-adjusted Cortisol ¹			Trauma Minutes After Sunrise x Log-adjusted Cortisol ²		
	β	<i>p</i> _{unadj}	<i>p</i> _{FDR}	β	<i>p</i> _{unadj}	<i>p</i> _{FDR}	β	<i>p</i> _{unadj}	<i>p</i> _{FDR}
<i>ARNTL</i>	1.2	0.080	0.42	0.23	0.072	0.19	-1.3	0.074	0.39
<i>ARNTL2</i>	0.33	0.60	0.97	-0.13	0.25	0.40	-0.45	0.47	0.78
<i>CLOCK</i>	0.44	0.48	0.92	-0.28	0.017*	0.089	-0.50	0.43	0.78
<i>CRY1</i>	0.67	0.29	0.76	0.053	0.64	0.73	-0.83	0.19	0.54
<i>CRY2</i>	0.042	0.95	0.98	0.0064	0.96	0.96	-0.094	0.88	0.98
<i>CSNK1E</i>	-0.22	0.74	0.98	0.032	0.79	0.83	0.019	0.98	0.98
<i>ESR1</i>	0.88	0.13	0.55	-0.067	0.53	0.68	-0.97	0.10	0.42
<i>ESR2</i>	1.3	0.043*	0.30	0.17	0.14	0.29	-1.4	0.029*	0.22
<i>FKBP5</i>	-0.014	0.98	0.98	0.25	0.044*	0.15	0.026	0.97	0.98
<i>NPAS2</i>	-0.24	0.72	0.98	-0.23	0.063	0.19	0.18	0.78	0.98
<i>NR1D1</i>	-0.48	0.45	0.91	-0.25	0.033*	0.14	0.46	0.48	0.78
<i>NR1D2</i>	-0.13	0.82	0.98	-0.25	0.016*	0.089	0.016	0.98	0.98
<i>PER1</i>	1.3	0.011**	0.23	0.71	1.4E-12**	2.9E-11**	-1.5	0.0025**	0.053
<i>PER2</i>	0.40	0.53	0.93	-0.081	0.66	0.73	-0.55	0.39	0.78
<i>PER3</i>	-0.021	0.97	0.98	-0.16	0.16	0.31	-0.030	0.96	0.98
<i>RORA</i>	0.56	0.35	0.82	-0.15	0.18	0.32	-0.74	0.23	0.54
<i>RORB</i>	0.16	0.81	0.98	0.072	0.55	0.68	-0.26	0.69	0.98
<i>TEF</i>	-1.4	0.026*	0.27	-0.32	0.0068*	0.071	1.4	0.031*	0.22
<i>TIMELESS</i>	0.80	0.23	0.69	-0.097	0.43	0.64	-0.87	0.20	0.54
<i>VIP</i>	-0.12	0.87	0.98	0.097	0.46	0.64	-0.018	0.98	0.98
<i>VIPR2</i>	-0.92	0.18	0.63	-0.21	0.098	0.23	0.85	0.21	0.54

p*<0.05; *p*<0.01

¹Main effects from models with time x cortisol interaction term

²Interaction term values only

FDR: False discovery rate (5% threshold)

All models adjust for three ancestry principal components, batch for genotyping and RNA, race, sex, age, BMI, education, pre-trauma PTSD symptom severity (PDS scores), clinician-rated trauma severity, interpersonal vs. non-interpersonal trauma type, and blood draw time

Table 4
Main Effects and Interactions Between Time of Trauma and Gene Expression in Predicting 6
Month PTSD Symptoms

Gene	Minutes			Gene			Minutes x Gene		
	β	p_{unadj}	p_{FDR}	β	p_{unadj}	p_{FDR}	β	p_{unadj}	p_{FDR}
<i>ARNTL</i>	6.5	0.047*	0.27	0.027	0.027*	0.19	-6.7	0.043*	0.33
<i>ARNTL2</i>	-0.24	0.68	0.79	0.077	0.59	0.80	0.15	0.80	0.83
<i>CLOCK</i>	-0.70	0.79	0.79	-0.070	0.61	0.80	0.60	0.82	0.83
<i>CRY1</i>	-0.87	0.77	0.79	0.037	0.79	0.92	0.77	0.79	0.83
<i>CRY2</i>	-2.2	0.46	0.74	-0.013	0.92	0.97	2.1	0.48	0.69
<i>CSNK1E</i>	-6.3	0.063	0.27	-0.21	0.15	0.53	6.1	0.067	0.33
<i>ESR1</i>	0.87	0.54	0.76	0.11	0.43	0.75	-0.98	0.49	0.69
<i>ESR2</i>	-1.1	0.43	0.74	-0.14	0.29	0.67	1.0	0.47	0.69
<i>FKBP5</i>	0.72	0.54	0.76	0.037	0.76	0.92	-0.83	0.48	0.69
<i>NPAS2</i>	1.7	0.072	0.27	0.024	0.86	0.95	-1.8	0.054	0.33
<i>NR1D1</i>	-2.6	0.074	0.27	-0.21	0.10	0.53	2.4	0.086	0.33
<i>NR1D2</i>	-1.6	0.42	0.74	-0.16	0.26	0.67	1.4	0.45	0.69
<i>PER1</i>	-0.80	0.59	0.77	-0.15	0.31	0.67	0.66	0.65	0.83
<i>PER2</i>	0.91	0.77	0.79	-0.004	0.98	0.98	-1.0	0.74	0.83
<i>PER3</i>	2.3	0.16	0.42	0.20	0.14	0.53	-2.4	0.14	0.37
<i>RORA</i>	-1.6	0.27	0.63	-0.11	0.43	0.75	1.5	0.30	0.69
<i>RORB</i>	-0.74	0.34	0.71	-0.076	0.57	0.80	0.63	0.41	0.69
<i>TEF</i>	-2.3	0.12	0.36	-0.13	0.32	0.67	2.2	0.14	0.37
<i>TIMELESS</i>	-4.3	0.0088**	0.18	-0.38	0.0035**	0.049*	4.2	0.010*	0.21
<i>VIP</i>	-2.0	0.078	0.27	-0.36	0.0047**	0.049*	1.9	0.095	0.33
<i>VIPR2</i>	-0.24	0.72	0.79	0.075	0.56	0.80	0.14	0.83	0.83

* $p < 0.05$; ** $p < 0.01$

FDR: False discovery rate (5% threshold)

All models adjust for three ancestry principal components, batch for genotyping and RNA, race, sex, BMI, age, education, interpersonal vs non-interpersonal trauma type, pre-trauma PTSD symptom severity (PDS scores), clinician-rated trauma severity, and blood draw time

Figure 1

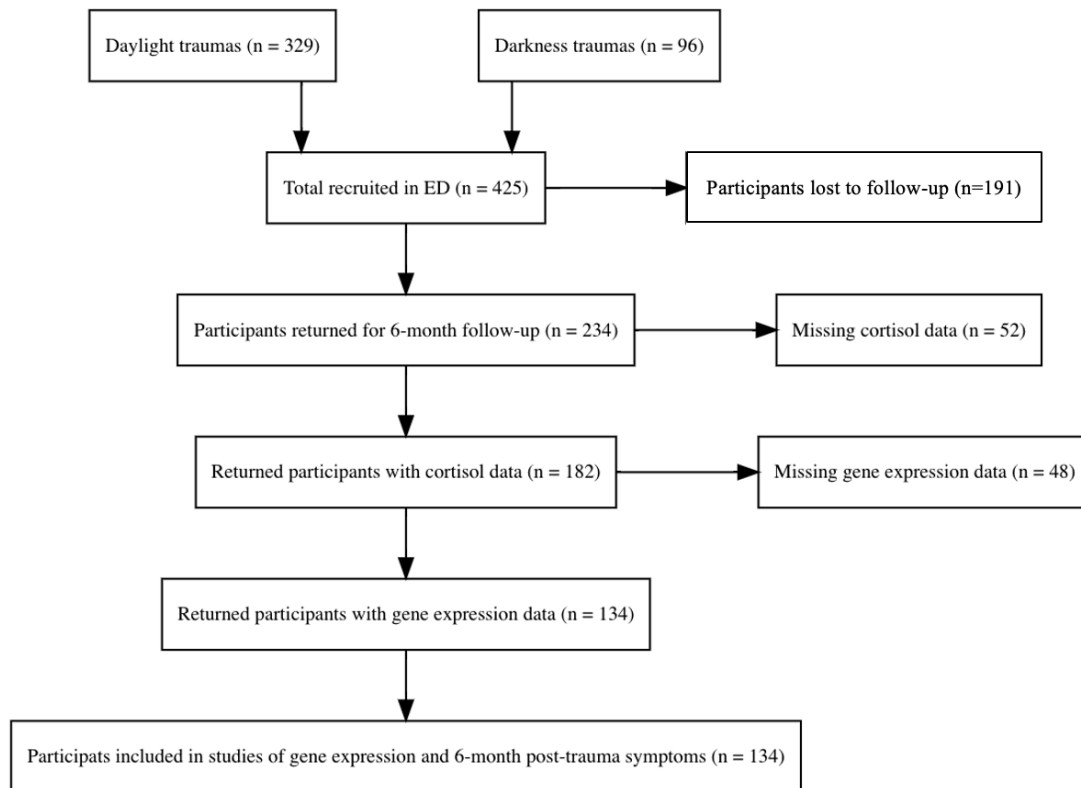


Fig 1. The number of participants included at each step of analysis was limited by available biometric data. The greatest possible number of participants was utilized for each step of the analysis, excluding participants with missing values at later steps.

Figure 2

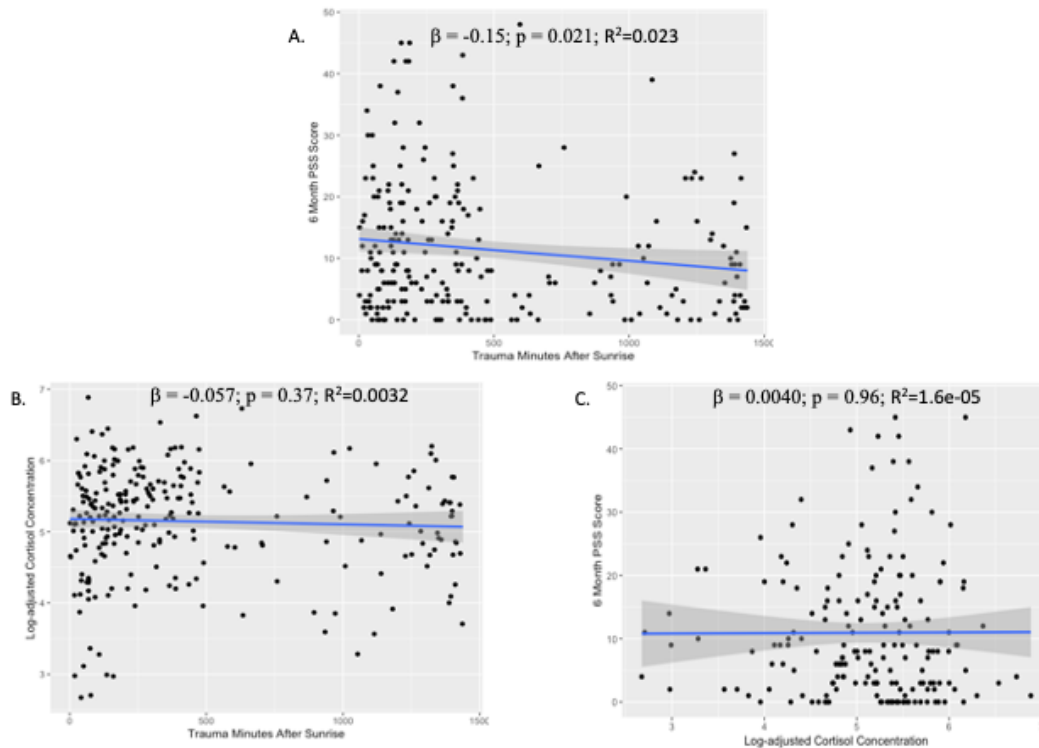


Fig 2. Negative correlation between trauma minutes after sunrise and 6-month PTSD symptoms without relationship between peritraumatic cortisol and trauma timing or PTSD symptoms.

Timing of the trauma, in reference to sunrise, predicted PTSD symptoms 6 months following trauma (A). Despite the endogenous rhythm of cortisol, no correlation was found between the time of trauma and the total post-trauma cortisol concentration (B). Moreover, cortisol concentration did not correlate with PTSD symptoms (C), suggesting that cortisol concentration alone does not explain the relationship between trauma timing and PTSD symptoms. For all panels, blue line represents linear regression approximations between x-axis variable and y-axis variable, without controlling for covariates. Shading represents 95% confidence interval around the regression line.

Figure 3

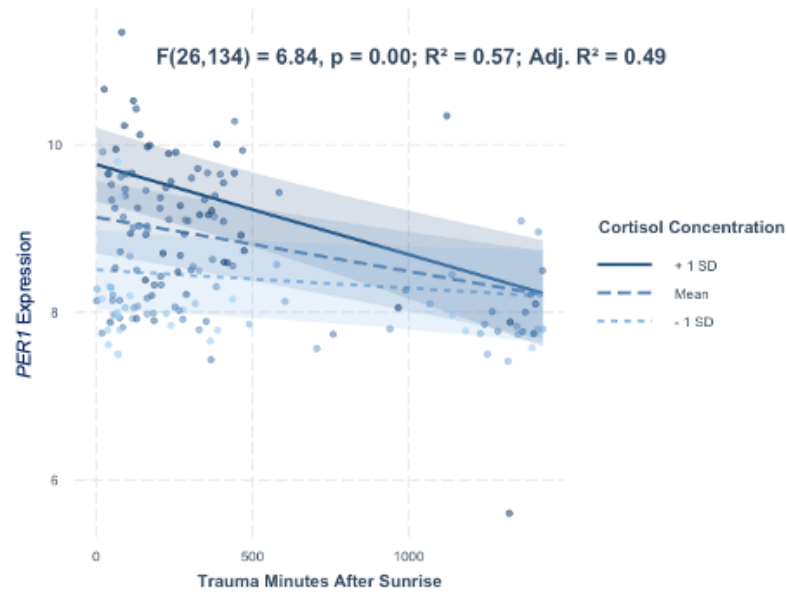


Fig 3. Cortisol moderates relationship between trauma timing and *PER1* expression. While at later trauma times cortisol concentration did not predict *PER1* expression, at earlier trauma times higher cortisol concentration correlated with higher *PER1* expression. Solid, long-dashed, and short-dashed blue lines are simple linear regression approximations of the relationship between the elapsed time since sunrise of the trauma and the whole blood expression of *PER1* at high cortisol concentration (one standard deviation above the mean), mean cortisol concentration, and low cortisol concentration (one standard deviation below the mean), respectively. Shading represents 95% confidence interval around the linear approximation of the corresponding color. Models are adjusted for three ancestry principal components, batch for genotyping and RNA, race, sex, age, education, pre-trauma PTSD symptom severity (PDS scores), clinician-rated trauma severity, and blood draw time.

Figure 4

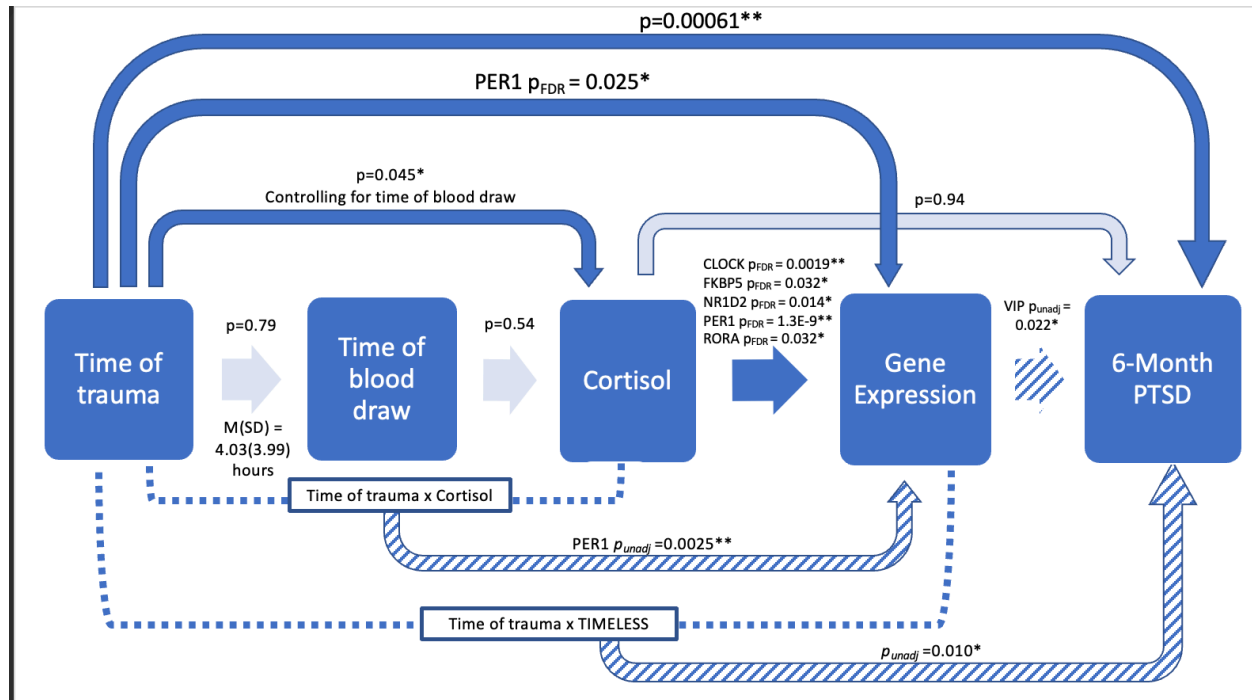


Fig 4. Summary of associations found between explored variables.