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Endogenous cortisol reactivity moderates the relationship between fear inhibition to safety signals and posttraumatic stress disorder symptoms

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ABSTRACT

Objective: Posttraumatic stress symptoms (PTSS) are commonly associated with impairments in extinguishing fear to signals previously associated with danger, and also with inhibiting fear to safety signals. Previous studies indicate that PTSS are associated with low cortisol activity, and cortisol is shown to facilitate fear extinction. Few studies have examined the influence of cortisol reactivity on fear extinction in PTSS.

Method: We used a standardized fear conditioning and extinction paradigm to investigate the relationship between fear extinction and endogenous salivary cortisol activity in participants with high PTSS ($n = 18$), trauma-exposed controls ($n = 33$), and non-trauma-exposed controls ($n = 27$). Skin conductance response (SCR) was used as an index of conditioned responding. Saliva samples were collected at baseline, and 20 min post-fear acquisition for basal and reactive cortisol levels, respectively.

Results: PTSS participants demonstrated a slower rate of extinction learning during the early extinction phase. A moderation analysis revealed that cortisol reactivity was a significant moderator between fear inhibition to the safety signal (CS−) during early extinction and PTSS, but not to the threat signal (CS+). Specifically, this interaction was significant in two ways: (1) participants with elevated cortisol reactivity showed lower PTSS as fear inhibition improved; and (2) participants with low cortisol reactivity showed higher PTSS as fear inhibition improved.

Conclusion: The findings of the present study show that the relationship between fear inhibition and cortisol reactivity is complex, and suggest that cortisol reactivity shapes safety signal learning in PTSS.

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1. Introduction

Posttraumatic stress disorder (PTSD) develops following a traumatic event that threatens physical integrity (American Psychiatric Association, 2013). Symptoms of PTSD include distressing intrusive memories, avoidance of trauma reminders, negative cognitions and mood, and hyperarousal. A prevailing model posits that persistent PTSD symptoms develop, in part, because of an impaired ability to extinguish a conditioned fear trace (e.g., Mineka and Oehlberg, 2008; Pitman et al., 2012). Stimuli present during the trauma become associated with fear and heightened arousal responses experienced during the event. After the event, benign stimuli act as triggers (conditioned stimuli; CS) for the fear response. PTSD-related impairments in fear extinction learning are well established (Blechert et al., 2007; Norrholm et al., 2011; Orr et al., 2000; Peri et al., 2000; Zuj et al., 2016a), and impaired fear extinction has been proposed as a central construct that links pre-trauma biological and cognitive vulnerability factors to PTSD (Zuj et al., 2016b). This is supported by evidence that impaired extinction learning prior to trauma exposure predicts subsequent PTSD following trauma (Guthrie and Bryant, 2006; Lommen et al., 2013; Orr et al., 2012). Further, exposure-based therapies for PTSD act on the principle of

extinguishing conditioned fear to trauma-related memory triggers (Milad et al., 2006).

Dysregulations in cortisol activity are commonly observed in patients with PTSD (Yehuda, 1997, 2009). The hypothalamic-pituitary-adrenal (HPA) axis is the premier neuroendocrine center involved in the neurochemical release of hormones and neuropeptides to combat stress. The end product of this neurochemical release, cortisol, binds to glucocorticoid receptors (GRs) in a negative feedback loop to inhibit the sympathetic nervous system and ongoing arousal (Yehuda, 1997), thus returning these neural systems to homeostasis. Alterations in the negative feedback system of the HPA axis have long been hypothesized in PTSD (e.g., Yehuda et al., 1991, 1993). After controlling for methodological differences (e.g., time-of-day, measurement type), a recent meta-analysis found that PTSD (and PTSD comorbid with major depressive disorder) was associated with lower daily cortisol output relative to non-trauma-exposed controls, and enhanced HPA feedback appears to be associated with general trauma exposure (Morris et al., 2012). The authors conclude that lower daily cortisol output may be a risk factor for the development of PTSD, rather than an effect of trauma exposure. Nevertheless, an earlier meta-analysis by Miller et al. (2007) noted that the relationship between cortisol activity and PTSD is complex, and further research in this area is required.

The effect of cortisol release (via pharmacological augmentation or acute stress tasks) on fear extinction learning has received little attention. Studies that have been conducted vary in their methodological design, for example the timing of cortisol administration (or stress induction) during conditioning and extinction paradigms (Merz et al., 2014). A recent study in healthy participants found that a stress-induction task impaired the retrieval of fear extinction memories, despite no deficit in fear acquisition or extinction 24 h earlier (Raio et al., 2014). Studies in rodents have found that glucocorticoid receptor agonists enhance the uptake of cortisol and facilitate fear extinction learning (Yang et al., 2006). These effects are partly due to actions on glutamate N-methyl-D-aspartate (NMDA) receptors in the amygdala (Yang et al., 2007), and many studies have shown glucocorticoid administration facilitates extinction (Barrett and Gonzalez-Lima, 2004; Cai et al., 2006; de Quervain et al., 2009).

Successful fear extinction is the underlying construct and goal of exposure therapy (Graham and Milad, 2011), and convergent evidence in clinical settings suggest that glucocorticoid administration improves response to exposure treatment for fear-related disorders. For example, clinical patients suffering from spider phobia who were administered hydrocortisone demonstrated significantly reduced fear after undergoing exposure therapy compared with placebo-controls (Soravia et al., 2006, 2014). Further, Yehuda et al. (2015) have shown that exposure therapy induces current, situational fear during treatment, which in turn increases patient drop out rates and that this can be countered with hydrocortisone administration in conjunction with prolonged exposure therapy. This reduces situational fear expression during the treatment session and thereby reduces drop out rates and enhances treatment benefit (Yehuda et al., 2015). In support, a recent study in patients with panic disorder and agoraphobia demonstrated that elevated endogenous cortisol significantly moderates greater symptom improvement to exposure therapy (Meuret et al., 2015). Together, these findings implicate that elevated cortisol levels facilitate the experimental and therapeutic extinction of fear.

Few studies have examined the influence of cortisol reactivity on fear extinction learning in the context of PTSD. Therefore, the current study used a discrimination fear conditioning and extinction paradigm to investigate the relationship between fear extinction and endogenous salivary cortisol reactivity in a sample with post-traumatic stress disorder symptoms (PTSS) and compared this to trauma-exposed, and non-exposed control groups without PTSS. Based on previous evidence, we considered two ways in which this relationship could manifest. First, greater cortisol reactivity may simply be associated with better fear extinction performance and lower PTSS. The second possibility is that cortisol reactivity may moderate the relationship between fear extinction learning and PTSS. That is, PTSS may be associated with significantly poorer fear extinction learning (e.g., Blechert et al., 2007; Norrholm et al., 2011), but this relationship may be stronger for participants with lower cortisol reactivity.

2. Method

2.1. Participants

Seventy-eight participants aged 18–63 years ($M = 28.1$ years, $SD = 11.8$ years; 35 males and 43 females) comprised three groups: PTSS ($n = 18$), trauma-exposed controls (TC; $n = 33$), and non-trauma-exposed controls (NTC; $n = 27$). Participants were classified on the basis of exposure to a criterion A stressor that threatened physical integrity (American Psychiatric Association, 2013) using the Traumatic Events Questionnaire (TEQ; Vrana and Lauterbach, 1994). Participants were exposed to a variety of environmental and interpersonal traumas: war exposure ($n = 4$), life-threatening accident ($n = 19$), natural disaster ($n = 24$), witness to serious injury or death ($n = 33$), assaulted or molested ($n = 22$), threatened or held captive ($n = 12$), and tortured or terrorist victim ($n = 1$). Mean years since trauma for the PTSS group was 10.5 years ($SD = 13.7$ years), and 9.8 years ($SD = 11.1$ years) for the TC group. The PTSD Checklist-Civilian version (PCL-C; Weathers et al., 1994) was used to estimate PTSS severity – participants who presented with at least 1 intrusive memory symptom, 3 avoidance symptoms, and 2 hyperarousal symptoms (defined as a score ≥ 3 on respective PCL-C items; National Center for Posttraumatic Stress Disorder, n.d.) were classified as showing clinical PTSS according to diagnostic criteria of the DSM-IV. Following these criteria, all PTSS participants had a PCL-C total greater than 40, and 61% ($n = 11$) of the PTSS group showed a PCL-C total greater than 50. The PCL-C for DSM-IV was used as testing began prior to the release of diagnostic instruments for the DSM-5. Participants were regarded as TC if they reported experience of a criterion A stressor, but did not reach the minimum possible cutoff of 30 for PTSD in general population samples (National Center for Posttraumatic Stress Disorder, n.d.). All participants in the TC group showed a PCL-C total score ≤29. Participants who reported no lifetime experience of a criterion A stressor were classified as NTC. Participants also completed the Depression Anxiety Stress Scale-21 item version (DASS; Lovibond and Lovibond, 1995). The Tasmanian Health and Medical Research Ethics Committee and the University of Tasmania Social Science Human Research Ethics Committee approved this study. All participants gave full informed consent prior to involvement.

2.2. Fear conditioning and extinction paradigm

The present study employed a differential fear conditioning and extinction paradigm used previously (Orr et al., 2000; Zuj et al., 2016a). Findings from a subset of the participants in the current study have been published elsewhere, which examined the impact of time-since-waking on fear extinction learning (Zuj et al., 2016a). The unconditioned stimulus (US) was a 500 ms mild electric shock delivered to the first interosseous muscle of the dominant hand, and set to a level considered "highly annoying, but not painful" by each participant prior to the task (Orr et al., 2000; Zuj et al., 2016a). Conditioned stimuli (CS) were red and blue circles presented individually for 12 s on a computer screen. The testing protocol included

four experimental phases: *habituation, acquisition, early extinction,* and *late extinction*. During *habituation*, participants were exposed to four trials of each colored circle (eight trials in total). After a short pause, participants were prompted to begin the *acquisition* phase when ready. During this phase, one of the colored circles (CS+) was followed by the US on all five trials (100% reinforcement schedule) while the other colored circle was not reinforced on any of the five trials (CS−; ten trials in total). There was a short interval of approximately 2–3 min post-acquisition, before participants completed the *early* and *late extinction* phases. The *early extinction* phase consisted of five trials of the CS+ (with no reinforcement) and five trials of the CS− (ten trials in total), followed by the *late extinction* phase, which mirrored early extinction. There was an approximately 1 min rest period between early and late extinction (Milad et al., 2005). Trial order was pseudo-random, with no more than two consecutive CS+ or CS− trials, and a variable inter-trial interval was used, ranging from 12 to 21 s ($M = 16$ s). All phases were completed in a single testing session. To ensure contingency awareness, participants were asked at the end of testing which colored circle was associated with the US. All participants reported accurate contingency awareness.

2.3. Skin conductance

Skin conductance level (SCL) was measured through a 22 mV$_{rms}$, 75 Hz constant-voltage coupler (FE116, ADInstruments) with bipolar electrodes on the intermediate phalange of the first and third fingers of the non-dominant hand, sampled at 512 Hz and stored at 64 Hz, and recorded in micro-siemens (μS). Skin conductance response (SCR) to the CS+ and CS− was calculated by subtracting the mean SCL during the 2 s prior to stimulus onset from the maximum SCL during the 12 s stimulus duration. SCR values were square-root transformed to normalize distributions (for negative SCR values, the absolute value was square-root transformed and the negative sign replaced).

2.4. Unconditioned stimulus expectancy ratings

During each 12 s stimulus presentation, participants were asked to rate their expectancy of the US on a 0–100 visual analogue scale (0 "certain no electrical stimulus"; 100 "certain electrical stimulus"; as used previously by Lommen et al., 2013).

2.5. Salivary cortisol

Saliva for cortisol was collected twice during testing. The first sample was collected upon arrival to the lab, and the second sample was collected approximately 20 min after the fear acquisition phase. Samples were immediately transferred to a freezer. Prior to assay, the saliva was thawed and centrifuged, and cortisol was measured using a commercially available ELISA assay (Salimetrics, USA) according to the manufacturers instructions. Salivary cortisol showed an assay sensitivity of 0.003 μg/dL. Intra-assay variability was 4.1%, and inter-assay variability was 4.6%. Cortisol data were square-root transformed to normalize distributions. A cortisol reactivity score was calculated by subtracting baseline levels from post-acquisition levels. To control for circadian variation in cortisol, testing was conducted between 12-6PM. Time-of-testing and hours-since-waking did not differ significantly between groups ($p = 0.114$, and $p = 0.610$, respectively).

2.6. Statistical analyses

Separate 3 (group) × 2 (CS) × 5 (trial) mixed-model analyses of variance (ANOVA) were conducted for each phase (with four trials for habituation and acquisition) to examine fear conditioning and extinction across groups. The first CS+/− trials during the acquisition phase were removed from analyses as the US had not been encountered at this stage, and no fear learning would have occurred (Zuj et al., 2016a) Analyses were identical for both the SCR and US-expectancy data. Greenhouse-Geisser corrections were made for within-subjects variables where necessary. Brown-Forsythe *F*-ratio adjustments were made where necessary, and pairwise comparisons were conducted with Bonferroni corrections or Games-Howell tests where appropriate. Moderation analyses were conducted using the PROCESS macro for SPSS (Model 1; Hayes, 2013). An alpha level of α = 0.05 was used for all tests of statistical significance. Effect sizes are reported as Cohen's *d* following the criteria of 0.2, 0.5, and 0.8 as small, moderate, and large effects, respectively (Cohen, 1988). Partial-eta squared (η_p^2) are reported as effect sizes for mixed-model ANOVAs.

3. Results

3.1. Descriptive and clinical data

Descriptive statistics, and additional inferential data are displayed in Table 1. One-way ANOVA revealed a significant between-group difference on age, $F(2, 42.51) = 4.15$, $p = 0.023$. While there was no significant age difference between the PTSS and TC groups ($p = 0.16$), and between the TC and NTC groups ($p = 0.47$), the PTSS group was, on average, significantly older than NTC participants ($p = 0.037$). As expected, there were significant between-group differences in PCL total scores, $F(2, 21.17) = 122.98$, $p < 0.001$. The PTSS group showed a significantly higher mean PCL

Table 1
Mean Scores and SDs for Demographic and Clinical Variables.

Measures	PTSD ($n = 18$)	TC ($n = 33$)	NTC ($n = 27$)	Test statistic	*P*-value
Demographic data					
-Age (years)	34.9 (15.0)	27.5 (10.1)	24.4 (9.7)	$F(2, 42.51) = 4.15$	0.023
-Sex	11F, 7M	14F, 19M	18F, 9M	$\chi^2(2) = 3.87$	0.15
PCL-C					
-Total	54.33 (11.81)	23.45 (3.85)	19.96 (2.52)	$F(2, 21.17) = 122.98$	<0.001
-Intrusive	3.38 (1.26)	0.19 (0.40)	0.00 (0.00)	$F(2, 58) = 132.30$	<0.001
-Avoidance	4.15 (1.82)	0.39 (0.67)	0.29 (0.59)	$F(2, 16.55) = 48.12$	<0.001
-Hyperarousal	3.77 (1.17)	0.48 (0.81)	0.18 (0.53)	$F(2, 25.09) = 74.56$	<0.001
DASS					
-Depression	9.78 (6.16)	2.27 (2.35)	1.63 (2.15)	$F(2, 24.67) = 23.94$	<0.001
-Anxiety	8.28 (4.10)	1.88 (1.85)	1.19 (1.66)	$F(2, 27.72) = 37.75$	<0.001
-Stress	13.11 (5.62)	5.00 (3.08)	2.59 (2.37)	$F(2, 30.92) = 37.51$	<0.001
AUDIT	7.06 (5.61)	6.27 (3.80)	6.22 (4.32)	$F(2, 45.78) = 0.21$	0.81

Note: PCL-C, PTSD Checklist; DASS, Depression Anxiety Stress Scale; AUDIT, Alcohol Use Disorders Identification Test.
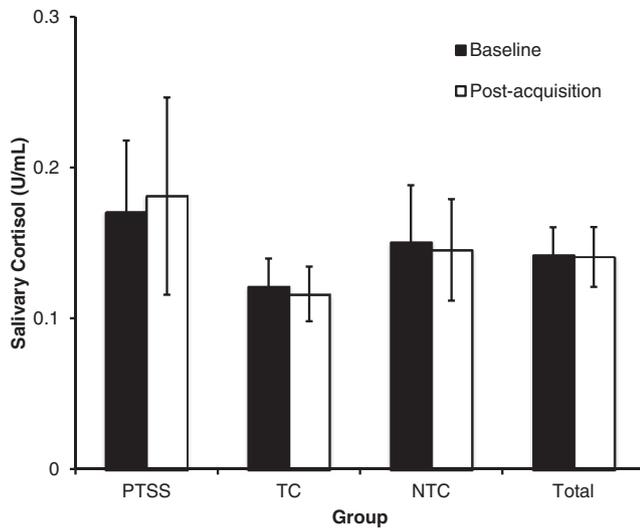
Fig. 1. Group and total levels of baseline and post-acquisition cortisol. On average, groups did not significantly differ, and there were no significant changes from baseline to post-acquisition. Figure shows raw salivary cortisol values. Error bars represent 95% confidence intervals.
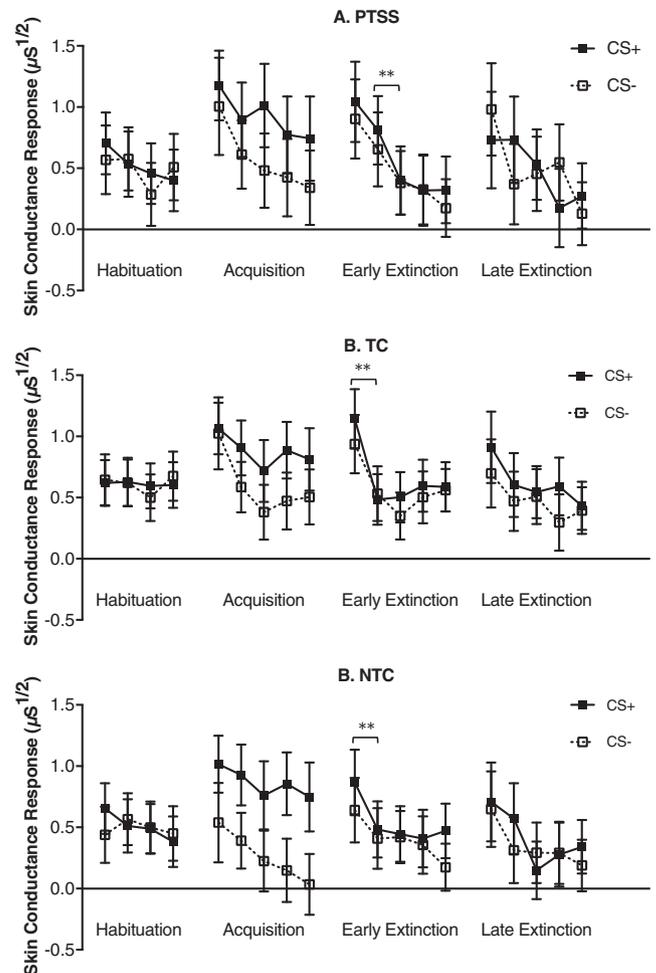

Fig. 2. Panel (A) displays SCRs to the CS+ and CS− during the fear conditioning and extinction paradigm for the PTSS group. Panel (B) displays SCRs for the TC group, and panel (C) displays SCRs for the NTC group. The first CS+/− trial of acquisition was omitted from all statistical analyses as the US had not yet been encountered, and no fear learning had occurred. The first trial of acquisition has been included in the figure for general information on the fear learning processes between PTSD, TC, and NTC groups. The NTC group displayed greater SCR to the CS+ compared to the CS− on trial 1 of acquisition, which was not anticipated. There was a significant group × trial interaction during the early extinction phase, with participants in the PTSD group showing a trend-level reduction from trial 1 to trial 2, and a significant reduction from trial 2 to trial 3, compared to the TC and NTC groups, who showed a significant reduction of SCRs from trial 1 to trial 2. Error bars display 95% confidence intervals of the mean. $\mu S^{1/2}$, SCR square-root transformed in micro-siemens. ** $p < 0.01$.

total than the TC and NTC groups ($ps < 0.001$), who also significantly differed ($p < 0.001$). One-way ANOVA revealed significant between-group differences on the depression, anxiety and stress subscales of the DASS (see Table 1 for descriptive and inferential statistics). Games-Howell post-hoc tests revealed that the PTSS group displayed significantly higher levels of depression, anxiety and stress than the TC and NTC groups (all $ps < 0.001$). The TC group also had significantly higher levels of stress than the NTC group ($p = 0.003$), with no significant differences on depression or anxiety between these two groups.

3.2. Salivary cortisol

Three (group) × 2 (time) repeated measures ANOVA revealed no significant change in cortisol levels from baseline to post-acquisition, $F(1, 73) = 0.06$, $p = 0.81$. Further, there was no significant group × time interaction, $F(2, 73) = 0.10$, $p = 0.91$. Raw baseline and post-acquisition cortisol levels are displayed in Fig. 1.

3.3. SCR amplitude data

3.3.1. Habituation

Mixed-model ANOVA showed a trend-level main effect of trial during the habituation phase, $F(2.85, 213.85) = 2.52$, $p = 0.06$, $\eta_p^2 = 0.032$, with SCR levels reducing across all trials. All further main effects and interactions were nonsignificant ($ps > 0.05$).

3.3.2. Acquisition

Mixed-model ANOVA showed a significant main effect of CS, $F(1, 75) = 67.01$, $p < 0.001$, $d = 0.83$, with significantly greater responding to the CS+ ($M = 0.84$, 95% CI[0.70, 0.97], $SD = 0.60$) compared to the CS− ($M = 0.38$ [0.27, 0.50], $SD = 0.49$) suggesting the acquisition of a conditioned fear response. There was also a significant main effect of trial, with SCRs declining across the acquisition phase, $F(2.85, 213.55) = 3.55$, $p = 0.017$, $\eta_p^2 = 0.045$. There were no further significant interactions, and no significant F-tests involving group.

3.3.3. Early extinction

During early extinction there was a significant main effect of CS, $F(1, 75) = 6.47$, $p = 0.013$, $d = 0.24$, indicating that participants continued displaying significantly greater SCRs in early extinction

to the CS+ ($M = 0.59$ [0.50, 0.69], $SD = 0.42$) compared to the CS− ($M = 0.49$ [0.39, 0.59], $SD = 0.43$). Fig. 2 shows that, on average, there was a reduction in SCR to the CS+/− from trial 1 to trial 5, as reflected in a significant main effect of trial, $F(3.58, 268.14) = 27.76$, $p < 0.001$, $\eta_p^2 = 0.270$. Further, there was a significant group × trial interaction, $F(7.15, 268.14) = 2.63$, $p = 0.012$, $\eta_p^2 = 0.065$. Tests of simple main effects revealed that the TC and NTC groups showed a significant reduction in SCRs from trial 1 to trial 2 ($ps < 0.001$), with no significant change in responding thereafter. The PTSS group, however, displayed a trend-level reduction in SCRs from trial 1 to trial 2 ($p = 0.051$), and a significant reduction from trial 2 to trial 3 ($p = 0.006$), suggestive of a slower rate of extinction learning that continued to decline from trial 2 to trial 3.

3.3.4. Late extinction

Mixed-model ANOVA revealed that the CS main effect was no longer significant, $F(1, 75) = 2.79$, $p = 0.10$, $d = 0.15$. A significant main effect of trial remained, $F(3.26, 244.66) = 13.93$, $p < 0.001$,

$\eta_p^2 = 0.157$, where responding to both the CS+ and CS− declined across the five trials (see Fig. 2). No further main effects or interactions were significant.

3.4. Threat expectancy ratings

3.4.1. Habituation
A $3 \times 2 \times 4$ mixed-model ANOVA revealed a significant main effect of trial during habituation, $F(2, 139.14) = 5.38$, $p = 0.006$, $\eta_p^2 = 0.071$, with US-expectancy ratings gradually declining as participants habituated to the task.

3.4.2. Acquisition
During acquisition, there were significant main effects of CS, $F(1, 69) = 235.42$, $p < 0.001$, $d = 3.04$, and trial, $F(3.50, 241.23) = 3.29$, $p = 0.016$, $\eta_p^2 = 0.046$, $\varepsilon = 0.874$. The successful acquisition of fear was evidenced by a significant CS $\times$ trial interaction, $F(3.50, 241.66) = 82.67$, $p < 0.001$, $\eta_p^2 = 0.545$, with differential responding to the CS+/− increasing throughout acquisition. That is, threat expectancy to the CS+ increased while responding to the CS− decreased, as shown in Fig. 3.

3.4.3. Early extinction
During early extinction, ANOVA revealed significant main effects of CS, $F(1, 70) = 46.96$, $p < 0.001$, $d = 0.97$, and trial, $F(2.53, 176.87) = 65.27$, $p < 0.001$, $\eta_p^2 = 0.483$, $\varepsilon = 0.632$. These main effects were superseded by a significant CS $\times$ trial interaction, $F(3.39, 236.96) = 7.16$, $p < 0.001$, $\eta_p^2 = 0.093$, $\varepsilon = 0.846$, with differential responding reducing across the early extinction phase (see Fig. 3).

3.4.4. Late extinction
Similar to early extinction, the late extinction phase showed a significant main effect of CS, $F(1, 70) = 18.10$, $p < 0.001$, $d = 0.47$. There was a significant trial main effect, $F(2.18, 152.72) = 39.40$, $p < 0.001$, $\eta_p^2 = 0.360$, $\varepsilon = 0.545$. ANOVA also revealed a significant CS $\times$ trial interaction, $F(3.43, 239.95) = 3.95$, $p = 0.006$, $\eta_p^2 = 0.053$, showing that the differential threat expectancy declined from trial 1 to trial 5 during the late extinction phase. Further, during late extinction there was a significant group main effect, $F(2, 70) = 8.98$, $p < 0.001$, $\eta_p^2 = 0.204$, with the PTSS group displaying higher mean US-expectancy ($M = 47.24$ [37.40, 57.07], $SD = 20.33$) than TC and NTC groups ($M = 23.07$ [15.78, 30.35], $SD = 20.33$, and $M = 23.80$ [15.69, 31.91], $SD = 20.33$, respectively).

3.5. Moderation analyses

As a significant group $\times$ trial interaction effect on SCR was only found during early extinction, and the three groups were all displaying similar levels of extinction learning by trial 3, the difference between trial 1 and trial 2 was calculated separately for the CS+ and the CS−, and entered into separate moderation analyses as the predictor variables (see Table 2, Models 1 and 2, respectively). Cortisol reactivity was entered as the moderator, with PCL total as the outcome variable, and age and depression were included as covariates. Using the predictor derived from the CS+ change score, there was a significant total model, $R^2 = 0.660$, $F(5, 70) = 27.18$, $p < 0.001$. However, as seen in Table 2, model 1, there was no significant effect of cortisol reactivity or CS+ change, and there was no significant moderation interaction between these variables. The main effects and moderation interaction remained nonsignificant after including age and depression as covariates.

Using the early extinction CS− change from trial 1 to trial 2 as the predictor, the model predicted a significant amount of variance in PCL total, $R^2 = 0.690$, $F(5, 70) = 31.08$, $p < 0.001$. There were no significant main effects of cortisol reactivity or CS− change. Critically, however, cortisol reactivity was a significant moderator of the
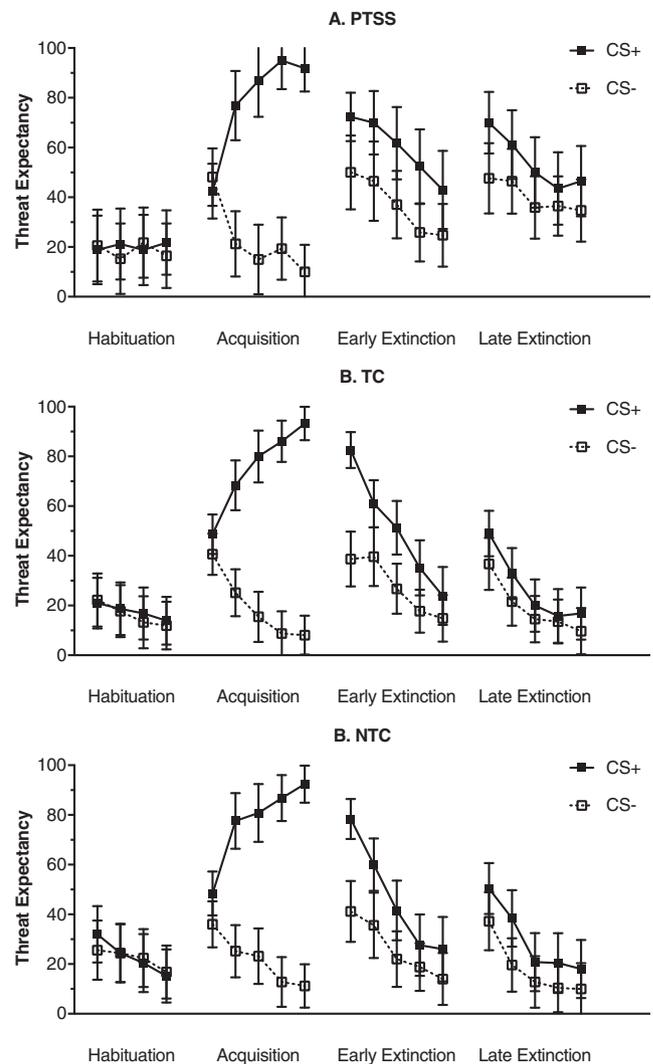

Fig. 3. Threat expectancy ratings for the CS+ and CS− across the differential conditioning and extinction paradigm are displayed separately for the PTSS group (A), TC group (B), and NTC group (C). The acquisition panels show clear understanding of the CS+/US contingency, with scores greater than 50 representing increased threat expectancy, and scores lower than 50 representing reduced threat expectancy. The early and late extinction panels show that threat expectancy of the US attenuated for all three groups. Error bars display 95% confidence intervals of the mean.

relationship between CS− change and PCL total (see Table 2, model 2), with age and depression included as covariates. As shown in Fig. 4, participants with greater cortisol reactivity showed a negative relationship between fear extinction learning and PTSS. That is, for participants with lower PTSS severity, high cortisol reactivity was associated with facilitated fear extinction learning to the CS−. This relationship was not evident at lower levels of cortisol reactivity: For participants with moderate levels of cortisol reactivity, fear extinction learning was not significantly related to PTSD symptoms. And, interestingly, for participants with low cortisol reactivity, the inverse relationship was found, with higher PTSD symptoms associated with better fear extinction.

4. Discussion

The aim of the current study was to investigate the relationship between fear extinction learning and endogenous cortisol reactivity in PTSS. We found that cortisol reactivity was a significant moderator of the relationship between fear extinction (specifically to the safety signal) and PTSS. This effect was demonstrated in two

Table 2
Linear Model of Predictors of PCL Total.

	b	SE b	t	P
Model 1				
-Constant	11.63 [6.02, 17.25]	2.82	4.13	<0.001
-Cortisol reactivity	3.99 [−20.48, 28.45]	12.27	0.33	0.75
-CS+ change T1–T2	−1.94 [−5.12, 1.24]	1.60	−1.21	0.23
-CS+ change × Cortisol reactivity	6.34 [−33.19, 45.88]	19.82	0.75	0.75
Model 2				
-Constant	11.65 [6.32, 16.99]	2.67	4.36	<0.001
-Cortisol reactivity	−0.81 [−23.79, 22.16]	11.52	−0.07	0.94
-CS− change T1–T2	0.53 [−2.06, 3.12]	1.30	0.41	0.69
-CS− change × Cortisol reactivity	−46.44 [−78.17, −14.71]	15.91	−2.92	.005*

Note: SE = standard error; Square brackets show 95% confidence intervals of *b*; * interaction is significant after age and depression were included in the model as covariates.
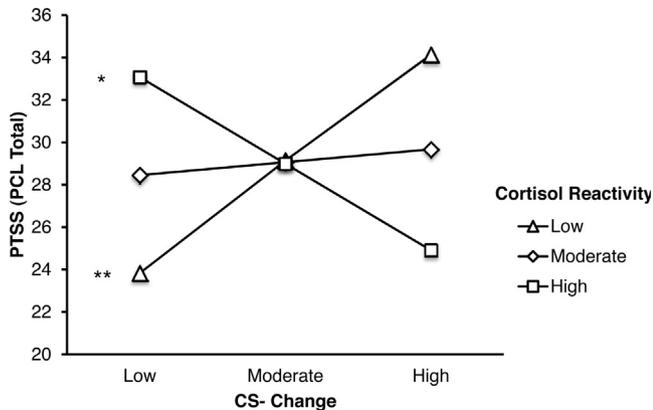

Fig. 4. The relationship between early extinction CS− change and PTSS was significant at lower levels (−1.5 *SD*) and higher levels (+1.5 *SD*) of cortisol reactivity. At moderate (mean) levels of cortisol reactivity, there was no significant relationship between fear extinction learning and PTSS. These findings suggest a positive relationship between PTSS and extinction learning when cortisol reactivity is low. Further, there is a negative relationship between PTSS and extinction learning when cortisol reactivity is high. * $p < 0.05$. ** $p < 0.01$.

ways. (1) High cortisol reactivity was associated with lower PTSS severity as fear extinction learning to the safety signal improved; and (2) low cortisol reactivity was associated with higher PTSS severity as fear extinction learning to the safety signal improved. Interestingly, this effect was only found with fear responding to the CS−, rather than the CS+.

In the present study, cortisol reactivity was a significant moderator between fear extinction to the CS− (but not the CS+) and PTSS. During the extinction phase(s) of a simple discrimination paradigm as employed in the current study, appropriate responding to the CS+ and CS− reflect extinction learning and fear inhibition, respectively (Jovanovic and Norrholm, 2011). Therefore, we speculate that the moderation analysis of the current study revealed cortisol reactivity to specifically interact with processes of fear inhibition in PTSS severity. Recent research implicates impaired fear inhibition to safety signals to be a key biomarker in PTSD (Jovanovic et al., 2012; Jovanovic and Norrholm, 2011). Previous research has shown patients with PTSD tend to show generalized conditioned fear responding from a reinforced CS+ to a non-reinforced CS− (Grillon and Morgan, 1999). This effect has previously been hypothesized to be attributable to either difficulty in learning the CS/US contingency, or an error in appropriately inhibiting fear despite accurate contingency awareness (Jovanovic et al., 2009). The findings of the present study support the latter, as all participants reported awareness of the CS/US contingency, a fact that is further supported by the self-reported threat expectancy ratings during the acquisition and early extinction phases (see Fig. 3). Further, we previously demonstrated that PTSD is associated with impaired fear extinction learning, which becomes worse with greater hours-since-waking

(Zuj et al., 2016a), and increased endogenous cortisol activity has recently been shown to mediate better treatment response in the morning in a sample of patients with panic disorder and agoraphobia (Meuret et al., 2016). In the current study, however, participants in the PTSS group showed a general impairment in fear responding to both CS+/− during early extinction, with no specific extinction deficit to either CS. Rather, the findings of the current study showed that cortisol reactivity interacts with fear inhibition to the CS− (but not extinction to the CS+) in a model of PTSS severity.

Regarding the influence of cortisol, the findings of the current study are supported by recent work showing that endogenous cortisol levels significantly moderate (Meuret et al., 2015) and mediate (Meuret et al., 2016) exposure-therapy success in patients with panic disorder and agoraphobia. Pharmacological studies have also demonstrated that dexamethasone suppression of HPA axis function significantly reduces exaggerated fear-potentiated startle in patients with PTSD, compared to traumatized controls without PTSD (Jovanovic et al., 2011). Interestingly, this finding was only revealed for fear-potentiated startle to the CS+ and not the CS−, suggesting a role for endogenous cortisol reactivity in fear inhibition. The findings of the current study, that endogenous cortisol reactivity is a moderator of fear inhibition, but not fear extinction learning, is a novel result, and requires further investigation. Additionally, glucocorticoid administration (significantly increasing cortisol output) in conjunction with exposure therapy for specific phobia significantly reduced symptom severity compared to the placebo control group in keeping with earlier research (de Quervain et al., 2009). Together, these findings suggest that cortisol augmentation and enhancing safety signal learning during the therapy process may improve treatment response. Although salivary cortisol has recently been used in similar research (Meuret et al., 2016, 2015), the collection of 24 h blood samples would provide a more accurate indication of the role of cortisol in fear extinction or inhibition.

The standardized differential fear conditioning and extinction paradigm used in the current study produced reliable fear conditioning and extinction learning. These findings are in line with previous studies showing PTSD-related impairments in extinction learning (Blechert et al., 2007; Norrholm et al., 2011; Orr et al., 2000; Peri et al., 2000), although some studies have failed to find specific extinction learning deficits in PTSD (Milad et al., 2008, 2009). The findings of the present study are consistent with the idea of "fear load", that PTSD is associated with significantly increased fear expression during the early trials of extinction learning (Norrholm et al., 2015, 2011). However, extinction learning in the current study occurred rapidly, with the PTSS group extinguishing conditioned fear responses by trial 3 of early extinction, and by trial 2 for the control groups with relatively small effect sizes. We speculate that this rapid extinction learning may be due to the use of a 100% reinforcement schedule during fear acquisition. While full reinforcement schedules are commonly used (e.g., Jovanovic et al., 2014; Orr et al., 2012, 2000), replicating the current study using

less predictable US-exposure (e.g., 60% reinforcement schedule, as used by Milad et al. (2007), Pace-Schott et al. (2013)) may slow extinction learning and increase the sensitivity to reveal effects.

The current finding that better fear extinction performance is associated with lower PTSS in the context of higher cortisol reactivity is consistent with the findings of a study that found glucocorticoid administration enhanced exposure therapy outcome in specific phobia (de Quervain et al., 2011). Exposure therapy is one of the most recommended treatments for PTSD (Foa et al., 2009) and acts by extinguishing conditioned fear memories associated with the trauma (Graham and Milad, 2011; Yehuda et al., 2015). Recently, Yehuda et al. (2015) found that hydrocortisone augmentation of exposure therapy resulted in significantly greater PTSD symptom reduction and patient retention compared to placebo. Due to increased PTSD symptom expression during initial exposure therapy sessions, patient drop-out rates are a serious issue (e.g., Jeffreys et al., 2014; Yehuda et al., 2015), and the results of the current study suggest that hydrocortisone augmentation of exposure therapy may have been effective in part due to the interaction between altered cortisol levels and correcting impaired fear inhibition processes.

While the primary finding of the moderation analysis is in line with previous research (e.g., Meuret et al., 2015), the moderation also revealed that for participants with low cortisol reactivity, greater PTSS was associated with better fear inhibition. This effect was not expected, and we speculate that low cortisol reactivity may act as a moderator of poor response to exposure therapy in clinical settings. That is, approximately 15–45% of patients still show diagnostic criteria for PTSD after prolonged exposure therapy (e.g., Mørkved et al., 2014; Powers et al., 2010). We suggest that prolonged exposure therapy may target key fear-related symptoms of PTSD, but may be ineffective in the treatment of other symptoms when HPA function is hypoactive, leading to ongoing need for treatment. Glucocorticoid administration may be beneficial in such situations. Alternatively, previous research in the glucocorticoid receptors of the rat nucleus of the solitary tract revealed a dose-response relationship with memory consolidation, whereby intermediate doses enhanced consolidation with no effects at low or high doses (Roozendaal et al., 1999). The moderation effect of low cortisol reactivity on the relationship between fear inhibition and PTSD symptoms was not anticipated, and preliminary animal evidence suggest that glucocorticoid receptors, and possibly cortisol activity, follow a U-curve dose-response relationship with learning and memory processes.

These effects notwithstanding, some limitations were present in the current study. First, we only examined the relationship between cortisol reactivity and fear extinction learning, and a previous pilot study suggests that a one-month course of low-dose cortisol administration in patients with PTSD resulted in reduced traumatic memory symptoms compared to placebo (de Quervain, 2008). Future research using a multi-day extinction learning and recall paradigm (e.g., Milad et al., 2008, 2009) may reveal important insights into this issue. Second, the PTSS group used in the current study also included participants with subclinical symptoms, and a sample of greater clinical severity are necessary to make robust implications for the interaction between cortisol and fear extinction in a clinical treatment setting. Finally, the use of self-report diagnostic instruments (and diagnostic tools for the DSM-IV) is a limitation of the current study, and future research would benefit from clinical diagnostic tools, such as the Clinician Administered PTSD Scale (CAPS) for the DSM-5.

In conclusion, we observed that endogenous cortisol reactivity is a significant moderator between the inhibition of fear responses to the CS− (but not the CS+) and PTSS. Our findings provide evidence that elevated cortisol influences the relationship between PTSS and fear inhibition, such that greater fear inhibition to a safety signal is associated with lower PTSS, and this relationship only occurs in participants experiencing greater cortisol reactivity. This finding provides a novel insight into the processes of fear inhibition, suggesting that endogenous cortisol reactivity moderates the relationship between fear inhibition to safety signals and PTSS.

Conflict of interest

Role of funding source

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References

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. Author, Washington, DC.

Barrett, D., Gonzalez-Lima, F., 2004. Behavioral effects of metyrapone on Pavlovian extinction. Neurosci. Lett. 371, 91–96.

Blechert, J., Michael, T., Vriends, N., Margraf, J., Wilhelm, F.H., 2007. Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. Behav. Res. Ther. 45, 2019–2033.

Cai, W.H., Blundell, J., Han, J., Greene, R.W., Powell, C.M., 2006. Postreactivation glucocorticoids impair recall of established fear memory. J. Neurosci. 26, 9560–9566.

Cohen, J., 1988. Statistical Power Analysis for the Behavioural Sciences, 2nd ed. Lawrence Erlbaum, Hillsdale, NJ.

de Quervain, D.J., Aerni, A., Schelling, G., Roozendaal, B., 2009. Glucocorticoids and the regulation of memory in health and disease. Front. Neuroendocrinol. 30, 358–370.

de Quervain, D.J., Bentz, D., Michael, T., Bolt, O.C., Wiederhold, B.K., Margraf, J., Wilhelm, F.H., 2011. Glucocorticoids enhance extinction-based psychotherapy. Proc. Natl. Acad. Sci. U. S. A. 108, 6621–6625.

de Quervain, D.J., 2008. Glucocorticoid-induced reduction of traumatic memories: implications for the treatment of PTSD. Prog. Brain Res. 167, 239–247.

Foa, E.B., Keane, T.M., Friedman, M.J., Cohen, J.A., 2009. Effective Treatments for PTSD: Practice Guidelines from the International Society of Traumatic Stress Studies, 2nd ed. Guilford, New York.

Graham, B.M., Milad, M.R., 2011. The study of fear extinction: implications for anxiety disorders. Am. J. Psychiatry 168, 1255–1265.

Grillon, C., Morgan, C.A., 1999. Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. J. Abnorm. Psychol. 108, 134–142.

Guthrie, R.M., Bryant, R.A., 2006. Extinction learning before trauma and subsequent posttraumatic stress. Psychosom. Med. 68, 307–311.

Hayes, A.F., 2013. Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach. The Guilford Press, New York.

Jeffreys, M.D., Reinfeld, C., Nair, P.V., Garcia, H.A., Mata-Galan, E., Rentz, T.O., 2014. Evaluating treatment of posttraumatic stress disorder with cognitive processing therapy and prolonged exposure therapy in a VHA specialty clinic. J. Anxiety Disord. 28, 108–114.

Jovanovic, T., Norrholm, S.D., 2011. Neural mechanisms of impaired fear inhibition in posttraumatic stress disorder. Front. Behav. Neurosci. 5, 44.

Jovanovic, T., Norrholm, S.D., Fennell, J.E., Keyes, M., Fiallos, A.M., Myers, K.M., Davis, M., Duncan, E.J., 2009. Posttraumatic stress disorder may be associated with impaired fear inhibition: relation to symptom severity. Psychiatry Res. 167, 151–160.

Jovanovic, T., Phifer, J.E., Sicking, K., Weiss, T., Norrholm, S.D., Bradley, B., Ressler, K.J., 2011. Cortisol suppression by dexamethasone reduces exaggerated fear responses in posttraumatic stress disorder. Psychoneuroendocrinology 36, 1540–1552.

Jovanovic, T., Kazama, A., Bachevalier, J., Davis, M., 2012. Impaired safety signal learning may be a biomarker of PTSD. Neuropharmacology 62, 695–704.

Jovanovic, T., Nylocks, K.M., Gamwell, K.L., Smith, A., Davis, T.A., Norrholm, S.D., Bradley, B., 2014. Development of fear acquisition and extinction in children: effects of age and anxiety. Neurobiol. Learn. Mem. 113, 135–142.

Lommen, M.J.J., Engelhard, I.M., Sijbrandij, M., van den Hout, M.A., Hermans, D., 2013. Pre-trauma individual differences in extinction learning predict posttraumatic stress. Behav. Res. Ther. 51, 63–67.

Lovibond, S.H., Lovibond, P.F., 1995. Manual for the Depression Anxiety Stress Scales, 2nd ed. Psychology Foundation, Sydney.

Mørkved, N., Hartmann, K., Aarsheim, L.M., Holen, D., Milde, A.M., Bomyea, J., Thorp, S.R., 2014. A comparison of narrative exposure therapy and prolonged exposure therapy for PTSD. Clin. Psychol. Rev. 34, 453–467.

Merz, C.J., Hermann, A., Stark, R., Wolf, O.T., 2014. Cortisol modifies extinction learning of recently acquired fear in men. Soc. Cogn. Affect. Neurosci. 9, 1426–1434.

Meuret, A.E., Trueba, A.F., Abelson, J.L., Liberzon, I., Auchus, R., Bhaskara, L., Ritz, T., Rosenfield, D., 2015. High cortisol awakening response and cortisol levels moderate exposure-based psychotherapy success. Psychoneuroendocrinology 51, 331–340.

Meuret, A.E., Rosenfield, D., Bhaskara, L., Auchus, R., Liberzon, I., Ritz, T., Abelson, J.L., 2016. Timing matters: endogenous cortisol mediates benefits from early-day psychotherapy. Psychoneuroendocrinology 74, 197–202.

Milad, M.R., Orr, S.P., Pitman, R.K., Rauch, S.L., 2005. Context modulation of memory for fear extinction in humans. Psychophysiology 42, 456–464.

Milad, M.R., Rauch, S.L., Pitman, R.K., Quirk, G.J., 2006. Fear extinction in rats: implications for human brain imaging and anxiety disorders. Biol. Psychol. 73, 61–71.

Milad, M.R., Wright, C.I., Orr, S.P., Pitman, R.K., Quirk, G.J., Rauch, S.L., 2007. Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol. Psychiatry 62, 446–454.

Milad, M.R., Orr, S.P., Lasko, N.B., Chang, Y., Rauch, S.L., Pitman, R.K., 2008. Presence and acquired origin of reduced recall for fear extinction in PTSD: results of a twin study. J. Psychiatr. Res. 42, 515–520.

Milad, M.R., Pitman, R.K., Ellis, C.B., Gold, A.L., Shin, L.M., Lasko, N.B., Zeidan, M.A., Handwerger, K., Orr, S.P., Rauch, S.L., 2009. Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol. Psychiatry 66, 1075–1082.

Miller, G.E., Chen, E., Zhou, E.S., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic–pituitary–adrenocortical axis in humans. Psychol. Bull. 133, 25–45.

Mineka, S., Oehlberg, K., 2008. The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. Acta Psychol. 127, 567–580.

Morris, M.C., Compas, B.E., Garber, J., 2012. Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. Clin. Psychol. Rev. 32, 301–315.

National Center for Posttraumatic Stress Disorder, 2016. Using the PTSD Checklist for DSM-IV (PCL) (n.d.) http://www.ptsd.va.gov/professional/assessment/adult-sr/ptsd-checklist.asp.

Norrholm, S.D., Jovanovic, T., Olin, I.W., Sands, L.A., Karapanou, I., Bradley, B., Ressler, K.J., 2011. Fear extinction in traumatized civilians with posttraumatic stress disorder: relation to symptom severity. Biol. Psychiatry 69, 556–563.

Norrholm, S.D., Glover, E.M., Stevens, J.S., Fani, N., Galatzer-Levy, I.R., Bradley, B., Ressler, K.J., Jovanovic, T., 2015. Fear load: the psychophysiological over-expression of fear as an intermediate phenotype associated with trauma reactions. Int. J. Psychophysiol. 98, 270–275.

Orr, S.P., Metzger, L.J., Lasko, N.B., Macklin, M.L., Peri, T., Pitman, R.K., 2000. De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. J. Abnorm. Psychol. 109, 290–298.

Orr, S.P., Lasko, N.B., Macklin, M.L., Pineles, S.L., Chang, Y., Pitman, R.K., 2012. Predicting post-trauma stress symptoms from pre-trauma psychophysiologic reactivity, personality traits and measures of psychopathology. Biol. Mood Anxiety Disord. 2.

Pace-Schott, E.F., Spencer, R.M.C., Vijayakumar, S., Ahmed, N.A.K., Verga, P.W., Orr, S.P., Pitman, R.K., Milad, M.R., 2013. Extinction of conditioned fear is better learned and recalled in the morning than in the evening. J. Psychiatr. Res. 47, 1776–1784.

Peri, T., Ben-Shakhar, G., Orr, S.P., Shalev, A.Y., 2000. Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. Biol. Psychiatry 47, 512–519.

Pitman, R.K., Rasmusson, A.M., Koenen, K.C., Shin, L.M., Orr, S.P., Gilbertson, M.W., Milad, M.R., Liberzon, I., 2012. Biological studies of post-traumatic stress disorder. Nature 13, 769–787.

Powers, M.B., Halpern, J.M., Ferenschak, M.P., Gillihan, S.J., Foa, E.B., 2010. A meta-analytic review of prolonged exposure for posttraumatic stress disorder. Clin. Psychol. Rev. 30, 635–641.

Raio, C.M., Brignoni-Perez, E., Goldman, R., Phelps, E.A., 2014. Acute stress impairs the retrieval of extinction memory in humans. Neurobiol. Learn. Mem. 112, 212–221.

Roozendaal, B., Williams, C.L., McGaugh, J.L., 1999. Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. Eur. J. Neurosci. 11, 1317–1323.

Soravia, L.M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehlert, U., Roozendaal, B., de Quervain, D.J., 2006. Glucocorticoids reduce phobic fear in humans. Proc. Natl. Acad. Sci. U. S. A. 103, 5585–5590.

Soravia, L.M., Heinrichs, M., Winzeler, L., Fisler, M., Schmitt, W., Horn, H., Dierks, T., Strik, W., Hofmann, S.G., de Quervain, D.J., 2014. Glucocorticoids enhance in vivo exposure-based therapy of spider phobia. Depress. Anxiety 31, 429–435.

Vrana, S.R., Lauterbach, D., 1994. Prevalence of traumatic events and post-traumatic psychological symptoms in a nonclinical sample of college students. J. Trauma. Stress 7, 289–302.

Weathers, F.W., Litz, B.T., Huska, J.A., Keane, T.M., 1994. The PTSD Checklist-Civilian Version (PCL-C) for DSM-IV. National Center for PTSD, Behavioral Sciences Division, Boston.

Yang, Y.L., Chao, P.K., Lu, K.T., 2006. Systemic and intra-amygdala administration of glucocorticoid agonist and antagonist modulate extinction of conditioned fear. Neuropsychopharmacology 31, 912–924.

Yang, Y.L., Chao, P.K., Ro, L.S., Wo, Y.Y., Lu, K.T., 2007. Glutamate NMDA receptors within the amygdala participate in the modulatory effect of glucocorticoids on extinction of conditioned fear in rats. Neuropsychopharmacology 32, 1042–1051.

Yehuda, R., Giller, E.L., Southwick, S.M., Lowy, M.T., Mason, J.W., 1991. Hypothalamic–pituitary–adrenal dysfunction in posttraumatic stress disorder. Biol. Psychiatry 30, 1031–1048.

Yehuda, R., Southwick, S.M., Krystal, J.H., Bremner, D., Charney, D.S., Mason, J.W., 1993. Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. Am. J. Psychiatry 150, 83–86.

Yehuda, R., Bierer, L.M., Pratchett, L.C., Lehrner, A., Koch, E.C., Van Manen, J.A., Flory, J.D., Makotkine, I., Hildebrandt, T., 2015. Cortisol augmentation of a psychological treatment for warfighters with posttraumatic stress disorder: randomized trial showing improved treatment retention and outcome. Psychoneuroendocrinology 51, 589–597.

Yehuda, R., 1997. Sensitization of the hypothalamic-pituitary-adrenal axis in posttraumatic stress disorder. Ann. N. Y. Acad. Sci. 821, 57–75.

Yehuda, R., 2009. Status of glucocorticoid alterations in post-traumatic stress disorder. Ann. N. Y. Acad. Sci. 1179, 56–69.

Zuj, D.V., Palmer, M.A., Hsu, C.K., Nicholson, E.L., Cushing, P.J., Gray, K.E., Felmingham, K.L., 2016a. Impaired fear extinction associated with PTSD increases with hours-since-waking. Depress. Anxiety 33, 203–210.

Zuj, D.V., Palmer, M.A., Lommen, M.J.J., Felmingham, K.L., 2016b. The centrality of fear extinction in linking risk factors to PTSD: a narrative review. Neurosci. Biobehav. Rev. 69, 15–35.