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Hydrocortisone suppression of the fear-potentiated startle response and posttraumatic stress disorder

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Summary This study examined the effects of oral administration of 20 mg hydrocortisone on baseline and fear-potentiated startle in 63 male veterans with or without PTSD. The procedure was based on a two-session, within-subject design in which acoustic startle eyeblink responses were recorded during intervals of threat or no threat of electric shock. Results showed that the magnitude of the difference between startle responses recorded during anticipation of imminent shock compared to "safe" periods was reduced after hydrocortisone administration relative to placebo. This effect did not vary as a function of PTSD group nor were there were any significant group differences in other indices startle amplitude. Findings suggest that the acute elevations in systemic cortisol produced by hydrocortisone administration may have fear-inhibiting effects. This finding may have implications for understanding the role of hypothalamic—pituitary—adrenal (HPA)-axis function in vulnerability and resilience to traumatic stress.
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Exaggerated startle responding, a symptom of posttraumatic stress disorder (PTSD; APA, 1994), is unique among psychiatric symptoms in the degree of correspondence between the clinical phenomenon experienced by patients and the behavioral analog (e.g., startle eyeblink amplitude) that can be recorded in clinical psychophysiology or animal laboratories. Over the past twenty years, neuroscientists and psychophysiologists have developed an advanced understanding of the neurocircuitry and neuromodulators of the startle response. Unfortunately, this new knowledge has not been fully explored in regard to the exaggeration of startle that commonly accompanies PTSD. The primary aim of this study was to attempt to clarify the mechanisms of exaggerated startle in PTSD by testing the hypothesis that startle amplitude is linked to activity of a key substrate of the stress response, the hypothalamic—pituitary—adrenal (HPA)-axis.

1. PTSD, the HPA-axis and startle

Because of its central role in the stress response, abnormalities in functioning of the HPA-axis have been a major focus of research on the neurobiology of PTSD (for reviews see:

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Yehuda, 2001, 1997; Kaskow et al., 2001). Activity in this system is initiated by release of corticotropin-releasing hormone (CRH) which triggers the production of downstream hormones and acts as a neurotransmitter in an elaborate network of interconnected neurons in the limbic system, brainstem, and cortex that are reactive to exogenous threat and challenge (for a review, see Lovallo and Thomas, 2000). CRH production is inhibited by cortisol which exerts negative feedback control over HPA-axis activity by binding to glucocorticoid receptors of the hypothalamus (McCann, 1988; Orth et al., 1992). In PTSD, there is evidence of elevated mean levels of central CRH (Bremner et al., 1997; Baker et al., 1999) and a heightened sensitivity to cortisol (i.e., as evidenced by response to dexamethasone; Yehuda et al., 1993, 1995a,b; Stein et al., 1997).

Research suggests possible links between activity of the HPA-axis, startle amplitude, and the phenomenon of exaggerated startle in PTSD. Animal studies have shown that increasing CRH levels has a potentiating effect on startle amplitude whereas increasing systemic cortisol exerts an inhibitory effect. Specifically, in rats, intracerebroventricular infusion of CRH produces a pronounced, dose-dependent enhancement of the startle response while this effect is blocked by pre-treatment with a CRH receptor antagonist (Swerdlow et al., 1989, 1986; Liang et al., 1992; Lee et al., 1994; Declan et al., 1998). In contrast, when corticosterone is injected intraperitoneally, or subcutaneously implanted, the startle response is attenuated (Sandi et al., 1996; Tanke et al., 2008) and antagonism of both the mineralcorticoid and glucocorticoid receptors, which produces a blockade of negative feedback on the HPA-axis, leads to an increase in startle amplitude (Korte et al., 1996).

Similar relationships have been found in studies of healthy human participants. Buchanan et al. (2001) conducted two experiments examining the effects of oral administration of hydrocortisone on amplitude of the acoustic startle reflex. Hydrocortisone is a semisynthetic derivative of the glucocorticoids which is chemically identical to cortisol (Harvey et al., 1992). Its administration causes rapid increases in circulating cortisol which readily passes the blood—brain barrier allowing it to reach receptors located throughout the brain including the limbic system as well as the hypothalamus and pituitary where it inhibits the release of CRH and ACTH, respectively (Won et al., 1986; DeBold et al., 1989). In their first experiment, Buchanan et al. administered either placebo or two levels of oral hydrocortisone (5 or 20 mg) to 12 healthy volunteers using a within-subject design over three experimental sessions. Results suggested a biphasic effect of hydrocortisone dosage: 20 mg led to a significant reduction of startle compared to 5 mg, though neither 5 mg nor 20 mg differed significantly from placebo. A similar effect was observed in a second experiment using a single session, between-subjects design in which 48 healthy volunteers received either placebo or 20 mg of hydrocortisone. In that one, there was a trend ($p = .052$) towards smaller startle in subjects treated with 20 mg compared to subjects received placebo. Similarly, Roemer et al. (2009) reported that pharmacologic suppression of cortisol with metyrapone exerted potentiating effects on startle eyeblink magnitude, and Miller and Gronfier (2006) found an inverse association between diurnal levels of cortisol and startle amplitude assessed in the sleep lab.

2. Exaggerated startle in PTSD

One factor that has received considerable attention in prior research on mechanisms of exaggerated startle in PTSD is *contextual anxiety* with investigators hypothesizing that the symptom is a context- or state-dependent phenomenon related to anxiety processes (Grillon and Morgan, 1999; Grillon et al., 1998a). This hypothesis follows from the seminal work of Davis et al. (1997, 1999) on the neurobiology of fear, anxiety, and startle demonstrating that startle amplitude is potentiated by exposure to both contextual threat (i.e., anxiety; as in returning to the location of previous aversive conditioning or during anticipation of a temporally remote aversive stimulus) and explicit threat (i.e., fear; as in anticipation of the imminent receipt of an aversive stimulus). In line with this, clinical research studies suggest that baseline startle differences between PTSD and non-PTSD samples are most reliably observed under test conditions involving the anticipation of future shocks that are not imminent (Grillon et al., 1998b; Morgan et al., 1995a,b). Exaggerated baseline startle is less commonly found when PTSD patients are tested in a safe context with no such threat (Grillon et al., 1998b; for a review, see Orr et al., 2004). Thus, given the aim of examining a possible link between cortisol levels and exaggerated startle in PTSD, in this study we employed a laboratory procedure designed to permit examination of the effects of both contextual vs. explicit threat on startle response amplitude and the possible modulating effects of hydrocortisone on both parameters of the startle response.

3. Cortisol and fear

Prior studies that have examined relationships between cortisol levels, fear-potentiated startle, and other indices of fear responding have yielded mixed results. On the one hand, many studies have shown positive correlations between indices of fear and levels of endogenous glucocorticoids (for a review, see Korte, 2001). For example, investigators have reported positive associations between fear-potentiated startle amplitude and cortisol levels during aversive conditioning in both rats (e.g., Campeau et al., 1997) and humans (Grillon et al., 2006).[1] Other evidence suggests that high doses of corticosterone potentiate freezing responses to a cue previously paired with shock (Corodimas et al., 1994) and several studies using a rat model have shown that high levels of corticosterone applied to the amygdala result in elevated CRF neuronal activation and long-lasting potentiation of anxiety-like behavior (Shepard et al., 2000, 2003). These effects may, however, reflect a chronic response to high level exposure and it is unknown whether they would generalize to acute effects at more moderate levels of cortisol.

Other research suggests that pharmacologically induced elevations in cortisol may exert anxiolytic effects on behavior. Two studies using fear conditioning paradigms have shown evidence of impaired electrodermal conditioning after

[1] It is noteworthy, though, that studies of endogenous cortisol and startle or fear conditioning do not unequivocally address cortisol's effects on the CNS because the endogenous levels themselves are a reflection of underlying amygdala activation.

hydrocortisone administration relative to placebo in male participants (Stark et al., 2006; Merz et al., 2010) and in the second of these studies, Merz et al. (2010) found reduced fMRI activation of the amygdala during processing of a CS + stimulus in individuals pre-treated with hydrocortisone. Similar evidence of a selective effect of hydrocortisone administration on activity of limbic structures was reported by Lovallo et al. (2010) who found reduced fMRI activation in the hippocampus and amygdala after drug administration in resting human participants. Compatible findings suggest that pre-treatment with hydrocortisone may reduce negative mood after a stressful movie or psychosocial stress (Reuter, 2002; Het and Wolf, 2007) and that cortisol administered prior to exposure to a phobic stimulus inhibits self-reported fear and heart rate increases in participants with social phobia and spider phobia (Soravia et al., 2006). Finally, evidence also suggests that hydrocortisone inhibits noradrenergic-induced panic symptoms (Vasa et al., 2009). In sum, though research on the relationship between systemic levels of cortisol and fear responding has yielded mixed results, a growing number of recent studies suggest that hydrocortisone administration may exert inhibitory effects on fear-related neurocircuitry and behavior.

4. Study aims and hypotheses

This study was designed to (a) clarify the relationship between cortisol levels and amplitude of the startle reflex and (b) investigate a possible association between the phenomena of exaggerated startle and low basal cortisol in PTSD. To do so, we tested the effect of oral administration of 20 mg hydrocortisone on baseline startle reflex amplitude and fear-potentiated startle in veterans with and without PTSD.

The primary study hypotheses for *baseline startle amplitude* were as follows:

(a) Replicating findings by Buchanan et al. (2001), we hypothesized that increasing systemic cortisol via hydrocortisone administration would result in a reduction of the overall magnitude of startle reflex relative to placebo.
(b) On the basis of research suggesting that the HPA-axis in PTSD is characterized by enhanced negative feedback of glucocorticoids (cf., Yehuda, 2001), we predicted that cortisol manipulations would exert more pronounced attenuation of overall startle amplitude in patients with PTSD relative to controls.

For *fear-potentiated startle amplitude*, prior studies suggested the following competing hypotheses:

(a) If corticosteroids stimulate CRH action to enhance fear (cf., Schulkin et al., 1998) then we expected to find enhanced fear-potentiated startle after hydrocortisone administration compared to placebo.
(b) Alternatively, if systemic elevations in cortisol exert anxiolytic effects on behavior then we expected to find an attenuating effect of hydrocortisone of the magnitude of the fear-potentiated startle response.

Finally, on the basis of prior research suggesting that PTSD patients may be hypersensitive to contextual anxiety cues, we predicted that participants with PTSD would show greater baseline startle than no PTSD control participants during conditions involving the distal anticipation of an aversive event.

5. Method

5.1. Participants

Participants were male veterans recruited from a database of individuals who had previously consented to be contacted for research studies at the National Center for PTSD in Boston or responded to study advertisements posted in Boston area U.S. Department of Veterans Affairs (VA) medical center facilities. Eligibility criteria included status as a male veteran of the U.S. military between the ages of 18 and 65 with a history of exposure to an adverse life event meeting the A1 criteria for the PTSD diagnosis as determined initially during a telephone screen and verified during a structured diagnostic interview. Veteran status was confirmed by the VA medical record. Medical exclusion criteria (i.e., conditions for which the administration of hydrocortisone or other study procedures would be contraindicated) included the presence of diabetes, herpes simplex in the eye, hypertension, myasthenia gravis, osteoporosis, peptic ulcers, renal insufficiency, tuberculosis, ulcerative colitis, any other serious illness such as cancer or HIV, taking medications including benzodiazepines, beta-blockers, blood thinners, diuretics, or any one ending in ''zole'', self-reported alcohol or substance dependence in the previous 30 days, or a clinical diagnosis of psychosis.

74 male veterans enrolled in the project. 11 were omitted from analysis due to an excessive number of unscorable startle responses ($n = 9$, as defined below), or because they were lost to follow-up and did not complete both study sessions ($n = 2$). The 63 participants who contributed to the final dataset ranged in age from 23 to 64 years ($M = 50.9$, $SD = 9.6$). All reported a history of trauma-exposure as determined by the Life Events Checklist (Gray et al., 2004). The index trauma on which the PTSD assessment was based was combat-related for 36 (57%) of participants and non-combat-related for the remaining 27 (43%). Participants were assigned to a PTSD ($n = 32$) or no PTSD group ($n = 31$) based on the results of administration of the Clinician-Administered PTSD Scale (CAPS; Blake et al., 1995) by a doctoral level psychologist using criteria specified in the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; APA, 1994). Positive symptoms were defined by CAPS intensity scores of 1 or greater and frequency scores of 2 or greater within the last month (frequency and intensity values range from 0 to 4; Weathers et al., 2001). 46 participants identified themselves as Caucasian, 13 as African-American, 3 as Hispanic, and 1 as Asian-American.

5.2. Procedure

Upon arrival, participants gave written informed consent, were administered a medical screening by a physician, and then interviewed to determine the possible presence of PTSD using the CAPS. Participants were then randomly assigned to receive either hydrocortisone or placebo during the first session on a double-blind basis. The second session (for

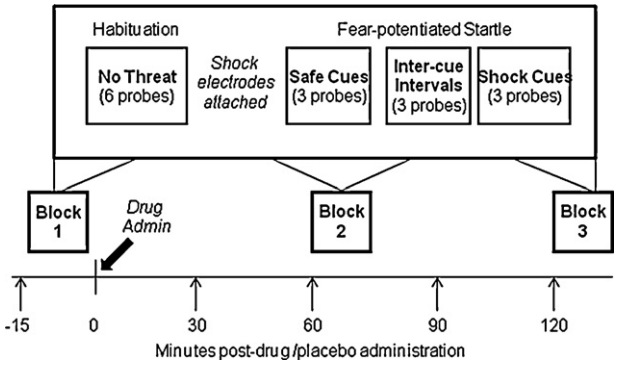

Figure 1 Schematic diagram of the experimental procedure.

the opposite drug condition) took place within one week of the first session, began at the same time of day, and replicated all of the laboratory procedures.

Each session consisted of three startle assessment blocks as depicted in Fig. 1. The first one was conducted just before the administration of the drug or placebo at 2:00 pm, the second and third assessment blocks were conducted 60 min and 120 min later, respectively. Each assessment block consisted of a *habituation period* and a *fear-potentiated startle period*.

Habituation periods were used to assess startle reactivity under conditions of no imminent threat of electric shock. Each one involved the presentation of 6 startle probes (described below) over headphones with inter-stimulus intervals of 25—35 s. During these periods, participants were instructed to simply sit still in dim light conditions with their eyes open. No visual stimuli were presented.

Fear-potentiated startle periods consisted of the presentation of 12 startle probes: 4 during the presentation of 60 s visual "safe" cues displayed on a computer monitor (a series of "O"s), 4 during 60 s visual "shock" cues (a series of asterisks [*], and 4 during 30 s inter-trial intervals (ITIs; i.e., between cues). The sequence of cue presentation was counterbalanced across two orders. Prior to initiating the procedure, participants were instructed that a visual cue would appear on a computer monitor in front of them with one type of cue representing a "threat period" and the other cue a "safe period". They were informed that they might receive an electric shock at any point while the threat cue was displayed and that they would receive "at least one but not more than three shocks" over the course of the procedure. One shock was delivered during the first block of each session. Shock electrodes were attached to the ring and the index fingers of the participant's right hand immediately prior to each fear-potentiated startle period.

After each block a saliva sample was collected and participants rated how anxious they felt during the previous shock and safe periods using a visual analog scale. They then rated their current mood using the Positive and Negative Affect Schedule (PANAS, present state version; Watson et al., 1988). Between blocks they also completed a series of self-report instruments. PTSD symptoms were assessed with the PTSD Checklist-Military version (PCL-M; Weathers et al., 1993) and the Mississippi Scale (Keane et al., 1988). Comorbid psychiatric symptoms were measured with the Beck Anxiety and Depression Inventories (BAI & BDI; Beck et al., 1961, 1988). Personality traits were assessed with the Multidimensional Personality Questionnaire-Brief Form (MPQ-BF; Patrick et al., 2002).

5.3. Study materials

Hydrocortisone (20 mg) and identical appearing placebo capsules were produced by the medical center pharmacy. 20 mg represents a physiological (as opposed to pharmacological) dosage, i.e., it produces an increase in cortisol comparable to that which would occur in response to naturally occurring stressors (Buchanan et al., 2001). The shock cue stimuli were presented on a color computer monitor and represented by a row of 72 pt graphical symbols, "O"s and asterisks (*), representing safe and threat periods, respectively. Acoustic startle probes consisted of 50 ms white noise bursts at 105 dB with an immediate rise time. The startle probes were generated by a Coulbourn Instruments (Allentown, PA) white noise generator (Model S81-02), amplified by an audio mixer-amplifier (Model S82-24), and transmitted through a set of headphones to both ears. Electrical shock stimuli were generated by a Coulbourn Instruments transcutaneous aversive finger stimulator (Model E13-22). The shocks were administered across the 2nd and 4th fingers of the participant's right hand for 500 ms, with an intensity of 5.0 mA and a frequency of 10 pulses per second.

5.4. Apparatus, recording, and data reduction

SuperLab software (Cedrus Corporation, 1999) was used for the timing of data acquisition and control of experimental stimuli. Sampling, digitization, and storage of EMG data were executed on a second computer using LabTech Notebook Pro software (Labtech Corporation, 2000). The initiation and termination of the physiological data collection was controlled by a transistor-transistor logic (TTL) signal from the computer running the SuperLab program.

Startle response. The eyeblink startle response was recorded using Beckman Ag/AgCl electrodes positioned over the orbicularis oculi muscle beneath the left eye. The raw EMG signal was amplified with a Coulbourn bioamplifier (Model S75-01) with low- and high-frequency cutoff values of 8 and 1000 Hz, respectively. The signal was rectified and integrated using a contour-following integrator (Model S76-01) with a time constant setting 20 ms. Digital sampling started 50 ms before the startle probe onset and ended 250 ms after the probe offset at 1000 Hz.

Saliva samples. Saliva samples were collected using cotton swabs (Salivette, Sarstedt, Germany), stored at −68 °C. Saliva samples were assayed at the Endocrine-Hypertension Laboratory of Brigham & Women's Hospital (Boston, MA). The assay had a sensitivity of <0.01 μg/dl, a range of 0.01—5.0 μg/dl (0.1—50.0 ng/mL), with 4—5% intra- and interassay coefficients of variation.

5.5. Data reduction and analysis

The startle response data were reduced off-line using a program developed by Curtin (1996) which scored startle-elicited blinks for magnitude and allowed scored responses to be visually inspected to control for artifacts before accepting them. A response was defined as the change from the mean EMG level during the 50-ms baseline period before the onset

of the startle stimulus to peak of activity between 40 and 150 ms after probe onset. Trials were rejected if the mean EMG level during the baseline was greater than 10 μV or if EMG levels during the baseline period were unstable (i.e., showed more than 5 μV change). For analyses of fear-potentiated startle effects we controlled for individual differences in overall blink magnitude by standardizing responses using a within-participant *z*-score transformation (cf. Berg and Balaban, 1999). Individual patterns of data were not altered by this transformation, but were merely placed on a common scale so that each participant contributed equally to the overall group pattern.

Hypotheses were tested using multivariate analysis of variance (MANOVA), with repeated measures treated as variates (Vasey and Thayer, 1987; Stevens, 1992) and effects were assessed for significance using the Wilks' Lambda statistic. General Threat effects were examined by comparing responses during habituation periods to those recorded during the ITIs of fear-potentiated startle periods. Specific Threat effects (i.e., conditions within fear-potentiated startle assessments) were examined by parsing this 3-level factor into orthogonal "linear" (i.e., threat vs. safe) and "quadratic" (i.e., threat/safe vs. inter-cue interval) contrasts-reflecting modulatory effects of threat and arousal/attention, respectively (cf., Vrana et al., 1988; Bradley et al., 1990; Patrick et al., 1993).

6. Results

6.1. Participant characteristics

Table 1 lists descriptive information for the two study groups. No significant group differences were found for age or race/ethnicity. The PTSD group scored higher than the non-PTSD group on all three measures of PTSD as well as on measures of general symptoms of depression and anxiety. On the MPQ-BF, participants in the PTSD group produced significantly higher scores on negative emotionality and lower scores on constraint compared to non-PTSD participants.

6.2. Salivary cortisol level

Effects of the hydrocortisone administration on salivary cortisol levels were examined using a 2 level Drug (hydrocortisone vs. placebo) × 3 level Block (0, 60, and 120 min post drug administration) × 2 level Group repeated measures MANOVA. Analyses revealed a main effect of Drug, $F(1, 58) = 161.61$, $p < .001$, with significantly higher mean cortisol levels observed in the hydrocortisone (Mean = 1.19 μg/dl, *SD* = 0.64) compared to placebo condition (Mean = 0.15 μg/dl, *SD* = .08). There was also a main effect of Time, $F(2, 57) = 68.81$, $p < .001$, with mean cortisol levels increasing from the first to the second and third blocks of the procedure. This effect was modified by Drug, $F(2, 57) = 84.03$, $p < .001$, indicating that cortisol increases were limited to the hydrocortisone condition. There were no significant main or interactive effects of Group.

6.3. Eyeblink startle EMG

General Threat effects and baseline startle amplitude. Effects of General Threat and hydrocortisone on raw startle amplitude were examined by performing a 4-way repeated measures MANOVA which included the 2 level Drug (hydrocortisone vs. placebo), 3 level Block (1st, 2nd, and 3rd), 2 level General Threat (habituation vs. shock threat ITIs) and

Table 1 Mean (and standard deviation) sample descriptive characteristics by diagnostic group.

Variable	Non-PTSD (*n* = 31)	PTSD (*n* = 32)	*T*-value
Demographic measures			
Age	50.9(8.6)	51.00 (10.5)	ns
Race/ethnicity (%)			
Caucasian	64.5	81.3	ns
African American	25.8	15.6	ns
Hispanic/other	9.7	3.1	ns
PTSD-related measures			
Combat exposure scale	11.3 (12.7)	13.9 (12.5)	ns
Clinician-Administered PTSD Scale	22.6 (16.6)	73.8 (22.1)	8.9***
Life Events Checklist	3.9 (2.1)	5.0 (2.6)	ns
Mississippi Scale	80.0 (17.4)	116.1 (17.8)	8.2***
PTSD Checklist	32.7 (11.6)	55.8 (12.5)	7.5***
Other psychopathology			
Beck Anxiety Inventory	7.4 (8.0)	13.2 (10.4)	2.5*
Beck Depression Inventory	9.8 (7.8)	20.9 (8.6)	5.3***
Multidimensional Personality Questionnaire (T scores)			
Positive emotionality	45.5 (11.6)	40.1 (12.1)	ns
Negative emotionality	54.2 (10.1)	64.3 (8.1)	4.4**
Constraint	47.7 (9.9)	41.9 (8.9)	2.5*

The LEC total score was based on the number of potentially traumatic events that the participant endorsed as "happened to me".
* $p < .05$.
** $p < .01$.
*** $p < .001$.

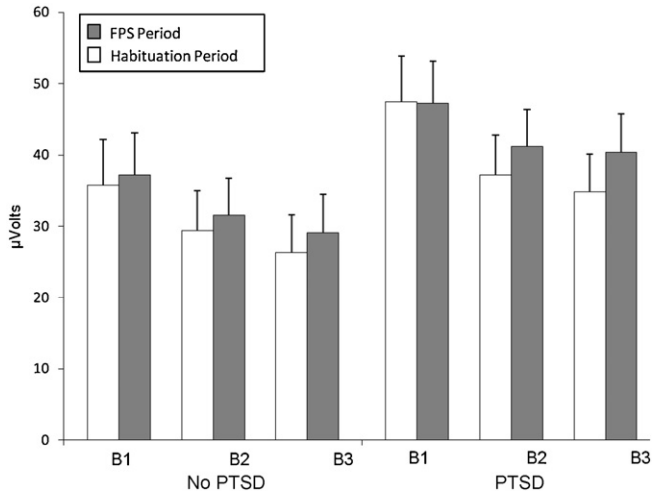

Figure 2 Startle amplitude by block, threat condition, and PTSD group. Bars represent mean blink amplitude during habituation vs. fear-potentiated startle periods (FPS) across the three blocks of the procedure. The figure depicts the general diminution of the startle response over the course of the experiment and a significant effect of threat condition. The latter indicates that startle blink amplitudes were greater during FPS periods compared to habituation trials when no shock threat was present.

Group (PTSD vs. no PTSD) factors. Results showed significant main effects of Block, $F(2, 60) = 17.31$, $p < .001$, reflecting a general diminution of the startle response over repeated presentations of the stimulus across the experiment. There was also a main effect of General Threat, $F(1, 61) = 5.62$, $p < .03$, indicating that startle blink amplitude was greater during the shock threat procedure while the shock electrodes were attached compared to the preceding habituation trials when no shock electrodes were attached. These effects are depicted in Fig. 2. There were no significant main or interactive effects involving Drug or Group. The latter implies that there was no evidence of exaggerated startle responding in the PTSD group, $F(1, 61) = 1.59$, $p = .213$ (partial eta[2] = .025).

Specific Threat effects. Effects of Specific Threat and hydrocortisone on startle amplitude were examined with a Drug × Block × 3 level Specific Threat (safe, iti, threat trials) × Group MANOVA. This revealed significant main effects of Block $F(2, 60) = 18.87$, $p < .001$, and Specific Threat $F(2, 60) = 36.66$, $p < .001$, but no significant main or interactive effects involving Group. Polynomial contrasts showed significant linear and quadratic trends for the Specific Threat factor, $F(1, 61) = 74.08$, $p < .001$, and $F(1, 61) = 6.75$, $p < .05$, respectively. The linear trend indicated that blink amplitude during viewing of shock cues were significantly greater than during safe cues. The quadratic effect indicated that the mean response during shock and safe trials was greater than during ITIs. In other words, the degree of startle attenuation during safe trials compared to ITIs was less than the magnitude of potentiation observed during threat compared to ITI.

Analyses also showed that the linear Threat effect was modified by a significant interaction with Block (2 way: $F(1, 61) = 4.42$, $p < .05$) and a trend towards a Drug × Block interaction (3 way: $F(1, 61) = 3.57$, $p = .06$). Since the effects of hydrocortisone on startle would only be evident in blocks 2 and 3 of the procedure (i.e., following drug administration) we next analyzed data from Block 1 (pre-drug administration) separately from the aggregated data from Blocks 2 and 3. Results for Block 1 showed a significant linear Specific Threat effect, $F(1, 61) = 51.03$, $p < .001$, indicating that startle responses were significantly greater during presentation of the shock compared to safe cues, but of course no significant interaction with Drug. In contrast, as shown in Fig. 3, during Blocks 2 and 3 (post-drug administration) there was a significant Drug × linear Threat interaction $F(1, 61) = 7.06$, $p < .01$, indicating that the magnitude of the fear potentiated startle effect was significantly attenuated in the hydrocortisone (linear trend $F(1, 62) = 19.25$, $p < .001$) compared to placebo condition (linear trend $F(1, 62) = 51.48$, $p < .001$).

6.4. Self-reported anxiety and mood

Effects of the Specific Threat manipulation on self-reported anxiety were assessed with visual analog scales. We exam-

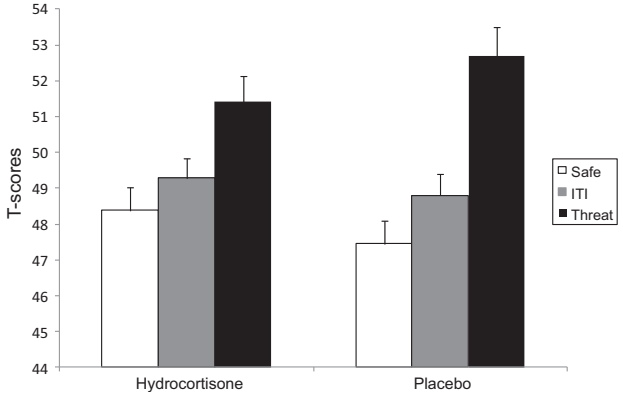

Figure 3 Fear potentiation by drug during Blocks 2 and 3.

[2] Effects of drug administration order (which was counterbalanced across subjects) was examined in an initial phase of data analysis but omitted from the final results because it did not interact significantly with any of the effects reported below.

ined these effects using a Drug × Block × 2 level Specific Threat (safe vs. threat) × Group repeated measures MANOVA. This analysis showed a significant effect of Specific Threat with higher ratings of anxiety reported during threat ($M = 4.7$, $SD = 2.5$ on a scale of 0—10) vs. safe ($M = 2.3$, $SD = 2.1$) periods, $F(1, 60) = 76.27 = p < .001$. There were no other significant main effects or interactions.

Similarly, PANAS mood ratings were examined with a Drug × Block × Group MANOVA. For negative affect (NA) ratings, results showed a significant main effect of Block, $F(2, 56) = 3.79$, $p < .03$, indicating that NA levels tended to decrease over the course of the procedure, and a significant main effect of Group, $F(1, 57) = 8.95$, $p < .01$ with higher levels of NA endorsed by the PTSD group. Positive affect (PA) ratings showed a similar decrease in total scores over the course of the three blocks, $F(2, 56)$ 13.82, $p < .001$, but no significant main or interactive effects of Drug or PTSD group.

7. Discussion

The primary finding of this study was that oral administration of 20 mg of hydrocortisone suppressed the magnitude of the fear-potentiated startle response in veterans with and without PTSD. Results of this two-session, within-subject design experiment showed that the size of the difference between startle responses recorded during shock vs. safe periods was attenuated following hydrocortisone administration compared to placebo. The acoustic startle reflex is reliably augmented during negative emotional states, including those elicited by cued anticipation of a noxious event (e.g., Grillon et al., 1991; Miller et al., 1999). It is thought to occur when there is a match between the defensive reaction to the startling noise probe stimulus and the ongoing defensive emotional state evoked by a foreground stimulus (e.g., a shock warning cue; Lang et al., 1990). Neuroanatomical research has shown this effect to dependent on the integrity of the pathway from the central nucleus of the amygdala — a key component of the subcortical defensive motivational system — to the startle reflex circuit, though other systems may exert modulatory effects as well (cf., Angrilli et al., 1996; Davis et al., 1999). Results of this study suggest, therefore, that hydrocortisone may inhibit activity in brain systems that mediate the fear-potentiated startle response, such as the amygdala.

Support for this conclusion comes from several prior studies. Merz et al. (2010) administered hydrocortisone or placebo to human participants prior to a differential fear conditioning procedure during which brain activity was recorded with fMRI. Results showed neural discrimination of CS+ and CS− stimuli in limbic regions including the left amygdala, right hippocampus, right insula, and bilaterally in the thalamus in the placebo group but these effects were blocked by hydrocortisone. Similar results were reported by Lovallo et al. (2010) who found reduced fMRI activation in the hippocampus and amygdala after hydrocortisone administration in resting human participants. Though the molecular mechanisms of these effects remain an open question, evidence points to inhibitory effects on levels of CRH and/or norepinephrine in these regions. The hippocampus and amygdala host a high density of cortisol receptors and CRH and norepinephrine are both well-established modulators of fear-reactivity and startle amplitude (Davis et al., 1999; van Stegeren et al., 2007).

Results of this study run contrary to prior evidence of positive associations between endogenous cortisol levels and fear-potentiated startle (e.g., Campeau et al., 1997; Grillon et al., 2006). However, evidence for a bi-phasic relationship between cortisol and fear conditioning (cf., Lupien and Lepage, 2001) raise the possibility that an inverted U-shaped relationship exists between cortisol levels and startle potentiation, i.e., small endogenous elevations of cortisol may enhance startle potentiation while larger exogenous increases (as in this study) inhibit fear-potentiation. This pattern may be related to the differential effects of cortisol level on glucocorticoid vs. mineralocorticoid receptors, respectively (for a review, see Korte, 2001). Alternatively, it is possible that high chronic levels of cortisol exert a sort of kindling effect at the amygdala that has long lasting effects (cf., Shepard et al., 2000, 2003) and that this process is distinct from the effects seen in the human following hydrocortisone administration. Thus, studying the effects of hydrocortisone dosage, duration, and receptor occupancy may provide useful avenues for future research on the relationship between HPA-axis function and fear responding.

Results also showed that while hydrocortisone exerted a suppressive influence on fear-potentiated startle, it had no corresponding effects on self-reported anxiety or mood. This finding suggests that the neuroendocrine factors that influence startle modulation during fear inductions may not map onto the body—brain interactions that subserve the subjective sense of fear, its interpretation, and its verbal expression via self report. Consistent with this interpretation, prior studies have shown that while laboratory stressors can be quite effective at evoking fear and stimulating cortisol increases, correlations between measures of these response systems may be modest or nonsignificant (e.g., al'Absi et al., 1997; Buchanan et al., 1999). Similarly, prior work has shown that certain pharmacologic manipulations (e.g., diazepam administration) may inhibit fear-potentiated startle without affecting self-reported emotion (Patrick et al., 1996).

Contrary to our a priori hypothesis, we found no significant main effects of hydrocortisone on overall startle amplitude. Thus, results of this study contrast with prior research by Buchanan et al. (2001) who reported significant effects of hydrocortisone on the overall amplitude of the startle response but no significant effects on the strength of fear-potentiation. Differences between study populations and designs and may have contributed to this discrepancy. Buchanan et al.'s study participants were healthy young men and women. In contrast, participants in this study were older male veterans and some studies suggest that hydrocortisone's suppressive effects on fear-conditioning may be specific to male participants (Stark et al., 2006; Merz et al., 2010). Buchanan et al. used still photographs to examine fear-potentiation whereas this study employed the threat of electric shock. A comparison of the magnitude of affect manipulations across the two studies suggest that the shock threat in this study yielded considerably stronger fear-potentiation effects (F-values for the linear threat effect in this study = 51.5, vs. 7.2 in Buchanan et al. (2001)). In addition, the hydrocortisone/placebo manipulation in the Buchanan et al. fear-potentiation study used a between-subject

design, whereas this one employed the more powerful within-subjects design.

7.1. PTSD group differences

Participants in this study were male veterans with a history of exposure to an adverse life event meeting the A1 criteria for the PTSD diagnosis (APA, 1994). Diagnoses were based on clinical interview and approximately half of the sample met criteria for a current diagnosis. Though inter-rater reliability for the CAPS diagnoses was not assessed, support for the validity of these classifications was provided by findings of significant PTSD group differences on two supplementary self-report measures of PTSD symptoms, measures of co-occurring anxiety and depressive symptoms, and a multidimensional measure of personality.

On the basis of prior research, we had expected to find evidence of low basal cortisol and exaggerated baseline startle amplitude in participants with PTSD, however, neither of these effects was found. With respect to cortisol, twenty years of clinical studies examining the relationship between cortisol levels and PTSD have yielded complicated and mixed results. Many investigations have found lower levels of cortisol under baseline or non-stressful conditions in individuals with PTSD compared to controls using both urinary (e.g., Yehuda et al., 1990, 1995a,b) and plasma samples (e.g., Boscarino, 1996; Olff et al., 2006). Investigators have also reported evidence for a reduced rise in salivary cortisol upon awakening in PTSD (Wessa et al., 2006; de Kloet et al., 2007) and patients with PTSD have been found in many studies to respond to dexamethasone administration with enhanced cortisol suppression, suggesting a heightened sensitivity to cortisol in PTSD (de Kloet et al., 2007; Stein et al., 1997). These findings are not uniform, however. Other studies have found higher levels of cortisol in individuals with PTSD compared to controls in samples drawn from urine (Pitman and Orr, 1990; Lemieux and Coe, 1995; Maes et al., 1998), plasma (Liberzon et al., 1999) and saliva (Lindley et al., 2004). Still others have shown no significant differences between groups differing in PTSD diagnostic status in salivary cortisol (Young and Breslau, 2004).

In an effort to integrate these discrepant findings, investigators have focused primarily on demographic differences between samples (e.g., age, sex, chronicity of PTSD, type of trauma) and variability in the methods used to assess cortisol, such as the time of day of sample collection, the source of the sample (e.g., plasma, urine, saliva, or CSF), or the conditions under which it was collected (baseline vs. pre- or post-psychological or physical stress). These factors account for a large proportion of variation in cortisol levels and a recent meta-analysis of over 100 studies linking stress to HPA-axis function showed that such factors contribute significantly to differential findings across studies (Miller et al., 2007). One such factor that may have contributed to our failure to find group differences in basal cortisol was the time of our assessment: between 2:00 and 4:00 pm. We chose this interval to minimize the influence of diurnal changes — basal cortisol levels are relatively low and stable during the afternoon — but statistical floor effects may have limited our ability to detect PTSD group differences in salivary cortisol.

Prior psychophysiological studies on exaggerated startle in PTSD have yielded similarly mixed results. Pole (2007) conducted a meta-analysis of 20 studies that compared samples of individuals with and without PTSD on measures of startle responding and found that the effect size for the eyeblink reflex, weighted to control for sample size differences, was a significant but modest ($r = .13$). Of those 20 studies, approximately half showed significant group differences in eyeblink startle amplitude with some showing quite large effects (i.e., $r > .50$; Morgan et al., 1995a, 1996). These findings suggest that one or more important moderating variables have not been consistently addressed by procedures used in past startle studies.

A relevant recent study suggests that a history of recurrent trauma and the presence of mood and anxiety disorder comorbidity may be important moderators associated with physiological blunting and dampened startle responding in individuals with PTSD (McTeague et al., 2010). McTeague and colleagues compared the psychophysiological response profiles of individuals with PTSD stemming from single vs. multiple traumas to control participants using a script-imagery procedure. Results showed that while single-trauma patients showed evidence of exaggerated startle compared to control subjects, those with higher magnitude recurrent traumas (who also endorsed more extreme anxiety and depressive comorbidity) showed blunted responses. It is conceivable, therefore, that the absence of PTSD group differences in this study was linked to the high number of lifetime traumas experienced by this sample of veterans and associated elevations on measures of negative emotionality, anxiety, and depression.

7.2. Study limitations and conclusions

The findings of this study should be evaluated in the context of its strengths and limitations. Primary strengths of the study included the two-session within-subject drug manipulation, the use of a sample of veterans with and without PTSD, and an experimental paradigm that permitted examination of several different facets of startle responding. Limitations included the modest size of the sample which may have limited power to detect group differences in baseline startle amplitude. Psychiatric diagnoses other than PTSD were not assessed so it was impossible to determine how comorbidity may have influenced observed findings. Also, since we only examined the effects of a 20 mg dose of hydrocortisone we were unable to evaluate how different levels of cortisol might influence startle responding.

In closing, the finding that oral administration of hydrocortisone suppressed the magnitude of the fear-potentiated startle response may have implications for understanding the role of cortisol in the development of PTSD. Several studies have shown individuals with low cortisol levels shortly after trauma to be at heightened risk for the development of PTSD. For example, Delahanty et al. (2000) measured urinary cortisol levels in motor vehicle accident victims during admission to a hospital trauma unit and assessed PTSD one month later. Results showed that participants subsequently diagnosed with acute PTSD exhibited lower urinary cortisol levels in the first 15 h after their accident than did those who did not meet diagnostic criteria.

Investigators have hypothesized that individuals with low basal cortisol may be at risk for the development of "supercondtioning" (Pitman, 1989) during traumatic stress exposure because they are unable to mount the level of cortisol response necessary for down-regulating the stress response (Yehuda et al., 1998). Other evidence consistent with the hypothesis that cortisol serves a protective function during exposure to acute stress includes findings indicating that hydrocortisone may be effective for the secondary prevention of PTSD after trauma exposure, possibly by compensating for low cortisol levels (Schelling et al., 2001). Finally, results of this study suggesting that hydrocortisone administration has fear-inhibiting effects could have implications for future efforts geared towards developing pharmacologic approaches to enhancing resilience to traumatic stress for military personnel and others at risk for exposure to extreme stress.

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Conflict of interest

None declared.

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