



Dexamethasone facilitates fear extinction and safety discrimination in PTSD: A placebo-controlled, double-blind study



Vasiliki Michopoulos^{a,b}, Seth D. Norrholm^{a,c}, Jennifer S. Stevens^a, Ebony M. Glover^d, Barbara O. Rothbaum^a, Charles F. Gillespie^a, Ann C. Schwartz^a, Kerry J. Ressler^{a,e}, Tanja Jovanovic^{a,*}

^a Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia

^b Yerkes National Primate Research Center, Atlanta, Georgia

^c Atlanta Veterans Affairs Medical Center, Mental Health Service Line, Decatur, Georgia

^d Department of Psychology, Kennesaw State University, Kennesaw, Georgia

^e Harvard/McLean Hospital, Boston, MA, United States

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ABSTRACT

Psychophysiological hallmarks of posttraumatic stress disorder (PTSD) include exaggerated fear responses, impaired inhibition and extinction of conditioned fear, and decreased discrimination between safety and fear cues. This increased fear load associated with PTSD can be a barrier to effective therapy thus indicating the need for new treatments to reduce fear expression in people with PTSD. One potential biological target for reducing fear expression in PTSD is the hypothalamic-pituitary-adrenal (HPA) axis, which is dysregulated in PTSD. Recent translational rodent studies and cross-sectional clinical studies have shown that dexamethasone administration and the resulting suppression of cortisol in individuals with PTSD leads to a decrease in the fear responses characteristic of PTSD. These data, taken together, suggest that dexamethasone may serve as a novel pharmacologic intervention for heightened fear responses in PTSD. We conducted a double-blind, placebo-controlled trial to test our hypothesis that dexamethasone administration and the concomitant suppression of HPA axis hyperactivity would attenuate fear expression and enhance fear extinction in individuals with PTSD. Study participants ($n = 62$) were recruited from Grady Memorial Hospital in Atlanta, GA. Participants were randomized to receive dexamethasone or placebo prior to fear conditioning and extinction, in a counterbalanced design (treatments separated by a week). Both PTSD- ($n = 37$) and PTSD+ ($n = 25$) participants showed significant startle increases in the presence of the danger signal during placebo and dexamethasone treatments (all $p < 0.05$). However, only PTSD- control participants showed decreases in fear-potentiated startle across extinction blocks during both conditions ($p's \leq 0.001$), with PTSD+ participants showing deficits in fear extinction and safety discrimination in the placebo condition. Notably, extinction and discrimination deficits in PTSD+ subjects were markedly reversed with dexamethasone ($p < 0.001$). These data suggest that dexamethasone may serve as a pharmacological agent with which to facilitate fear extinction and discrimination in individuals with PTSD.

1. Introduction

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric disorder that occurs in some individuals after exposure to a traumatic life event and increases individual vulnerability to adverse health outcomes across military and civilian populations (Dedert et al., 2010). Improving the present understanding of the neurobiological underpinnings of PTSD is crucial to the discovery of more effective treatment options for individuals suffering from this chronic and debilitating

disorder. PTSD is a heterogeneous disorder consisting of avoidance, re-experiencing, and hyperarousal symptoms along with negative symptoms of cognition and mood. The hallmark psychophysiological characteristic of PTSD is dysregulation of the normal fear response. More specifically, individuals with PTSD consists have an exaggerated fear response (termed fear load, see (Norrholm et al., 2015)), impaired inhibition of conditioned fear (Jovanovic and Ressler, 2010), and deficient fear extinction (Galatzer-Levy et al., 2016; Norrholm et al., 2011; Peri et al., 2000). Critically, the increased fear load associated with

* Corresponding author at: Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, 49 Jesse Hill Jr. NE, Atlanta, 30303, Georgia.
E-mail address: tjovano@emory.edu (T. Jovanovic).

PTSD (Norrholm et al., 2015) may interfere with treatment and the achievement of remission in individuals who are unresponsive or incompletely responsive to conventional treatments for PTSD. As a consequence, there is an unmet need for interventions that address this gap in treatment efficacy.

One potential biological target for reducing fear in PTSD is the hypothalamic-pituitary-adrenal (HPA) axis and the actions of glucocorticoids, both of which are dysregulated in those with PTSD (Yehuda, 2009). While studies characterizing basal cortisol levels in PTSD have been equivocal (Meewisse et al., 2007), enhanced glucocorticoid negative feedback inhibition of the HPA axis in response to a dexamethasone suppression test has been repeatedly described in PTSD (Yehuda et al., 2004a). This enhanced suppression of endogenous cortisol in response to dexamethasone administration in individuals with PTSD occurs in tandem with lower levels of the GR co-chaperone FKBP5 (Yehuda et al., 2009a), and increased concentrations of corticotropin-releasing hormone (CRH) (Baker et al., 2005; de Kloet et al., 2008b) and glucocorticoid receptors (GRs, (Matic et al., 2013)) that can facilitate augmented glucocorticoid sensitivity (Yehuda et al., 2004a). Importantly, dexamethasone administration and the resulting suppression of cortisol in individuals with PTSD also results in a reduction of heightened fear responses that is characteristic of the PTSD (Jovanovic et al., 2010; Jovanovic et al., 2011). Because peripheral levels of cortisol have not been consistently associated with PTSD, the current status of the literature has been focused on glucocorticoid receptor sensitivity, rather than hormone levels (Yehuda et al., 2009a; Yehuda et al., 2004a). Thus, the current study used dexamethasone suppression of cortisol, which specifically targets GR hypersensitivity as a mechanism of deregulation fear responses in PTSD, rather than absolute values of baseline or suppressed cortisol levels. We hypothesize that dexamethasone suppression prior to extinction may serve to clamp the HPA hypersensitivity and increased glucocorticoid sensitivity previously reported to increase PTSD risk.

While these data suggest that dexamethasone may serve as a pharmacological intervention for heightened fear responses in PTSD, no studies to date have tested dexamethasone's ability to modulate fear responses or facilitate fear extinction in those with PTSD in a within-subjects design. Thus, in the current study, we undertook a double-blind, placebo-controlled trial to test the hypothesis that dexamethasone administration and the concomitant suppression in cortisol would result in decreased fear expression and enhanced fear extinction in individuals with PTSD.

2. Methods

2.1. Participants

Study participants were recruited from Grady Memorial Hospital in Atlanta, GA between June 2011 and December of 2013. Study participants were English-speaking men and women between the ages of 18 and 65 years who were enrolled in the Grady Trauma Project, a large project assessing trauma exposure and clinical symptoms in a low

income urban population. Exclusion criteria included pregnancy, positive urine toxicology (tetrahydrocannabinol, benzodiazepines, cocaine, and opiates), hearing impairment as assessed by an audiometer (Grason-Stadler, Model FS1710), active psychosis and major medical illnesses as determined by a medical professional during a history and physical examination. Upon determining eligibility for the study and obtaining consent for the current study, participants were randomly assigned to drug condition and intervention order. All study procedures were reviewed and approved by the Emory Institutional Review Board and the Grady Hospital Research Oversight Committee.

2.2. Psychological assessment

As part of the Grady Trauma Project, all participants provided self-reported levels of PTSD symptoms using the modified PTSD Symptom Scale (PSS), a psychometrically valid, 17-item self-report measure assessing PTSD symptoms. For the present study, the PSS was used to determine the categorical definition of PTSD status based on DSM-IV criteria (APA, 2000), if participants reported any level of severity of at least one re-experiencing symptom, three avoidance and/or numbing symptoms, and two hyperarousal symptoms (Falsetti et al., 1993). Because the PSS is a self-report measure and not a structured diagnostic interview, this should be considered as a probable PTSD diagnosis (Binder et al., 2008). The Traumatic Events Inventory (TEI), was used to assess lifetime adult trauma history by detailing the frequency and type of trauma(s) experienced by study participants (Gillespie et al., 2009). Total level of trauma exposure was measured by a sum score reflecting the total number of different types of trauma (e.g., car accident, sexual assault, and natural disaster) to which a participant had been exposed over the course of their life. The Childhood Trauma Questionnaire (CTQ, (Bernstein et al., 1997)) is a 25-item, self-report inventory assessing three domains of childhood abuse (sexual, physical, and emotional), and two domains of childhood neglect (physical and emotional). The CTQ was used to capture childhood trauma exposure that occurred at or before 18 years of age. The Beck Depression Inventory (BDI) – II is a 21-item self-report measure assessing depressive symptoms in the last two weeks (Beck et al., 1996). Demographic information included participant sex, age, self-identified race, education, and income.

2.3. Experimental design

A double-blind, crossover design study was conducted with two groups (Group: PTSD+, PTSD-) and two within-subjects treatment conditions (Drug: placebo, DEX; see Fig. 1). To suppress endogenous cortisol levels in the DEX condition, a single 0.5 mg dose of dexamethasone was taken by the participant the night prior to the study visit as previously described to specifically target GR hypersensitivity as a mechanism of deregulation of fear responses in PTSD, rather than absolute values of baseline or suppressed cortisol levels (Jovanovic et al., 2010; Koopmans et al., 1992; Yehuda et al., 2004b). The treatment conditions were separated by one week and counterbalanced to

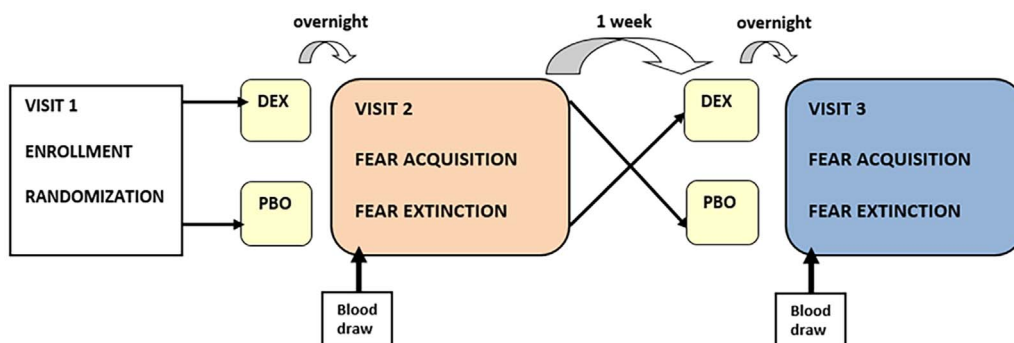


Fig. 1. Diagram of the design for the current randomized, placebo-controlled, double-blind study to assess the effects of dexamethasone (DEX) versus placebo (PBO) on fear responses in individuals with (PTSD+) and without PTSD (PTSD-).

control for order effects. Each condition consisted of fear-potentiated startle test (the testing visit included fear acquisition and fear extinction assessment as outlined below). Participants were randomized by study pharmacist to order of treatment condition (placebo vs. dexamethasone) and received a pill from the hospital pharmacy and told to take it at 11 p.m. the night prior to the next visit. The study coordinator also called the participant to remind them to take the pill the day prior to their scheduled visit. The hospital pharmacist maintained a randomization list, and was not involved in any of the other study procedures. The next day, a blood sample was collected at 8 a.m. and the fear-potentiated startle session was conducted. Upon completion of the startle visit, the participant was scheduled for the next visit one week later and given the second pill. Both participants and assessors were blind to treatment condition throughout the study.

2.4. Fear-potentiated startle paradigm

FPS was measured by the relative increase in the acoustic startle reflex in the presence of conditioned stimuli that were paired with aversive unconditioned stimuli (Jovanovic et al., 2012). Differential fear conditioning included two distinct cues: the reinforced conditioned stimulus (CS+, also referred to as the danger signal) and a non-reinforced conditioned stimulus (CS-, also referred to as the safety signal).

2.4.1. Startle testing

As previously described (Jovanovic et al., 2012), the eyeblink component of the acoustic startle response was measured by electromyography (EMG) recordings of the right *orbicularis oculi* muscle with two 5-mm Ag/AgCl electrodes filled with electrolyte gel. The startle probe was a 108-dB (A) SPL, 40 ms burst of broadband noise with near instantaneous rise time, delivered binaurally through headphones. The startle response data was acquired using Biopac MP150 for Windows (Biopac Systems, Inc.) as described in our previous studies. All data was sampled at 1000 Hz and amplified with a gain of 5000 using the EMG module of the Biopac system. The acquired data were rectified and filtered between 28 Hz and 500 Hz using MindWare software (MindWare Technologies, Ltd.) and exported for statistical analyses. A maximum amplitude of the eyeblink muscle contraction 20–200 ms after presentation of the startle probe was used as a measure of the acoustic startle response.

2.4.2. Fear acquisition

The fear acquisition session consisted of an initial habituation phase wherein CS's were presented without any reinforcement (Norrholm et al., 2015). The acquisition phase consisted of three blocks with four trials of each type of CS (reinforced conditioned stimulus, CS+; non-reinforced conditioned stimulus, CS-; noise probe alone, NA) for 12 trials per block and a total of 36 trials. Both CS's were colored shapes (i.e. blue square, purple triangle) presented on a computer monitor for six seconds each. The CS's differed between the two study visits and were counterbalanced across subjects. The unconditioned stimulus (US; aversive stimulus) was a 250-msec air blast of 140-psi intensity to the larynx that has been shown in our previous studies to produce a robust fear-potentiated startle response (Norrholm et al., 2015). The air blast was delivered from a compressed air tank via polyethylene tubing and controlled by a solenoid switch. The inter-trial intervals throughout the acquisition phase were randomized to be between nine and 22 s in duration.

2.4.3. Fear extinction

As previously described (Norrholm et al., 2015), the fear extinction session occurred ten minutes after acquisition. Neither the CS+ nor the CS- was paired with the US. The extinction session consisted of four blocks of four trials of each type (NA, CS+ (unreinforced), and CS-) in each block. The first two trials of fear acquisition and extinction were categorized as early and the last two trials of each session were

categorized as late fear acquisition and extinction as previously described (Norrholm et al., 2015).

2.5. Cortisol and dexamethasone sampling and assays

On the morning of each FPS visit a blood sample was collected and stored at -80°C until time of assay. Cortisol suppression due to dexamethasone was assessed by subtracting post-dexamethasone cortisol concentrations from cortisol concentrations the morning after placebo treatment. Plasma cortisol and dexamethasone were assayed at the Yerkes National Primate Research Center Biomarkers Core using mass spectrometry (Franke et al., 2011). Dexamethasone was assayed in order to verify compliance.

2.6. Statistical analyses

Sociodemographic data were analyzed using *t*-tests and chi-square test. Data were analyzed using mixed-model analysis of variance (ANOVA) to assess differences in startle magnitude during late acquisition between NA and CS+ as a within-subjects factor during acquisition with PTSD diagnosis as a between-groups variable. FPS was calculated as the difference in startle magnitude between NA and each CS. Discrimination between danger and safety cues during late acquisition was tested in an ANOVA comparing FPS to CS+ and CS- and PTSD diagnosis. A separate ANOVA was used to compare FPS during early CS+ and late CS+ during extinction, again with PTSD diagnosis as between-groups variable. The ANOVAs were repeated for each condition (placebo and dexamethasone). Group differences were analyzed with and without co-varying for trauma exposure and depression symptoms to see whether these variables accounted for the observed differences in startle measures. Correlations were used to assess whether cortisol levels were associated with dexamethasone-induced differences in fear acquisition and extinction. Based on our previous work using the same fear extinction paradigm, a sample size of $n = 24$ per group is necessary to detect a decrease in fear during extinction with 80% power and 0.05 alpha. The data were analyzed using SPSS (v.24) and alpha level was set at $p \leq 0.05$ for statistical significance.

3. Results

3.1. Sociodemographics

Sixty-eight individuals met inclusion criteria, were enrolled and completed the current study. Five participants (2 PTSD- and 3 PTSD+) were not compliant in taking the study medication the night prior to the study visit. Of the remaining 63 participants, 24 participants received a diagnosis of PTSD (PTSD+) and 39 participants did not (PTSD-; Table 1). There were no significant differences between groups with respect to age, sex, race, education, or income (all $p > 0.05$; see Table 1). While both groups of participants reported exposure to trauma during their lifetime, individuals with PTSD had significantly higher rates of childhood and adult trauma, as well as greater PTSD and depressive symptoms (all $p < 0.001$; Table 1).

3.2. Cortisol and dexamethasone concentrations

Assays for dexamethasone revealed that dexamethasone levels were undetectable during the placebo condition and averaged 1.13 ± 0.15 ng/mL during the dexamethasone condition across all participants, with no differences between PTSD groups. Assays for cortisol revealed that mean cortisol levels were 2.17 ± 0.42 $\mu\text{g}/\text{dL}$ during the placebo condition and 0.70 ± 0.32 $\mu\text{g}/\text{dL}$ during the dexamethasone condition across all participants. There was no significant difference in baseline cortisol levels between PTSD- and PTSD+ participants ($p = 0.82$). Administration of dexamethasone significantly suppressed cortisol levels ($F = 295.11$, $p < 0.001$), but did so in a

Table 1
Mean ± SEM and frequency of participant sociodemographic characteristics broken down by PTSD status. Asterisks denote significant differences between PTSD- and PTSD+ participants ($p < 0.001$).

	PTSD- (n = 39)	PTSD+ (n = 24)	p-value
Trauma and Symptoms			
CTQ Total	35.79 ± 2.09	52.87 ± 3.02	< 0.001*
TEI Total Experienced	2.74 ± 0.28	5.25 ± 0.49	< 0.001*
PSS Total	7.87 ± 1.17	26.67 ± 2.07	< 0.001*
BDI Total	12.69 ± 1.55	24.33 ± 2.53	< 0.001*
Demographics			
	Percent (%)	Percent (%)	
Age	42.41 ± 2.05	43.08 ± 2.24	0.83
Race			0.69
African American	94.9	95.8	
Other	5.1	4.2	
Sex			0.62
Male	30.8	25.0	
Female	69.2	75.0	
Education			0.74
< High School (HS)	10.8	17.4	
HS or GED	45.9	39.1	
> HS	43.3	43.5	
Monthly Income			0.30
< \$500	54.1	73.9	
\$500–\$999	13.5	8.7	
> \$1000	32.4	17.4	

manner independent of PTSD diagnosis ($p = 0.53$). The percent cortisol suppression due to dexamethasone administration did not differ between those without ($59.0\% \pm 5.3$) and with PTSD ($60.1\% \pm 6.9$). Controlling for depression symptoms or degree of trauma exposure did not change the results.

3.3. Fear acquisition

ANOVA with startle magnitude and PTSD diagnosis during the placebo condition showed a significant increase in startle to the CS+ relative to the NA trials ($F = 12.40, p = 0.001$) demonstrating that fear to the CS+ was acquired, with no interaction or main effect of PTSD diagnosis (Fig. 2). During the dexamethasone condition, both groups again showed a significant increase in startle magnitude to the CS+ ($F = 24.74, p < 0.001$), with no effect of PTSD diagnosis (Fig. 2). Comparison of FPS (difference in startle magnitude from CS to NA) to

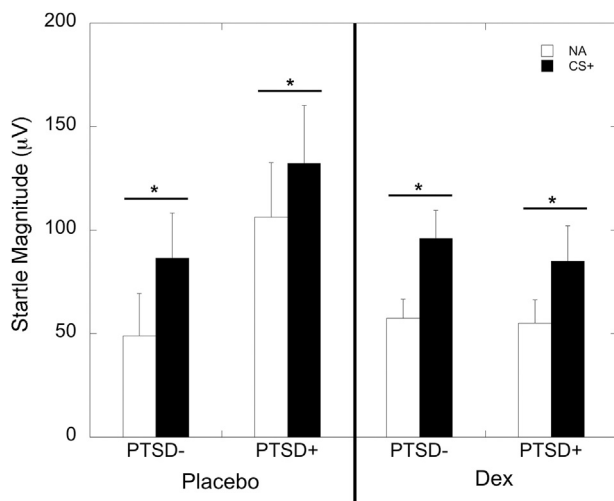


Fig. 2. Mean ± SEM of fear-potentiated startle (FPS) responses to noise alone (NA) and danger signal (CS+). Both PTSD- and PTSD+ participants showed increased startle response to the danger signal compared to the noise alone during both the placebo and dexamethasone (DEX) treatment conditions.

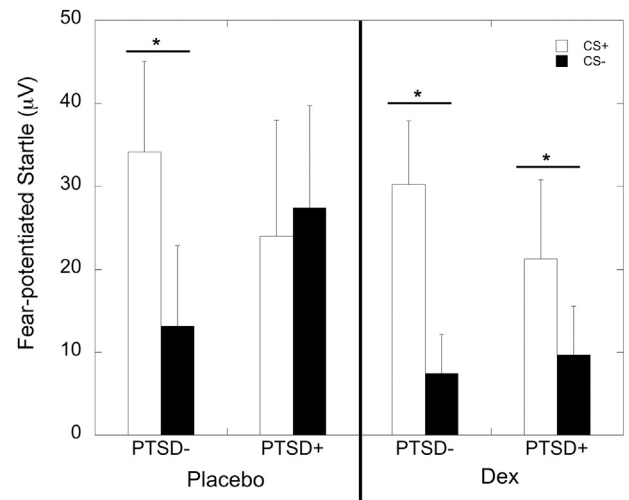


Fig. 3. Mean ± SEM of fear-potentiated startle (FPS) responses to danger (CS+) and safety (CS-) signals during the fear acquisition paradigm. PTSD- participants showed significant discrimination between the safety and danger signals during both the placebo and dexamethasone (DEX) treatment conditions. In contrast, PTSD+ subjects only showed discrimination during dexamethasone treatment.

CS+ and CS- during placebo revealed an interaction between trial type and PTSD diagnosis ($F = 5.32, p = 0.025$), with the PTSD- group showing a significantly lower FPS response to the CS- than to the CS+ ($F = 10.48, p = 0.003$), while the PTSD+ group did not ($F = 0.16, p = 0.69$). After dexamethasone, all participants showed lower FPS to the CS- than to the CS+ ($10.62, p = 0.002$), with no interaction effect with PTSD (Fig. 3). We examined correlations between the percent cortisol suppression and post-dexamethasone FPS to CS+ and CS-, separately in each PTSD group, and found a negative correlation between FPS to danger and cortisol suppression in the PTSD- group ($r = -0.52, p = 0.005$), but not in the PTSD+ group ($r = 0.15, p = 0.55$). FPS to the CS- was not correlated with cortisol suppression. The order of dexamethasone or placebo administration did not influence fear acquisition and did not interact with PTSD status (all $p > 0.05$).

3.4. Fear extinction

ANOVA with FPS to CS+ during early vs. late extinction during the placebo condition showed a main effect of PTSD ($F = 5.08, p = 0.028$), but no effect of extinction. The effect of PTSD remained significant after co-varying for trauma exposure and depression symptoms ($p = 0.021$). When the two groups were analyzed separately, the PTSD- group showed a significant decrease in FPS from early to late extinction ($F = 16.05, p < 0.001$), whereas the PTSD+ group did not ($F = 0.06, p = 0.81$; Fig. 4). In the dexamethasone condition, there was a significant effect of extinction ($F = 41.79, p < 0.001$), but no main or interaction effect with PTSD diagnosis. Separate analyses in each group showed that both groups decreased in FPS from early to late extinction ($F = 23.29, p < 0.001$ in PTSD- and $F = 20.64, p < 0.001$ in PTSD+; Fig. 4). Cortisol suppression due to dexamethasone was not associated with the decreased FPS during the late extinction blocks in those with ($r = -0.09, p = 0.73$) or without PTSD ($r = 0.14, p = 0.46$). The order of dexamethasone or placebo administration did not influence fear extinction and did not interact with PTSD status (all $p > 0.05$).

4. Discussion

The current double-blind, placebo-controlled crossover trial indicated that administration of dexamethasone resulted in facilitation of fear discrimination and extinction specifically in individuals with PTSD. Dexamethasone administration resulted in suppression of cortisol concentrations, suggesting that HPA actions may be partially responsible

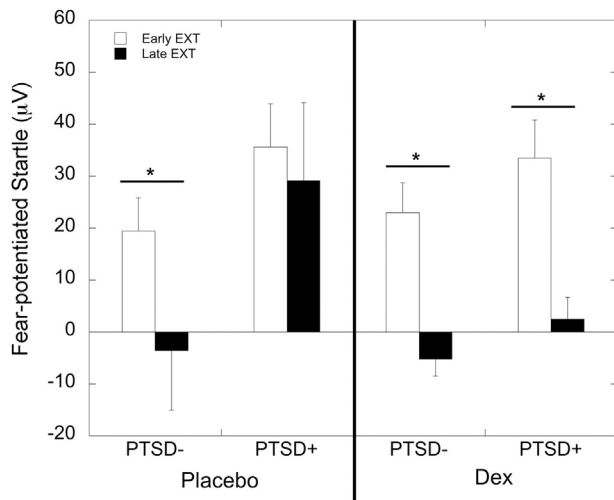


Fig. 4. Mean \pm SEM of fear-potentiated startle (FPS) responses during early and late blocks of the fear extinction paradigm. PTSD- participants showed significant decreases in FPS in response to the danger signal during both the placebo and dexamethasone (DEX) treatment conditions. In contrast, PTSD+ subjects only showed fear extinction during dexamethasone treatment.

for fear extinction deficits observed in PTSD (Milad et al., 2008). Translating these experimental findings to the clinical arena, our results suggest that dexamethasone administration may improve the efficacy of extinction-based therapies such as prolonged exposure for traumatized individuals with PTSD.

The finding of dexamethasone facilitation of extinction supports recent results from a translational rodent model of PTSD-like behavior in which dexamethasone administration enhanced fear extinction and extinction retention in a dose-dependent manner (Sawamura et al., 2016). Additional studies in rodents have shown the efficacy of dexamethasone in decreasing FPS and contextual fear during fear extinction paradigms (Yang et al., 2006). Furthermore, enhanced fear extinction upon dexamethasone treatment in rodents occurred in tandem with decreases in mRNA expression of *FKBP5*, a gene which codes for a co-chaperone of GR implicated in the pathophysiology of PTSD (Binder et al., 2008), within the amygdala along with alterations in *FKBP5* epigenetic regulation (Sawamura et al., 2016). A recent cross-species examination of fear extinction implicates *FKBP5* as integral to extinction mechanisms in rodents and humans (Galatzer-Levy et al., 2016). Thus, it is possible that the dexamethasone effects on *FKBP5* regulation of GR are central to the extinction enhancement observed here. Finally, even though dexamethasone has high affinity for the glucocorticoid receptors, it may also influence *FKBP5* interactions with limbic mineralocorticoid receptors that have been implicated in PTSD to mediate corticosteroid response (Matic et al., 2014). Future studies are necessary to determine the effects of dexamethasone on *FKBP5* expression in individuals with PTSD.

While the current study found that dexamethasone facilitated fear extinction and safety discrimination between danger and safety cues in those with PTSD, it did not have a significant effect on fear acquisition (see Fig. 2). Therefore, dexamethasone rescued extinction deficits associated with PTSD in a relatively specific manner. There were no differences on the influence of dexamethasone on fear conditioning between the PTSD and control groups. The inability to discriminate danger and safety cues in PTSD may, in part, arise from an over-generalization of stimuli and increased arousal (Jovanovic et al., 2012). We have previously shown in a naturalistic, between-group study that dexamethasone administration and parallel reductions in endogenous cortisol levels eliminate exaggerated FPS to danger cues in individuals with PTSD (Jovanovic et al., 2011). Additionally, administration of hydrocortisone, another exogenous GR agonist, prior to fear conditioning impairs eyeblink conditioning in individuals with PTSD

(Vythilingam et al., 2006) and normalizes increased amygdala activation in individuals with PTSD as assessed by glucose metabolism PET (Yehuda et al., 2009b). In addition, GR activation facilitates fear memory consolidation (Roosendaal et al., 2009) and GR antagonism attenuates dysregulated fear responses in a rodent model of PTSD (Kohda et al., 2007). Importantly, glucocorticoid receptors are abundant in the amygdala, a region of the brain critical for fear conditioning whose hyperactivity has been consistently shown in those with PTSD (Shin et al., 2006), and thus can modulate the expression of fear as previously shown in rodents and non-human primates (Roosendaal et al., 2008). These studies are consistent with our finding that cortisol suppression was associated with less fear to the danger signal in controls. While our results show that a single, low dose of dexamethasone specifically facilitates fear extinction and safety discrimination in PTSD, future studies are necessary to characterize whether higher doses, different types (i.e. hydrocortisone) and longer duration treatments of corticosteroids also influence fear acquisition in PTSD.

Suppression of cortisol due to dexamethasone treatment occurred in parallel with facilitation of fear extinction in those with PTSD. However, the degree of cortisol suppression was not associated with fear extinction in individuals with PTSD in the current study. Previous studies indicate that baseline and post-dexamethasone concentrations of ACTH, but not cortisol, are correlated with FPS to danger signals in a fear discrimination task, but only in PTSD+ individuals (Jovanovic et al., 2010). Importantly, other HPA signals have been implicated in enhanced fear responses in animal models. Elevated CRH concentrations are associated with increased fear responses (Kalin and Takahashi, 1990) and anxiety (Sutton et al., 1982), including the startle response (Keen-Rhinehart et al., 2009) and enhanced fear conditioning (Roosendaal et al., 2002; Swerdlow et al., 1989) in rodents and in monkeys. CRH hypersensitivity has been particularly associated with survivors of childhood trauma (de Kloet et al., 2008a; Heim et al., 2008), as is very common in our cohort. Increased concentrations of CRH also occur in individuals with PTSD (Baker et al., 2005; de Kloet et al., 2008b) in tandem with augmented GR levels (Matic et al., 2013) and lower levels of the GR co-chaperone *FKBP5* (Yehuda et al., 2009a) that can facilitate augmented glucocorticoid sensitivity in PTSD (Yehuda et al., 2004a). Our current findings suggest that dexamethasone-mediated stabilization of HPA hyperactivity at multiple levels may contribute to the decreased fear and enhanced extinction effects.

A separate but potentially important mechanism of stress-related fear and extinction deficits in PTSD is the immune system. Multiple lines of evidence from separate samples and studies indicate that PTSD is associated with increased inflammation (Michopoulos et al., 2015; Passos et al., 2015; Plantinga et al., 2013). Importantly, heightened immune activation in the form of elevated C-reactive protein (CRP) has also been associated with increased FPS and hyperarousal symptoms (Michopoulos et al., 2015; Passos et al., 2015). Because GR activity can directly modulate peripheral inflammation levels, dexamethasone administration may suppress the heightened immune activation associated with PTSD and fear responses. Future studies are necessary to characterize the effects of dexamethasone administration on pro-inflammatory markers such as CRP and cytokines, and how dexamethasone-induced suppression of the immune system is associated with dexamethasone's facilitation of fear discrimination and extinction.

In the present study, dexamethasone suppressed cortisol levels similarly in those with and without PTSD. This result does not replicate previous reports of enhanced glucocorticoid negative feedback inhibition of the HPA axis in PTSD (Yehuda et al., 2004a). One reason for this may be due to the fact that we did not exclude participants with comorbid depression symptoms that have been shown to be associated with diminished glucocorticoid negative feedback inhibition of the HPA axis (McEwen, 2008; Yehuda et al., 2004b); however co-varying for depression symptoms did not change the lack of group difference. Another reason for a lack of a main effect of PTSD on dexamethasone

suppression may be that the PTSD- group also reported high rates of trauma exposure, even though not as high as those that met PTSD diagnosis. Trauma exposure itself has been found to have similar effects as PTSD on cortisol suppression following dexamethasone administration in combat veterans compared to non-traumatized controls (de Kloet et al., 2007), suggesting that our research cohort may have a ‘ceiling effect’ in this phenotype due to level of total trauma exposure. Additionally, recent findings indicate that single nucleotide polymorphisms within the *FKBP5* gene that have been associated with increased PTSD risk (Binder et al., 2008) may be direct contributing factors to the hypersensitivity of the HPA axis (Klengel et al., 2013). Thus, assessing the role of genetic variation within *FKBP5* in the future may help disentangle the relationship between PTSD and glucocorticoid negative feedback.

Taken together, the results generated from our double-blind, placebo-controlled study indicate that dexamethasone facilitates fear extinction and safety discrimination in those with PTSD compared to placebo. Limitations of the current study include the use of self-report PSS to determine a probable PTSD diagnosis based upon DSM-IV criteria and the inclusion of both traumatized and non-traumatized controls in the PTSD- group. Additionally, the lack of full medical histories did not allow us to control for other psychiatric (i.e. bipolar disorder) and medical (i.e. body mass index) co-morbidities that are known to influence HPA axis function and glucocorticoid sensitivity (Maripuu et al., 2014; Pasquali et al., 2002). Follow-up studies are necessary to replicate the current findings and expand the generalizability of the results to other populations of traumatized individuals as our urban study sample consisted primarily of African Americans of low socioeconomic status.

Importantly, our data suggest that dexamethasone may serve as a pharmacological agent with which to facilitate extinction-based interventions such as exposure therapy delivered during cognitive behavioral therapy (CBT) or virtual reality exposure therapy (VRE). The efficacy of such an approach has recently been highlighted by studies showing that D-cycloserine, an NMDA receptor partial agonist, facilitates fear extinction in rodent models of PTSD (Davis et al., 2006) and augments exposure therapy for PTSD (Rothbaum et al., 2014). A similar approach should be tested to examine whether dexamethasone enhances treatment of PTSD using fear-extinction based therapies exposure therapy. A recent study used hydrocortisone as an adjunct to prolonged exposure therapy and found greater treatment retention and decreased GR sensitivity in responders (Yehuda et al., 2015), emphasizing that agents targeting the glucocorticoid system have great potential in PTSD treatment. The current study points to improved fear regulation as a mechanism for these promising treatment approaches.

Contributors

Authors Jovanovic, Norrholm, Ressler and Rothbaum designed and organized the studies and wrote the protocols. Authors Michopoulos, Stevens and Glover collected the data. Authors Michopoulos and Jovanovic undertook the statistical analyses. Authors Michopoulos, Stevens, Glover, Jovanovic, Norrholm, Ressler and Rothbaum contributed to the analysis and interpretation of the data. Authors Michopoulos and Jovanovic wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Disclosure

Dr. Rothbaum owns equity in Virtually Better, Inc. that creates virtual reality products. The terms of this arrangement have been reviewed and approved by Emory University in accordance with its conflict of interest policies. Dr. Rothbaum has funding from Wounded Warrior Project, Department of Defense Clinical Trial Grant No.W81XWH-10-1-1045, “Enhancing Exposure Therapy for PTSD: Virtual Reality and Imaginal Exposure with a Cognitive Enhancer” and

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