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Sleep and Hypothalamic Pituitary Adrenal Axis Responses to Metyrapone in Posttraumatic Stress Disorder

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Abstract

Disturbed sleep is a core feature of posttraumatic stress disorder (PTSD), characterized in part by decreased delta power sleep that may result from stress-related alterations in corticotropin releasing factor (CRF), hypothalamic pituitary adrenal axis (HPA) regulation and glucocorticoid signaling. Overnight HPA axis response mediating sleep disturbances in men and women with PTSD was examined using a metyrapone challenge. Metyrapone blocks cortisol synthesis, removing negative feedback, and increases the release of hypothalamic CRF and pituitary adrenocorticotropic hormone (ACTH). Laboratory-based polysomnography was used to monitor the sleep of 66 medically healthy, medication-free men and pre-menopausal follicular phase women including 33 with chronic PTSD (16 women and 17 men) and 33 age- and sex-matched controls (14 women and 19 men) over 3 consecutive nights. Participants completed an overnight metyrapone challenge after an adaptation and baseline night of sleep and ACTH was obtained by repeated blood sampling. Metyrapone resulted in a greater increase in ACTH and greater decreases in cortisol and delta spectral power sleep in PTSD subjects compared to controls, and a greater increase in ACTH in women compared to men. There was no sex difference in metyrapone effects on delta power sleep, and no significant metyrapone by PTSD by sex interactions with either ACTH or delta power sleep. Regression analyses indicated that a greater increase in ACTH response was associated with a greater decrease in delta power sleep response in PTSD subjects, but no such relationship was found in controls. The PTSD group difference was similar in men and women. These results suggest that stress-related alterations of the HPA axis in PTSD may

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contribute to sleep difficulties. Therapeutics that target the HPA axis may offer promise as a potential future treatment for PTSD and related sleep difficulties.

Keywords

Posttraumatic Stress Disorder; Sleep; ACTH; Metyrapone; Sex Differences

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a disabling consequence of trauma, manifested by recurrent and distressing re-experiencing of the traumatic event, avoidance of trauma reminders, and hyperarousal symptoms, seen in increased startle reactivity and impaired sleep that result from central and autonomic nervous system alterations (American Psychiatric Association, 2013; Hendrickson and Raskind, 2016). Disturbed sleep is one of the most prevalent PTSD symptoms, with patients reporting difficulties with non-restorative sleep, frequent awakenings and nightmares, and a debilitating impact on many domains of functioning (Hoge et al., 2007). A meta-analysis of objective sleep studies reported alterations in sleep duration and patterns in PTSD, including increased Stage 1 sleep and decreased slow wave sleep (SWS) (Kobayashi et al., 2007). Studies using spectral analysis, a powerful method that examines quantitative sleep microarchitecture, have shown that delta band spectral power, which is most prominent in slow wave NREM sleep, is decreased in PTSD (Neylan et al., 2003; Otte et al., 2007; Richards et al., 2013). Delta power sleep is driven by thalamocortical oscillations and thought to both represent the bioenergetic recovery process of cortical wake activity and replay phenomenon in models of procedural memory consolidation (Genzel et al., 2014). Functions of delta power sleep include homeostatic recovery, glucose metabolism and other fundamental biological processes (Tasali et al., 2008). Deficiencies in delta power sleep associated with PTSD may have important consequences for cognition and health.

Stress-related alterations in corticotropin releasing factor (CRF) and the hypothalamic-pituitary-adrenal (HPA) axis may account for alterations in delta power sleep in PTSD. CRF, which functions as a neurotransmitter in extrahypothalamic sites (e.g., amygdala, locus ceruleus (LC), bed nucleus of the stria terminalis (BNST)), has an arousing effect on the cortex (Reviewed in (Zorrilla and Koob, 2004)). CRF is also the primary hypothalamic neuropeptide involved in the control of adrenal secretion of cortisol. Pulsatile CRF release in the hypothalamus leads to adrenocorticotropic hormone (ACTH) release in the pituitary gland, which then stimulates adrenal cortisol release. CRF, ACTH and cortisol levels are lowest in the first few hours of sleep when delta power sleep activity is maximal (Friess et al., 1995). CRF pulses increase after 4 to 5 hours of sleep and reach peak activity at the beginning of the wake period (Gallagher et al., 1973). The rise in overall activity of the HPA axis prepares the organism for food intake, regulates changes in energy metabolism, and promotes optimal neural activity required for initiating wakeful behavior (McEwen, 1995). Studies have found an inverse relationship between delta power sleep and pulsatile cortisol release (e.g., (Vgontzas et al., 1999)). The apparent relationship between peripheral cortisol levels and delta power sleep is likely to be driven by activity of hypothalamic CRF,

supported by findings that exogenous cortisol infusion, which reduces CRF, increases delta power sleep (Friess et al., 1994).

PTSD is associated with increases in both hypothalamic and extra-hypothalamic CRF activity. CRF mediates anxiety and fear behaviors via signaling with CRF1 receptors located in the amygdala, LC, BNST, and cerebral cortex (See (Zorrilla and Koob, 2004) for review). Several studies have found higher levels of CRF in cerebrospinal fluid (CSF) in PTSD, reflecting mainly extrahypothalamic sources (Baker et al., 1999; Bremner et al., 1997; Sautter et al., 2003). Elevated peripheral CRF measured in plasma was found in veterans with PTSD compared to combat-exposed veterans without PTSD and healthy controls (de Kloet et al., 2008). Increased CRF may explain the increased ACTH and cortisol response to psychological challenge observed in individuals with PTSD (Bremner et al., 2003). However, the findings have been mixed. For example, dexamethasone suppression-CRH stimulation resulted in a blunted ACTH response in PTSD, which was interpreted to indicate possible down-regulation of CRF receptors from chronically elevated hypothalamic CRF release (Strohle et al., 2008).

Metyrapone challenge remains the strongest stimulus for endogenous CRF release that exists in the field. Metyrapone, a medication used for the diagnosis of adrenal insufficiency, blocks cyp11B1 (11ß-hydroxylase) and hence blocks the conversion of 11-deoxycortisol to cortisol. This results in reduced cortisol synthesis and increased levels of 11-deoxycortisol (Fiad et al., 1994). Reduced cortisol concentrations result in removal of glucocorticoid negative feedback to the underlying drive of the paraventricular nucleus of the hypothalamus to release CRF, but has no impact on extrahypothalamic CRF (Kalin et al., 1987). Metyrapone causes a 12-fold amplification of secretory ACTH release and results in increased awakenings and reduced delta power sleep (Jahn et al., 2003).

In two previous studies, we found that the sleep and endocrine responses to metyrapone (i.e., decrease in delta power and increase in ACTH, respectively) were significantly diminished in PTSD compared to controls. Further, in the whole sample, greater decline in delta power sleep activity was significantly correlated with greater increases in 11-deoxycortisol and ACTH measured the morning before and after metyrapone administration (Neylan et al., 2003; Otte et al., 2007), providing initial evidence of alterations in the brain response to increased hypothalamic CRF. However, metyrapone blocks cortisol synthesis for approximately 4 hours, and the endocrine response was assessed 9 hours after metyrapone was administered. Although PTSD subjects had a significantly smaller increase in ACTH compared to controls, it is unclear whether the two groups had the same immediate peak and recovery of the ACTH response proximal to metyrapone administration. In order to better understand the effect of the neuroendocrine challenge on delta power sleep in PTSD, the present study examined the effects of metyrapone on ACTH using repeated sampling of nocturnal hormone activity during sleep recordings in men and women with chronic PTSD compared to healthy controls. We tested the hypothesis that the ACTH response to metyrapone would be associated with a decreased delta power sleep response and that the relationship between ACTH and delta power sleep responses would be moderated by having PTSD. Due to previous findings of sex differences in sleep architecture and in ACTH

responses to metyrapone (Inslicht, 2014; Richards et al., 2013), we also examined sex differences in these relationships.

MATERIALS AND METHODS

The present study used a cross-sectional, 2 × 2 design (PTSD/control × female/male) involving 66 medically healthy, non-medicated adults aged 19–39 years in an inpatient sleep laboratory. The study sample was comprised of 33 individuals with current chronic PTSD (16 women and 17 men; 48% female) and 33 control subjects (14 women and 19 men; 42% female). This sample was drawn from a larger study of 93 participants. We excluded data from 10 participants due to side effects or an inadequate metyrapone dose and from 17 participants due to missing data related to difficulties in blood collection over the night or poor quality sleep EEG recordings. Chronic PTSD was defined by fulfillment of DSM-IV criteria for chronic PTSD on the Clinician-Administered PTSD Scale (CAPS) and a CAPS score >40.

Female participants were premenopausal (having at least one menstrual period in past 12 months) as determined by medical screening interview, and were scheduled during the follicular phase of the menstrual cycle within 10 days following the onset of menses. Exclusion criteria included extreme morning or evening tendencies or irregular sleep wake schedules as documented by actigraphy and sleep diary; a diagnosis of sleep apnea; history of traumatic brain injury, presence of neurologic disorders or systemic illness; use of psychiatric, anticonvulsant, antihypertensive, sympathomimetic, steroidal, statin or other prescription medications; anemia, recent blood donation in the past 2 months, obesity (defined as BMI >30); prominent suicidal or homicidal ideation; alcohol abuse or dependence in the prior 2 years; substance abuse or dependence in the previous year; any psychiatric disorder with psychotic features; bipolar disorder or obsessive compulsive disorder; and pregnancy. Exclusion criteria for control subjects also included a lifetime history of major depressive disorder (MDD) or panic disorder.

Nocturnal blood sampling was obtained as a part of a 3-night polysomnography sleep study (night 1= adaptation, nights 2–3= pre- and post-metyrapone administration) conducted in a Clinical Research Center (CRC). Habitual sleep onset time, calculated from the actigraphy and sleep diary data was used to determine the timing of procedures on the CRC. Subjects were instructed to maintain the same sleep onset time during the week of actigraphy monitoring as well as during the CRC admission. All subjects were alcohol and drug-free and restricted to having one optional cup of caffeinated coffee each morning. Two hours before habitual sleep onset, a catheter was inserted in an antecubital vein for repeated sampling of blood on nights 2 and 3 (5.5 ccs every 15 min providing 32 samples for each night). Assays for ACTH and cortisol were performed on every other sample, resulting in 16 pre- and 16 post-metyrapone measures. Blood was sampled for up to 8 h following the final dose of metyrapone (M = 6.5 h; SD = 1.9 h). A single additional blood sample was obtained in the morning, at habitual wake time, to measure ACTH, cortisol, and 11-deoxycortisol.

Subjects were allowed to walk on the CRC, but were instructed to avoid vigorous activity. They were allowed to watch TV, play games and talk to staff. All subjects were provided

meals at 8:00 AM, 12:00 noon, and 5:30 PM. Prior to the 3rd night of polysomnography subjects were given metyrapone as described below. The timing of the doses were adjusted so that the last dose occurred at the subject's diary and actigraphy determined habitual bedtime. Nocturnal blood sampling provided the opportunity to evaluate the association between acute HPA axis and sleep responses to metyrapone over the course of the night. This research was approved by the Committee on Human Research at the University of California, San Francisco. All participants provided written informed consent before participating in any study procedures.

Measures

Demographics.—Self-report questionnaires were used to gather demographic data.

Psychiatric Diagnoses and Trauma History.—The CAPS was used to assess current and lifetime PTSD (Blake et al., 1995). The CAPS assesses the frequency and intensity of PTSD symptoms corresponding to the re-experiencing, avoidance and hyperarousal symptoms described in the DSM-IV diagnostic criteria. Diagnosis of PTSD was based on symptoms experienced in the previous month, which were associated with the participant's self-identified worst traumatic event.

The **Structured Clinical Interview for DSM-IV, Non-Patient edition (SCID-NP)** was used to diagnose all other psychiatric disorders, including MDD (Spitzer et al., 1992). The type of trauma exposure and age of occurrence was assessed using the **Life Stressor Checklist- Revised** interview (Wolfe et al., 1996).

Metyrapone Challenge and Biological Assays.—On the day after the second night on the GCRC, subjects were given an oral dose of metyrapone of 750mg every 4 hours starting at 12 hours before habitual sleep (HS) onset, for a total of 3 doses (12h, 8h, 4h before HS), and one dose of 2.5g at HS along with 30ccs of an antacid to minimize gastrointestinal upset. The schedule of four doses every 4 hours ending at bedtime adheres to the standard overnight metyrapone test used for evaluating pituitary reactivity. Given our particular focus on slow wave sleep and HPA axis activity, this schedule was selected to ensure that metyrapone was absorbed and had time to inhibit the enzymatic conversion of 11-deoxycortisol to cortisol and for the elimination of cortisol to exert an effect on CRF. The 2.5 mg dose given at bedtime was intended to eliminate any group differences in residual adrenal cortisol release and is similar to doses used in several other studies (Jahn et al., 2003; Yehuda et al., 1996).

Two hours before habitual sleep onset, a catheter was inserted in an antecubital vein for repeated sampling of blood on nights 2 and 3 (5.5 ccs q 15 minutes providing up to 32 samples for ACTH (16 pre- and 16 post-metyrapone)). Blood was sampled for up to 8 hours following the final dose of metyrapone (M = 6.5 hours; SD = 1.9 hours).

Cortisol.—Blood was collected using an EDTA tube and plasma (q 30 minutes) was separated by centrifugation. Plasma levels of cortisol were measured using the Access Chemiluminescent Immunoassay (Beckman Coulter, Fullerton, CA) at the Specialized Assay Core, Brigham and Women's Hospital.

ACTH.—Blood was collected using an EDTA tube and plasma was separated by centrifugation. Plasma levels of ACTH were measured using a commercially available immunoradiometric assay (DiaSorin Inc., Stillwater, Minnesota) at the Specialized Assay Core, Brigham and Women's Hospital. The DiaSorin ACTH immunoradiometric assay is designed to detect whole molecule ACTH and is a more sensitive method for the detection of ACTH in plasma than a radioimmunoassay.

Polysomnographic Measurement.—Polysomnography recordings were obtained with ambulatory polysomnography (Nihon Kohden Trackit Ambulatory Recording System). The parameters recorded included an electroencephalogram (EEG) at leads C3, C4, O1 and O2, left and right electrooculograms (EOG), submental electromyogram (EMG), bilateral anterior tibialis EMGs, and electrocardiogram (EKG) in accordance with standardized guidelines (Rechtschaffen, 1968). Electrode impedance was set at < 5 kohm at the start of the recording. The EEG and EOG leads were referenced to linked mastoids. Raw EEG signals were filtered and amplified, then digitized at 256 Hz and recorded to a removable hard disk in European Data Format (EDF) file format. The low frequency and high frequency hardware filters on the recorder were single pole analog filters with 3 db points at 0.5 Hz and 100 Hz. Pass Plus was utilized for both visual scoring and quantitative EEG analysis of the digitized polysomnography data.

Power Spectral Analysis.—Pass Plus (Delta Software) analytic software was used to measure sleep activity in all frequency bands delta through gamma from the C3 electrode by power spectral analysis (PSA). The C4 electrode was used if there was excessive artifact. A limitation of Pass Plus is that artifact removal is accomplished by removal of whole epochs tagged with artifact. This has the potential to introduce additional confounds given the removal of typically longer bouts of uncontaminated EEG. Therefore, epochs were tagged for slow and fast artifact for additional analyses. Primary analyses were conducted with all epochs and then checked for the impact of removal of epochs with slow and fast artifact. Removal of fast artifact (for bandwidths alpha and above) and slow artifact (for bandwidths delta and theta) did not significantly impact our findings in NREM sleep. All results are therefore reported without removal of epochs containing artifact. Pass Plus applied a 5μV smoothing constant to eliminate spurious waves caused by electrical jitter. PSA was conducted on all epochs of NREM and REM sleep. Epochs visually scored as wake were not included in these analyses. Visual scoring was conducted by one of the authors, a highly experienced registered polysomnography technician who was blind to PTSD status, but not to pre- vs. post- metyrapone status. Pass Plus was used to perform Fast Fourier Transformation analysis on 4.0 second Welch tapered windows with 2 second overlap, yielding 15 windows per 30-second epoch. Power spectra for delta (1–4 Hz) were analyzed to address our primary hypothesis with respect to delta power sleep.

Statistical Analysis

The two groups defined by PTSD status were compared on demographic and clinical characteristics using t-tests for continuous variables and chi-squared tests for categorical variables. ACTH (ng/ml), cortisol (μg/ml), and delta sleep spectral power density (μV^2) were natural log transformed to normalize their distributions. ACTH measures were obtained up

to 16 times per night (30 min apart). Mean delta sleep power was integrated over each 30 min period to provide sleep measures commensurate with the ACTH measurement time points. Separate random-intercepts linear mixed models were used to predict ACTH and mean delta power per epoch, respectively, from metyrapone status, PTSD, sex, and their full interactions.

To assess the relationship between metyrapone effects on ACTH and on sleep, mean transformed ACTH and delta spectral power values were calculated for each subject averaged over each night, pre- and post- metyrapone administration. A linear regression analysis was conducted on baseline-adjusted change in whole-night delta power sleep predicted from baseline-adjusted change in whole-night ACTH, sex, PTSD status, and their full interactions. The model was parameterized using the delta power sleep difference score (post- minus pre-metyrapone nights) as the outcome, with pre-metyrapone delta power sleep as a covariate. This model is equivalent to using baseline-adjusted post-metyrapone delta power sleep as the outcome, but provides a more interpretable regression coefficient in terms of the effects of predictors on difference scores. Similarly, both ACTH pre-post difference scores and the ACTH pre-treatment scores were included as predictors. Again, this redundancy in parameterization does not affect the model fit, but provides a regression coefficient for the baseline-adjusted ACTH response score to facilitate interpretation.

RESULTS

Demographic Data and Clinical Characteristics.

Sample characteristics are presented in Table 1. There were no significant differences in sex or race/ethnicity distribution between PTSD and control subjects. As expected, PTSD subjects had higher CAPS scores and rates of current MDD. As per the exclusion criteria, no control subjects met criteria for current MDD. Six control subjects (3 men and 3 women) reported a lifetime history of a traumatic criterion A1 event, but all had a current and lifetime CAPS score of zero. Among women, 2 of the 14 controls and 5 of the 16 PTSD participants reported taking hormonal birth control pills, but there were no differences by group, $X^2(1, N = 30) = 1.20$, $p = .273$.

Potential Confounders.

There were no significant associations between BMI, smoking, current MDD, and use of hormonal birth control on baseline-adjusted delta power sleep and ACTH, therefore these variables were not included in further analyses. MDD was associated with the delta power sleep response (lower delta power on night 3 even after adjusting for night 2; $p = .006$), and childhood trauma was associated with ACTH response (higher ACTH on night 3 adjusted for night 2; $p = .006$). However, both of these variables were highly correlated with PTSD status: All 6 people with MDD were in the PTSD+ group, and 14 out of the 17 with childhood trauma were in the PTSD+ group. Since both depression and childhood trauma were highly confounded with PTSD, these variables were not included in further analyses.

Endocrine and Sleep Effects of Metyrapone.

Table 2 shows the mean values and statistical contrasts for the sleep and endocrine measures in each group before and after metyrapone administration. As expected, metyrapone administration led to significant suppression of nocturnal cortisol and enhancement of nocturnal ACTH. Metyrapone also significantly reduced mean delta sleep power. Furthermore, as seen in Table 2, we observed significant interactions of metyrapone with PTSD driven by greater effects of metyrapone on ACTH, cortisol, and delta spectral power sleep in the PTSD subjects. Nocturnal endocrine data before and after metyrapone for PTSD subjects and controls is shown in Figures 1 and 2. Specifically, compared to controls, PTSD subjects had a greater decrease in cortisol (Fig 1), and a greater increase in ACTH (Fig 2.). PTSD subjects also had a greater decrease in delta spectral power in response to metyrapone (Table 2). Examination of other sleep bands indicated that while there was increased beta power on the pre-metyrapone night in the PTSD group, there was no differential group response to metyrapone in any of the sleep bands outside of delta (all p's for interaction terms >.286; supplementary Table 1). Metyrapone caused an increase in power in the Beta1, Beta2, and Gamma frequency bands.

Additional analyses examining sex differences indicated that there was a greater overall nocturnal ACTH response in women (M=1.65, 95% CI [1.54, 1.76], SD=1.08) compared to men (M=1.04, 95% CI [0.94, 1.14], SD = 0.67), leading to a significant metyrapone by sex interaction $F(1, 1636) = 65.39$, $p < .001$. There was no significant difference in delta power sleep response in women (M =−8.1, 95% CI [−7.9, −8.3], SD = 20.5) compared to men (M = −13.1, 95% CI [−13.3, −12.9], SD = 24.9), metyrapone by sex interaction $F(1, 1636) = 1.19$, $p = .275$. There were also no significant 3-way metyrapone X PTSD X sex interactions for either ACTH or delta power, $F(1, 1636) = 2.22$, $p = .137$ for ACTH; $F(1, 1526) = 0.76$, $p = .383$ for delta power.

Examination of single point awakening hormone levels indicated that metyrapone administration resulted in the reduction of awakening morning cortisol from M=4.9, SD=0.31, to M=3.67, SD = 0.36, $F(1, 55) = 412.63$, $p < .001$, while awakening 11-deoxycortisol was enhanced from M=0.76 to M=4.99, $F(1, 55) =3396.57$, $p < .001$. Awakening ACTH increased from M=3.4 to M=5.6, $F(1, 63) = 384.9$, $p < .001$. Furthermore, PTSD subjects compared to controls showed elevated awakening 11-deoxycortisol levels on both days, but there was no differential response to metyrapone, $F(1, 55) = .02$, $p = .899$). There were also no group differences in ACTH or cortisol pre- or post-metyrapone. The awakening ACTH response was marginally greater in women (M=2.37, 95% CI [1.98, 2.76], SD=1.00) than in men (M=2.00, 95% CI [1.63, 2.36], SD=0.90), interaction $F(1, 63) = 2.98$, $p = .089$.

Relationship between ACTH response and Sleep.

Figure 3 shows the relationship between metyrapone-related change in mean delta power and change in ACTH. The linear regression analysis showed that this relationship differs between PTSD and controls, ACTH by group interaction $F (1, 56) = 10.11$, $p = .002$. Within-group contrasts showed that the relationship was negative in PTSD but not in controls, B =

−0.27 (SE = 0.07), p < .001 for PTSD and B = 0.07 (SE. = 0.08), p = .375 for controls. This PTSD group difference was not moderated by sex, interaction F (1, 58) = 0.00, p = .999.

DISCUSSION

Our findings indicate that metyrapone resulted in a greater decrease in nocturnal cortisol, greater increase in ACTH, and greater decrease in delta power in sleep of PTSD subjects compared to controls. Consistent with our hypotheses, we found that PTSD moderated the association between the ACTH and delta power sleep response to metyrapone. Specifically, we found that a greater increase in ACTH was associated with a greater decrease in delta power sleep in PTSD subjects, but no such relationship in controls. These findings highlight alterations of the HPA axis in PTSD that may account for sleep disturbances that are so common in this disorder.

There are several potential explanations that may account for group differences in hormonal response. Metyrapone, by blocking the conversion of 11-deoxycortisol to cortisol, results in decreased cortisol and increased 11-deoxycortisol (Fiad et al., 1994). In healthy individuals, it is expected that CRH and ACTH levels would increase in response to decreasing cortisol levels because of reduced feedback inhibition. Our findings of a greater decrease in cortisol, and a greater increase in ACTH in PTSD subjects suggests even greater responsiveness of underlying HPA axis drive when eliminating naturally circulating cortisol by administration of metyrapone. Our findings of elevated 11-deoxycortisol the morning after both nights, and numerically higher cortisol pre-metyrapone in PTSD suggest some marginal increased adrenal activity in producing cortisol or possibly decreased clearance of cortisol/11-deoxycortisol. The greater effect of metyrapone in PTSD on the HPA axis could also be explained increased sensitivity to glucocorticoid signaling in PTSD which could produce a greater hypothalamic release of corticotropin releasing factor (CRF) and/or pituitary release of ACTH. Our findings are consistent with numerous studies that found enhanced responsiveness of glucocorticoids in PTSD (reviewed in (Daskalakis et al., 2013)). There could also be PTSD effects on sensitivity of CRF signaling at the level of the pituitary. The increased 11-deoxycortisol and the numerically, though non-significantly, increased cortisol pre-metyrapone in PTSD in the setting of virtually identical levels of ACTH suggest that the adrenal gland shows increased responsiveness to ACTH signaling or reduced clearance in the PTSD group.

Polymorphisms of genes that regulate both CRF and glucocorticoid receptor activity have been associated with PTSD, suggesting that individual variability in HPA axis regulation may be associated with risk for PTSD (reviewed in (Castro-Vale et al., 2016)). While the precise mechanism has yet to be determined, overall, our findings suggest that blocking cortisol produced a greater biological response in PTSD. Given this, it is plausible to consider that therapeutics that target the HPA axis, potentially by blocking CRF or by reducing glucocorticoid signaling, may offer promise as a potential future treatment for PTSD and related sleep difficulties. Although, a recently completed clinical trial of a CRF antagonist in PTSD did not produce an overall greater response relative to placebo, a subgroup of subjects who were GG homozygotes for the CRF1 SNP rs110402 who had history of childhood abuse did show greater response to the drug (Dunlop BW, In Press).

While the precise mechanism accounting for the effect of metyrapone on delta power sleep is not entirely delineated, one possible pathway may be through the LC. PVN CRF neurons project to the LC, where CRF type-1 receptors (CRF1) are expressed (reviewed in (Zitnik, 2016)). Metyrapone administration results in c-fos induction in the LC, indicating neuronal activation (Rotllant et al., 2002). Administration of a CRF1-antagonist prevents the effects of CRF on LC neurons (Schulz et al., 1996). Thus, CRF projections from the PVN to the LC may be a point of integration between neurohormonal and neurotransmitter CRF systems (Valentino et al., 1992). The LC is strongly implicated in arousal and waking, with evidence of interactions with suprachiasmatic nucleus (SCN) circuitry that drives circadian rhythms in sleep–wake cycles (Aston-Jones et al., 2007; Schwartz and Kilduff, 2015). It is plausible that stimulation of the LC by CRF is of sufficient magnitude to increase arousal signaling and reduce delta power sleep. While we considered an alternative explanation in which PTSD subjects may have been more arousable, resulting in greater awakenings during blood collection, we did not find any evidence of an effect of PTSD on sleep maintenance in this study or in number of awakenings on the pre-metyrapone night in our previous report of this data (Richards et al., 2013).

Our findings of a greater ACTH response and decreased delta power sleep response to metyrapone in PTSD subjects compared to controls contrasts with our previous studies in which we found that the increase in ACTH and decrease in delta power sleep responses to metyrapone were significantly diminished in individuals with PTSD compared to controls (Neylan et al., 2003; Otte et al., 2007). We considered the possibility that this difference may be related to differences in the timing and measurement of hormonal responses. Our initial studies determined ACTH from blood samples that were drawn at a single time point, delayed to the morning after an overnight metyrapone test (Neylan et al., 2003; Otte et al., 2007). In the present study, we were interested in capturing nocturnal hormonal responses in relation to sleep, and so used values that were based on ACTH values and delta power spectral energy values over the whole night which was a methodological strength over the previous studies. An additional notable difference in methodology is that in the previous studies only 3 grams (4 doses of 750 mg) of metyrapone were administered compared to 4.75 grams in this study (3 doses of 750 mg and 2.5 grams at lights out). In the current and past studies (Neylan et al., 2003; Otte et al., 2007), there was no differential effect of metyrapone in PTSD on the suppression of the single measure of morning cortisol. The differential effect of metyrapone on cortisol suppression was only observable with nocturnal sampling. Jahn previously reported a dose response effect on both ACTH and SWS (Jahn et al., 2003). It is possible that lower doses used in our prior studies resulted in rebound of cortisol, which could have led to some recovery of SWS similar to what has been demonstrated in experimental infusion of cortisol. PTSD subjects may have been more sensitive to this higher dose of metyrapone, resulting in lower nocturnal cortisol than controls arising from greater inhibition of the enzymatic conversion of 11-deoxycortisol to cortisol (Born et al., 1991).

Another potential difference in studies was in the age of participants. Our previous studies consisted of male Vietnam combat veterans who were in their late forties and pre-menopausal female civilians in their mid-thirties (Neylan et al., 2003; Otte et al., 2007). In this study, participants were relatively younger, with a mean age of 30 in both sexes. A large

body of evidence has shown that delta power sleep, measured by visual sleep scoring or by quantitative analysis, diminishes with age (For review see (Landolt and Borbely, 2001)).

While our results indicated a sex difference in ACTH response to metyrapone, sex was not found to moderate PTSD group differences in either ACTH response or delta power sleep response, as indicated by non-significant group by sex by metyrapone interactions. Sex also did not moderate the relationship between ACTH response and delta power sleep response, as indicated by a non-significant group by sex interaction in the regression model predicting delta power sleep response from ACTH response to metyrapone. However, it is important to consider that we had limited power to detect three-way interactions in this study. Moreover, all women in this study participated during the follicular phase of their menstrual cycle when estrogen and progesterone are relatively low and several women in this study were on hormonal birth control (2 controls and 5 PTSD women). More robust sex differences may emerge in studies that examine women at other menstrual phases when estrogen and progesterone levels are higher, and with larger study samples.

There are several additional limitations to consider. Six participants with PTSD also met criteria for depression, and it is possible that depression may impact both CRF and sleep. Fourteen participants with PTSD and three controls reported childhood trauma. Since childhood trauma was also associated with ACTH response, it may account for the effect of PTSD on ACTH. Our relatively small sample size might have reduced our power to detect a significant interaction between PTSD and sex on our outcomes. Further, we are not able to confirm whether our findings are a result of alterations in glucocorticoid, CRF, or ACTH signaling or receptor sensitivity or clearance.

Strengths of this study include nocturnal sampling of hormone activity during sleep across the night, allowing us to confirm that the effect of metyrapone on delta power sleep and hormones during the active phase of metyrapone’s action. Although, limited by power constraints, equally balanced groups of women and age-matched men with and without PTSD allowed us to explore the interaction of PTSD and sex on the relationship between ACTH and sleep responses to metyrapone.

To summarize, our data indicates that PTSD status was associated with a greater increase in ACTH, a greater decrease in cortisol and in delta power sleep in response to metyrapone compared to controls. We also found that PTSD status moderated the association between the ACTH response and delta power sleep response to metyrapone. Our findings may reflect increased hypothalamic CRF, which could result from increased CRF receptor sensitivity and/or increased negative feedback inhibition in PTSD. Implications of these findings are that pharmacological agents that target the HPA axis, such as CRF or GR antagonists, may have therapeutic effects on PTSD-related sleep disturbances. In fact, there may be greater benefits on sleep in individuals experiencing PTSD than in those without any psychiatric conditions. Further studies are needed in the realm of other hormones (i.e., growth hormone, progesterone) that are affected by metyrapone and may modulate sleep.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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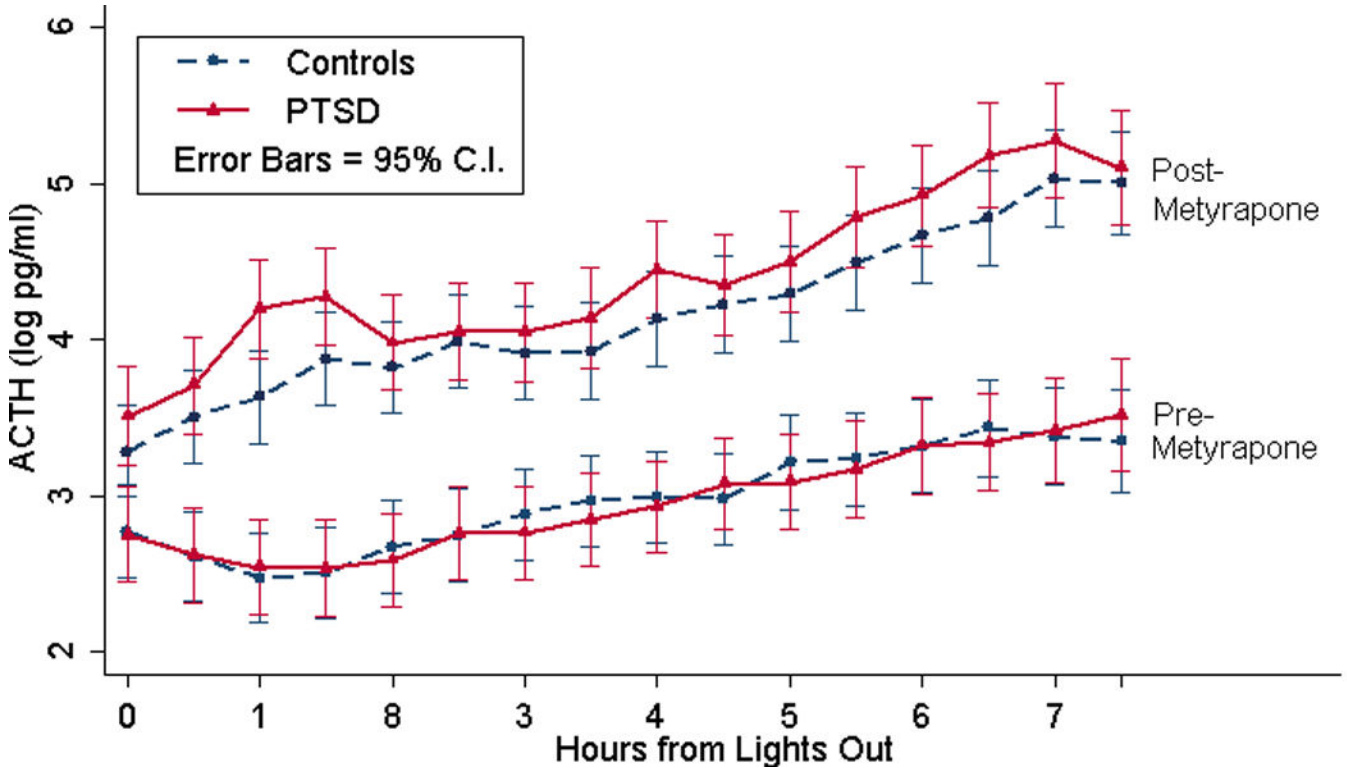

Fig 1.

Time course of mean cortisol level during pre- and post-metyrapone nights, by PTSD status

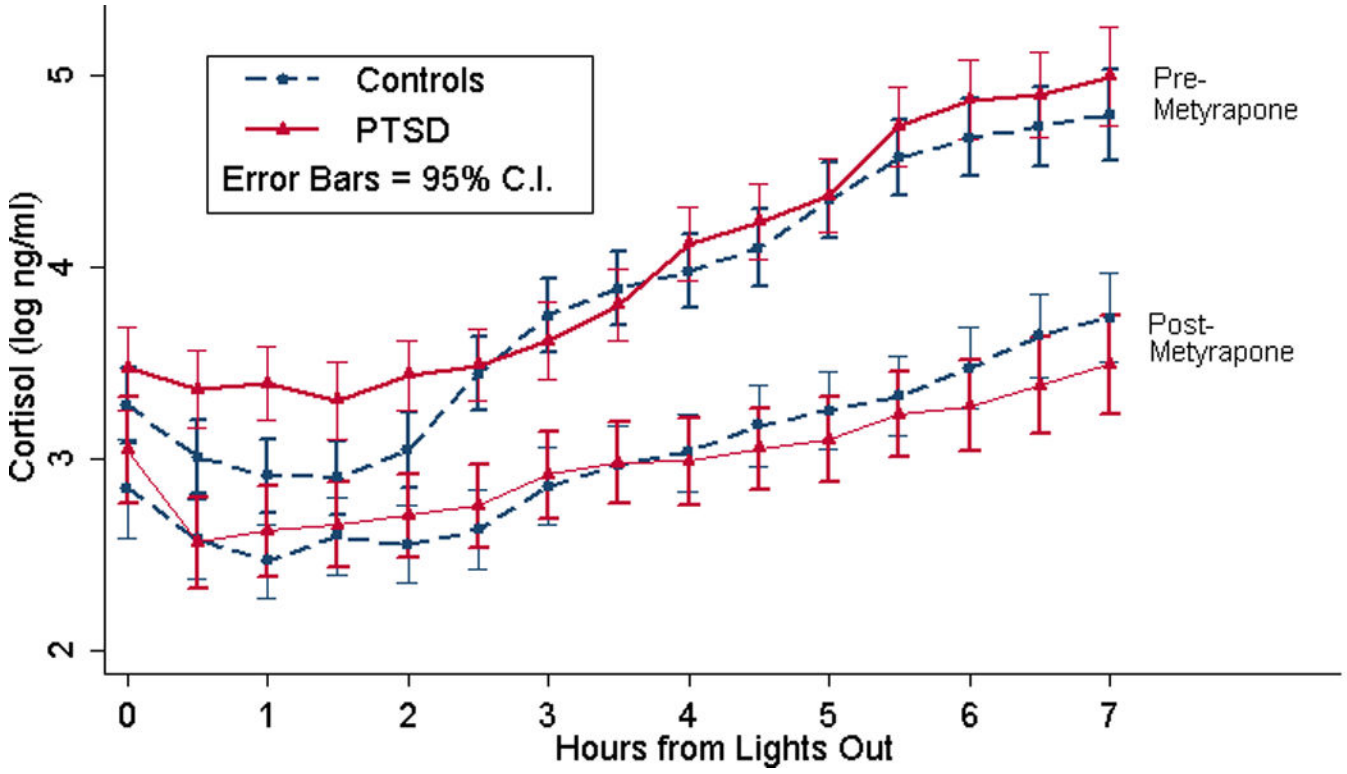

Fig 2.
Time course of mean ACTH level during pre- and post-metyrapone nights, by PTSD status

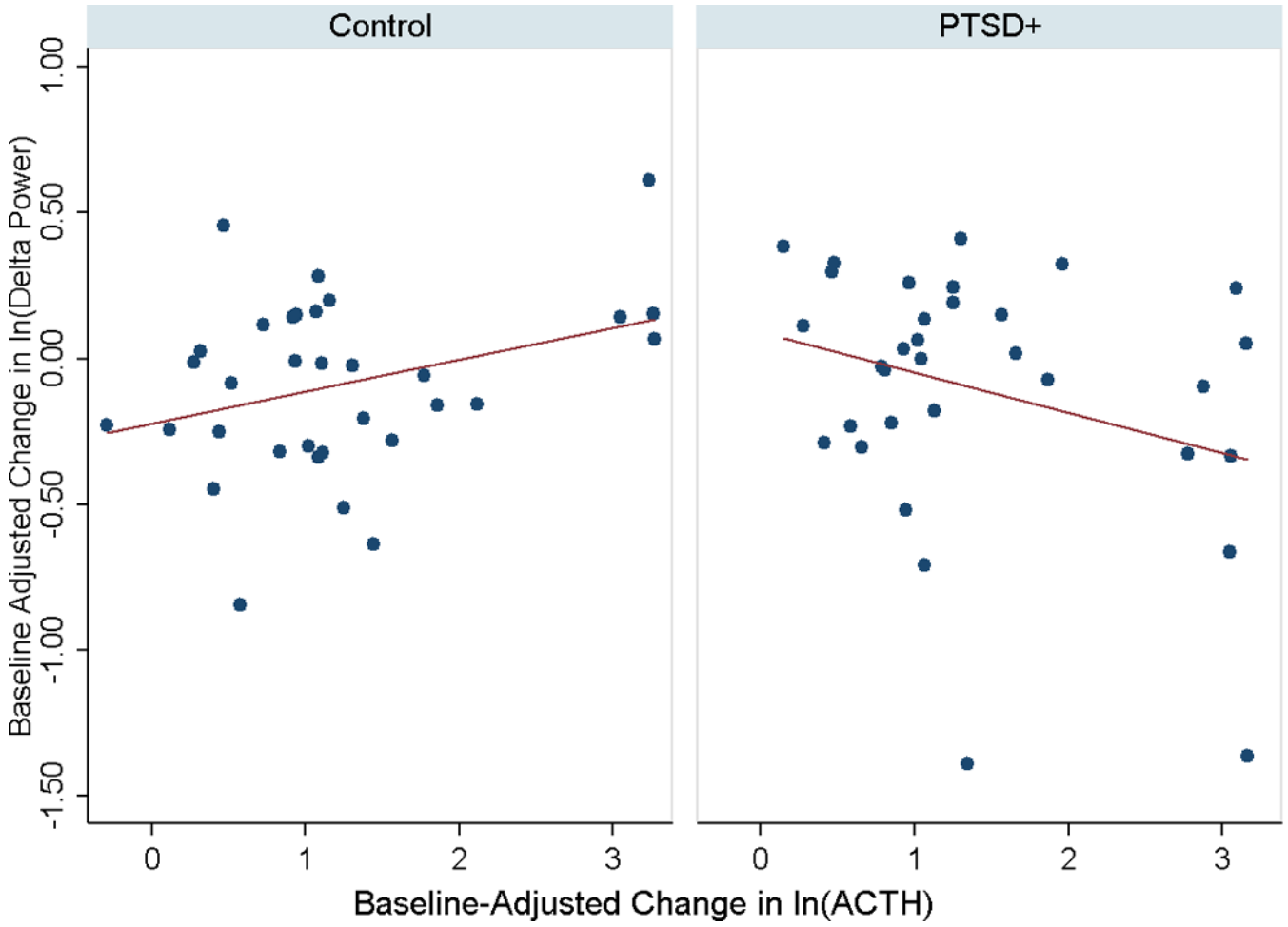

Fig 3.
Relationship of metyrapone-related change in delta power sleep to change in ACTH, by PTSD status

Table 1.

Demographic and clinical characteristics by PTSD status.

	Control (N=33)	PTSD+ (N=33)	P-value
Female Gender	14 (42%)	16 (49%)	.621
Age	30.3 (8.4)	29.6 (5.3)	.715
Education (Years)	15.3 (2.2)	15.3 (2.0)	.931
Minority vs. Caucasian	9 (27%)	15 (45%)	.125
Current CAPS score (Mean ± SD)	0.0 ± 0.0	52.5 ± 13.7	.000
Current MDD	0 (0%)	6 (18%)	.000
BMI	24.3(3.5)	26.5(4.8)	.022
Smoking status	7 (21%)	8 (24%)	.769
Hormonal Birth Control (Among women)	2 (14%)	5 (31%)	.273

CAPS: Clinician Administered PTSD Scale; MDD: Major Depressive Disorder; BMI: Body Mass Index

Table 2.

Endocrine and sleep variables pre- and post-metyrapone.

	Control (N=33)		PTSD+ (N=33)		Pre-Post Metyrapone Contrast[1]			Group Contrast[1]			Metyrapone X Group Interaction[1]		
	Pre-metyrapone Mean (SD)	Post-metyrapone Mean (SD)	Pre-metyrapone Mean (SD)	Post-metyrapone Mean (SD)	F	d.f.	p	F	d.f.	p	F	d.f.	p
Delta spectral power (ln(μV^2))	2.25 (0.39)	2.15 (0.41)	2.15 (0.36)	1.89 (0.54)	7.32	1, 1526	.007	4.18	1, 1526	.041	0.68	1, 1526	.007
ACTH (ln(pg/ml))	2.91 (0.36)	4.13 (0.89)	2.92 (0.51)	4.34 (1.11)	1318.57	1, 1636	.000	0.23	1, 1636	.628	6.14	1, 1636	.013
Cortisol (ln (ng/ml))	3.82 (0.87)	2.99 (0.58)	3.97 (0.85)	2.95 (0.48)	574.78	1, 1446	.000	1.17	1, 1446	.279	5.92	1, 1446	.015

[1]ACTH, delta power, and cortisol contrasts are based on linear mixed models with random subject effects and repeated measures on night (pre/post metyrapone) and timepoint (up to 16 per night). Means and SD's are based are based on raw subject-level data.