Effects of Metyrapone on Hypothalamic-Pituitary-Adrenal Axis and Sleep in Women with Post-Traumatic Stress Disorder

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Background: Metyrapone blocks cortisol synthesis which results in removal of negative feedback, a stimulation of hypothalamic corticotropin releasing factor (CRF) and a reduction in delta sleep. We previously reported a diminished delta sleep and hypothalamic-pituitary-adrenal (HPA) response to metyrapone in men with post-traumatic stress disorder (PTSD). In this study, we aimed to extend these findings to women.

Methods: Three nights of polysomnography were obtained in 17 women with PTSD and 16 controls. On day 3, metyrapone was administered throughout the day up until bedtime. Plasma adrenocorticotropic hormone (ACTH), cortisol, and 11-deoxycortisol were obtained the morning following sleep recordings the day before and after metyrapone administration.

Results: There were no significant between-group differences in hormone concentration and delta sleep at baseline. Relative to controls, women with PTSD had decreased ACTH and delta sleep responses to metyrapone. Decline in delta sleep was associated with the magnitude of increase in ACTH across groups.

Conclusions: Similar to our previous findings in men, the ACTH and sleep electroencephalogram response to metyrapone is attenuated in women with PTSD. These results are consistent with a model of downregulation of CRF receptors in an environment of chronically increased CRF activity or with enhanced negative feedback regulation in PTSD.

Key Words: Corticotropin-releasing-hormone, cortisol, HPA axis, posttraumatic stress disorder, sleep

Sleep disturbances are among the most common symptoms of post-traumatic stress disorder (PTSD). Delta sleep is believed to be the primary marker for sleep homeostasis and the restorative function of sleep (Borbely and Achermann 2000). Three studies have shown that PTSD is associated with decreased visually scored delta sleep (Glaubman *et al.* 1990; Kramer and Kinney 1988; Neylan *et al.* 2003). This contrasts with a larger number of studies of PTSD that have found no differences in visually scored delta sleep (Breslau *et al.* 2004; Mellman *et al.* 1995a, 1995b, 1995c, 1997; Ross *et al.* 1994a, 1994b; Woodward *et al.* 1996a, 1996b, 1996c). However, since visually scored delta sleep typically represents a small fraction of total sleep time, most studies lack the power to detect significant group differences. To date, only two studies examined quantitative sleep microarchitecture (e.g. delta power) in PTSD. Both of these studies documented that subjects with PTSD compared to controls had significantly decreased quantitative delta sleep (Neylan *et al.* 2003; Woodward *et al.* 2000). Decreased delta sleep activity has been shown in a number of studies to be associated with increased hypothalamic corticotropin-releasing factor (CRF) release (Steiger 2002). There are consistent results of elevated CRF in the cerebrospinal fluid in PTSD patients (Baker *et al.* 1999; Bremner *et al.* 1997).

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Metyrapone reduces cortisol levels and eliminates cortisol-mediated feedback inhibition of the HPA axis by blocking the enzymatic conversion of 11-deoxycortisol to cortisol. This leads to stimulation of hypothalamic CRF and adrenocorticotropic hormone (ACTH) and subsequent accumulation of 11-deoxycortisol (Fiad *et al.* 1994). In humans, metyrapone reduces delta sleep activity (Jahn *et al.* 2003; Neylan *et al.* 2003; Wagner *et al.* 2005). Metyrapone administration in rats resulted in electroencephalogram (EEG) activation and reduced sleep time which was mediated by the rise of hypothalamic CRF, as opposed to the drop in corticosterone (Burade *et al.* 1996). EEG activation and loss of sleep time was reversed by anti-CRF antibodies. Consistent with a model of downregulation of CRF receptors in an environment of chronically increased CRF activity in PTSD, we have previously shown that male PTSD patients had a diminished decrease of delta sleep and an attenuated ACTH increase to metyrapone (Neylan *et al.* 2003). Despite possibly elevated hypothalamic CRF activity, many but not all studies showed low cortisol in PTSD subjects. Thus, it is possible that PTSD is associated with enhanced negative feedback of the HPA axis, or reduced adrenal output, or a combination of these two mechanisms (for review, see Yehuda 2002). While enhanced negative feedback would lead to a small ACTH to cortisol ratio, reduced adrenal capacity would be expected to yield a large ratio of ACTH to cortisol release.

Age and gender have been shown to influence both HPA activity (Kudielka *et al.* 2004; Otte *et al.* 2005a) and sleep patterns (Armitage and Hoffmann 2001). In a comprehensive analysis of seven studies including 177 participants, premenopausal women had slightly lower 24-hour mean cortisol levels than men in the same age range, primarily because of lower morning maxima (Van Cauter *et al.* 1996). Furthermore, premenopausal women showed less delta sleep and a significantly greater decrease in delta activity from the first to the second half of the night (Antonijevic *et al.* 1999). Given these documented gender effects on sleep and HPA activity, we aimed to extend our earlier

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findings in middle-aged men by examining sleep, particularly quantitative delta sleep, before and after metyrapone administration in 17 younger women with PTSD without substance abuse in the last two years and 16 age and gender matched controls. Based on our results in middle-aged men with PTSD, we hypothesized that 1) compared to controls, women with PTSD would show a diminished ACTH and delta sleep response to metyrapone, 2) across groups the delta sleep response would be correlated with the ACTH response, and 3) women with PTSD would have less delta sleep at baseline.

Methods and Materials

Participants

Medically healthy female subjects were recruited from web-based and newspaper advertisement and from the San Francisco Veterans Affairs Medical Center PTSD Outpatient Program. All subjects provided written informed consent. The study protocol and consent form were approved by the Committee on Human Research at the University of California, San Francisco (UCSF). Seventeen PTSD subjects and 16 controls completed three nights of polysomnography in the General Clinical Research Center at UCSF. Subjects were included in the PTSD group if they met DSM-IV criteria for current PTSD as assessed by the Clinician Administered PTSD Scale (CAPS) (Blake *et al.* 1995). Subjects filled out the Beck Depression Inventory (Beck *et al.* 1961) and the Impact of Event Scale-Revised (Weiss and Marmar 1996) after the sleep recordings. All women were tested during the follicular phase of the menstrual cycle (day 3-10). Subjects spent the three days on the General Clinical Research Center at UCSF. They were allowed to have one cup of coffee in the morning. Naps during the day were not allowed.

Subjects were excluded if they met criteria for alcohol or substance abuse or dependence within the past two years, lifetime criteria for dementia, delirium, schizophrenia, schizoaffective disorder, bipolar disorder, panic disorder, or obsessive compulsive disorder as assessed by the Structured Clinical Interview for DSM-IV (SCID-P; First *et al.* 1996). Current and lifetime major depression were not exclusion criteria. Medical exclusion criteria included any history of neurological disease, current systemic illness affecting central nervous system function, or use of any medication affecting the brain. All subjects were free of any psychiatric medications for at least two months prior to participation.

Sleep Recording

Ambulatory polysomnography (Oxford MR95 digital recorder, Oxford Instruments, Clearwater, Florida) was used to monitor three nights of sleep. Subjects adhered to a stable sleep-wake schedule at their habitual times. The first night of polysomnography was used as an adaptation night and was not utilized in the analyses. The parameters recorded included an EEG at leads C3 and C4, right and left electrooculograms (EOG), submental electromyogram (EMG), and an electrocardiogram (EKG) in accordance with standardized guidelines. An oximeter (Respironics Cricket, Monroeville, Pennsylvania) was used to screen for obstructive sleep apnea (OSA). The cutoff criterion for apnea was 10 desaturation events per hour in bed, which has been shown to have a sensitivity of 98% and specificity of 48% in detecting OSA (Series *et al.* 1993). Subjects who screened positive for obstructive sleep apnea were excluded. All sleep was imported into Pass Plus (Delta Software, St. Louis, Missouri) analytic software and visually scored per 30-sec epochs (Rechtschaffen and Kales 1968). Delta sleep activity was analyzed by period amplitude analysis (PAA) using the Pass Plus (Delta Software) analytic software. Integrated amplitude of .3 to 4.0 Hz activity per 30 sec epoch was analyzed by Non-Rapid Eye Movement (NREM) cycles across the night following the technique described by Feinberg *et al.* (1987), Feinberg *et al.* (1991), and Travis *et al.* (1991). Epochs scored as wake were not included in these analyses. Movement artifact was visually monitored and recorded and not included in the analyses.

Neuroendocrine Measures

On the morning after the second night, blood was collected by venipuncture and plasma was assayed for ACTH, 11-deoxycortisol, and cortisol. Subjects orally received 750 mg of metyrapone along with 30 cc's of an antacid every 4 hours for a total of 4 doses prior to the third night of the study. The timing of the doses was adjusted so that the last dose occurred at the subject's self-reported habitual bedtime. The following morning, 8 to 9.5 hours after the last metyrapone dose, another blood sample was collected to measure ACTH, 11-deoxycortisol, and cortisol. Subjects withheld food and fluids for 9 hours prior to the two morning blood draws.

Statistical Analyses

Differences in demographic characteristics between patients and controls were compared using *t*-tests for continuous variables and chi-square tests for dichotomous variables. The effect of metyrapone on visually scored sleep stages, quantitative delta sleep as indexed by period amplitude analysis, and plasma cortisol, ACTH, and 11-deoxycortisol was analyzed by a repeated measures analysis of variance (ANOVA) with condition (metyrapone administration) as a within subjects factor and group membership as a between subjects factor. The relationships among the changes in delta sleep, cortisol, 11-deoxycortisol, and ACTH were analyzed by 2-tailed Pearson correlations. All values were expressed as mean and standard deviation.

Results

Demographic Variables

There were no differences between patients and controls regarding age, body mass index (BMI), alcohol use, and smoking (Table 1). Among the 17 women with PTSD, three had a diagnosis of current major depression, and 13 had a diagnosis of lifetime major depression.

Table 1. Demographic and Psychometric Data in Patients and Healthy Controls

	PTSD (n = 17)	Controls (n = 16)	p Value
Demographics			
Age	36 (10)	35 (9)	.75
BMI	27 (5)	25 (5)	.14
Smokers[a]	2	1	.99
Psychopathology			
CAPS	56 (16)	.5 (1)	<.001
IES-R	1.05 (.78)	.14 (.30)	<.001
BDI	9.8 (6.2)	3.4 (3.6)	<.001
Current Depression	3	0	.23
Lifetime Depression	13	0	<.001

Data are mean (SD) unless otherwise indicated. PTSD, post traumatic stress disorder; BMI, Body Mass Index; CAPS, Clinician Administered PTSD Scale; IES-R, Impact of Event Scale-Revised; BDI, Beck Depression Inventory.
[a]Number.