Title: Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones

Author: Mary Ann C. Stephens Pamela B. Mahon Mary E. McCaul Gary S. Wand

PII: S0306-4530(15)30047-0
DOI: http://dx.doi.org/doi:10.1016/j.psyneuen.2015.12.021
Reference: PNEC 3161

To appear in:

Received date: 25-8-2015
Revised date: 23-11-2015
Accepted date: 17-12-2015

Please cite this article as: Stephens, Mary Ann C., Mahon, Pamela B., McCaul, Mary E., Wand, Gary S., Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones.Psychoneuroendocrinology http://dx.doi.org/10.1016/j.psyneuen.2015.12.021

Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones

Mary Ann C. Stephens * [1], Pamela B. Mahon * [2], Mary E. McCaul [1], and Gary S. Wand ** [1,3]
The Johns Hopkins University School of Medicine
Baltimore, Maryland USA 21205

[1] Department of Psychiatry and Behavioral Sciences
[2] Mood Disorders Center, Department of Psychiatry and Behavioral Sciences
[3] Department of Medicine
* Joint first authors
** Senior author

Mary Ann C. Stephens, Ph.D.
550 N. Broadway, Suite 115
Baltimore, MD 21205 USA
+1-410-955-9525
+1-410-614-7003 (fax)
mchutua1@jhmi.edu

Pamela B. Mahon, Ph.D.
600 N. Wolfe St, Phipps 300
Baltimore, MD 21287 USA
+1-410-955-1382
+1-410-614-3676 (fax)
pbelmon2@jhmi.edu

Mary E. McCaul, Ph.D.
550 N. Broadway, Suite 115
Baltimore, MD 21205 USA
+1-410-955-9526
+1-410-614-7003 (fax)
betsymc@jhmi.edu

Gary S. Wand, M.D.
720 Rutland Ave.
Ross Building, Rm 863
Baltimore, MD 21205 USA
+1-410-614-0056
+1-410-955-0841 (fax)
gwand@jhmi.edu

HIGHLIGHTS

- Healthy young men and women (in the follicular phase of the menstrual cycle) completed the Trier Social Stress Test.
- Post-stress hypothalamic-pituitary-adrenal (HPA) axis hormone responses were greater in men than women.
- Pre-stress testosterone levels were negatively associated with post-stress salivary cortisol response in men.
- Pre-stress progesterone levels were negatively associated with post-stress ACTH and cortisol responses in women.
- Pre-stress progesterone in men and estradiol in women were not associated with post-stress HPA axis response.

Summary

Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis influences the risk for developing stress-related disorders. Sex-dependent differences in the HPA axis stress response are believed to contribute to the different prevalence rates of stress-related disorders found in men and women. However, studies examining the HPA axis stress response have shown mixed support for sex differences, and the role of endogenous sex hormones on HPA axis response has not been adequately examined in humans. This study utilized the largest sample size to date to analyze the effects of biological sex and sex hormones on HPA axis social stress responses. Healthy, 18- to 30- year-old community volunteers (N=282) completed the Trier Social Stress Test (TSST), a widely used and well-validated stress-induction laboratory procedure. All women (n=135) were tested during the follicular phase of their menstrual cycle (when progesterone levels are most similar to men). Adrenocorticotropic hormone (ACTH) and cortisol measures were collected at multiple points throughout pre- and post-TSST. Testosterone and progesterone (in men) and progesterone and estradiol (in women) were determined pre-TSST. Following the TSST, men had greater ACTH and cortisol levels than women. Men had steeper baseline-to-peak and peak-to-end ACTH and cortisol response slopes than women; there was a trend for more cortisol responders among men than

women. Testosterone negatively correlated with salivary cortisol response in men, while progesterone negatively correlated with ACTH and cortisol responses in women. These data confirm that men show more robust activation of the HPA axis response to the TSST than do women in the follicular phase of the menstrual cycle. Testosterone results suggest an inhibitory effect on HPA axis reactivity in men. Progesterone results suggest an inhibitory effect on HPA axis reactivity in women. Future work is needed to explain why men mount a greater ACTH and cortisol response to the TSST than do women during the follicular phase of the menstrual cycle.

Keywords: Cortisol; Progesterone; Testosterone; TSST; Stress; Sex Differences

1. Introduction

Stress-related psychiatric syndromes, such as anxiety, depression, and substance use disorders, are believed to share common biological mechanisms that include dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Girdler et al., 2012; Petrowski et al., 2010; Stephens and Wand, 2012; Young et al., 2004). The HPA axis is a major component of the neuroendocrine system that is activated in response to stressors to help restore homeostasis. Corticotropin releasing factor and arginine vasopressin are released from the paraventricular nucleus of the hypothalamus and control the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary which, in turn, stimulates the adrenal cortex to secrete glucocorticoid hormones, mainly cortisol in humans.

The HPA axis hormonal response to psychosocial stress appears to be moderated by sex. A series of studies with small to moderate sample sizes have documented sex-moderating effects in response to the Trier Social Stress Test (TSST), a well-validated, standardized protocol that induces psychosocial stress

(Kirschbaum et al., 1993). Cumulative evidence over the past 16 years generally shows that men have greater HPA axis activation in response to the TSST than women (see Foley and Kirschbaum, 2010 and Allen et al., 2014 for review); however, these differences may depend on the menstrual phase in which women are tested and on whether free (salivary) or total (blood) cortisol is measured (e.g., Childs et al., 2010a; Duchesne et al., 2012; Kirschbaum et al., 1999). Sex differences in TSST responses have not yet been confirmed within a single, well-powered study.

Sex-related differences in HPA axis stress reactivity may contribute to differences in vulnerability toward specific disorders between men and women. This view is supported by research showing that the prevalence of mood disorders (such as anxiety, major depressive and post-traumatic stress disorders) is almost twice as common in women as men whereas substance use disorders is twice as common in men as women (Compton et al., 2007; Girgus and Yang, 2015; Tolin and Foa, 2006). Further, HPA axis response to psychosocial stressors has been shown to differ between men and women, particularly in depressive disorders (Bagley et al., 2011; Chopra et al., 2009). The mechanisms involved in regulating HPA axis stress responses, how they differ in men compared with women, and how they are associated with or protective in developing stress-related pathologies remain unclear. It is important to elucidate these mechanisms to facilitate and inform prevention and treatment efforts.

Preliminary research supports the potential role of sex hormones in determining the sex-related HPA axis stress response. In men, acute doses of progesterone suppressed cortisol response to the TSST (Childs et al., 2010b). Recently, Juster et al. (2016) reported that sex differences in cortisol response to the TSST were attenuated when analyses were adjusted for baseline progesterone, testosterone and estradiol levels. Although their sample of women was large (n=144), it comprised a group with heterogeneous hormonal status (normal cycling, post-menopausal, or on hormonal contraceptives), many of whom had current or past psychiatric histories. Only two previous studies examined healthy young adults and considered pre-TSST endogenous levels of sex hormones; these studies found no effect on

subsequent cortisol response to the TSST in follicular phase women (n=14; Altemus et al., 2001), men (n=23) and luteal phase women (n=18; Schoofs and Wolf, 2011). The small sample sizes in the latter two studies, and confounds within the recent larger study, precluded drawing any definitive conclusions about the effects of endogenous circulating sex hormones on HPA axis reactivity. Thus, using the TSST in the largest sample of healthy individuals to date, the current study sought to confirm whether the HPA axis response differs by sex, and examine the extent to which pre-TSST sex hormones relate to the magnitude of the HPA axis response.

Whereas many of the earlier TSST studies examined menstrual phase as a surrogate marker of hormonal influence, we examined the effect of endogenous circulating levels of testosterone and progesterone (in men) and progesterone and estradiol (in women) at the onset of the stressor. We tested women during the follicular phase of the menstrual cycle, when progesterone levels are most comparable to men (Schumacher et al. 2014). The study objectives were to (1) compare the HPA axis responses in young men and women in greater detail than previously completed, and (2) determine the effects of circulating sex hormone levels on HPA axis responsivity. First, we hypothesized that men will have a greater ACTH and cortisol response than women to the TSST. Based on extensive preclinical literature and preliminary human studies, we also hypothesized that sex hormone levels at the onset of stress will negatively correlate with HPA axis hormone response in both men and women (Handa and Weiser, 2014; Juster et al., 2016).

2. Methods

2.1. Recruitment

We recruited healthy adults, aged 18–30 years, to participate in a stress response study through newspaper advertisements and posted flyers throughout the Baltimore metropolitan region. Initial

screening was done by telephone and then in person at the Johns Hopkins University School of Medicine (JHU). Participants gave written informed consent after complete description of the study. The study was approved by the JHU School of Medicine Institutional Review Board. Participant assessments included a medical history, physical examination, blood chemistry profile, complete blood count, alcohol breathalyzer test, and urine toxicology screen. DSM-IV axis I psychiatric diagnoses were determined by a Master's level interviewer administering The Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-II; Bucholz et al., 1994). The SSAGA-II is a comprehensive research diagnostic assessment designed to obtain a detailed history of current and past psychiatric disorders. Developed for the Collaborative Study on the Genetics of Alcoholism (COGA; Begleiter et al., 1995), it is specifically designed to differentiate commonly occurring co-morbid conditions by identifying the ages of onset and recency of diagnoses, and by distinguishing symptoms due to alcohol and drug use from those observed in affective, conduct, or antisocial personality disorders. The SSAGA-II has been shown to have good intra- and inter-rater reliability and validity in clinical as well as general population samples (Bucholz et al., 1994, 2006; Hesselbrock et al., 1999).

Exclusion criteria included: (a) current medical conditions and/or use of prescription medications, (b) diagnosis of any DSM-IV Axis I disorder, (c) use of any psychoactive medication within the past 30 days, (d) treatment in the last 6 months with antidepressants, neuroleptics, sedative hypnotics, glucocorticoids, appetite suppressants, estrogens, opiates, or dopamine medications, (e) seizure disorder or history of closed head trauma, (f) self-reported drinking of more than 30 drinks per month in women or 60 drinks per month in men, (g) drug-positive urine screens at intake or on procedure day, (h) nicotine dependence measured by the Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991), and (i) pregnancy, or use of hormonal birth control methods in women.

Participants in this study were included in an earlier report that examined the association of cortisol responses to the TSST with allelic variants in the CRHR1 and FKBP5 genes (Mahon et al., 2013). A

subset of these participants was also analyzed for sex differences in HPA axis reactivity in an earlier report (Uhart et al., 2006). For this study, we included only women with serum progesterone levels <3 ng/mL obtained on the day of the TSST session.

2.2. Psychometric instruments at intake assessment

Upon eligibility assessment, participants completed the Trait component of the State–Trait Anxiety Inventory (STAI) which contains 20 items that are scored from 1 (not at all) and 4 (very much so), and yields a total summary score ranging from 20 from 80. Higher scores on the STAI-Trait scale indicates greater tendency to respond to stressful circumstances with increased anxiety (Spielberger et al., 1983). Participants also completed the Life Experiences Survey (LES), which lists 47 non-traumatic major life events (Sarason et al., 1978). For each event that the participant had experienced in the last 12 months, the participant rated the event as (a) whether they viewed it as being positive or negative, and (b) the perceived impact of the event on their life at the time of the occurrence. Ratings range from either extremely negative (-3) to extremely positive (+3). The sum of the impact ratings for positive events provides a positive impact score and the sum of the impact ratings for negative events provides a negative impact score. Adding the two values provides a total change score.

2.3. Session Procedures

All participants underwent a modified version of the TSST (Kirschbaum et al., 1993) as described in Mahon et al. (2013). Women were scheduled to complete the TSST within 14 days after onset of menses. Prior to the psychosocial stress session, participants were instructed to fast beginning the night before and to refrain from the use of any alcohol, illicit drugs, or over the counter medications for 48 hrs. prior to the session. Participants reported to the study room at 1000 hrs. A toxicology screen was performed and participants were disqualified if positive. Women also submitted to urine pregnancy

testing and were discontinued if positive. Between 1000 and 1015 hrs., participants had an intravenous catheter inserted into a forearm vein, and consumed a calorie-controlled breakfast to reduce cortisol response variability due to differences in food intake (Foley and Kirschbaum, 2010). Participants then sat in a quiet room until testing began. Serum progesterone levels that were obtained prior to the TSST were used to confirm that women were tested during the follicular phase.

2.3.1. Trier Social Stress Test (TSST)

The TSST began at 1330 hrs. Participants were given audio-taped instructions for the TSST speech and mental arithmetic performance tasks. Participants were told they would be taking on the role of a job applicant for the position of hospital administrator and must deliver a 5-min speech that will convince a panel of interviewers they were the best candidate for the job. They were also told they would perform a 5-min oral mental arithmetic challenge that would be judged on speed and accuracy. They were given 10 min to mentally prepare for the tasks, and then escorted to another room. Participants were instructed to stand at one end of a long table with two interviewers sitting at the other end. One interviewer asked the participant to describe his/her qualifications for the job, while the other operated a video camera. Participants were expected to utilize the entire 5 min for the speech and were prompted as needed by the interviewers. For the mental arithmetic task, participants were told to repeat a four-digit number after the interviewer, subtract 13 from it, and speak their answer aloud. The participant was asked to start again if they made a mistake. After the tasks, participants were escorted back to the study room and asked to sit quietly.

2.3.2. Passive TSST

Approximately one week before the active TSST session, each participant underwent a “passive” TSST session. The purpose of this session was to acclimate participants to the intravenous line and session

procedures and to minimize the possibility that baseline cortisol levels prior to the TSST would be elevated from exposure to a novel environment. The passive session procedures were identical to the TSST session except the TSST procedure was omitted (data not shown).

2.3.3. Subjective Anxiety Measure

Participants completed the STAI-State scale which measures transitory subjective feelings of tension, apprehension, nervousness, and worry. The questionnaire contains 20 items that are scored from 1 (not at all) and 4 (very much so). The total sum scores range from 20 to 80, with higher scores indicating greater anxiety (Spielberger et al., 1983). Participants completed the STAI-State immediately pre- and post-TSST.

2.4. Hormone collection and assays

Saliva and blood samples were obtained 30, 15 and 1 min pre-TSST (Time 0), and at 25 min (immediately after the arithmetic task), 40, 55, 70, and 85 min post Time 0. Salivettes were used to collect saliva for cortisol measurement. Salivary and plasma cortisol, and plasma ACTH were assayed at all of the time points indicated above. Plasma testosterone, progesterone and estradiol were assayed at 30, 15, and 1 min pre-TSST. All assays were performed in our laboratory by RIA (Diagnostics Product Co., Los Angeles, CA) using a model 1470 counter (PerkinElmer, Shelton, CT). The inter- and intra-assay coefficients of variation for all assays are less than 10%. For the study sample, values for salivary and serum cortisol were positively correlated at 0.79 for peak change from baseline and 0.85 for area under the curve (AUCi; see below).

2.5. Outcome Measures

2.5.1.HPA-Axis Hormone Response

Baseline HPA axis hormone levels were calculated as the mean of the three baseline blood draws 30, 15 and 1 min before the start of the TSST. Our primary outcomes were post-TSST ACTH, and salivary and cortisol levels at each time point. Additional outcomes included four response components each for ACTH, salivary cortisol, and plasma cortisol: (1) Peak change from baseline ("change"), defined as the highest level reached after completing the TSST, minus baseline.; (2) AUCi, calculated by trapezoidal approximation with respect to the increase from the baseline value (Pruessner et al. 2003); (3) Baseline-to-peak ascending slope; and (4) Peak-to-end descending slope of the response curve. Thus, a total of 12 HPA-axis curve components were examined.

2.5.2.Cortisol Responders

Not all individuals mount an ACTH and cortisol response to the TSST. This finding identified a need to establish a hormone response that was above the level of normal pulsatile cortisol secretory activity that would distinguish responders from non-responders. We applied the criteria of Miller et al. (2013), where a threshold response value of >15.5% fulfilled the objective of separating responders from non-responders.

2.5.3.Pre-TSST Sex Hormone Levels

The baseline sex hormone values used in the analyses were the means of the three baseline blood draws 30, 15 and 1 min before the start of the TSST.

2.6. Statistical Methods

Demographic and clinical characteristics of interest were tested for sex differences using *t*-tests for continuous variables and χ^2 tests for categorical variables. Our primary analysis was a repeated measures analysis, testing for differences in response by gender across the time points using methods described in

Uhart et al (2006). Briefly, the HPA-axis hormone measure at each time point after the TSST was treated as the outcome in a generalized linear model, using generalized estimating equations (GEE) to take into account the within-individual correlation residuals arising from repeated measurements for each individual. The model included a contrast for gender difference, an indicator variable for each time point, terms for the interaction between gender and each time point and was adjusted for baseline hormone level prior to the TSST. An autoregressive correlation structure was specified and robust variance estimation was used.

When the repeated measures analysis showed evidence of a difference in response over time by gender, we additionally performed tests of 12 HPA-axis curve components by gender and for correlation between the HPA-axis curve components and sex hormones. Examination of normal quantile plots showed non-normality of residuals for the association between HPA-axis curve components and gender, as well as for the correlations between HPA-axis curve components and sex hormones. Transforming the variables did not resolve this issue. Therefore, nonparametric methods were employed. We used Wilcoxon-Mann-Whitney tests to examine differences in the distributions of the 12 HPA-axis curve components by gender. For proportion of cortisol responders, we used χ^2 to test for sex differences. For correlations between sex hormones (progesterone and testosterone in men and progesterone and estradiol in women) and each of the 12 HPA-axis curve components, we used Spearman's rank correlations. All analyses were conducted in STATA, version 13.0.

ACTH was available in 134 men and 114 women, salivary cortisol in 99 men and 112 women, and serum cortisol in 142 men and 132 women. Based on the overlapping subsets, most measures of the HPA axis curve components (i.e., ACTH, and salivary and serum cortisol; AUCi, peak change, and baseline-to-peak ascending slopes) were positively correlated at 0.6 or better; the peak-to-end descending slopes were negatively correlated with the other measures at -0.46 or better. Sex hormones measured in men were moderately correlated (progesterone and testosterone; rho=0.35, p<0.0001), as were sex hormones

measured in women (progesterone and estradiol; rho=0.35, p<0.0001). Using matSpD (http://neurogenetics.qimrberghofer.edu.au/matSpD/), we determined the 12 HPA-axis curve components constituted 4 effectively independent tests. Therefore, we considered our threshold for significance to be p=0.0125 (p=0.05/4).

3. **Results**

3.1. *Sample Characteristics*

A total of 282 participants were included in this study. Demographic and baseline rating scale measures for the total study sample, and for men versus women are shown in Table 1. Since trait anxiety, negative life experiences, and race have been associated with differences in HPA-axis response in previous studies (e.g., Armbruster et al., 2011; Hostinar et al., 2014; Jezova et al., 2004), we explored the impact of these characteristics on our data. Women were less likely to be Caucasian (χ^2 =5.10, p=0.024), reported significantly greater negative life experiences (z=2.14, p=0.0332) and scored higher on STAI-Trait anxiety (z=2.0, p=0.0322) compared with men. We found no correlation between these demographic variables and any of the HPA axis and sex hormone measures (all p>0.20), thus, we report unadjusted analyses.

3.2. *Sex Differences in Response to TSST*

3.2.1.*HPA Axis Stress Hormones*

Repeated measures analysis showed differences in response over time by sex, with men having greater response to the TSST on ACTH (χ^2=31.26, p<0.0001), serum cortisol (χ^2=55.04, p<0.0001) and salivary cortisol (χ^2=23.86, p=0.0002) measures. Post-TSST time points with significant interactions with sex occurred at 25 min for ACTH; 25 and 40 min for salivary cortisol; and 25, 40, and 55 min for serum cortisol (all p<0.05; Figure 1). With respect to the HPA axis curve components, men had greater change and AUCi responses than women for ACTH (Change: $Median_{men}$=13.4, $Median_{women}$=5.3, z=4.31, p<0.0001;

AUC: $Median_{men}$=371.5, $Median_{women}$=145.0, z=3.34, p=0.0008), salivary cortisol (Change: $Median_{men}$=0.21, $Median_{women}$=0.09, z=2.79, p=0.0053; AUC: $Median_{men}$=6.32, $Median_{women}$=2.29, z=3.15 p=0.0016), and serum cortisol (Change: $Median_{men}$=7.1, $Median_{women}$=4.9, z=3.30 p=0.001; AUC: $Median_{men}$=270.1, $Median_{women}$=161.0, z_{AUC}=3.14 p_{AUC}=0.0017). Also, men compared with women had steeper ascending and descending response slopes for ACTH and salivary and serum cortisol (all p<0.0039). We then plotted the frequency of participants who experienced their peak response at each time point. There was a trend for men to have an earlier time to peak than women on salivary (t_{280}=-1.85, p=0.0658) but not serum cortisol or ACTH (data not shown).

There was a trend for more men to be cortisol responders than women (salivary: 81.8% vs 67.9%, respectively, χ^2 =5.38, p=0.02; serum: 87.3% vs 78.0%, respectively, χ^2 =4.16, p=0.04). When comparing responders and non-responders, responders were more likely to be older (t_{272}=-4.55, p<0.0001); there were no differences between responders and non-responders by race, STAI Trait or State anxiety, or LES scores. Females responders had lower median progesterone levels than female non-responders (χ^2=6.84, p=0.009). There were no other differences in sex hormone levels between responders and non-responders.

3.2.2.STAI-State Anxiety

Pre-TSST mean (SD) scores for men and women were 44.3 (5.3) and 43.1 (5.8), respectively (ns). Mean change in score from pre- to post-TSST for men and women were -1.8 (3.7) and -2.4 (4.4), respectively (ns).

3.3. *Pre-TSST Sex Hormones and HPA Axis Stress Hormone Responses*

Table 2 shows the Spearman's rho and p-values for correlations between baseline sex hormones and HPA axis response. In men, the mean (SD) and range of pre-TSST testosterone levels were 489 (159) and 118-1033 ng/mL; rank order for pre-TSST testosterone and rank order salivary cortisol response were negatively correlated (ρ_{change}=-0.30, p_{change}=0.0027; ρ_{AUC}=-0.26, p_{AUC}=0.0093). The mean (SD) and range of pre-TSST progesterone levels were 1.0 (0.3) and 0.38-2.1 ng/mL; there were no rank-order associations between pre-TSST progesterone and HPA-axis hormone response in men. In women, the mean (SD) and range of pre-TSST progesterone was 0.8 (0.4) and 0.21-2.83 ng/mL; rank order for pre-TSST progesterone was negatively correlated with rank order serum cortisol response (ρ_{change}=-0.26, p_{change}=0.0027; ρ_{AUC}=-0.30, p_{AUC}=0.0006). The mean (SD) and range of pre-TSST estradiol was 42 (35) and 6.7-193.7 ng/mL; in contrast with progesterone, there were no rank-order associations between pre-TSST estradiol and HPA-axis hormone response in women.

4. Discussion

This study assessed whether healthy young men and women in the follicular phase of the menstrual cycle differ in HPA axis hormone response to a standardized psychosocial stressor, and whether sex hormones are associated with the HPA axis response. Our results confirmed that men and women differ in HPA axis reactivity to stress, with men showing a greater stress hormone response than women. These findings concur with those previously observed in several smaller studies (Childs et al., 2010a; Hidalgo et al., 2014; Kirschbaum et al., 1999; Uhart et al., 2006). We also found that circulating sex hormones may be associated with the magnitude of subsequent cortisol stress response in a sex-dependent manner. To our knowledge, this is the first study to examine associations between pre-TSST sex hormones and HPA axis response in a sample of healthy young men and women in the follicular phase of the menstrual cycle.

The large sample size of the current study enhanced power not only to detect differences in HPA axis hormone output with both salivary and serum cortisol, but also allowed us to compare additional dynamic aspects of HPA axis hormone response, thereby extending the literature on sex differences in acute stress responses. Upon deconstruction of the stress hormone response curve, men showed steeper ascending and descending slopes of ACTH and cortisol levels than women. Further, there was a trend for an earlier peak response in men than in women ($p=0.065$), reflecting more rapid onset and deactivation of the HPA axis. The physiological significance of this minor sex difference in rapid onset and termination of the HPA axis response remains uncertain. However, rapid activation of the HPA axis (ascending slope) generally signifies a healthy and competitive response to stress. To restore homeostasis, circulating glucocorticoids must act rapidly to mobilize energy stores and alter a number of stress related behaviors (Papadimitriou and Priftis, 2009). Likewise, a rapid decline from peak response back to baseline (descending slope) following termination of stress also indicates a healthy stress hormone profile that is maintained by sensitive glucocorticoid-mediated negative feedback circuits (Sapolsky et al., 2000). Faster and greater mounted cortisol response in men may predict resistance to habituation upon repeated exposures to stress (Kirschbaum et al., 1995). This resistant may have once served an adaptive purpose by mobilizing energy stores needed to physically escape future stressors. Rapid mobilization of energy may have benefits in the short term, however, long term exposure to stress with prolonged exposure to cortisol may increase vulnerability toward developing stress-related pathologies.

On the other hand, women had an attenuated HPA axis response compared to men resulting in less exposure to stress-induced cortisol. One plausible explanation for this difference is that women may be less sensitive to the hormonal activation effects of the TSST. For example, cortisol response in women compared with men has been found to be greater during social rejection tasks but lower during achievement tasks (such as the TSST; Stroud et al., 2002). Another explanation is that exposure to negative life experiences may chronically activate the HPA axis (Kudielka and Wust, 2010), and when coupled with

elevated trait anxiety (STAI-Trait measure), may result in a blunted cortisol response to acute stress due to allostatic changes in the HPA axis (Jezova et al., 2004; McEwen, 2007). However, this explanation seems less likely since women in our sample had just slightly elevated LES negative experiences scores (7.2) compared with normative data from college men and women (4.7 and 5.6, respectively; Sarason et al. 1978), and lower STAI-Trait scores (30.4) compared with normative data from working men and women (35.6 and 36.2, respectively; Spielberger et al., 1983). Further, we found no associations between STAI-Trait or LES scores and stress hormone responses.

In contrast to the increase in biological stress markers, the TSST did not increase subjective anxiety overall as measured with the STAI-State scale. It is possible that participants did not find the TSST subjectively stressful. Alternatively, we may have captured anticipatory anxiety which then subsided after termination of stress. von Dawans et al. (2011) showed that STAI-State scores peaked from 30 to 10 minutes before TSST administration and began decreasing after the TSST was completed. Indeed, the items on the STAI-State reflect subjective feelings of apprehension, tension, nervousness, worry and activation/arousal of the autonomous nervous system; these may be transient feelings that likely abate once the stressor is removed. Since we measured only one pre-TSST time point, the reason for this finding remains unclear.

With respect to stress and sex hormone relationships, we report the novel and important finding that pre-stress levels of endogenous sex hormones may modestly impact HPA axis reactivity and may do so in a sexually dimorphic manner. Preclinical work has shown that androgens and estrogens influence HPA function, in part, by modulating adrenal (Kitay, 1965), anterior pituitary (Coyne and Kitay, 1969, 1971; Viau and Meaney, 2004) and hypothalamic functions (Viau and Meaney, 2004), although the mechanisms by which these gonadal hormones influence HPA function are not yet known (Handa and Weiser, 2014). First, we report the novel finding that testosterone levels in men were negatively correlated with salivary cortisol response to the TSST. While Juster et al. (2016) reported no correlation between pre-TSST

testosterone and post-TSST cortisol, their sample size of men was smaller (n=60) suggesting that the study may have been underpowered. The correlations we observed were -0.3 and -0.26 which, with a sample size of 147 men, is considered to be in the moderate range. Our findings are consistent with animal models showing that gonadectomy causes a more robust corticosterone response to stress in male rodents, induced by increased CRH expression and decreased corticosteroid-binding globulin levels (Seale et al., 2004a; Viau and Meaney, 2004). Moreover, this disinhibition of the corticosterone response is reversible with testosterone replacement (Seale et al., 2004b; Viau and Meaney, 2004). In humans, Bedgood and colleagues (2014) who reported that pre-stress cortisol levels negatively correlated with post-TSST testosterone peak response. Thus, our study supports and further extends the literature in humans on the inverse relationships of testosterone and cortisol in response to stress.

We also predicted an inverse relationship between pre-stress progesterone levels and HPA axis hormone response. This relationship was observed with the serum cortisol measurement among women, with correlations in the moderate range as well (-0.26 and -0.30). Similarly, we observed that responders had lower median progesterone levels than non-responders among the women but not men. The main source of progesterone for men and women in the follicular phase comes from the adrenal gland which is regulated by ACTH and produces comparable levels between men and women (Schumacher et al., 2014). Progesterone is believed to modulate the adaptive response to stress, mainly through the effect of its neurosteroid metabolite allopregnanolone on GABAA receptor activity (Wirth 2011). Indeed, progesterone administration dampens psychological and cortisol responses to stress in both men and women (Childs et al., 2010b; Soderpalm et al., 2004). Thus, we did not anticipate these divergent effects of progesterone by sex. On the other hand, stress increases progesterone levels that strongly correlate with stress-induced cortisol levels in men, but not in cycling women (Wirth et al. 2007). Presumably, this stress-induced progesterone release serves to deactivate cortisol response. This homeostatic mechanism might override any association that pre-TSST progesterone has with post-TSST cortisol levels in men. It is

important to note, however, that Juster et al. (2016) found a negative correlation in men between progesterone and cortisol response just as we had with women. The men in that study differed from the present study in that they were generally older (average age, 39) and more diverse in drug use and mental health history. Thus, the mechanisms through which progesterone may moderate sex differences requires further study and should consider the impact of other sex-related hormones or factors that we did not measure, such as sex hormone binding globulins as well as neuroactive metabolites of progesterone.

Finally, contrary to our predictions, no significant correlations were detected between estradiol levels and HPA axis response in follicular phase women. We based our hypothesis on preclinical work demonstrating that estradiol inhibits HPA axis reactivity, and also stimulates the production of CBG and lowers unbound cortisol levels (Dayas et al., 2000; Minni et al., 2014). Also, some TSST studies showed that healthy women using oral contraceptives, which contain high levels of estradiol, had cortisol responses lower than men and similar to follicular women (e.g, Cornelisse et al., 2011; Kirschbaum et al., 1999; but not Klumbies et al., 2014). Given this evidence, the lack of association in this study was somewhat surprising. With respect estradiol, the main difference in this and other studies is that oral contraceptives effectively maintained high steady levels of this hormone. In contrast, natural dynamic changes of estradiol occurred in the present study. During the early follicular phase, levels start low and relatively stable, then begin to rise 5- to 8-fold until levels peak at ovulation. Since we did not target a period of relatively stable estradiol levels (e.g., early follicular phase) and tested women throughout the 2-week follicular phase, estradiol concentrations consequently varied widely from 6.7 to 193.7 ng/mL. It may be difficult to capture associations with subsequent cortisol response when estradiol is measured during these rapidly changing levels. This idea is supported by the recent findings of Juster et al. (2016) who reported that cortisol stress responses were negatively associated with baseline estradiol levels but the finding was driven primarily by women on oral contraceptives. Repeated measures of estradiol in

response to the TSST may help determine more precise interaction between estradiol and cortisol stress response.

The findings from this study, with a sample size much larger than previous studies, provides more definitive evidence that men show greater HPA axis reactivity to acute psychosocial stress than women in the follicular phase of the menstrual cycle. It is important to note that not all studies comparing men and women have observed sex differences in cortisol response to the TSST, although the results generally lean towards men showing a greater response than women. Reasons for the negative findings remain unclear. To that end, Table 3 summarizes 22 TSST experiments in healthy young men and women within similar age range to the present study. Sex differences were more likely observed with salivary cortisol, which measures unbound free cortisol (about 10% of total cortisol), than with blood, which measures bound and unbound (total) cortisol. Indeed, Allen et al. (2014) posit that the mixed findings with respect to sex differences may be attributed to salivary versus serum cortisol assessment; Foley and Kirschbaum (2010) argue that acute stress effects of the TSST is best characterized by using salivary cortisol. In our large study, sex differences were evident with both salivary and serum cortisol measures. However, despite a strong correlation between salivary and serum cortisol in our study, cortisol levels in both fluids did not always correlate with sex hormone levels. The reason for this is unclear.

Another contributor to the mixed findings in the literature is that a large number of studies in Table 3 either did not examine HPA axis responses by specific menstrual phase or based menstrual phase on participant self-report (Duchesne et al., 2012; Hidalgo et al., 2014; Villada et al., 2014), which is imprecise confirmation of menstrual cycle phase (Wideman et al., 2012). Unlike the follicular phase, which is characterized by relatively stable progesterone and rising estradiol levels, the luteal phase is characterized by widely fluctuating levels of both female sex hormones. These differences in hormonal environment may influence HPA axis reactivity in ways that are not yet clear. For example, studies comparing men and women in the luteal versus follicular phases on cortisol reactivity have been mixed:

Luteal phase women have responded to the TSST similarly to men in some (Kirschbaum et al. 1999; Kudielka et al. 2004; Rohleder et al. 2001) but not all (Childs et al. 2010a; Schoofs et al. 2011) studies, and they have responded with greater (Espin et al. 2013; Kirschbaum et al. 1999), similar (Childs et al. 2010a) or reduced (Maki et al. 2015) cortisol compared with women in the follicular phase. Accurate determination of menstrual phase could further clarify the conditions under which men and women differ in stress response. Nevertheless, cortisol reactivity may be determined partly by sex and further modified by hormone levels across the menstrual cycle. Therefore, future research should include biological confirmation of sex hormone levels and their potential impact when examining HPA axis function relative to sex and differences in normal cycling women.

There are caveats regarding our findings on endogenous sex hormones and HPA axis stress response. First, the relationship between pre-TSST sex hormone levels and subsequent ACTH and cortisol responses is modest and the physiological significance of this finding is yet to be determined. Second, the TSST can induce sex hormone secretion (e.g., Lennartsson et al., 2012; Childs et al. 2010a), which has a dynamic regulatory negative feedback effect on the HPA axis, thus a stronger relationship between ACTH/cortisol and sex hormones may have been found if we examined sex hormone responses during the TSST. Third, bioavailability of testosterone and estradiol is regulated by sex hormone binding globulin (SHBG) and progesterone by CBG. Future work in this area could measure these carrier proteins to determine their effect on and from stress, as well as inform interpretation and physiological significance of study outcomes. Finally, our sex comparison is only valid for women in the follicular phase of the menstrual cycle where progesterone levels are similar to levels in men but considerably less than levels during the luteal phase of the cycle.

5. Conclusions

The mechanisms linking stress to disease states in humans are not fully understood, but stress-induced glucocorticoid changes are recognized as having a major influence. We were able to confirm that findings from several small studies that HPA axis response to stress were sex-dependent. Fine-grained analyses of the cortisol curve components allowed us to determine that response dynamics were not similar between men and women in the follicular phase of the menstrual cycle and, as a consequence, men are exposed to higher levels of cortisol during the TSST. Our findings are consistent with the observation that men and women have a different risk for certain diseases. Women are more likely to develop autoimmune diseases (McCarty et al., 1995) and disorders that have been linked to underactivity of the HPA axis such as PTSD and panic disorder (e.g., Petrowski et al., 2010), whereas men are more likely develop diseases such as diabetes mellitus and cardiovascular disease that are thought to be secondary to high glucocorticoid allostatic load which results from over activation of the stress system (McEwen, 1998). We also extended the literature by examining whether circulating gonadal steroid levels modified ACTH and cortisol responses to the TSST. Consistent with animal data, pre-stress progesterone in follicular phase women and testosterone in men were inversely related to stress-induced cortisol. Contrary to animal data, pre-stress progesterone in men and estradiol in follicular phase women were not associated with HPA axis response. Thus, the mechanisms that contribute to sex differences in hormone response remain unclear, but progesterone levels may play an important role for women during the follicular phase. Further research is necessary to elucidate the complex interdependent nature of psychological stress, the HPA axis and gonadal steroids and how these relationships may underlie sex differences in stress-linked disorders.

ROLE OF FUNDING

The NIH had no further role in study design; in the collection, analyses and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENT

This research was supported by the National Institutes of Health (NIH): NIMH grant R01MH076951 (GSW), NIAAA grant K05AA020342 (GSW), and NIDA grant R01DA021779 (MEM). We thank the staff of the Integrated Program for Substance Abuse Research for conducting the research procedures

References

Allen, A.P., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., 2014. Biological and psychological markers of stress in humans: Focus on the Trier Social Stress Test. Neuroscience & Biobehavioral Reviews 38, 94-124.

Altemus, M., Redwine, L.S., Leong, Y.M., Frye, C.A., Porges, S.W., Carter, C.S., 2001. Responses to laboratory psychosocial stress in postpartum women. Psychosomatic Medicine 63, 814-821.

Armbruster, D., Mueller, A., Strobel, A., Lesch, K.P., Brocke, B., Kirschbaum, C., 2011. Predicting cortisol stress responses in older individuals: Influence of serotonin receptor 1A gene (HTR1A) and stressful life events. Horm Behav 60, 105-111.

Bagley, S.L., Weaver, T.L., Buchanan, T.W., 2011. Sex differences in physiological and affective responses to stress in remitted depression. Physiol Behav 104, 180-186.

Balodis, I.M., Wynne-Edwards, K.E., Olmstead, M.C., 2010. The other side of the curve: examining the relationship between pre-stressor physiological responses and stress reactivity. Psychoneuroendocrinology 35, 1363-1373.

Bedgood, D., Boggiano, M.M., Turan, B., 2014. Testosterone and social evaluative stress: The moderating role of basal cortisol. Psychoneuroendocrinology 47, 107-115.

Begleiter, H., Reich, T., Hesselbrock, V., Porjesz, B., Li, T., Schuckit, M., Edenberg, H., Rice, J., 1995. The collaborative study on the genetics of alcoholism. Alcohol Health and Research World 19, 228-228.

Bucholz, K.K., Cadoret, R., Cloninger, C.R., Dinwiddie, S.H., Hesselbrock, V.M., Nurnberger, J.I., Jr., Reich, T., Schmidt, I., Schuckit, M.A., 1994. A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. J Stud Alcohol 55, 149-158.

Bucholz, K.K., Nurnberger, J.I., Jr., Kramer, J.R., Hesselbrock, V.M., Schuckit, M.A., Bierut, L.J., 2006. Comparison of psychiatric diagnoses from interview reports with those from best-estimate procedures. Journal of studies on alcohol 67, 157-168.

Childs, E., Dlugos, A., De Wit, H., 2010a. Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. Psychophysiology 47, 550-559.

Childs, E., Van Dam, N.T., de Wit, H., 2010b. Effects of acute progesterone administration upon responses to acute psychosocial stress in men. Exp Clin Psychopharm 18, 78-86.

Chopra, K.K., Ravindran, A., Kennedy, S.H., Mackenzie, B., Matthews, S., Anisman, H., Bagby, R.M., Farvolden, P., Levitan, R.D., 2009. Sex differences in hormonal responses to a social stressor in chronic major depression. Psychoneuroendocrinology 34, 1235-1241.

Compton, W.M., Thomas, Y.F., Stinson, F.S., Grant, B.F., 2007. Prevalence, correlates, disability, and comorbidity of DSM-IV drug abuse and dependence in the United States: results from the national epidemiologic survey on alcohol and related conditions. Archives of general psychiatry 64, 566-576.

Cornelisse, S., van Stegeren, A.H., Joels, M., 2011. Implications of psychosocial stress on memory formation in a typical male versus female student sample. Psychoneuroendocrinology 36, 569-578.

Coyne, M.D., Kitay, J.I., 1969. Effect of Ovariectomy on Pituitary Secretion of ACTH. Endocrinology 85, 1097-1102.

Coyne, M.D., Kitay, J.I., 1971. Effect of Orchiectomy on Pituitary Secretion of ACTH. Endocrinology 89, 1024-1028.

Dayas, C.V., Xu, Y., Buller, K.M., Day, T.A., 2000. Effects of chronic oestrogen replacement on stress-induced activation of hypothalamic-pituitary-adrenal axis control pathways. Journal of Neuroendocrinology 12, 784-794.

Duchesne, A., Tessera, E., Dedovic, K., Engert, V., Pruessner, J.C., 2012. Effects of panel sex composition on the physiological stress responses to psychosocial stress in healthy young men and women. Biol Psychol 89, 99-106.

Espin, L., Almela, M., Hidalgo, V., Villada, C., Salvador, A., Gomez-Amor, J., 2013. Acute pre-learning stress and declarative memory: impact of sex, cortisol response and menstrual cycle phase. Hormones and Behavior 63, 759-765.

Foley, P., Kirschbaum, C., 2010. Human hypothalamus-pituitary-adrenal axis responses to acute psychosocial stress in laboratory settings. Neurosci Biobehav Rev 35, 91-96.

Gaffey, A.E., Wirth, M.M., 2014. Stress, rejection, and hormones: Cortisol and progesterone reactivity to laboratory speech and rejection tasks in women and men. F1000Res 3, 208.

Girdler, S.S., Lindgren, M., Porcu, P., Rubinow, D.R., Johnson, J.L., Morrow, A.L., 2012. A history of depression in women is associated with an altered GABAergic neuroactive steroid profile. Psychoneuroendocrinology 37, 543-553.

Girgus, J.S., Yang, K., 2015. Gender and depression. Current Opinion in Psychology 4, 53-60.

Handa, R.J., Weiser, M.J., 2014. Gonadal steroid hormones and the hypothalamo–pituitary–adrenal axis. Frontiers in Neuroendocrinology 35, 197-220.

Hatzenbuehler, M.L., McLaughlin, K.A., 2014. Structural stigma and hypothalamic-pituitary-adrenocortical axis reactivity in lesbian, gay, and bisexual young adults. Ann Behav Med 47, 39-47.

Heatherton, T.F., Kozlowski, L.T., Frecker, R.C., Fagerstrom, K.O., 1991. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. British journal of addiction 86, 1119-1127.

Hesselbrock, M., Easton, C., Bucholz, K.K., Schuckit, M., Hesselbrock, V., 1999. A validity study of the SSAGA--a comparison with the SCAN. Addiction 94, 1361-1370.

Het, S., Schoofs, D., Rohleder, N., Wolf, O.T., 2012. Stress-induced cortisol level elevations are associated with reduced negative affect after stress: indications for a mood-buffering cortisol effect. Psychosomatic Medicine 74, 23-32.

Hidalgo, V., Almela, M., Villada, C., Salvador, A., 2014. Acute stress impairs recall after interference in older people, but not in young people. Horm Behav 65, 264-272.

Hostinar, C.E., McQuillan, M.T., Mirous, H.J., Grant, K.E., Adam, E.K., 2014. Cortisol responses to a group public speaking task for adolescents: Variations by age, gender, and race. Psychoneuroendocrinology 50, 155-166.

Jezova, D., Makatsori, A., Duncko, R., Moncek, F., Jakubek, M., 2004. High trait anxiety in healthy subjects is associated with low neuroendocrine activity during psychosocial stress. Progress in Neuro-Psychopharmacology and Biological Psychiatry 28, 1331-1336.

Juster, R.-P., Raymond, C., Desrochers, A.B., Bourdon, O., Durand, N., Wan, N., Pruessner, J.C., Lupien, S.J., 2016. Sex hormones adjust “sex-specific” reactive and diurnal cortisol profiles. Psychoneuroendocrinology 63, 282-290.

Kelly, M.M., Tyrka, A.R., Anderson, G.M., Price, L.H., Carpenter, L.L., 2008. Sex differences in emotional and physiological responses to the Trier Social Stress Test. Journal of Behavior Therapy and Experimental Psychiatry 39, 87-98.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosomatic Medicine 61, 154-162.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76-81.

Kirschbaum, C., Prussner, J.C., Stone, A.A., Federenko, I., Gaab, J., Lintz, D., Schommer, N., Hellhammer, D.H., 1995. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. Psychosomatic Medicine 57, 468-474.

Kitay, J.I., 1965. Depression of Adrenal Corticosterone Production in Oophorectomized Rats. Endocrinology 77, 1048-1052.

Klumbies, E., Braeuer, D., Hoyer, J., Kirschbaum, C., 2014. The reaction to social stress in social phobia: discordance between physiological and subjective parameters. PLoS One 9, e105670.

Kudielka, B.M., Buske-Kirschbaum, A., Hellhammer, D.H., Kirschbaum, C., 2004. HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. Psychoneuroendocrinology 29, 83-98.

Kudielka, B.M., Wust, S., 2010. Human models in acute and chronic stress: assessing determinants of individual hypothalamus-pituitary-adrenal axis activity and reactivity. Stress 13, 1-14.

Lennartsson, A.K., Jonsdottir, I.H., 2011. Prolactin in response to acute psychosocial stress in healthy men and women. Psychoneuroendocrinology 36, 1530-1539.

Lennartsson, A.K., Kushnir, M.M., Bergquist, J., Billig, H., Jonsdottir, I.H., 2012. Sex steroid levels temporarily increase in response to acute psychosocial stress in healthy men and women. Int J Psychophysiol 84, 246-253.

Mahon, P.B., Zandi, P.P., Potash, J.B., Nestadt, G., Wand, G.S., 2013. Genetic association of FKBP5 and CRHR1 with cortisol response to acute psychosocial stress in healthy adults. Psychopharmacology (Berl) 227, 231-241.

Maki, P.M., Mordecai, K.L., Rubin, L.H., Sundermann, E., Savarese, A., Eatough, E., Drogos, L., 2015. Menstrual cycle effects on cortisol responsivity and emotional retrieval following a psychosocial stressor. Hormones and Behavior.

Maruyama, Y., Kawano, A., Okamoto, S., Ando, T., Ishitobi, Y., Tanaka, Y., Inoue, A., Imanaga, J., Kanehisa, M., Higuma, H., Ninomiya, T., Tsuru, J., Hanada, H., Akiyoshi, J., 2012. Differences in salivary alpha-amylase and cortisol responsiveness following exposure to electrical stimulation versus the Trier Social Stress Tests. PLoS One 7, e39375.

McCarty, D.J., Manzi, S., Medsger, T.A., Jr., Ramsey-Goldman, R., LaPorte, R.E., Kwoh, C.K., 1995. Incidence of systemic lupus erythematosus. Race and gender differences. Arthritis and rheumatism 38, 1260-1270.

McEwen, B.S., 1998. Protective and damaging effects of stress mediators. The New England journal of medicine 338, 171-179.

McEwen, B.S., 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 87, 873-904.

Miller, R., Plessow, F., Kirschbaum, C., Stalder, T., 2013. Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: evaluation of salivary cortisol pulse detection in panel designs. Psychosomatic Medicine 75, 832-840.

Minni, A.M., de Medeiros, G.F., Helbling, J.C., Duittoz, A., Marissal-Arvy, N., Foury, A., De Smedt-Peyrusse, V., Pallet, V., Moisan, M.P., 2014. Role of corticosteroid binding globulin in emotional reactivity sex differences in mice. Psychoneuroendocrinology 50, 252-263.

Papadimitriou, A., Priftis, K.N., 2009. Regulation of the Hypothalamic-Pituitary-Adrenal Axis. Neuroimmunomodulation 16, 265-271.

Petrowski, K., Herold, U., Joraschky, P., Wittchen, H.U., Kirschbaum, C., 2010. A striking pattern of cortisol non-responsiveness to psychosocial stress in patients with panic disorder with concurrent normal cortisol awakening responses. Psychoneuroendocrinology 35, 414-421.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916-931.

Rohleder, N., Schommer, N.C., Hellhammer, D.H., Engel, R., Kirschbaum, C., 2001. Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. Psychosom Med 63, 966-972.

Sapolsky, R.M., 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57, 925-935.

Sarason, I.G., Johnson, J.H., Siegel, J.M., 1978. Assessing the impact of life changes: development of the Life Experiences Survey. Journal of consulting and clinical psychology 46, 932.

Schoofs, D., Pabst, S., Brand, M., Wolf, O.T., 2013. Working memory is differentially affected by stress in men and women. Behav Brain Res 241, 144-153.

Schoofs, D., Wolf, O.T., 2011. Are salivary gonadal steroid concentrations influenced by acute psychosocial stress? A study using the Trier Social Stress Test (TSST). International Journal of Psychophysiology 80, 36-43.

Schumacher, M., Mattern, C., Ghoumari, A., Oudinet, J.P., Liere, P., Labombarda, F., Sitruk-Ware, R., De Nicola, A.F., Guennoun, R., 2014. Revisiting the roles of progesterone and allopregnanolone in the nervous system: Resurgence of the progesterone receptors. Progress in Neurobiology 113, 6-39.

Seale, J.V., Wood, S.A., Atkinson, H.C., Bate, E., Lightman, S.L., Ingram, C.D., Jessop, D.S., Harbuz, M.S., 2004a. Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. Journal of Neuroendocrinology 16, 516-524.

Seale, J.V., Wood, S.A., Atkinson, H.C., Harbuz, M.S., Lightman, S.L., 2004b. Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-

induced hypothalamic-pituitary-adrenal axis activity of male and female rats. Journal of Neuroendocrinology 16, 989-998.

Shalev, I., Lerer, E., Israel, S., Uzefovsky, F., Gritsenko, I., Mankuta, D., Ebstein, R.P., Kaitz, M., 2009. BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. Psychoneuroendocrinology 34, 382-388.

Soderpalm, A.H., de Wit, H., 2002. Effects of stress and alcohol on subjective state in humans. Alcohol Clin Exp Res 26, 818-826.

Spielberger, C., Gorsuch, R., Lushene, R., Vagg, P., Jacobs, G., 1983. Manual for the state-trait anxiety inventory. Consulting Psychologists' Press, Palo Alto, Calif.

Stephens, M.A., Wand, G., 2012. Stress and the HPA Axis: Role of Glucocorticoids in Alcohol Dependence. Alcohol Res 34, 468-483.

Stroud, L.R., Salovey, P., Epel, E.S., 2002. Sex differences in stress responses: social rejection versus achievement stress. Biological Psychiatry 52, 318-327.

Tolin, D.F., Foa, E.B., 2006. Sex differences in trauma and posttraumatic stress disorder: A quantitative review of 25 years of research. Psychological Bulletin 132, 959-992.

Uhart, M., Chong, R.Y., Oswald, L., Lin, P.I., Wand, G.S., 2006. Gender differences in hypothalamic-pituitary-adrenal (HPA) axis reactivity. Psychoneuroendocrinology 31, 642-652.

Viau, V., Meaney, M.J., 2004. Testosterone-dependent variations in plasma and intrapituitary corticosteroid binding globulin and stress hypothalamic-pituitary-adrenal activity in the male rat. J Endocrinol 181, 223-231.

von Dawans, B., Kirschbaum, C., Heinrichs, M., 2011. The Trier Social Stress Test for Groups (TSST-G): A new research tool for controlled simultaneous social stress exposure in a group format. Psychoneuroendocrinology 36, 514-522.

Villada, C., Hidalgo, V., Almela, M., Salvador, A., 2014. Individual Differences in the Psychobiological Response to Psychosocial Stress (Trier Social Stress Test): The Relevance of Trait Anxiety and Coping Styles. Stress Health.

Wideman, L., Montgomery, M.M., Levine, B.J., Beynnon, B.D., Shultz, S.J., 2012. Accuracy of Calendar-Based Methods for Assigning Menstrual Cycle Phase in Women. Sports Health: A Multidisciplinary Approach, 1941738112469930.

Wirth, M.M., 2011. Beyond the HPA Axis: Progesterone-Derived Neuroactive Steroids in Human Stress and Emotion. Front Endocrinol (Lausanne) 2, 1-14.

Wirth, M.M., Meier, E.A., Fredrickson, B.L., Schultheiss, O.C., 2007. Relationship between salivary cortisol and progesterone levels in humans. Biological Psychology 74, 104-107.

Young, E.A., Abelson, J.L., Cameron, O.G., 2004. Effect of comorbid anxiety disorders on the hypothalamic-pituitary-adrenal axis response to a social stressor in major depression. Biol Psychiatry 56, 113-120.

Youssef, F.F., Dookeeram, K., Basdeo, V., Francis, E., Doman, M., Mamed, D., Maloo, S., Degannes, J., Dobo, L., Ditshotlo, P., Legall, G., 2012. Stress alters personal moral decision making. Psychoneuroendocrinology 37, 491-498.

Figure 1. Mean HPA-axis hormone levels over time for men and women in the follicular phase of the menstrual cycle. Times prior to the task are designated in negative minutes, times after the task as positive. Standard error bars are shown. Total samples sizes were 147 males and 135 females.

* significant interaction with sex ($p<0.05$).

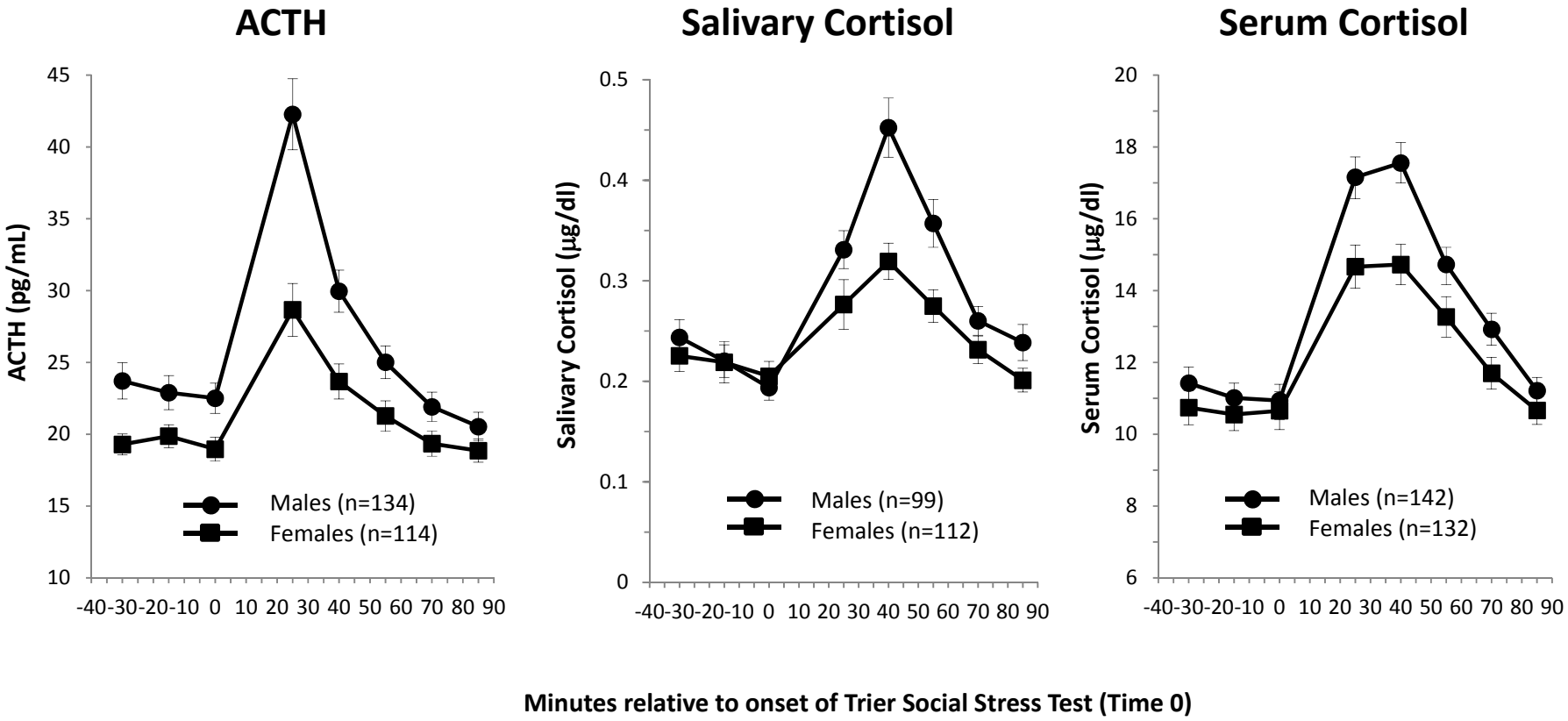

Table 1. Demographic characteristics and rating scales

	Total (N=282)	Men (N=147)	Follicular Women (N=135)	p-value
Age (mean(SD))	23.6 (3.3)	23.6 (3.2)	23.6 (3.3)	0.9898
Race (% Caucasian)	69.9	76.3	64.0	**0.0240**
STAI Trait (mean(SD))	29.5 (6.8)	28.7 (6.4)	30.4 (7.1)	**0.0322**
LES Negative impact score (mean(SD))	6.3 (6.2)	5.6 (5.8)	7.2 (6.5)	**0.0332**
LES Positive impact score (mean(SD))	12.0 (8.2)	11.5 (8.2)	12.6 (8.3)	0.2638
LES Total impact score (mean(SD))	5.7 (9.8)	5.9 (10.0)	5.4 (9.6)	0.6912

STAI: Spielberger State-Trait Anxiety Inventory; LES: Life Experiences Survey

* T-tests were used with continuous variables, chi-square tests with dichotomous variables. Tests significant at the 0.05 threshold are in bold.

Table 2. Correlations between pre-TSST sex hormone levels and post-TSST HPA-axis stress hormone responses in men and women

	ACTH		Salivary Cortisol		Serum Cortisol	
	Change	AUCi	Change	AUCi	Change	AUCi
<u>Men</u>						
Testosterone	-0.12 (0.1798)	-0.10 (0.2390)	**-0.30 (0.0027)**	**-0.26 (0.0093)**	-0.12 (0.1555)	-0.10 (0.2167)
Progesterone	0.01 (0.9154)	-0.04 (0.6732)	-0.14 (0.1602)	-0.12 (0.2343)	-0.05 (0.5417)	-0.06 (0.4941)
<u>Follicular Women</u>						
Estradiol	0.01 (0.9339)	0.05 (0.6207)	0.02 (0.8276)	0.05 (0.6022)	-0.07 (0.4543)	-0.06 (0.4798)
Progesterone	-0.22 (0.0175)	-0.23 (0.0143)	-0.21 (0.0304)	-0.21 (0.0262)	**-0.26 (0.0027)**	**-0.30 (0.0006)**

Shown are Spearman's rho (p-value). Correlations significant at the 0.0125 threshold are in bold.

Change = highest magnitude post-TSST minus the average of the three baseline pre-TSST levels

AUCi = area under the curve, calculated by trapezoidal approximation with respect to the increase from baseline (Pruessner et al. 2003)

Table 3. Comparisons between healthy young men and women on cortisol response to Trier Social Stress Test (TSST).

	Sample Sizes					Cortisol Outcome	
Author	Men	Women				Salivary	Serum/Plasma
		Follicular	Luteal	OCP	Unspecified		
Hidalgo et al., 2014	18	17				M>FW	
Lennartsson and Jonsdottir, 2011	30	15					ns
Uhart et al., 2006 [a]	55	27					M>FW
Villada et al., 2014	18	17				ns	
Childs et al., 2010a	28	29	23			M>FW,LW	M>FW,LW
Duchesne et al., 2012	25	21	19			ns	
Kirschbaum et al., 1999 [a]	20	19	21	21		LW,M>FW,OCP	ns
Kudielka et al., 2004	20		21			ns	ns
Rohleder et al., 2001	27		18			ns	
Schoofs et al., 2011 [b]	39		42			M>LW	
Balodis et al., 2010	29				58	M>W	
Gaffey and Wirth, 2014	70				61	ns	
Hatzenbuehler and McLaughlin, 2014	34				40	M>W	
Het et al., 2012 [b]	148				84	M>W	
Kelly et al., 2008	30				32		ns
Maruyama et al., 2012	92				57	ns	
Schoofs et al., 2013 [b] (experiment 1)	27				26	M>W	
Schoofs et al., 2013 [b] (experiment 2)	64				40	ns	
Shalev et al., 2009	46				51	M>W	
Youssef et al., 2012	30				35	ns	
Cornelisse et al., 2011	23			39	15	M>W,OCP	
Klumbies et al., 2014	41			16	21	ns	ns

M: Men; W: Women; FW: Women in the follicular phase; LW: Women in the luteal phase; OCP: oral contraceptive pills

[a] ACTH also examined; men had greater stress-induced ACTH response than women

[b] Between-group design; sample size includes participants in both stress versus non-stress conditions