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Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase

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Abstract

The prevalence of stress disorders differs between men and women. An understanding of how men and women vary in acute stress responses may help to understand these sex differences. We compared responses to the TSST and a control task in healthy men ($N=28$) and women tested in two phases (Follicular $N=29$, Luteal $N=23$) of the menstrual cycle. Men exhibited greater cortisol responses to stress than women in either phase. Luteal women exhibited the greatest subjective and allopregnanolone responses to stress, whereas follicular women exhibited blunted noradrenaline responses. Partial correlations controlling for group differences revealed that individuals who were most sensitive to the subjective effects of stress exhibited the largest salivary cortisol, noradrenaline, and allopregnanolone responses and the smallest progesterone responses to stress. We discuss our findings in the context of sex differences in the prevalence of stress-linked disorders.

Descriptors

TSST; Stress; Sex differences; Cardiovascular; Hormones; Mood

Stress contributes to many diseases, including not only cardiovascular, infectious, autoimmune, and mood disorders (al'Absi & Wittmers, 2003; Godbout & Glaser, 2006; Harbuz, Richards, Chover-Gonzalez, Marti-Sistac, & Jessop, 2006; Krishnan & Nestler, 2008; Reagan, Grillo, & Piroli, 2008) but also substance use disorders and relapse to drug abuse (Sinha, 2008). Inter-individual variation in responses to stress are likely to contribute to risk for these stress-linked disorders. For example, there is evidence that men and women vary in responses to acute stress (Kajantie & Phillips, 2006), and men and women also differ in the prevalence of stress-linked disease (Carter-Snell & Hegadoren, 2003). Thus, investigating sex differences in responses to acute stress may advance our understanding of the pathogenesis of stress-related disease.

Acute stress initiates a complex sequence of physiological and psychological events that may differ between men and women. First, activation of the sympathetic nervous system causes release of catecholamines and subsequent increases in heart rate and blood pressure,

and activation of the hypothalamic-pituitary-adrenal (HPA) axis, causes secretion of corticotrophic releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and glucocorticoids including cortisol (Chrousos & Gold, 1992; Herman et al., 2003; Lopez, Akil, & Watson, 1999; Munck, Guyre, & Holbrook, 1984). Acute stress also induces emotional reactions such as increases in anxiety and irritability, which may occur independently of sympathetic or HPA axis responses. There is some debate about whether the sympathetic, HPA axis, and subjective components of the stress response are distinct events or aspects of a coordinated integrated response (Schommer, Hellhammer, & Kirschbaum, 2003; Schlotz et al., 2008). However, most studies of responses to acute stress focus upon only one or two of these measures, making it difficult to study the relationship between measures. Thus, studies that include each of these components of the stress response are needed to investigate their interrelationships and to understand how variation in stress responses, including sex differences, influences the progression toward disease.

Studies of sex differences in stress responses have yielded mixed results (see Kajantie & Phillips, 2006 for review) in part because of differences in the procedures used to invoke stress between laboratories (Dickerson & Kemeny, 2004; Kajantie & Phillips, 2006; Krantz & Manuck, 1984; Kudielka & Kirschbaum, 2005; Lundberg, 2005; Stroud, Salovey, & Epel, 2002; Uhart, Chong, Oswald, Lin, & Wand, 2006). Recently, a single standardized procedure, the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993a), has been adopted by many laboratories and facilitates comparisons across sites. The TSST incorporates elements of social-evaluative threat (public speech) and achievement (mental arithmetic) and increases cortisol, heart rate, blood pressure, and subjective reports of anxiety amongst men and women alike (Dickerson & Kemeny, 2004; Kudielka et al., 2004a; Williams, Hagerty, & Brooks, 2004). In direct comparisons of men and women, there is some evidence that women experience more pronounced emotional responses to the TSST and similar tasks (Steptoe, Fieldman, Evans, & Perry, 1996; Kirschbaum et al., 1999; Kelly, Tyrka, Anderson, Price, & Carpenter, 2008) whereas men exhibit larger blood pressure responses (Lepore, Allen, & Evans, 1993; Steptoe et al., 1996; Matthews, Gump, & Owens, 2001). However, sex differences in heart rate and HPA axis responses to speech tasks are less consistent. Some studies reported no sex differences in heart rate responses (Kirschbaum et al., 1999; Kelly et al., 2008), whilst others report that women exhibit greater heart rate increases (Kudielka et al., 2004b). In addition, in some studies ACTH (Kudielka et al., 1998; Kirschbaum et al., 1999; Uhart et al., 2006), plasma cortisol (Kudielka et al., 1998; Uhart et al., 2006), and salivary cortisol (Kirschbaum, Wust, & Hellhammer, 1992; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995a; Kirschbaum, Pirke, & Hellhammer, 1995b; Steptoe et al., 1996; Nicolson, Storms, Ponds, & Sulon, 1997; Kirschbaum et al., 1999, men vs. follicular women) responses are greater in men whereas others have found no sex differences in ACTH (Kudielka et al., 2004a; Back et al., 2008), plasma cortisol (Kirschbaum et al., 1999, men vs. luteal women; Kudielka et al., 2004a; Back et al., 2008; Kelly et al., 2008), or salivary cortisol (Girdler, Turner, Sherwood, & Light, 1990; Sgoifo et al., 2003; Kudielka et al., 2004a) responses. Together, these findings suggest that men and women may respond differently on certain stress response measures.

Comparing stress responses in men and women is complicated by variations related to menstrual cycle. Several studies have investigated responses to cognitive and stressful

challenges during different phases of the menstrual cycle (Polefrone& Manuck, 1988; Stoney, Owens, Matthews, Davis, & Caggiula, 1990; Weidner& Helmig, 1990; Tersman, Collins, & Eneroth, 1991; Sita& Miller, 1996; Litschauer et al., 1998; Sato & Miyake, 2004; Carter& Lawrence, 2007; Hlavacova, Wawruch, Isonova, & Jezova, 2008), although only a few have employed the TSST or a similar speech task (Kirschbaum et al., 1995a; Kirschbaum et al., 1999; Pico-Alfonso et al., 2007). In general, these studies suggest that cardiovascular, subjective, and cortisol responses to stress do not vary across the menstrual cycle (Kirschbaum et al., 1995a; Kirschbaum et al., 1999; Pico-Alfonso et al., 2007), except for one study which found greater salivary cortisol responses to stress during the luteal phase (Kirschbaum et al., 1999). Thus, additional data are needed to assess the full profile of stress responses in women in different phases of the menstrual cycle.

The present study therefore sought to examine acute stress responses in healthy men and women, and in women in relation to menstrual cycle phase, upon a comprehensive range of physiological and subjective responses to the TSST. Based upon previous reports of responses to the TSST (see earlier), we hypothesized that men would exhibit greater cardiovascular and hormonal responses to stress whereas women, especially those tested in the luteal phase, would exhibit greater subjective discomfort.

Materials and Methods

Participants

Healthy non-smoking men ($n=28$) and women ($n=52$) aged 18–32 years were recruited from the University and surrounding community by flyers, newspaper, and university website advertisements without regard to race or ethnicity. Individuals who used regular medications (including hormonal contraceptives) and those with serious medical conditions, current or prior diagnosis of Axis I disorder (APA, 1994) including drug abuse or dependence, less than a high school education (for the purposes of completing standardized questionnaires), a body mass index outside of 19–26 kg/m², high or low blood pressure (as defined by the U.S. Centers for Disease Control and Prevention), an abnormal electrocardiogram, or those who worked night shifts were excluded. Cigarette smokers were excluded since smoking alters responses to the TSST (al'Absi et al., 2003; Kirschbaum et al., 1993b; Childs & de Wit, 2009). Women who self-reported significant, i.e., problematic, premenstrual affective symptoms or an irregular cycle (abnormal menstruation in comparison to their normal cycle) within the last 3 months were excluded. Participants signed a consent form which stated that the aim of study was to investigate the effects of alertness upon mood and physiology. They were told that they would complete short verbal tasks during each session but were not told the specific nature of the tasks until they had completed the study.

Design

This study used a mixed within- (Stress vs. No stress) and between- subjects (Men $N=28$ vs. Follicular Women $N=29$ vs. Luteal Women $N=23$) design. Power analyses, based upon the inter-individual variation in stress responses seen in previous studies, indicated that group sizes of 20 would be sufficient to detect significant differences. Volunteers participated in two experimental sessions in which they underwent the TSST or a control task in

randomized order. The sessions were conducted between 9 am and 1 pm at least 48 hours apart. Women were randomly assigned to menstrual phase condition; follicular women were tested between days 3 and 10 of their cycle, luteal women were tested between two and 10 days after a positive urine ovulation test. A 7-day time window was used for each phase so that women could schedule both experimental sessions within a single cycle and sessions were scheduled as near to the beginning of the time window as possible. Plasma levels of estrogen and progesterone at the start of each session were used to verify self-reported menstrual cycle phase (Griffin & Ojeda, 1996). As expected, plasma levels of estrogen were significantly higher in the luteal phase (99.6 ± 8.6 pg/ml) than the follicular phase (32.9 ± 2.5 pg/ml, $t(16)=7.4$ $p<.001$ $d=1.9$), as were levels of progesterone [luteal 5.3 ± 0.8 ng/ml, follicular 0.4 ± 0.2 ng/ml, $t(28)=6.1$ $p<.001$ $d=1.6$].

Procedure

The University of Chicago Hospital's Institutional Review Committee for the use of human subjects approved the protocol. Participants were tested individually in patient rooms at the University of Chicago Hospital's General Clinic Research Center (GCRC) where they stayed overnight before each session to standardize sleeping and eating. Participants awoke at 7:30 am, ate breakfast at 8 am (including their usual serving of caffeine), catheters were inserted at 8:30 am and baseline measures obtained at 9:40 am (baseline measures). The sessions were conducted based upon standardized procedures as previously described (Childs & de Wit, 2009). Briefly, the TSST consisted of 5min public speaking and 5 min arithmetic (serial subtraction) performed in front of two examiners, who were unknown to the participant, and a video camera. Throughout the TSST, participants could see their own image portrayed on a television monitor. During the control condition (also 10 min), participants spoke to the Research Assistant about their interests and hobbies without a video camera. Inclusion of the control task allowed us to identify responses that are specific to psychosocial stress, not simply cognitive challenge or postural changes. Participants were given 10 min to prepare for each task. Participants were escorted to an adjacent room to perform the tasks which always began at 10 am. Intravenous catheters, for collection of blood samples for hormonal analysis, were placed 70 min prior to collection of baseline samples. Cardiovascular and hormonal measures were obtained 20min before and at 0, 5, 10, 20, 30, 60, 90, and 120min after the tasks. Self-reported mood was assessed 20 min before and at 0, 10, 20, 30, 60, 90, and 120 min after the tasks. The subjective questionnaires were completed within 5 min at each time point so individuals had an opportunity to rest between completions of the questionnaires. We and others have used these standardized questionnaires to assess mood repeatedly with no adverse effects. At the end of the second session, participants were fully debriefed about the specific purposes of the study and received payment (\$200).

Cardiovascular Measures

Participants wore a Polar chest band and monitor that recorded heart rate continuously (Mini-Logger, Mini Mitter/Respironics, Bend, OR) and blood pressure was measured at repeated intervals using a monitor (Critikon Dinamap Plus Vital Signs Monitor, GE Healthcare Technologies, Waukesha, WI).

Hormonal Measures

Saliva samples were collected using Salivette cotton wads. The GCRC Core Laboratory at the University of Chicago determined levels of cortisol in saliva and plasma (Saliva=Salimetrics LLC [State College, PA], sensitivity=0.003 ug/dL; Plasma=Immulite/Immulite 1000 Cortisol [Siemens Healthcare Diagnostics, Deer-field, IL], sensitivity=0.2 ug/dL). The University of Chicago Medical Endocrinology Laboratory determined levels of progesterone in plasma (Immulite 2000 Progesterone, sensitivity=0.1 ng/ mL). Dr. Richard Hauger at the University of California, San Diego, determined levels of plasma allopregnanolone according to the methods of Purdy et al., 1990. Mayo Medical Laboratories (Rochester, MN) determined levels of plasma catecholamines and estrogen [Test codes 8532 and 200255, sensitivity: noradrenaline=70 pg/mL, estradiol 15 pg/mL]. Unfortunately, plasma samples collected for ACTH assay were spoiled after a freezer malfunction, and insufficient data was obtained for meaningful statistical analysis.

Subjective Measures

Subjective mood was measured using the Profile of Mood States (POMS; McNair, Lorr, & Droppleman, 1971). The 72-item POMS checklist yielded eight subscales; Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor, Fatigue, Confusion, Friendliness, Elation.

Statistical Analysis

Demographic characteristics of the three groups were compared using one-factor analysis of variance (ANOVA, continuous variables) and Pearson's chi-square test (categorical variables). Baseline differences between the groups were assessed using one-factor (Group) ANOVA upon the average of the pre-task values during each session (since there were no significant session differences or effects of session order).

The effects of the tasks upon cardiovascular, hormonal, and subjective data across time were assessed using three-factor (Task and Time as within-subjects factors, Group as between-subjects factor) ANOVA for repeated measures. Analyses were conducted upon mean absolute scores at each time point. For continuous heart rate data, the mean heart rate during successive 10-min periods was calculated. Since there were differences at baseline for some measures, three-factor (Task*Time*Group) repeated measures ANOVAs were also performed upon data expressed as a change from the pre-task baseline at each time point and two-factor (Task*Group) repeated measures ANOVAs were performed upon peak change scores, i.e., the maximum positive or negative change from the pretask baseline. Any differences between the analyses are detailed in the results section. Effect sizes are reported using partial eta squared (η_p^2) for analyses of variance; 0.01, 0.06, and 0.14 are considered, respectively, small, medium, and large effect sizes.

All analyses were conducted using SPSS version 14 (SPSS Inc., Chicago, IL) for Windows. Missing cases (due to equipment malfunction, problem with sample collection) were deleted list wise, leading to smaller sample sizes for some analyses. Due to budgetary constraints, analysis of plasma noradrenaline, progesterone, and allopregnanolone was performed in a subset of participants, thus subject numbers for these analyses were smaller (Men $N=20$, Follicular women $N=18$, Luteal women $N=14$). Repeated measures ANOVAs were

performed with Greenhouse Geisser correction when violations of sphericity were detected. All significant findings remained significant when thus adjusted, and therefore we report degrees of freedom from uncorrected analyses.

Results

Demographic Characteristics

Most participants were European American college students in their early twenties (Table 1). Men consumed more alcoholic drinks per week than luteal women [$F(2,79)=5.5$ $p<.01$, Bonferroni *post hoc* $p<.01$] but not follicular women (Bonferroni *post hoc* $p>.05$), and more men reported having ever used marijuana than women in either phase [Men vs. Follicular women Pearson $\chi^2(2)=9.5$ $p<.01$, Men vs. Luteal Women $\chi^2(2)=21.7$ $p<.001$]. The groups did not differ upon other demographic characteristics.

Baseline Differences

Before the tasks, men exhibited higher systolic blood pressure [Group $F(2,77)=30.5$ $p<.001$] than women tested in either phase (Bonferroni *post hoc* test $p<.05$ for heart rate, $p<.001$ for systolic blood pressure). Men also exhibited significantly higher diastolic blood pressure [Group $F(2,77)=4.2$ $p<.05$] than luteal women (Bonferroni *post hoc* test $p<.05$) but not follicular women (Bonferroni *post hoc* test $p<.06$). Luteal women demonstrated significantly higher levels of plasma progesterone [Group $F(2,50)=47.9$ $p<.001$] and allopregnanolone [Group $F(2,48)=27.7$ $p<.001$] than follicular women (Bonferroni *post hoc* test $ps<.001$) and men (Bonferroni *post hoc* test $ps<.001$). At baseline, luteal women scored higher upon the POMS Tension- Anxiety [Group $F(2,76)=4.6$ $p<.05$] and Fatigue scales [Group $F(2,76)=6.4$ $p<.006$] than follicular women (Bonferroni *post hoc* tests, respectively, $p<.05$, $p<.01$) but not men (Bonferroni *post hoc* tests, respectively, $p=.09$, $p>.3$).

Effects of Stress

Cardiovascular measures—In comparison to the control task, the TSST significantly increased heart rate [Task*Time $F(5,290)=7.0$ $p<.001$ $\eta_p^2=0.11$], systolic [Task*Time $F(6,462)=4.5$ $p<.001$ $\eta_p^2=0.06$] and diastolic blood pressure [Task*Time $F(6,462)=5.5$ $p<.001$ $\eta_p^2=0.07$] similarly in all three groups [Figure 1; Task*Time*Group heart rate $F(10,290)=0.9$ $p=.5$ $\eta_p^2=0.03$; systolic blood pressure $F(12,462)=1.6$ $p=1.6$ $\eta_p^2=0.04$; diastolic blood pressure $F(12,462)=0.5$ $p=.9$ $\eta_p^2=0.01$]. Luteal women exhibited significantly higher heart rate overall in comparison to men [Group $F(2,58)=3.8$ $p<.05$ $\eta_p^2=0.12$, Bonferroni *post hoc* test $p<.05$] but not follicular women (Bonferroni *post hoc* test $p>.3$). In line with differences at baseline, men exhibited significantly higher systolic blood pressure in comparison to women tested in either phase [Group $F(2,77)=41.3$ $p<.001$ $\eta_p^2=0.52$, Bonferroni *post hoc* test $p<.001$] and significantly higher diastolic blood pressure than luteal women [Group $F(2,77)=4.0$ $p<.05$ $\eta_p^2=0.10$, Bonferroni *post hoc* test $p<.05$] but not follicular women (Bonferroni *post hoc* test $p=.1$). Analysis of change from baseline and peak change scores did not alter the results.

Hormonal measures—Salivary cortisol responses to the TSST differed among the groups [Task*Time*Group $F(12,438)=1.8$ $p<.05$ $\eta_p^2=.05$, Figure 2]. Simple effects analyses revealed that there was a greater, more significant difference between cortisol levels after the stressful and non-stressful tasks in men [Task $F(1,24)=16.5$ $p<.001$ $\eta_p^2=0.41$] than in women tested in either the follicular [Task $F(1,28)=4.3$ $p<.05$ $\eta_p^2=0.13$] or luteal phases [Task $F(1,21)=6.2$ $p<.05$ $\eta_p^2=0.23$]. There was a trend to a similar difference between groups in levels of plasma cortisol after the tasks, although this did not reach significance [Task*Time*Group $F(12,420)=1.6$ $p<.09$ $\eta_p^2=0.04$, Figure 2]. Analysis of change from baseline and peak change scores did not alter the results for salivary cortisol responses, however, there was a significant Task*Time*Group interaction for plasma cortisol change scores [$F(10,365)=2.8$ $p<.01$ $\eta_p^2=0.07$]. Simple effects analyses revealed that only men exhibited a significant cortisol response to the TSST [Task*Time $F(5,135)=6.5$ $p<.001$ $\eta_p^2=0.27$; effect sizes for main effect of task, respectively, 0.09 and 0.15 for follicular and luteal women, $ps>.1$].

There were significant group differences in the effects of the tasks upon plasma noradrenaline [Task*Time*Group $F(8,188)=2.0$ $p<.05$ $\eta_p^2=0.08$, Figure 3]. Simple effects analyses revealed a significant main effect of task upon noradrenaline among men [Task $F(1,19)=13.0$ $p<.01$ $\eta_p^2=.41$] and luteal women [Task $F(1,12)=5.6$ $p<.05$ $\eta_p^2=0.32$] but not among follicular women [Task $F(1,16)=1.3$ $p>.2$ $\eta_p^2=0.08$]. Similarly, there were group differences in the effects of the tasks upon plasma progesterone [Task*Time*Group $F(10,230)=1.9$ $p<.05$ $\eta_p^2=0.08$] and allopregnanolone [Task*Time*Group $F(10,225)=2.1$ $p<.05$ $\eta_p^2=0.09$, Figure 3]. The TSST significantly increased plasma progesterone among men [Task $F(1,19)=18.6$ $p<.001$ $\eta_p^2=0.50$] and follicular women [Task $F(1,14)=5.0$ $p<.05$ $\eta_p^2=0.26$] but not luteal women [Task $F(1,13)=2.2$ $p>.1$ $\eta_p^2=0.14$]. Stress significantly influenced plasma allopregnanolone among luteal women [Task*Time $F(5,55)=2.8$ $p<.05$ $\eta_p^2=0.20$] and there was a trend to decreased allopregnanolone among follicular women [Task*Time $F(1,17)=4.2$ $p=.055$ $\eta_p^2=0.20$] but levels among men were unchanged [Task $F(1,17)=0.02$ $p>.8$ $\eta_p^2=0.001$]. Analysis of change from baseline and peak change scores for noradrenaline, progesterone, and allopregnanolone did not alter the results.

Subjective measures—The TSST significantly increased scores upon the POMS Anger-Hostility [$F(5,380)=14.2$ $p<.001$ $\eta_p^2=0.16$], Tension-Anxiety [Task*Time $F(5,380)=22.1$ $p<.001$ $\eta_p^2=0.23$], Confusion [Task*Time $F(5,380)=10.7$ $p<.001$ $\eta_p^2=0.12$], and Depression-Dejection subscales [Task*Time $F(5,380)=7.9$ $p<.001$ $\eta_p^2=0.09$], and decreased scores upon the POMS Elation [Task*Time $F(5,380)=14.7$ $p<.001$ $\eta_p^2=0.16$], Friendliness [Task*Time $F(5,380)=20.3$ $p<.001$ $\eta_p^2=0.21$], and Vigor subscales [Task*Time $F(5,380)=5.5$ $p<.001$ $\eta_p^2=0.07$]. There were differences between groups in the effects of stress upon the Anger-Hostility [Task*Time*Group $F(10,380)=1.9$ $p<.05$ $\eta_p^2=0.05$] and Depression-Dejection [Task*Time*Group $F(10,380)=2.1$ $p<.05$ $\eta_p^2=0.05$] scales (Figure 4). *Post hoc* analyses revealed that women in the luteal phase exhibited more prolonged increases in ratings of anger and hostility than women in the follicular phase and men; luteal women exhibited significantly elevated Anger-Hostility scores for 20 min after the TSST whereas scores for men and follicular women were not significantly different from baseline 10min after the task (see Figure 4). In contrast, simple effects analyses revealed that women

in the follicular phase did not exhibit stress-induced changes in ratings of feeling depressed [Task*Time $F(5,140)=1.1$ $p>.3$ $\eta_p^2=0.04$] whereas stress increased scores among men [Task*Time $F(5,130)=6.5$ $p<.001$ 0.20] and luteal phase women [Task*Time $F(5,110)=3.0$ $p<.05$ 0.12].

In line with differences at baseline, luteal women scored higher overall upon the POMS Tension-Anxiety subscale [Group $F(2,76)=11.9$ $p<.001$ $\eta_p^2=0.24$] than the other groups (Bonferroni *post hoc* test $ps<.01$). Luteal women also scored higher upon the POMS Confusion scale [Group $F(2,76)=6.1$ $p<.01$ $\eta_p^2=0.14$] after each task than follicular women (Bonferroni *post hoc* test $p<.01$) but not men.

Analysis of peak change from baseline scores confirmed these findings; women tested in the luteal phase exhibited significantly larger increases in Anger [Group $F(2,76)=4.7$ $p<.01$ $\eta_p^2=0.11$], Depression [Group $F(2,76)=6.6$ $p<.01$ $\eta_p^2=0.15$] and Anxiety [Group $F(2,76)=6.1$ $p<.01$ $\eta_p^2=0.14$] than follicular women (Bonferroni *post hoc* $ps<.01$) and men (Bonferroni *post hoc* $ps<.05$ for Depression and Anxiety only, $p>.1$ for Anger).

Correlational analyses—Partial correlations between the outcome measures controlling for Group revealed some interesting relationships. As expected, salivary and plasma cortisol responses to stress were correlated ($r^2=0.64$ $p<.001$), as were systolic and diastolic blood pressure responses ($r^2=0.35$ $p=.05$) and systolic blood pressure responses were correlated with heart rate responses ($r^2=0.40$ $p<.05$). Subjective sensitivity to stress was associated with hormonal responses; negative mood was positively correlated with salivary cortisol (Elation $r^2=-0.37$ $p<.05$, Fatigue $r^2=0.40$ $p<.05$, Anxiety $r^2=0.3$ $p=.08$), allopregnanolone (Anxiety $r^2=0.36$ $p<.05$, Anger $r^2=0.44$ $p<.05$, Depression $r^2=0.34$ $p<.06$) and noradrenaline (Anger $r^2=0.36$ $p<.05$) responses, and negatively correlated with progesterone responses (Anger $r^2=-0.36$ $p<.05$, Friendliness $r^2=0.38$ $p<.05$, Depression $r^2=-0.33$ $p<.07$). Allopregnanolone responses to stress were not significantly associated with progesterone responses ($r^2=0.06$ $p=.76$), although they were positively correlated with noradrenaline responses ($r^2=0.37$ $p<.05$) and marginally positively correlated with diastolic blood pressure responses ($r^2=0.33$ $p=.06$).

Discussion

Sex differences in physiological or subjective responses to acute stress may contribute to sex differences in the prevalence of stress-related disorders. In this study, we found evidence of both sex and menstrual cycle-related differences in cardiovascular, hormonal, and subjective responses to stress. First, men exhibited greater increases in serum and salivary cortisol compared to women tested in either phase. Second, women tested during the luteal phase exhibited larger allopregnanolone responses to stress and expressed greater feelings of Anger and Depression than men and women tested in the follicular phase. Furthermore, women tested in the luteal phase exhibited greater heart rate responsivity and anxiety overall. Finally, whereas men and women tested in the luteal phase exhibited stress-induced increases in noradrenaline and negative mood, compared to the control task, women tested in the follicular phase did not.

It has been suggested that responses to stress differ fundamentally between men and women. Taylor et al. (2000) proposed that men typically exhibit a so-called “fight-or-flight” response to stress, characterized by sympathetic and HPA axis responses, whereas women tend to exhibit a “tend-and-befriend” response, which is more favorable for protecting offspring in the face of a threat. The marked difference in cortisol responses to stress between men and women supports this idea; greater cortisol responses in men may reflect a physical preparedness to “fight” or “flee.” In addition, men exhibited a more pronounced increase in plasma noradrenaline compared to women tested in the follicular (but not luteal) phase. Conversely, women, especially those tested during the luteal phase, were more reactive to stress on subjective self-report measures, consistent with the idea that stress affects psychosocial responses more in women than in men. Sex differences in cortisol and noradrenaline responses to stress may have had evolutionary significance and conferred sex-specific advantages for survival, allowing males to mount a physical response to threatening stimuli. However, today, stressors do not typically affect survival and may be more subtle and repetitive, and sex differences in responses to acute stress may provide clues regarding sex differences in the prevalence of stress-linked disorders. For example, chronic inflammatory disease has been linked to hypoactivity of the HPA axis (Harbuz, 2002) and primarily affects women (Whitacre, 2001; Lockshin, 2006; Fairweather, Frischno-Kiss, & Rose, 2008). Thus, blunted adrenocortical responses to acute stress among women may contribute to a heightened susceptibility to some autoimmune diseases.

Some of our findings are in line with previous findings. That is, some investigators have reported blunted salivary or plasma cortisol responses to the TSST in women compared to men (Kirschbaum et al., 1992; Kirschbaum et al., 1995a; Kirschbaum et al., 1995b; Kirschbaum et al., 1999, follicular women vs. males; Uhart et al., 2006) and other studies have reported more pronounced emotional responses to stress in women (Steptoe et al., 1996; Kirschbaum et al., 1999; Kelly et al., 2008). Further, studies have reported greater basal levels and stress-induced increases in plasma allopregnanolone among women in the luteal, compared to the follicular phase (Girdler et al., 2001; Girdler, Beth Mechlin, Light, & Morrow, 2006). These cycle-related differences may be due to a higher availability of progesterone, a precursor of allopregnanolone, in luteal women. Finally, women in the luteal phase appear to exhibit greater heart rate responses to stressors including mental stress (Manhem, Jern, Pilhall, Shanks, & Jern, 1991), shock avoidance (Hastrup & Light, 1984), and the cold pressor test (Tersman et al., 1991) compared to women in the follicular phase, possibly because of elevated estrogen levels during the luteal phase (Pollard, Pearce, Rousham, & Schwartz, 2007).

A novel finding in this study was that the TSST increased plasma progesterone among men and women tested in the follicular phase. Other studies have reported increases in progesterone in men and women after emotion-arousing stimuli (Schultheiss, Wirth, & Stanton, 2004; Wirth & Schultheiss, 2006; Wirth, Meier, Fredrickson, & Schultheiss, 2007), yet this is among the first demonstrations of stress-induced increases in progesterone in humans. Previously, we (Childs & de Wit, 2009) reported significant increases in progesterone among men, and now we extend this finding to women tested in the follicular phase. The stress-induced increases in progesterone were particularly evident during the luteal phase, although the cycle-related difference, i.e., that luteal women appeared to

exhibit larger increases, did not reach significance probably because of the large variability and high background levels of progesterone. Stress-induced increases in progesterone have also been reported in animal studies (Barbaccia et al., 1996). It is thought that progesterone is released from the adrenals upon stress-induced HPA axis activation (Genazzani et al., 1998) and that this may be a homeostatic mechanism to counteract the effects of stress (Barbaccia et al., 1996).

Partial correlations controlling for group differences revealed interesting relationships among the stress response measures. Individuals who exhibited the greatest subjective sensitivity to stress also exhibited the greatest salivary cortisol, noradrenaline, and allopregnanolone responses to stress. These results support those of a recent study, which found evidence of cross-correlations between emotional and endocrine responses to the TSST (Schlotz et al., 2008). This suggests that systems mediating hormonal release after stress also mediate psychological changes. Another interesting finding of the correlational analyses was that the increase in progesterone after stress was not correlated with increases in allopregnanolone. It has been suggested that progesterone released after stress is the source of allopregnanolone, which serves as a counter-regulatory mechanism to limit the effects of stress (Barbaccia et al., 1996). Instead, our results showing dissociation between progesterone and allopregnanolone suggest that progesterone is not the source of stress-induced allopregnanolone, but that release of allopregnanolone is more directly linked to activation of the sympathoadrenal system. Indeed, a more direct relation between activation of stress systems and allopregnanolone might be a more efficient mechanism to limit the physiological and psychological effects of stress.

The present study has some limitations. First, we were unable to analyze levels of plasma ACTH, which would have provided important information about whether pituitary sensitivity to ACTH is different in men and women (Kirschbaum et al., 1999). In addition, our sample was mainly Caucasian, which limits application of the results to a broader population. This is of particular concern because some studies have reported ethnic differences in stress responses (Sherwood, May, Siegel, & Blumenthal, 1995; Mills et al., 1996; Ahwal et al., 1997; Harshfield et al., 2007; Chong et al., 2008). Finally, for scheduling reasons, stress sessions were conducted in the morning at a time when cortisol levels are declining. While baseline levels of cortisol did not differ in men and women, other studies have reported a higher cortisol awakening response among women (Kunz-Ebrecht et al., 2004; Maina et al., 2007,) and inspection of cortisol levels between the groups during the control condition illustrates a steeper decline across the session among women. Thus, it is possible that greater increases in cortisol might have been observed in women if testing had occurred later in the day. Nevertheless, women did exhibit a significant cortisol response relative to the non-stressful control condition, i.e., the slope of the decline was significantly less steep after stress. Indeed, it is a strength of the study that a control condition was included in the design and we are able to dissociate the effects of stress from effects of cognitive challenge or postural changes. For example, there were significant increases in noradrenaline exhibited by all groups in the control condition that likely reflect the influences of postural changes (Mlynarik et al., 2007).

In conclusion, we found evidence of sex- and menstrual cycle-related differences in acute responses to mild speaking stress. To the extent that responses to acute stress may predict stress-linked diseases, the sex differences in stress responses reported here may provide clues regarding sex differences in the long-term adverse consequences of stress. A major strength of this study was the inclusion of a range of measures across the physiological and psychological domains. This provided a complete profile of variations in responses to the stress, and notably allowed us to demonstrate correlations among the different stress measures after taking sex- and cycle-related differences into account.

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Figure 1.

Changes in heart rate (A) and systolic (B) and diastolic (C) blood pressure after the TSST (filled symbols) and control task (open symbols) for men ($N=28$), follicular women ($N=29$) and luteal women ($N=23$). Data represent mean $\pm$ SEM for i) heart rate averaged across successive 10-min periods, and ii) blood pressure as a function of time (Task preparation from -20 to -10 min, task performance from -10 to 0 min).

Figure 2.

Changes in plasma (A) and salivary (B) cortisol after the TSST (filled symbols) and control tasks (open symbols) for men ($N=28$), follicular women ($N=29$) and luteal women ($N=23$). Data represent mean $\pm$ SEM as a function of time after the task (Task preparation from -20 to -10 min, task performance from -10 to 0 min).

Figure 3.

Changes in plasma noradrenaline (A), progesterone (B), and allopregnanolone (C) after the TSST (filled symbols) and control tasks (open symbols) for men ($N=20$), follicular women ($N=18$) and luteal women ($N=14$). Data represent mean $\pm$ SEM as a function of time after the task (Task preparation from -20 to -10 min, task performance from -10 to 0 min).

Figure 4.

Changes in POMS Anger (A), Depression (B), and Anxiety (C) after the TSST (filled symbols) and control tasks (open symbols) for men ($N=28$), follicular women ($N=29$) and luteal women ($N=23$). Data represent mean $\pm$ SEM as a function of time after the task (Task preparation from -20 to -10 min, task performance from -10 to 0 min). Asterisks represent a significant difference from pre-task baseline value at -20 min (Student's paired t -test corrected for multiple comparisons, $p < .05$).

Table 1

Demographic Characteristics of Participants

Demographic characteristic	Male	Female Follicular	Female Luteal
<i>N</i>	28	29	23
Age (mean $\pm$ SEM)	21.8 $\pm$ 0.7	22.1 $\pm$ 0.8	21.6 $\pm$ 0.7
BMI (mean $\pm$ SEM)	22.4 $\pm$ 0.4	22.3 $\pm$ 0.4	22.2 $\pm$ 0.3
Education (%)			
High school	7	17	13
Partial college degree	68	52	57
College degree	21	28	26
Advanced degree	4	3	4
Full time student (%)	75	69	83
Race (%)			
European American	54	52	74
African American	21	38	5
Asian American	18	3	17
Other	7	7	4
Current drug use (mean $\pm$ SEM)			
Alcoholic drinks/wk *	4.4 $\pm$ 1.0	2.2 $\pm$ 0.6	1.2 $\pm$ 0.4
Caffeine drinks/wk	4.5 $\pm$ 1.5	5.7 $\pm$ 1.6	8.3 $\pm$ 2.8
Lifetime drug use (% ever used)			
Stimulants	7	7	0
Sedatives	0	3	0
Hallucinogens	14	14	0
Opiates	36	45	30
Marijuana *	89	62	30
Inhalants	4	3	0

Notes: Asterisks indicate a significant difference between males and females tested in each phase, $p < .001$.

* indicates a significant group difference (see Results section: Demographic Characteristics).