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Psychophysiological and neuroendocrine responses to laboratory stressors in women: Implications of menstrual cycle phase and stressor type☆

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ABSTRACT

This study assessed stressor and menstrual phase effects on psychophysiological and neuroendocrine responses to laboratory stressors in freely cycling women (*N* = 78, ages 18–45). Participants performed counterbalanced stressors [Paced Auditory Serial Addition Test (PASAT) or cold pressor test (CP)] during their follicular and luteal menstrual cycle phases between 1:00 and 3:00 p.m. to control for cortisol rhythm. Participants rested 30-min, performed the stressor, and then recovered 30-min while electrocardiography continuously monitored heart rate (HR). Systolic (SBP) and diastolic blood pressure (DBP), salivary cortisol, and state anxiety were assessed at timed intervals. HR, SBP, and cortisol varied more over the course of luteal than follicular phase testing. A three-way interaction revealed state anxiety reactivity was greater with the PASAT during the follicular phase. DBP showed equal and persistent reactivity with both stressors during both cycle phases. Results extend the stressor-specific HPAA hypothesis and have important methodological implications for women's biopsychology research.
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Understanding the intricacies of cardiovascular reactivity remains a critical area of women's health research given the links between cardiovascular reactivity and heart disease; the latter being the leading cause of death among American women (Centers for Disease Control and Prevention [CDC], 2004). One pathway through which the cumulative effects of cardiovascular reactivity lead to heart disease is hypertension (Carroll et al., 2001; Manuck et al., 1990), a condition worsened by frequent cortisol exposure (McEwen and Stellar, 1993). Given the presence of estrogen receptors in the heart and the direct influences of steroid hormones on the cardiovascular system (Hirshoren et al., 2002), researchers have explored the effects of the menstrual cycle on women's stress-induced cardiovascular reactivity (Miller and Sita, 1994; Sato et al., 1995; Sita and Miller, 1996; Stoney et al., 1990; Tersman et al., 1991). Yet, despite several attempts to explicate menstrual cycle phase effects on psychophysiological and neuroendocrine responses to laboratory stressors in women, findings remain equivocal, with some studies reporting greater cardiovascular reactivity during the luteal cycle phase compared to the follicular phase (e.g., Manhem et al., 1991; Sato et al., 1995; Tersman et al., 1991), and others reporting greater reactivity during the follicular cycle phase (Miller and Sita, 1994). Still other studies reveal no evidence of cycle phase effects (Stoney et al., 1990; Weidner and Helmig, 1990).

Our ability to effectively draw conclusions from these discrepant findings is limited by methodological variations among studies. One such variant is the means by which cycle phase is determined. In some studies, cycle phase is estimated from a participant's subjective report of the first day of their last menses (Polefrone and Manuck, 1988). This calendar method is problematic given the inter- and intra-individual variability of follicular phase duration. Other studies assess cycle phase through measured progesterone and/or estrogen levels (Manhem et al., 1991; Miller and Sita, 1994; Pollard et al., 2007). Although very

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precise, this method is costly and has contributed to small sample sizes and reduced statistical power for assessing cycle phase effects.

Collective interpretation of results is also limited by inconsistent operational definitions and measurement of physiological and psychological responses to laboratory stressors in women. For example, when assessing physiological reactivity, some researchers use direct measures of heart rate (HR) and blood pressure (BP; Weidner and Helmig, 1990), while others use assessments of underlying hemodynamics (Sita and Miller, 1996) or HR variability (Sato et al., 1995) to operationally define reactivity. Neuroendocrine assessments also vary with some studies adding catecholamine changes to the operational definition of stress reactivity (Litschauer et al., 1998; Stoney et al., 1990), while others measure hypothalamic–pituitary–adrenal axis activity (HPAA) via changes in cortisol levels (Kirschbaum et al., 1999). Assessments of psychological responses to laboratory stressors are similarly inconsistent. For example, Miller and Sita (1994) included questionnaires to assess post-stressor state anxiety and anger reports, though much of the research in this area has neglected to assess this psychological response to a stressor.

A third problem that plagues research on reactivity to laboratory stressors is the varied nature and number of the stressor tasks employed. Laboratory stressors have included well-validated cognitive challenges (e.g., math calculations) and physical tasks (e.g., cold pressor test), but they have also included study-specific stressors with no known psychometric properties (Sato et al., 1995). Further, some researchers use only one stressor type repeated over multiple tests introducing the potential confound of habituation (Collins et al., 1985), while other researchers use multiple stressors (Miller and Sita, 1994). The varied nature of stressor tasks may explain why the literature to date does not evince clear cycle phase effects on psychophysiological or neuroendocrine responses to laboratory stressors; responses may be specific to the kind of stressor that is utilized (e.g., cognitive vs. physiological). Non-significant findings may be the result of an inadequate stressor task rather than non-reactivity. For example, recent evidence suggests that among the many acute psychological stressors utilized in research, those involving a motivated performance task characterized by an evaluative and/or uncontrollability component produce significant cortisol responses (Dickerson and Kemeny, 2004), and this HPAA responsivity may be affected by menstrual cycle phase (Kajantie and Phillips, 2006). However, systematic evaluations of this stressor-specific HPAA hypothesis in studies accounting for menstrual phase effects on stress reactivity and recovery in freely cycling women are scant.

Considering the aforementioned limitations, our purpose was to systematically investigate women's psychophysiological and neuroendocrine reactivity and physiological recovery during the follicular and luteal menstrual cycle phases using two well-validated and counterbalanced laboratory stressors of similar duration in a repeated measures design. Further, we used an endocrine assessment of ovulation to determine cycle phase, and tested enough healthy and freely cycling women to exceed power requirements. Given the equivocal findings reported in the extant literature, we did not test directional hypotheses but rather posited three research questions. First, in response to laboratory stressors, does psychophysiological reactivity measured by HR, BP, and state anxiety reports differ by cycle phase or stressor type? Second, in response to laboratory stressors, does neuroendocrine stress reactivity measured by salivary cortisol differ by cycle phase or stressor type? Third, after laboratory stressors, does HR and BP recovery differ by cycle phase or stressor type?

1. Method

1.1. Power analyses

Power analyses were performed with G-Power 3.0.3 (Faul et al., 2007). Given the inconsistent findings reported in the literature, we set our effect size input parameters as small-moderate with $f^2 = .15$, $\alpha = .05$, and $1 - \beta = .80$. For two groups and five repetitions (detailed below) a sample size of 56 was needed. For these same analyses with three repetitions for cortisol reactivity, the sample size needed to achieve the same power was 74.

1.2. Participants

Following approval from the University Institutional Review Board, participants were recruited via local advertisements. Eligible participants self-identified as: (a) 18–45 years of age (not premenarcheal or menopausal); (b) non-smokers; (c) not taking hormones, medications, or having undergone a medical procedure known to affect the natural menstrual cycle; (d) not pregnant, nursing, or amenorrheic and reported having a cycle length of 21–40 days; (e) not taking medications known to affect the stress response (including psychotropics); (f) free from chronic physical and mental health conditions (e.g., hypertension, known arrhythmias, obesity, major depression); (g) not wearing braces or dental apparatuses that might affect salivary sampling; (h) able to read and write English; and (i) able to come to our lab for two, one-hour research sessions. Interested participants were instructed to call our lab for screening, which involved confirming eligibility criteria and assessing if a traumatic event had occurred in the participants life in the past 2 months. Since upward of 10% of cycles may be anovulatory (Swain et al., 1974), we incorporated planned missingness strategies (Schafer and Olsen, 1998) into our study, which included pulsing our advertising throughout the study and screening continuously until our projected sample size was met. Participants were paid $75.00 for completing all parts of the study; otherwise, partial remuneration was issued commensurate with level of completion.

1.3. Apparatus and measures

1.3.1. Cardiovascular measures

BP was measured with an automatically inflated sphygmomanometer (Dinamap, 1846: Critikon, Inc., Tampa, FL). HR was continuously measured with electrocardiography via the online chart recorder system Powerlab (Powerlab 800; ADinstruments, Boulder, CO).

1.3.2. Salivary cortisol

HPAA reactivity was measured by salivary cortisol. Saliva samples were collected at three time points throughout the laboratory session (see Fig. 1 for timing) with a 10 mm × 37 mm cotton pledget (Salimetrics, LLC, State College, PA) following the collection advice offered by Salimetrics (2009a) and described by Granger et al. (2007). Samples were kept on ice throughout the laboratory session and subsequently frozen at −20 °C until shipped on dry ice via over-night mail to the Salimetrics Lab for assay. Samples were analyzed using high sensitivity enzyme immunoassay specifically designed and validated by Salimetrics for the quantitative measurement of salivary cortisol. The inter-assay coefficient of variation (CV) was 3.75–6.41%, the intra-assay CV was 2.9%, and the lower limit of sensitivity was <0.003 μg/dl for our samples.

1.3.3. State anxiety

To be consistent with other studies that measured psychological aspects of stress reactivity in response to laboratory or naturalistic stressors (e.g., Choi and Salmon, 1995; Dimitriev et al., 2008; Lewis et al., 2007; O'Donovan and Hughes, 2008; Renaud and Blondin, 1997; Summer et al., 1999), we employed a measure of state anxiety. Self-reported state anxiety was assessed pre and post stressor task via the state portion of the Spielberger State/Trait Anxiety Inventory (STAI-S; Spielberger et al., 1983). In completing this assessment, women rated their present moment feelings including tension, upset, and nervousness on a 4-point scale ranging from (1) *not at all* to (4) *very much so*. Spielberger et al. (1983) reported acceptable internal reliability for the state measure (alpha = .86–.95), and test–retest reliability coefficients for time intervals of one hour to 104 days that were moderate ($r = .16$–.62), as expected with transitory emotional states.

1.3.4. Demographic and health information

Participants provided information on their age, ethnicity, perceived cycle normality and length, cigarette and alcohol use, as well as height and weight for calculation of body mass index. Participants were also queried on their past and current level of regular physical activity and if they had ever received a diagnosis of premenstrual syndrome or premenstrual dysphoric disorder (PMS/PMDD). The US Public Health Service definition of regular physical activity was provided to participants, which states that people are regularly active if they do either of the following: (a) moderate-intensity activities for at least 30 min on at least five days of the week, or (b) vigorous-intensity activities for at least 20 min on at least three days of the week. Moderate-intensity physical activity includes such things as brisk walking (as if you are going some place, 3–4.5 mph or 14.3–20 min per mile), lawn

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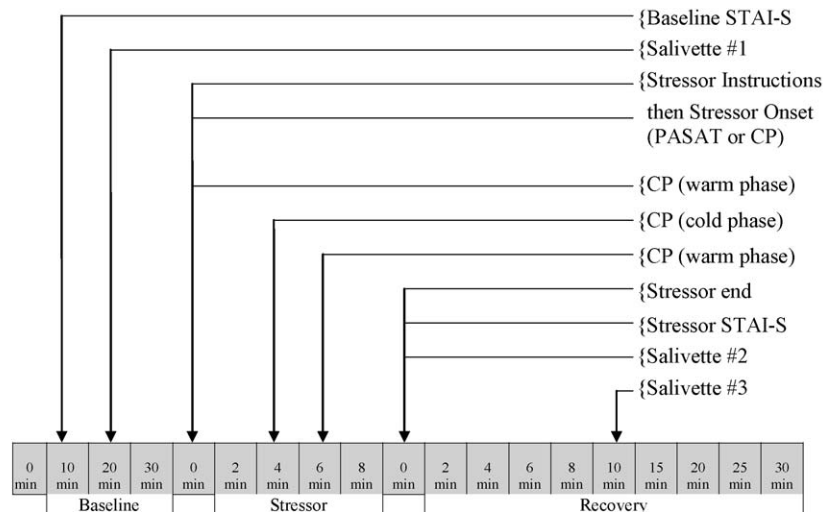

Fig. 1. Timeline of data collection for laboratory sessions.
Note: (a) The 0-min interval signifies the start of a new period within the laboratory session; (b) blood pressure was taken at each time interval above, with the exception of the 0-min demarcation; (c) heart rate was monitored continuously throughout the laboratory session; (d) counting menses onset as day 1, the follicular laboratory session occurred during days 5–9 post-menses onset and treating ovulation as day zero, the luteal laboratory session occurred during days 7–10 post-ovulation. STAI-S = State portion of the State-Trait Anxiety Inventory; PASAT = Paced Auditory Serial Addition Test; CP = Cold Pressor.

mowing with a motorized mower, dancing, swimming, and bicycling on level terrain. Vigorous-intensity physical activity includes such things as very rapid walking (faster than 4.5 mph), jogging, lawn mowing with a non-motorized mower, wood chopping, high-impact aerobic dancing, continuous lap swimming, and bicycling uphill. Participants indicated if they were regularly physically active six months prior to the study and during the previous month.

1.4. Procedure

Laboratory sessions occurred between 1:00 and 3:00 p.m. to control for the diurnal rhythm in cortisol levels. In accordance with the saliva collection advise published online by Salimetrics (2009a) prior to each laboratory testing session, participants were reminded to avoid: (a) alcohol/tobacco within 24-h of testing (note: sample self-identified as non-smokers), (b) eating, drinking (except water), or brushing their teeth within one hour of testing, (c) heavy exercise the morning of testing, and (d) over the counter medications such as acetaminophen (e.g., Tylenol), ibuprofen (e.g., Advil, Motrin) or cold medicines on the morning of testing. Upon arrival to the laboratory, participants were queried whether they complied with these restrictions. Violations of these restrictions led to rescheduling testing for the participant's next menstrual cycle.

The timing of the laboratory sessions was based on menstrual cycle phase. Counting menses onset as day 1 the follicular cycle laboratory session occurred during days 5–9 post-menses onset, which correspond to the mid-follicular phase and a relatively steady and moderate level of ovarian estradiol. Following ovulation, the luteal phase session was scheduled. Ovulation was determined with a home urine test (Answer Quick: Scantibodies Laboratory, Inc.), which measures the surge of leutenizing hormone with over 98% accuracy. Participants were trained on the testing procedure during their first laboratory session. This training involved reading the step-by-step instructions with a researcher, learning how to read the test results by comparing the test line to the reference line and viewing sample test sticks. Women performed the ovulation test at home according to these instructions at the same time of day. Days of testing were based on each woman's typical cycle length per product instructions. Participants called the lab following a positive result and brought the positive test stick with them to their luteal laboratory session for confirmation of ovulation by a researcher. Treating ovulation as day zero, the luteal laboratory session occurred during days 7–10 post-ovulation, which corresponds to the mid-luteal phase and the high point of ovarian estradiol and progesterone release within this phase. The selection of these testing time frames is consistent with previous research investigating the stress response across the menstrual cycle (Collins et al., 1985; Hastrup and Light, 1984; Kirschbaum et al., 1999; Manhem et al., 1991).

All participants completed follicular phase testing first and luteal phase testing second. Although we could have randomized testing between subjects by cycle phase, there was a noteworthy tradeoff to exercising control of "order" in this type of work that was governed by the menstrual cycle itself and guided our decision to test women over one healthy cycle beginning with the follicular phase. Specifically, a woman's cycle begins with menses. During that phase, ovarian follicular cells mature into the endocrine tissues that will sustain the follicular phase of the cycle. After ovulation, those same cells undergo luteinization, generating the endocrine tissue that will sustain the luteal phase of the cycle. By monitoring the start of a cycle with day one of menses followed by ovulation and the subsequent menses onset, the follicular and luteal phases can be accurately determined in women. To randomize the cycle start phase would require monitoring all of these phases in each woman for at least two cycles to capture a luteal phase confirmed with ovulation and subsequent menses onset. Moreover, the next phase for a woman who started testing during her luteal phase would actually occur during a completely different cycle governed by a completely new set of endocrine tissues, confounding cycle phase with time. Since our goal was to systematically investigate psychophysiological and neuroendocrine reactivity and physiological recovery during the follicular and luteal menstrual cycle phases, we followed women across one healthy cycle to assess differences during the two phases of one cycle.

Although cycle phase was not counterbalanced between participants, the stressor type was counterbalanced with half of the participants performing a cognitive stressor at their follicular laboratory session and a physical stressor at their luteal laboratory session, or vice versa.

Participants provided informed consent, and completed the demographic questionnaire at their first laboratory session. The protocol for each laboratory session began with having participants sit in a semi-reclined chair in a temperature controlled (65–70 °F) dimly lit room while relaxing music played. The researcher then provided a brief overview of the protocol and secured the physiological monitoring apparatus used to measure BP and HR.

Fig. 1 depicts the timeline for data collection at each laboratory session. Testing began with a 30-min baseline period while BP was taken at 10-min intervals. Participants completed the STAI-S at 10-min, and provided a saliva sample at 20-min. Saliva was collected with a cotton pledget (Salimetrics, LLC, State College, PA). Participants inserted the pledget in their mouth and rolled it around for two minutes, at which time they spat the pledget into a vial. Vials were stored at −20 °C in our laboratory and remained frozen until prepared for shipment to Salimetrics where the samples were analyzed. HR was continuously monitored throughout the entire session via Powerlab supported ECG.

At the end of baseline, the stressor period began. The stressor period lasted approximately 8 min with BP taken at 2-min intervals. The two stressors used were the physiological cold pressor task (CP; Cuddy et al., 1966) and the cognitive Paced Auditory Serial Addition Test (PASAT; Gronwall, 1977). The CP involved submerging one's hand into warm water (35–37 °C) for 4-min followed by submerging the same hand into cold water (1–3 °C) for as long as tolerable or 2-min total. A final 4-min warm-water phase followed. The cold water and subsequent warm-water submersion phases are the stress inducing phases of the CP, totaling approximately 6 min. The PASAT involved four trials of 50 single digits presented via audiocassette with increasing pace. The digit presentation rate increased over the four trials with verified rates of 2.4, 2.0, 1.6, and 1.2 s per digit, respectively. Following standardized protocol instructions and practice trials to ensure that participants understood the task, participants audibly added each newly presented digit to the one immediately preceding it while answers were recorded. Each participant finished the entire tape regardless of performance. The total length of the PASAT audiocassette is 8 min; however, the length of the test

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minus the instruction period is approximately 6 min, which is comparable in length to the stress inducing phases of the CP test.

Immediately post-stressor, a second STAI-S was completed and a second saliva sample was collected. Participants remained reclined for 30-min of recovery while a third saliva sample was collected 10 min after stressor completion. As demonstrated by Kirschbaum et al. (1999) in women tested during either the follicular or luteal cycle phases, salivary cortisol levels peaked in response to a laboratory stressor approximately 10-min after task completion and then proceeded to recover. Similar observations in timing are described by Dickerson and Kemeny (2004). This delayed peak can be explained by the time needed for cortisol to diffuse into saliva. As our goal was to assess only reactivity in cortisol, we collected our samples 10-min into baseline, immediately after completing the stressor task, and 10-min following stressor task completion. During recovery, HR was continuously monitored. To monitor BP recovery, we continued to take BP at 2-min intervals (as was done during the stressor phase) for the first 10 min of recovery switching to 5-min intervals after that.

Following the second stress testing session, participants received a take-home packet that included payment information along with the Life Events Questionnaire (LEQ; Brugha and Cragg, 1990). The LEQ assessed the occurrence of events deemed stressful by most individuals (e.g., death of spouse) within the past year. This assessment allowed us to determine if any major stressors occurred during participation and confirmed the preliminary assessment made at screening. Participants were instructed to complete this packet and return it by mail within three days of completing this second stress test. In an effort to estimate regular cyclicity, participants' payments were released after receiving this packet and a final phone call indicating the start of participants' next menstrual period following the second laboratory session.

1.5. Statistical procedures

1.5.1. Missing data

In light of recent arguments on the value of imputing missing data prior to statistical analyses (Buhi et al., 2008) we chose to impute according to the following. First, women who completed follicular testing but failed the ovulation test and subsequently dropped out ($n = 13$) did not systematically differ from those that persisted on demographic variables or group assignment; however, we did not impute luteal data for these women as they may have experienced an anovulatory cycle or an abnormally long follicular phase. As we could not determine the cause of anovulation, we made a conservative choice and opted for reducing the overall sample size as this small loss of subjects had minimal consequences on power in this study.

Second, after correcting for the above attrition, missingness for an additional 9 participants who dropped due to a loss of interest after confirmed ovulation was determined to be unrelated to group assignment or demographic variables. Yet again, we opted for a conservative approach and chose not to impute luteal stress testing values for these women as individual stress responses would not be captured. Even with this conservative approach, our remaining sample of 78 women exceeded our power requirements.

Aside from attrition, minimal data loss resulted from researcher or equipment or assay failure. Two BP assessments were lost due to improper inflation timing of the BP cuff. There were four cortisol samples that were missed due to researcher error (forgetting to sample during testing or improper sample labeling). This missingness was not related to group assignment, demographic variables, or time of testing. Since there was no theoretical justification not to impute but a statistical justification to impute, we used NORM (Schafer, 1999) to perform multiple imputations for these six cases. All iterations converged within 10 imputations (Schafer and Olsen, 1998). Resultant values were replaced in SPSS and the remaining analyses performed. It is noteworthy, however, that analyses were performed with and without imputed values and the same pattern of results emerged. Specifically, imputation did not render any additional analyses significant over the non-imputed analyses performed.

1.5.2. Data exploration and preliminary analyses

Data reduction was applied to HR and BP assessments for reactivity and recovery analyses. Single means were generated for baseline and stressor performance and recovery was reduced to three ten-minute blocks. This data reduction resulted in means for five time points which were: (a) Baseline, (b) Stressor, (c) first 10-min (Recovery 1), (d) second 10-min (Recovery 2), and (e) third 10-min of recovery (Recovery 3). No reductions were applied to cortisol or state anxiety data.

Prior to imputation and statistical analyses, data were assessed for normality and homogeneity of variance (HOV) with univariate box plots, bivariate scatterplots, and with Kolmogorov–Smirnov and Levene tests. As this study employed a repeated measures design, compliance with test assumptions was assessed for each variable at each time point for comparison. Of the variables studied, normality and HOV assumptions were violated at various time points for HR and BP only. All violations of normality were the result of positive skew; therefore, none of the values were reflected prior to performing log transformations and imputations on BP values. These transformations removed normality and HOV assumption violations. The sphericity assumption was met for all analyses.

We assessed the randomization procedure by evaluating mean group differences on demographic and health information as well as baseline measures from the first lab session. No significant group differences on any variables were observed and as such were not covaried in subsequent analyses. LEQ results were not related to any of the outcome measures nor did the order of stressor performance affect results independent of cycle phase of testing. Therefore, remaining analyses involved a mixed model RM-ANOVA with two within group factors and one between group factor. The first within group factor, *cycle phase*, had two levels: (a) follicular and (b) luteal. The second within group factor, *time across stress testing*, (henceforth referred to as *time*) had five levels: (a) Baseline, (b) Stressor, (c) Recovery 1, (d) Recovery 2, and (e) Recovery 3. The between group factor, *stressor type*, had two levels: (a) PASAT and (b) CP. We report statistically significant results with effect sizes for significant contrasts on focused effects using the formula:

$$r = \mathrm{sqrt}\, F(1, df_R)/F(1, df_R) + df_R$$

where df_R is the residual degrees of freedom for the model.

2. Results

Of the 115 women screened, 100 satisfied inclusion criteria and were randomly assigned in equal numbers to perform one of the two stressor tests at their first lab session. Thirteen participants did not complete the study due to cycle failure/anovulation (i.e., they failed the ovulation test). Three of these anovulatory women agreed to perform a second ovulation test during their next cycle, yet subsequently dropped out of the study due to a loss of interest. An additional nine women dropped following a positive ovulation test due to a loss of interest. The resultant sample included 78 women who ovulated and completed the entire study. Three women self-identified as failing to comply with pre-lab test restrictions (2 at follicular and 1 at luteal testing) and rescheduled testing for their next cycle. Their data are included here. As reported in Table 1, the sample included an ethnically diverse group of reproductively aged, freely cycling women not suffering from PMS or PMDD.

2.1. Physiological reactivity and recovery

To facilitate visual inspection of the results, physiological reactivity and recovery for HR and BP using actual values are depicted in Fig. 2. Because the main effect of stressor type was not statistically significant for HR or BP, the graphs in Fig. 2 are collapsed across stressor type to facilitate inspection of menstrual cycle phase effects. Yet, given the data inspection results for all time points included in the HR and BP repeated measures analyses, HR and BP values were log transformed for analyses.

Table 1
Characteristics of study participants ($N = 78$).

Characteristic	*n*	%
Race/ethnicity*		
Black American	6	8
American Indian/Alaskan	4	5
Asian/Pacific Islander	12	15
Hispanic	3	4
White	51	65
Other	1	1
Age (years)		
18–21	39	50
22–25	15	19
26–29	10	13
30–33	6	8
34+	8	10
Health-Related Variables		
PMS/PMDD Diagnosis (yes)	0	0
Physically Active—6 months ago	66	87
Physically Active—presently	55	74
Characteristic	***Mean***	***SD***
Cycle length (in days)	29.8	3.8
BMI	22.9	3.4

Note: * One person chose not to complete the ethnicity portion of the demographic sheet accounting for the remaining 1%. PMS = Premenstrual Syndrome, PMDD = Premenstrual Dysphoric Disorder, BMI = Body Mass Index.

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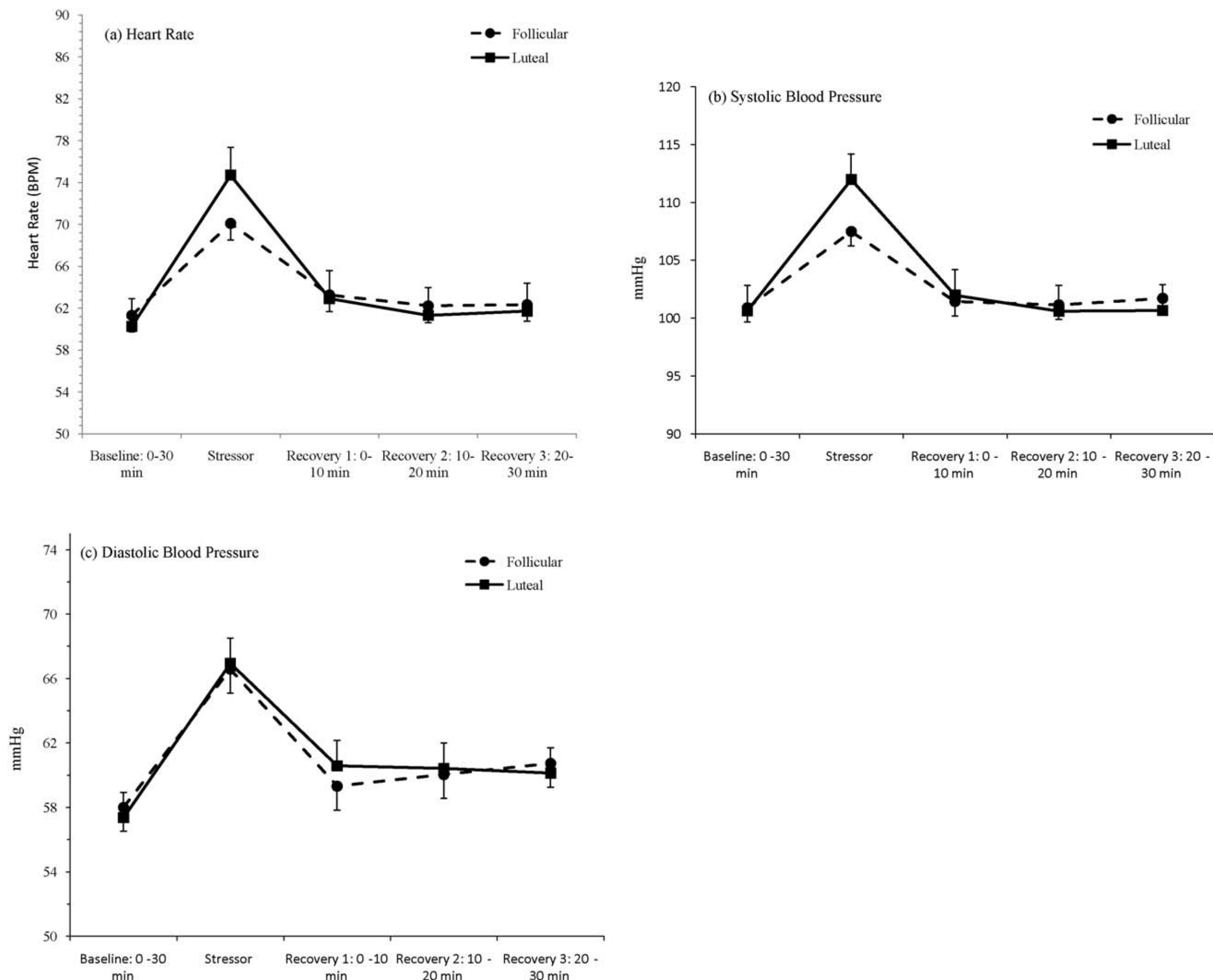

Fig. 2. Heart rate and blood pressure responses during the follicular and luteal phases of the menstrual cycle collapsed across stressor type.
Note: (a) Points represent mean heart rate in beats per minute (BPM); (b) points represent mean systolic blood pressure in millimeters of Mercury (mmHg); (c) points represent mean diastolic blood pressure in mmHg. For (a)–(c) vertical lines depict standard errors of the means. Baseline constituted the first 30-min of recording; stressors were on average 8 min long as detailed in the methods section; 30-min of recovery followed the stressor and are depicted in the above graphs in 10-min intervals. Counting menses onset as day 1, the follicular laboratory session occurred during days 5–9 post-menses onset and treating ovulation as day zero, the luteal laboratory session occurred during days 7–10 post-ovulation. According to the National Institutes of Health (2008) normal baseline HR values are 60–100, normal SBP is <120 and normal DBP is <80.

2.2. Heart rate

Of the two within subject factors (time and cycle phase), statistically significant main effects in HR were observed for time only, $F(4, 304) = 183.32$, $p < .001$. Contrasts revealed that this effect was due to differences between each time point and baseline, $F(1, 76) = 308.52$, $p < .001$, $r_{\text{baseline to stressor}} = .91$; $F(1, 76) = 46.96$, $p < .001$, $r_{\text{baseline to recovery1}} = .62$; $F(1, 76) = 7.69$, $p < .01$, $r_{\text{baseline to recovery2}} = .30$; $F(1, 76) = 8.18$, $p < .01$, $r_{\text{baseline to recovery3}} = .31$. The between subject main effect of stressor type was non-significant, indicating that without other factors considered, the type of stressor task performed did not affect HR or BP responses (discussed next).

The highest order interaction that was statistically significant was the two-way cycle phase by time interaction, $F(4, 304) = 17.04$, $p < .001$. Contrasts revealed that only the first interaction term was significant, $F(1, 76) = 30.92$, $p < .001$, $r_{\text{baseline to stressor, follicular vs. luteal}} = .54$. Thus, HR reactivity was greater during the luteal phase compared to the follicular phase independent of stressor type.

2.3. Systolic blood pressure (SBP)

The main effect of time was statistically significant, $F(4, 304) = 79.00$, $p < .001$. Contrasts revealed that this effect was due to differences between baseline and stressor, $F(1, 76) = 106.64$, $p < .001$, $r_{\text{baseline to stressor}} = .76$, and between baseline and the first point of recovery, $F(1, 76) = 7.62$, $p < .01$, $r_{\text{baseline to recovery1}} = .30$.

The highest order interaction that was statistically significant was the time by cycle phase interaction, $F(4, 304) = 9.74$, $p < .001$. Contrasts revealed that only the first interaction term was significant, $F(1, 76) = 15.16$, $p < .001$ $r_{\text{baseline to stressor, follicular vs. luteal}} = .41$ and as such the cycle phase effect on SBP reactivity was greater during the luteal phase compared to the follicular phase independent of stressor type.

2.4. Diastolic blood pressure (DBP)

The within subject main effect of time was the only statistically significant effect observed for DBP, $F(4, 304) = 103.17$, $p < .001$. The contrasts revealed that this effect was due to differences between each time point and baseline $F(1, 76) = 186.15$, $p < .001$, $r_{\text{baseline to stressor}} = .84$; $F(1, 76) = 48.10$, $p < .001$, $r_{\text{baseline to recovery1}} = .62$; $F(1, 76) = 43.70$, $p < .001$, $r_{\text{baseline to recovery2}} = .61$; $F(1, 76) = 71.35$, $p < .001$, $r_{\text{baseline to recovery3}} = .71$. There was a nearly significant cycle phase by time interaction, $F(4, 304) = 2.47$, $p = .05$, which contrasts revealed was due to a larger deviation from baseline at Recovery 1 during the luteal phase compared to the follicular phase,

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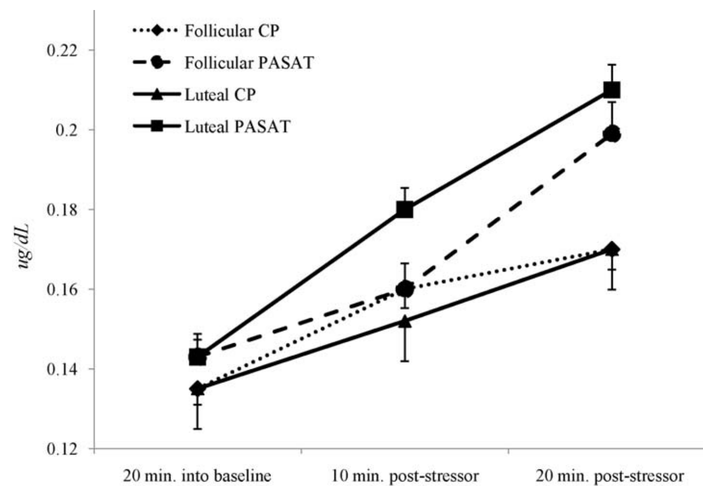

Fig. 3. Salivary cortisol responses during the follicular and luteal phases of the menstrual cycle displayed by laboratory stressor type.
Note: Points represent mean salivary cortisol values in microgram per deci-liter (μg/dl); vertical lines depict standard errors. Counting menses onset as day 1, the follicular laboratory session occurred during days 5–9 post-menses onset and treating ovulation as day zero, the luteal laboratory session occurred during days 7–10 post-ovulation. Values fall within the expected salivary free cortisol range for adult females, ages 21–50 samples during 1:00–3:00 p.m. as reported by Salimetrics (2009b), the lab contracted to perform cortisol assays in the present study. PASAT = Paced Auditory Serial Addition Test; CP = Cold Pressor.

$F\ (1,\ 76) = 11.26$, $p < .01$, $r_{\text{baseline to recovery1, follicular vs. luteal}} = .36$. Based on the statistically significant main effect of time, DBP did not recover in the time period of testing during either phase. All other interactions were non-significant.

2.5. Neuroendocrine reactivity

Mean cortisol values for each time point of sampling are depicted in Fig. 3. A statistically significant main effect of time was observed, $F\ (2,\ 152) = 77.42$, $p < .001$, which contrasts revealed was due to differences between each time point and baseline, $F\ (1, 76) = 35.60$, $p < .001$, $r_{\text{baseline to stressor1}} = .56$; $F\ (1,\ 76) = 102.85.88$, $p < .001$, $r_{\text{baseline to stressor2}} = .76$. The main effect of stressor type was also nearly significant with higher PASAT than CP values, $F\ (1, 76) = 4.06$, $p = .05$, $r_{\text{stressor}} = .23$. The three-way interaction of cycle phase by time by stressor was statistically significant, $F(4, 152) = 5.42$, $p < .01$ resulting from greater PASAT than CP levels in the second stressor sample (i.e., Stressor 2 in Fig. 3) compared to baseline during luteal phase testing, $F\ (1,\ 76) = 7.76$, $p < .01$, $r_{\text{baseline to stressor2, follicular vs. luteal, PASAT vs. cold pressor}} = .30$. Thus, cortisol reactivity was greatest in response to the PASAT during the luteal phase.

So that these findings can be interpreted in accordance with Dickerson and Kemeny's (2004) meta-analytic findings on salivary cortisol responses, we also calculated the *d* statistic using the effect size formula: $d = (M_{poststressor} - M_{prestressor})/SD_{prestressor}$, where 0.20 indicates a small effect, 0.50 indicates a moderate effect, and 0.80 a large effect. Results revealed moderate to large effects for both stressor tasks. Specifically, during the follicular phase testing session, the CP *d* calculated for Stressor 1 was .67 and for Stressor 2 was 1.6 (Fig. 3). During the luteal phase, the CP *d* for Stressor 1 was .59 and for Stressor 2 was .98. The PASAT *ds* during the follicular phase were .39 for Stressor 1 and .77 for Stressor 2, and during the luteal phase were .55 for Stressor 1 and 1.11 for Stressor 2.

2.6. State anxiety reactivity

Mean STAI-S reports at baseline and immediately following the stressor tasks are depicted in Fig. 4. The only statistically significant main effect was for time, $F(1, 76) = 109.15$, $p < .001$, $r_{\text{baseline to stressor}} = .77$. A significant three-way interaction was observed, $F\ (1,\ 76) = 88.18$, $p < .001$, $r_{\text{baseline to stressor, follicular vs. luteal, CP vs. PASAT}} = .73$, indicating that the PASAT led to more state anxiety reactivity

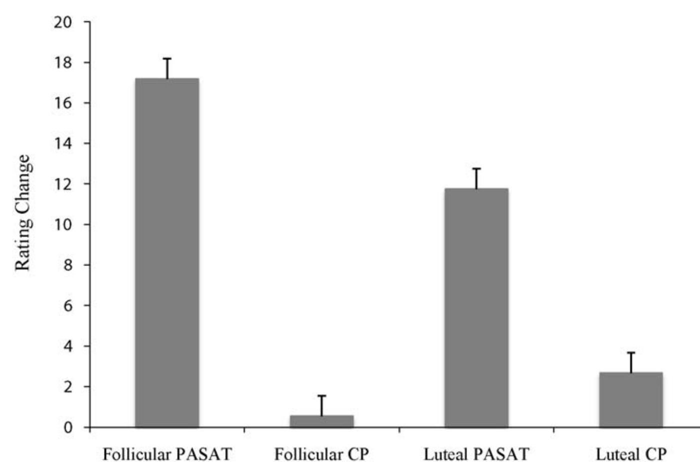

Fig. 4. Mean rating changes in self-reported state anxiety (STAI-S) in response to laboratory stressors during the follicular and luteal phases of the menstrual cycle.
Note: Bars represent mean rating changes from baseline in self-reported State Anxiety (i.e., STAI-S) immediately following the stressor; vertical lines depict standard errors. Counting menses onset as day 1, the follicular laboratory session occurred during days 5–9 post-menses onset and treating ovulation as day zero, the luteal laboratory session occurred during days 7–10 post-ovulation. The possible score range on the STAI-S is 20–80 for women 19–69 years of age; Spielberger et al. (1983) reported normative data as (M = 38.76, SD = 11.95) in college students and (M = 35.20, SD = 10.61) in working adults. PASAT = Paced Auditory Serial Addition Test; CP = Cold Pressor.

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than the CP during the follicular phase compared to the luteal phase, however in both phases the PASAT produced the greatest reactivity.

3. Discussion

The present study fills an important gap in women's biopsychology research by systematically investigating psychophysiological and neuroendocrine responses during the luteal and follicular menstrual cycle phases using well-validated stressor techniques. We examined three research questions: (a) In response to laboratory stressors, does psychophysiological reactivity measured by HR, BP, and state anxiety differ by menstrual cycle phase or stressor type?; (b) In response to laboratory stressors, does neuroendocrine reactivity measured by salivary cortisol differ by cycle phase or stressor type?; and (c) After a laboratory stressor, does HR and BP recovery differ by cycle phase or stressor type?

With respect to our first research question, we found that participant's HR and SBP responses were significantly more reactive during the luteal phase compared to the follicular phase of their menstrual cycle, results that corroborate the findings of Tersman et al. (1991) and Manhem et al. (1991). Conversely, none of the two-way interactions for HR or BP were affected by the type of stressor (i.e., PASAT vs. CP test). These results suggest it is unlikely that the differences in cardiovascular response reported across prior studies (e.g., Miller and Sita, 1994; Sato et al., 1995; Stoney et al., 1990) are fully attributable to the use of different stressors.

Further, we found that state anxiety reactivity was greatest in response to the PASAT during the follicular phase. Importantly, during the follicular phase the CP test produced little reported anxiety (i.e., the mean change in state anxiety in response to the CP was less than one unit of measure on the STAI-S scale), however, significant increases in physiological and neuroendocrine reactivity for the CP existed. This dissociation between psychological and physiological reactivity to the CP is interesting and cannot be explained by the duration of the CP task since the difference in the mean length of time women left their hands in the cold water during the follicular and luteal phases was non-significant.

The dissociations among psychological, physiological, and neuroendocrine responses to laboratory stressors are not unique to our study. Early research demonstrated that HR and skin conductance were not correlated with increases in subjective states of stress in women as indicated by the STAI (Holroyd et al., 1978). Such observations were capitalized upon during the late seventies and eighties to develop various mind–body approaches to reduce the stress response (e.g., biofeedback), in part by decreasing disembodiment or a disconnected mind–body state. More recently, Galantino et al. (2005) demonstrated that while an 8-week mindfulness-based stress reduction program led to a significant reduction in perceived stress levels as indicated via pen and paper assessments, salivary cortisol did not significantly change over the course of the program. Our observation that there are menstrual cycle ramifications in women's psychological, physiological, and neuroendocrine responses to laboratory stressors certainly calls for further investigation.

We found that the magnitude of reactivity for DBP did not differ by cycle phase or stressor type. While these findings are consistent with Stoney et al. (1990), Sato et al. (1995), and Weidner and Helmig (1990) who observed similar BP reactivity during both cycle phases they refute those of Miller and Sita (1994) who found higher DBP reactivity during the follicular phase. Incongruent DBP findings among studies are not unique to women's psychobiology research. Such inconsistencies have contributed to debates over the importance of DBP in predicting health-related outcomes such as hypertension (Tin et al., 2002). Since such predictability from DBP is greater in young adults (50 years and younger (Franklin, 2007)) studying DBP reactivity in women of reproductive age remains a worthwhile endeavor.

With respect to our second research question, we observed greater salivary cortisol reactivity during the luteal phase in women that performed the PASAT. These findings are consistent with the stressor-specific HPAA hypothesis, which suggests that stressors involving motivated performance tasks with evaluative components produce more reliable cortisol reactivity than other types of stressors (Dickerson and Kemeny, 2004). Yet, based on our observations this is specific to luteal phase testing in freely cycling women.

This stressor type effect on luteal phase salivary free cortisol reactivity likely reflects the combined effects of estrogen and cortisol binding globulin (CBG) activity on free cortisol. Kirschbaum et al. (1999) and Kajantie and Phillips (2006) report that the cortisol reactivity responses in women are related to three factors: (a) the menstrual cycle phase during which they are assessed, (b) the sampling medium, and (c) exogenous hormone usage. When salivary free cortisol reactivity is assessed, freely cycling women tested in their luteal phase show greater responses than women tested during their follicular phase, and women tested in their follicular phase subsequently demonstrate more reactivity than women taking oral contraceptives (OC). Conversely, when total cortisol in plasma was assessed, OC users showed the greatest cortisol response and cycle phase responses in freely cycling women did not differ. Kajantie and Phillips (2006) suggest that the differences observed in OC users are due to exogenous estrogen increases in CBG, which thereby decreases available free cortisol for analyses in saliva. Since few studies systematically investigate stressor and menstrual cycle effects on cortisol in freely cycling women, our findings offer a timely contribution to this area of research.

With respect to our third research question regarding HR and BP recovery, we found that DBP failed to recover to baseline within the 30-minute post-stressor monitoring periods irrespective of cycle phase or stressor type. This delayed post-stressor recovery provides timely support for the growing evidence that impaired cardiovascular recovery predicts future hypertension (Steptoe and Marmot, 2005, 2006). Based on evidence from the Whitehall psychobiology study investigating inflammatory and hemostatic responses to laboratory stressors and psychosocial risk factors to such responses in men and women, Steptoe and Marmot (2006) suggest that delayed post-stress blood pressure recovery serves as a marker for prolonged hemostatic responses that directly influence cardiovascular disease pathogenesis. Considering this evidence with our findings, freely cycling women who experience delayed post-stressor recovery in DBP may be particularly vulnerable if they experience repeated stressors. What remains to be elucidated is whether small differences leading to statistically significant delays in recovery, such as those we observed, can accumulate over time if they are repeated over cycles in women.

Limitations to this study exist. First, not randomizing cycle phase, albeit for sound biological reasons, may have introduced uncontrolled ordering effects. Yet, our results do not argue for systematic practice or habituation effects as reactivity in all measures that were observed during the follicular cycle phase were subsequently observed during the luteal phase. Second, we collected only three saliva samples to measure reactivity. Our choice in sample number was based on the findings of Kirschbaum et al. (1999) who demonstrated that salivary cortisol reactivity peaked 10-min after participants began performing the stressor task, which was the Trier Social Stress Task. Given these findings, our budget constraints, and our goals of only studying reactivity, we opted to collect our last reactivity

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sample 10-min after stressor completion. However, it is possible that additional samples may have revealed further reactivity. Additional research is needed to test this possibility.

In conclusion, the results of this study reflect the need for considering menstrual cycle phase of testing when assessing psychophysiological and neuroendocrine responses to laboratory stressors in women. Moreover, controlling for stressor type is relevant in studies of state anxiety reactivity and HPAA responses measured by salivary cortisol in women. A complement to these findings would be investigations of the inter-relationships among psychophysiological and neuroendocrine stressor responses and variables previously linked with perceived stress in women such as premenstrual symptoms (Lustyk and Gerrish, 2010), a history of abuse or other trauma (Lustyk et al., 2007; Widman et al., 2005), irritable bowel syndrome (Levy et al., 1997), or disordered eating (Ball and Lee, 2000). Given the intimate link between stress and cardiovascular health in women (Cohen et al., 1998), more research that explores physiological responses to stressors across menstrual cycle phases is needed. Our results revealed more luteal phase reactivity in HR and SBP and the absence of recovery (in 30-min) in DBP. These findings have implications for cardiovascular health as repeated acute and persistent stress responses tax the heart muscle, which may have life threatening consequences. Given the frequency with which cognitive stressors present themselves in the daily lives of women, time will tell if interventions aimed at reducing the body's specific responses to such stressors will provide targeted health benefits for women.

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