Neurobiology of Stress 3 (2016) 96–104

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: http://www.journals.elsevier.com/neurobiology-of-stress/

Stress-induced increases in progesterone and cortisol in naturally cycling women

Alexandra Ycaza Herrera*, Shawn E. Nielsen, Mara Mather

Davis School of Gerontology, University of Southern California, 3715 McClintock Ave, Los Angeles, CA 90089, United States

ARTICLE INFO

Article history:
Received 11 September 2015
Accepted 9 February 2016
Available online 11 February 2016

Keywords:
Progesterone
Cortisol
Stress
Menstrual cycle
Hormones
Women

ABSTRACT

Studies with animals of both sexes show that the adrenal glands release progesterone in addition to cortisol in response to stress. However, little is known about the progesterone response to stress in naturally cycling women. We investigated the effect of stress on estradiol, progesterone, and cortisol levels in women during the follicular phase of the menstrual cycle. We found that physical stress (the cold pressor test) had no effect on estradiol levels, but increased progesterone and cortisol. We also found positive correlations between baseline progesterone and cortisol levels, as well as between the change in progesterone and cortisol before and after water exposure in both the stress and control sessions. Mediation analyses revealed during the stress session, the change in progesterone from baseline to 42-min post-stress onset was mediated by the magnitude of change in cortisol levels across the same time span. Overall, these findings reveal that progesterone released in response to stress as observed in animals and men extends to women during the low ovarian output follicular phase of the menstrual cycle, and that the mechanism of release may be similar to the mechanism of cortisol release.

1. Introduction

The levels of bioavailable, salivary cortisol observed in response to a stressor varies between sexes and across the menstrual cycle in women (Kirschbaum et al., 1992 Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005). For example, women in the luteal phase of the menstrual cycle (moderate estradiol and high progesterone levels) and men exhibit comparable salivary cortisol increases to social stress, while women in the follicular phase (low estradiol and low progesterone) and women on oral contraceptives (low ovarian output of estradiol and progesterone) exhibit significantly smaller salivary cortisol responses to the same social stressor (Kirschbaum et al., 1999). More recent studies show that hormonal contraceptives also attenuate the salivary cortisol response to physical stress compared to naturally cycling women (Nielsen et al., 2013b) and more specifically, luteal women (Nielsen et al., 2014).

One interpretation of these findings is that higher progesterone (P) levels during certain phases the menstrual cycle leads to greater free cortisol levels in response to stress. Other work supports such an interpretation. For example, at least one group of women (those with induced hypogonadism via administration of the gonadotropin releasing hormone agonist lupron) exhibited amplified cortisol responses to exercise stress when also administered progesterone but not estradiol (Roca et al., 2003). However, the interpretation that P amplifies cortisol response to stress fails to acknowledge that the adrenal glands also secrete P and that the influence of menstrual cycle fluctuations of P on cortisol response to stress may be masking whether and how adrenal P may be responding to stress. The effect of stress on adrenal output in animals and men has shown that the adrenal glands secrete not only cortisol, but also P, in response to stress (Fajer et al., 1971 Brown et al., 1976b; Deis et al., 1989 Breier and Buchanan, 1992; Cooper et al., 1995 Elman and Breier, 1997; Duncan et al., 1998 Romeo et al., 2004; Romeo et al., 2006), with limited work examining the effect in women (Childs et al., 2010; Gaffey and Wirth, 2014). This P release during stress is of importance for studies examining menstrual cycle influences on the stress response, as many studies average P values across multiple time points in order to determine average cycle-related P levels during an experimental session (Nielsen et al., 2013a; Nielsen et al., 2014; Petersen et al., 2014). If women also experience adrenal release of P in response to stress, then this adrenal release of P in response to stress may contribute to the pattern of greater bioavailability of cortisol in response to stress during high progesterone phases of the menstrual cycle

* Corresponding author.
E-mail address: ycaza@usc.edu (A.Y. Herrera).

http://dx.doi.org/10.1016/j.ynstr.2016.02.006

(Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005). Despite this possibility, little work has tested the relationship between estradiol (E2), P, and the cortisol response to stress in young, naturally cycling women.

In the present study, we aimed to investigate the influence of baseline P on cortisol responses and to test the effect of stress exposure on E2, P, and cortisol levels in response to a physical stressor (Cold Pressor Test; CPT) in naturally cycling women during the early and late follicular phase of the menstrual cycle. Another neglected factor when drawing conclusions regarding the relationship between P and cortisol during the luteal phase of the menstrual cycle is the concomitant increase in E2 also experienced during the luteal phase. We thus elected to test women during the low-P follicular phase of the menstrual cycle, which abolished the concerns for accompanying changes in E2 and the ability to investigate whether P and cortisol shared a similar relationship when P fluctuations are much smaller as observed during the follicular phase than when P levels are much higher as during the luteal phase (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005).

By investigating the P and cortisol relationship during the low-P follicular phase of the menstrual cycle, we made the following hypotheses. First, we hypothesized that both salivary cortisol and salivary P would increase in response to CPT exposure. Second, we hypothesized that baseline salivary cortisol and baseline salivary P would be positively correlated. Finally, based on the aforementioned observed associations between high P and higher levels of stress-induced bioavailable cortisol during the luteal phase of the menstrual cycle (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005), we hypothesized that baseline P levels would mediate the cortisol response to CPT exposure, such that higher baseline salivary P levels would account for larger cortisol responses to CPT.

2. Materials and methods

2.1. Participants

Thirty-three naturally cycling undergraduate females from the University of Southern California (18–24 years) participated in this study. Participants attended four sessions after first providing informed consent. Two sessions occurred during the Early Follicular phase (EF; days 1–5, with day 1 being the first day of menses) and two occurred during the Late Follicular phase (LF; days 8–12), order counterbalanced. Twenty-seven women completed all four sessions. Cycle regularity was defined as menses regularly occurring between 25 and 31 days. Women were determined to be regular if they self-reported their prior two cycles as falling within the range of 25–31 days during a phone interview that occurred prior to their participation. During the phone interview, women also reported the expected start date of their next menses. Women were then seen for their first of four sessions upon confirming the start of that post-phone interview menses. The average age of the participants was 20.8 ± 1.8 years (range: 18–24 years) and the average years of education was 14.8 ± 1.8 years (range: 12–18); 77.8% were of non-Hispanic ethnicity and 22.2% of Hispanic ethnicity, and race breakdown was 55.6% Asian, 18.5% Caucasian, 7.4% biracial, 14.8% other, while 3.7% declined to state.

Participants were free from heart disease, peripheral vascular disease, diabetes, Reynaud's phenomenon, cryoglobulinemia, vasculitis, lupus, tingling or numbness in the hands and/or feet, and any other serious chronic illness. They were non-smokers, not using beta-blocker or corticosteroid-based medications, or psychoactive drugs, and had never been pregnant. Former hormonal contraception users had stopped using hormonal contraception at least 6 months before participation.

Participants completed one stress and one control session in both the EF and LF phases, order counterbalanced. Most women first seen during the EF phase completed all 4 sessions within the same menstrual cycle, whereas women first seen during the LF phase completed their 4 sessions across two consecutive menstrual cycles. Three women were seen across more than 2 menstrual cycles due to schedule conflicts.

2.2. Salivary hormone measurements

All sessions were conducted in the afternoons between 1200 and 1900 h, with no session starting later than 1730 h. To ensure stable hormone levels prior to collection of the baseline saliva samples, participants were asked to refrain from exercise and food/drink (except water) within one hour, sleep within two hours, and caffeine and alcohol within three hours of their session start time. The general protocol for all sessions was (see Fig. 1): arrive, drink 8 oz. of water, saliva sample 1 (baseline; minimum of 10 min after finishing water), CPT, saliva sample 2 fifteen minutes after CPT onset (15m-post-stress), behavioral tasks, and saliva sample 3 after all behavioral tasks had been completed, or an average of forty-two minutes after CPT onset (42m-post-stress). While part of a larger behavioral study examining the effects of stress on working memory and emotional memory processes, this study focused only on cortisol, P, and E2 responses to CPT stress, thus behavioral data are not reported here (although timing of tasks is also displayed in Fig. 1).

Salivary samples are a reliable source for determining biologically available, unbound, levels of hormones (Vining et al., 1983; Tunn et al., 1992). Participants passively drooled saliva into a collection tube for each sample. Cortisol levels were measured in all three saliva samples, and P and E2 in the first and last samples. Due to the common practice of determining E2 and P levels by averaging two sample measurements in menstrual cycle studies (Nielsen et al., 2013a, 2014; Petersen et al., 2014), we wanted to test the first and last samples to see whether and how stress affects P and E2. Samples were stored at 0 °C until all data collection was completed, at which time saliva was assayed to determine hormone levels.

Salivary levels of cortisol, 17β-estradiol, and progesterone were measured using Salimetrics, LLC (State College, PA) ELISA kits and measured optically using Molecular Devices, LLC SpectraMax M3 Multi-mode Microplate Reader (Sunnyvale, CA). The inter- and intra-assay variations for cortisol (8.16%; 12.3%), 17β-estradiol (4.12%; 16.2%), and progesterone (11.7%; 19.9%) were within the expected ranges from our lab.

2.3. Stress manipulation

The CPT was used to induce a stress response and has been shown to reliably induce cortisol secretion (Lighthall et al., 2009, 2012; Mather et al., 2010). Participants immersed their non-dominant hand, up to the wrist, in ice water (0–5 °C at time of immersion) for up to three minutes. Participants completed a minimum of one minute with their hand immersed in ice water. Participants unable to complete at least 60 consecutive seconds in the ice water were allowed to remove and re-immerse their hand until they accumulated at least 60 s with their hand in the water. Eighteen participants successfully kept their hands immersed in the stressful ice water for 3 min. Of the nine remaining participants, 4 kept their hands immersed for at least one minute, but fewer than 3 min, and 5 participants removed and re-immersed their hands until accumulating at least 1 min in the ice water. The control condition replaced the ice water with warm water

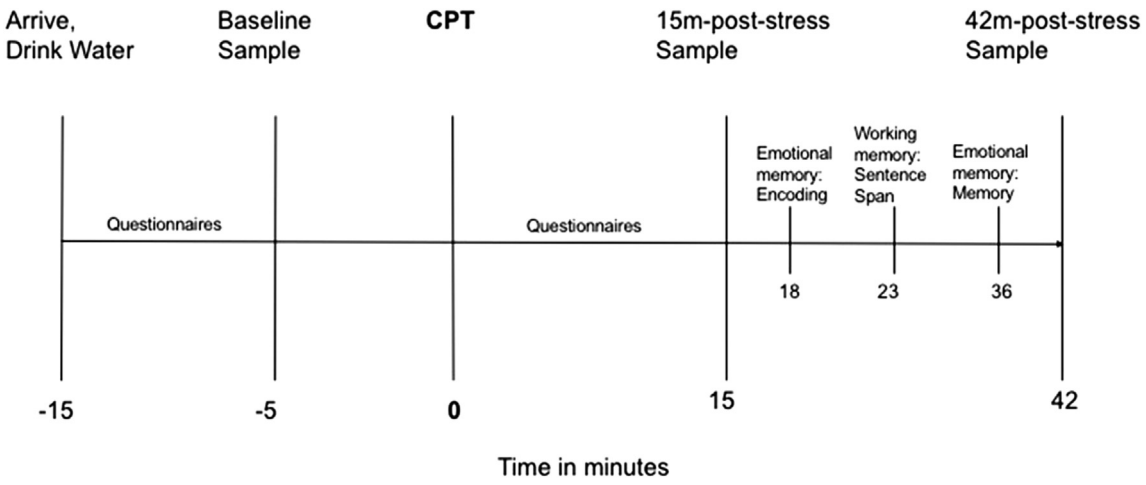

Fig. 1. Timeline for all sessions. General protocol and timeline for stress and control sessions, including cognitive tasks not reported here.

(37–40 °C at time of immersion). All but one participant kept their hands immersed in the warm water control for the full 3 min. Order of the stress and control condition was counterbalanced in each phase, such that condition was randomized for the first session within a phase and then participants received the alternate condition during the second session within a particular phase. In order to better control for anticipatory stress levels, participants were told they may receive alternate water conditions at each session or the same water condition at each session, depending on random assignment.

2.4. Statistical analysis

A preliminary 2 (phase: EF vs. LF) x 2 (session: stress vs. control) repeated-measures ANOVA on baseline hormone levels found no main effects of phase on E2, $F(1,25) = 1.472$, $p > .05$, or P, $F(1,26) = .762$, $p > .05$, no main effect of session on E2, $F(1,25) = .591$, $p > .05$, or P, $F(1,26) = .124$, $p > .05$, and no phase by session interactions for E2, $F(1,25) = .336$, $p > .05$, or P, $F(1,26) = .259$, $p > .05$. Additional preliminary 2 (session: first and second ice water exposure; i.e., stress exposures) x 3 (time: baseline vs. 15m-post-stress vs. 42m-post-stress) repeated measure ANOVA revealed an expected significant main effect of time, $F(2,52) = 7.693$, $p < .05$, with no main effect of whether it was their first or second stress session on cortisol response, $F(1,26) = .046$, $p > .05$, or session by time interaction, $F(2,52) = .608$, $p > .05$. As a result, we collapsed across EF and LF phases to examine the effect of stress on E2, P, and cortisol regardless of phase. This was achieved by averaging baseline samples from stress sessions in EF and LF and baseline samples from the control sessions in EF and LF, and so on for the 15m-post-stress and 42m-post-stress samples. One participant was removed from the E2 analyses for insufficient saliva during the baseline sample. There was enough in the baseline sample for cortisol and P, so the participant is included in all progesterone and cortisol analyses.

To examine the effect of stress on cortisol across all three sample points and on P and E2 using baseline and 42m-post-stress saliva samples in the control and stress sessions, we conducted a series of repeated-measures ANOVAs. Partial eta squared effect sizes and p-values for Fisher's least significant difference (LSD) post-hoc pairwise comparisons are also reported.

To further explore the possibility that higher endogenous P levels might affect cortisol response to stress, we created High and Low P groups using a median split, which provided the benefit of comparing higher versus lower P levels during the low-P follicular phase while avoiding concomitant differences in E2 between groups, $t(24) = .502$, $p = .620$. We then conducted a mixed-model ANOVA to examine potential effects of high versus low P levels on cortisol response after CPT exposure.

We used Pearson's correlations to examine the direction of relationship between baseline levels of P and cortisol, as well as the direction of the relationship between the change in cortisol and P after stress exposure. Finally, in order to test the possibility that baseline P levels influence the magnitude of bioavailable cortisol responses as was suggested by menstrual cycle studies comparing luteal and follicular phases (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005), we ran mediation analyses to determine whether the magnitude of change in P and cortisol after stress accounted for the change from baseline in one another. Mediation was tested using PROCESS for SPSS (Hayes, 2013), which tested mediation via calculation of 5000 bias-corrected bootstrap 95% confidence intervals. P-values for each pathway in the mediation models are reported, as well as the CI for overall model significance.

Significance for all analyses providing p-values was set at $p \leq .05$. Significance for the overall fit of the mediation models produced using PROCESS was set as CI not containing 0.

3. Results

3.1. Cortisol levels increase in response to CPT during the follicular phase of the menstrual cycle

A 2 (stress: CPT vs. control) × 3 (time: baseline vs. 15m-post-stress vs. 42m-post-stress) repeated measures ANOVA on salivary cortisol levels revealed a marginally significant main effect of stress, $F(1,26) = 3.326$, $p = .080$, $\eta_p^2 = .113$, a significant main effect of time, $F(2,52) = 5.757$, $p < .01$, $\eta_p^2 = .181$, and a significant stress × time interaction showing that CPT exposure increased cortisol levels, whereas warm water exposure did not, $F(2,52) = 10.040$, $p < .001$, $\eta_p^2 = .279$; see Fig. 2. Post-hoc Fisher's LSD pairwise comparisons revealed that in the stress session, women experienced significant increases in cortisol levels from baseline to 15m-post-stress ($p < .001$) followed by significant decreases from 15m-post-stress to 42m-post-stress ($p < .01$), such that cortisol levels were no longer significantly different from baseline by 42m-post-stress ($p > .1$; see Fig. 2). The only significant effect of time observed during the control session was a

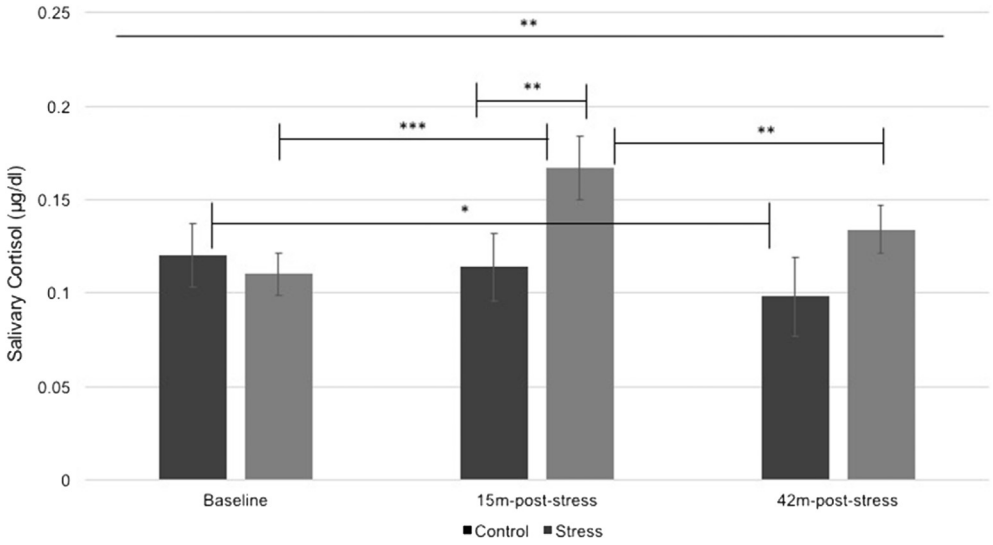

Fig. 2. Cortisol levels increase in response to cold pressor stress during the follicular phase of the menstrual cycle. Cortisol levels during the stress and control sessions in women during the follicular phase of the menstrual cycle. A significant stress session × sample time point interaction is indicated by the top line. Significant pairwise differences between Baseline and 15m-post-stress during the stress session, 15m-post-stress and 42m-post-stress during the stress session, Baseline and 42m-post-stress during the control session, and between the control and stress sessions for the 15m-post-stress onset sample time point, are also indicated. ***: $p < .001$, **: $p < .01$, *: $p < .05$.

significant decrease from baseline to 42m-post-stress ($p < .05$). Additional pairwise comparisons showed the expected effects that women did not differ in their baseline cortisol levels between stress and control sessions and that women had significantly higher cortisol levels at 15m-post-stress during the stress session compared with the control session ($p < .01$; see Fig. 2). The decrease in cortisol levels experienced by women from 15m-post-stress to 42m-post-stress during the stress session resulted in women exhibiting similar cortisol levels at 42m-post-stress in the two sessions, although women did show a trend toward higher cortisol levels during the stress session ($p > .09$; see Fig. 2).

3.2. Progesterone levels increase in response to CPT during the follicular phase of the menstrual cycle

A 2 (stress: CPT vs. control) × 2 (time: baseline vs. 42m-post-stress) repeated measures ANOVA on P levels failed to uncover a main effect of time, $F(1,26) = .084$, $p > .7$, $\eta_p^2 = .003$, but did uncover a marginally significant main effect of stress, $F(1,26) = 4.054$, $p = .055$, $\eta_p^2 = .135$ and revealed a significant stress × time interaction showing P levels increased in response to CPT exposure, and decreased in response to warm water exposure, $F(1,26) = 7.466$, $p < .05$, $\eta_p^2 = .223$; see Fig. 3. Post-hoc Fisher's LSD pairwise comparisons revealed that women did not differ in their baseline P levels between stress and control sessions ($p > .7$), but that women had significantly higher P levels at 42m-post-stress during the stress session than at the same time point during the control session ($p < .01$; see Fig. 3). This significant difference between P levels at 42m-post-stress was driven by a non-significant increase in P after CPT exposure in the stress session and non-significant decrease in P after warm water exposure in the control session.

3.3. Baseline progesterone levels influenced cortisol levels at all time points, and marginally affected cortisol differences in the stress and control sessions

To investigate whether low and high P levels within a typically low-P phase of the menstrual cycle differentially affected cortisol response to stress, we used a median split to create high and low P groups and conducted a 2 (stress: CPT vs. control) × 2 (time: baseline vs. 15m-post-stress) × 2 (P: high vs. low) mixed-model ANOVA. The analysis failed to uncover a main effect of stress, $F(1,25) = 3.002$, $p > .09$, $\eta_p^2 = .107$, but did uncover a main effect of time, $F(1,25) = 8.825$, $p < .01$, $\eta_p^2 = .261$, a main effect of P, $F(1,25) = 12.225$, $p < .01$, $\eta_p^2 = .328$, and a marginally significant 3-way interaction, $F(1,25) = 4.040$, $p = .055$, $\eta_p^2 = .139$; see Fig. 4, with the high P group showing higher cortisol levels. Post-hoc Fisher's LSD pairwise comparisons revealed that the high P group displayed higher baseline cortisol levels in the stress session ($p < .05$) and in the control session ($p < .01$), and still exhibited higher cortisol levels at 15m-post-stress in both the stress session ($p < .01$) and the control session ($p < .05$). Additional pairwise comparisons revealed that only the high P group experienced significant 15m-post-stress increases in cortisol during the stress session ($p < .001$).

3.4. Baseline progesterone and cortisol levels, as well as change in both hormone levels across time, were positively correlated

To better examine the relationship between P and cortisol levels and responses, we conducted a series of correlation analyses. Levels of baseline P and cortisol were positively correlated in both the stress, $R^2(25) = .485$, $p < .001$ (see Fig. 5a), and control sessions, $R^2(25) = .348$, $p = .001$ (see Fig. 5b). Additional analyses were completed on the change in hormone levels (42m-post-stress onset minus baseline) for P and cortisol; these analyses were limited to the 42m-post-stress onset since this was the only post stress time point available for both hormones. These analyses revealed a significant positive correlation between the change in P and cortisol during the stress session, $R^2(25) = .418$, $p < .001$ (see Fig. 6a), suggesting that both hormones responded in a similar fashion after stress exposure. This same significant positive correlation between change scores was observed in the control session, $R^2(25) = .562$, $p < .001$ (see Fig. 6b), suggesting that P and cortisol follow a similar trajectory both after stress exposure and in the absence of stress exposure.

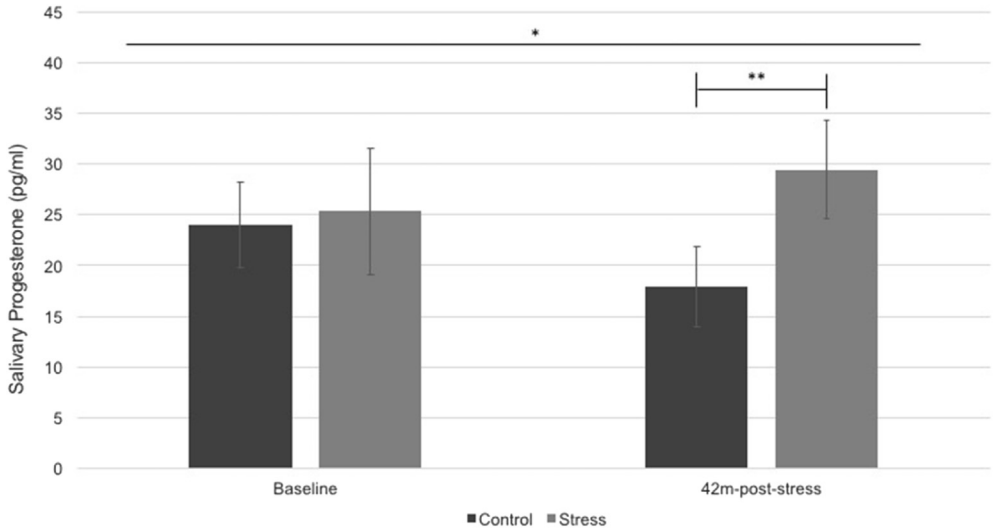

Fig. 3. Progesterone levels increase in response to cold pressor stress during the follicular phase of the menstrual cycle. Progesterone levels during the stress and control session at Baseline and 42m-post-stress. A significant stress session × sample time point interaction is indicated by the top line. A significant difference between control and stress session during the 42m-post-stress sample time point is also indicated. **: $p < .01$, *: $p < .05$.

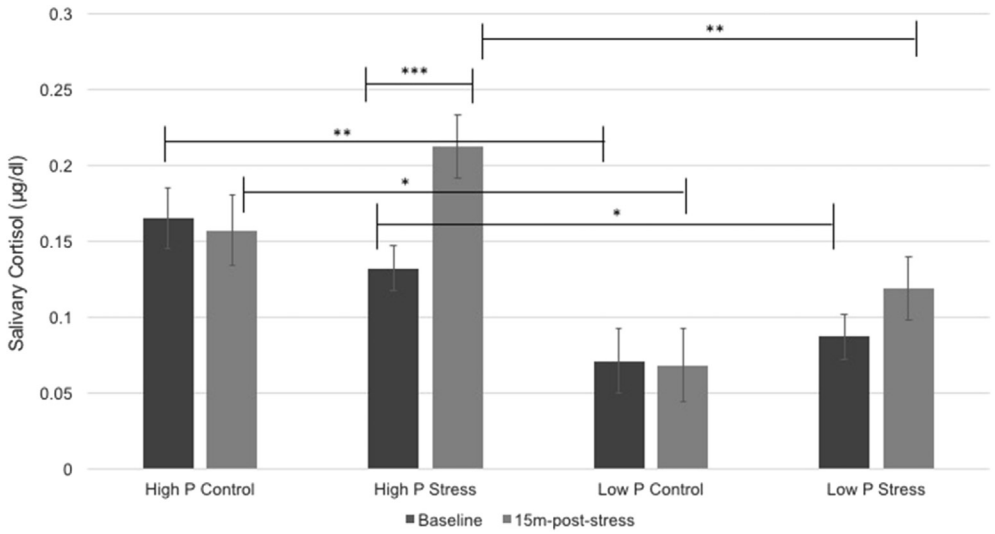

Fig. 4. Baseline progesterone levels were associated with pre- and post-stress cortisol levels. Cortisol response from baseline to 15m-post-stress during the stress and control sessions in women with high baseline P and low baseline P determined by a median split. The 3-way interaction between stress session × sample time point × P group was marginally significant ($p = .055$). The high P group had significantly higher cortisol levels at each time point during the stress and control sessions. Only the high P group exhibited a significant increase in cortisol during the stress session. ***: $p < .001$, **: $p < .01$, *: $p < .05$.

3.5. Cortisol response from baseline to 42m-post-stress mediated the change in progesterone across the same time span

To better examine these positive associations between baseline P and cortisol levels, and change in these hormones across time, we conducted a series of mediation analyses using PROCESS in SPSS. Because of the influence of baseline P level on cortisol levels throughout the sessions, we first analyzed whether the magnitude of change in P levels (42m-post-stress minus baseline) during the stress session mediated the raw change in cortisol from baseline to 42m-post-stress (see Fig. 7a). This analysis revealed that while baseline cortisol influenced both the magnitude of change in P ($p < .05$) and cortisol levels 42m-post-stress ($p < .01$), and that P change scores influenced cortisol levels 42m-post-stress ($p < .01$), the overall model failed to show that P change was significantly mediating cortisol increase during the stress session (CI for indirect effect of magnitude of P change on change in cortisol from baseline to 42m-post-stress: −.6994, .0164; see Fig. 7a).

Conversely, analysis examining the magnitude of change in cortisol (42m-post-stress minus baseline) during the stress session on raw change in P levels from baseline to 42m-post-stress during the stress session (see Fig. 7b) revealed that baseline P influenced the magnitude of change in cortisol during the session ($p < .01$) and P levels 42m-post-stress ($p < .001$). It also revealed that magnitude of cortisol change influenced P levels 42m-post-stress ($p < .05$), and that the overall model was significant (CI for indirect effect of magnitude of cortisol change on change in P from baseline to 42m-post-stress: −.4042, −.0330; see Fig. 7b), suggesting that the magnitude of cortisol change from baseline to 42m-post-stress mediates the increase in P from baseline to 42m-post-stress after stress exposure.

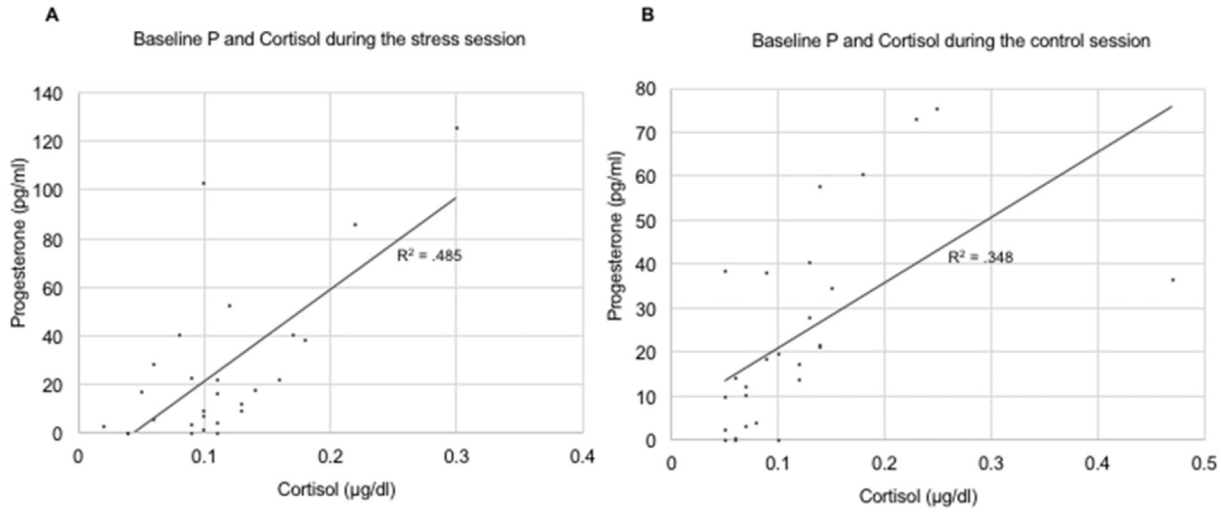

Fig. 5. Correlations between Baseline P and Cortisol. A) Baseline P and baseline cortisol levels during the stress session. Baseline levels of both hormones were positively correlated during the stress session: $p < .001$. B) Baseline P and baseline Cortisol levels during the control session. Baseline levels of both hormones were positively correlated during the control session: $p = .001$.

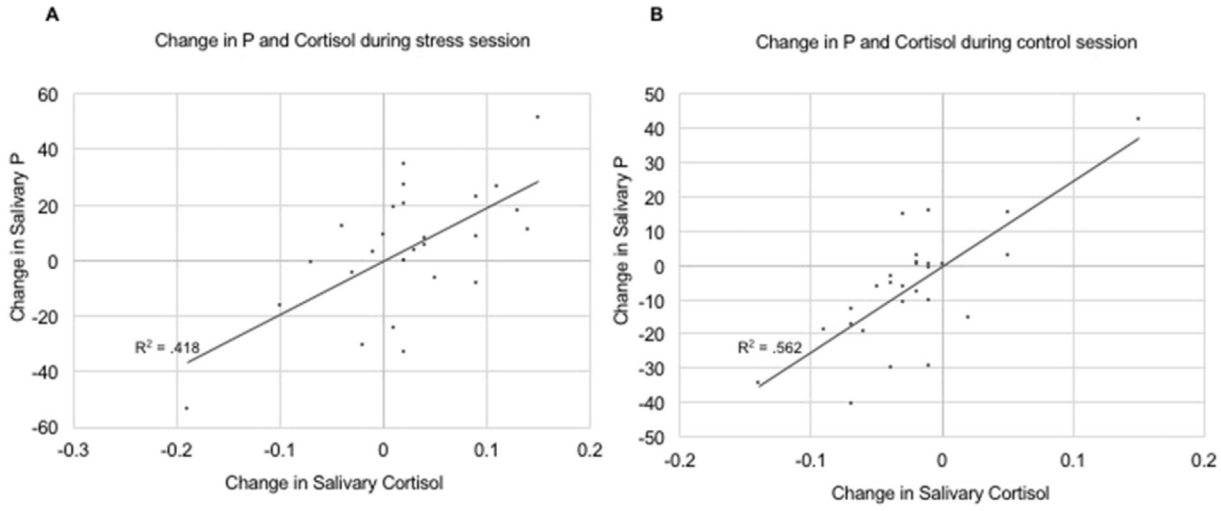

Fig. 6. Correlations between Change in P and Cortisol Levels. A) Change in P levels (42m-post-stress minus baseline) and change in cortisol levels (42m-post-stress minus baseline) during the stress session. Magnitudes of change in both hormones were positively correlated during the stress session: $p < .001$. B) Change in P levels (42m-post-stress minus baseline) and change in cortisol levels (42m-post-stress minus baseline) during the control session. Magnitudes of change in both hormones were positively correlated during the control session: $p < .001$.

3.6. 17β-estradiol levels do not change in response to CPT during the follicular phase of the menstrual cycle

In accordance with our hypotheses, a 2 (stress: CPT vs. control) × 2 (time: baseline vs. 42-min post-stress onset) repeated measures ANOVA revealed no effect of CPT on E2 levels in either session, $F(1,25) = 2.638$, $p = .117$, nor were baseline or post-stress responses of E2 and cortisol correlated with one another.

4. Discussion

This study examined the influence of natural baseline P levels on cortisol response to a stressor and tested the effects of stress on E2, P, and cortisol levels. Determining the P response to stress in naturally cycling women can be difficult due to the hormone fluctuations throughout the menstrual cycle. For example, while work in rodents demonstrated that adrenal P is at its lowest levels when ovarian P is at its peak from the evening of estrus to the evening of metestrus (Holzbauer and Godden, 1974), other work showed that a relationship between P and cortisol could be detected in a group of women with low ovarian P output as a result of hormonal contraception use (Wirth et al., 2007). The pattern of ovarian P output influencing adrenal P output in female rodents and women suggests that women should experience smaller P responses to stress during the high-P luteal phase of the menstrual cycle and greater P responses to stress during the low-P follicular phase of the menstrual cycle. Thus, in order to account for this influence of ovarian P output on adrenal P output, we tested women during the follicular phase of the menstrual cycle, when ovarian P output is low.

As hypothesized, and expected by findings showing a relationship between P and cortisol in women using hormonal contraception and therefore with low ovarian P output (Wirth et al., 2007), we found that higher baseline P levels during the low-P follicular phase were associated with higher baseline levels of

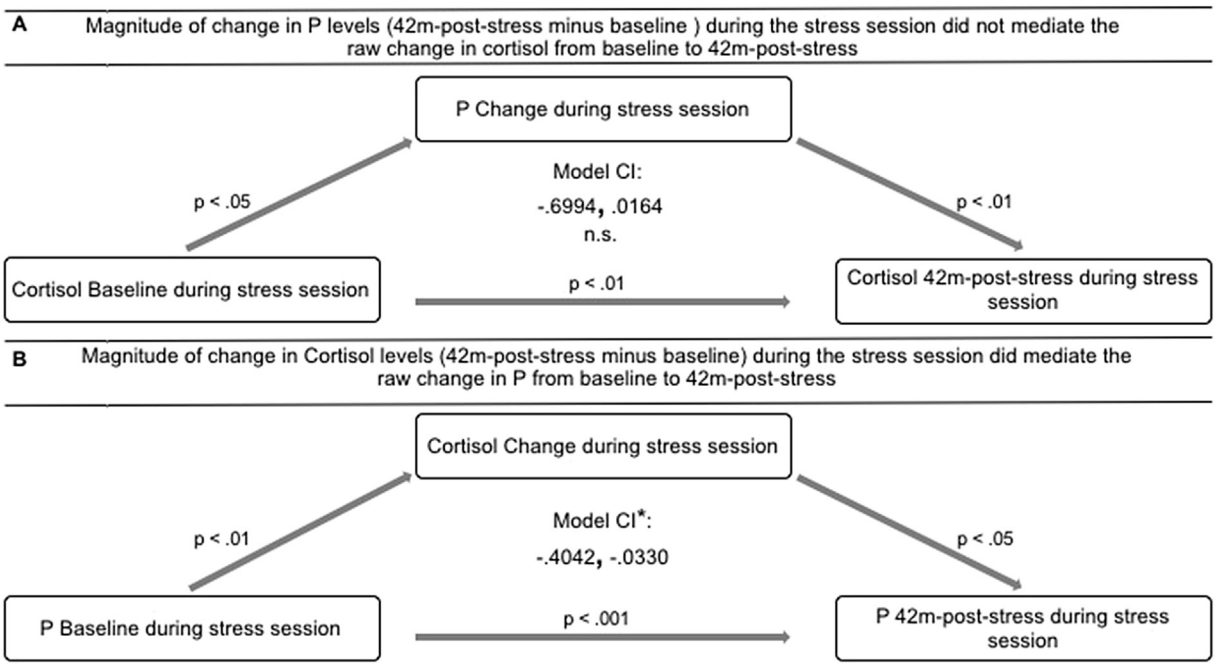

Fig. 7. Mediation models for the relationship between cortisol and progesterone responses to stress. Mediation models of analyses conducted using PROCESS in SPSS for A) the influence of the magnitude of change in P during the stress session on the raw change of cortisol from baseline to 42m-post-stress. Despite all pathways being significant, the overall model of P mediation of cortisol response was not significant; B) the influence of the magnitude of change in cortisol during the stress session on the raw change of P from baseline to 42m-post-stress. In addition to all pathways being significant, the overall model of cortisol mediation of P response was significant.

free cortisol and greater levels of bioavailable cortisol in response to a stressor. This pattern may result from at least two mechanisms. First, P is a precursor in cortisol biosynthesis, which may mean that when women have higher levels of circulating P (i.e., during the luteal phase of the menstrual cycle) more cortisol may be synthesized. Additionally, progesterone competitively binds to corticosteroid binding globulin, reducing the amount of cortisol able to bind to the protein and potentially resulting in higher levels of bioavailable cortisol (for review see, Brien, 1981). These two factors may explain why women experience greater levels of bioavailable cortisol in response to stress during the luteal phase of the menstrual cycle compared to women using hormonal contraception or during low P phases of the naturally cycling menstrual cycle (Kirschbaum et al., 1999 Kudielka and Kirschbaum, 2005; Nielsen et al., 2013b; Nielsen et al., 2014 but see Gaffey and Wirth, 2014 findings that hormonal contraception was associated with higher cortisol levels after cortisol administration).

Also as hypothesized, we found no effect of stress on E2, nor did correlation analyses uncover any relationship between E2 and cortisol in either session. The lack of E2 influence remained constant when additional analyses examined the effects of high vs low baseline E2 levels on both P and cortisol response to stress, such that mixed-model ANOVAs failed to find any difference in P response pattern or cortisol response pattern between the high and low E2 groups. This pattern of findings for E2 is expected given the findings that the adrenal glands produce relatively low amounts of E2 compared to P and compared to ovarian production, as observed in chicks (Tanabe et al., 1979), as well as reports in post-menopausal women showing that ACTH administration does not result in increased adrenal output of E2 (Greenblatt et al., 1976).

The relationship between P and free cortisol in both sessions is not surprising given the other similarities the two hormones share. For instance, work in female rodents show P and cortisol follow a similar diurnal rhythm, with adrenal P peaking in the early morning and then reaching its lowest values in the afternoon (Mann and Barraclough, 1973). Further, the mechanism of adrenal P release is similar to the signaling mechanism for glucocorticoid release, with rises in adrenocorticotropin hormone leading to release of both hormones from the adrenals, as evidenced in rodents (Feder and Ruf, 1969 Resko, 1969; Piva et al., 1973; Brown et al., 1976a), and in humans (Genazzani et al., 1998). Also like glucocorticoids, adrenal P release is inhibited by dexamethasone administration in rodents (Resko, 1969).

Based on findings presented here showing that baseline P levels are associated with cortisol levels across sessions and cortisol response to a stressor, we were expecting to find that the magnitude of change in P levels during the stress session would mediate the change in bioavailable cortisol during the same session. This pattern would suggest that the strength of the cortisol response to stress is influenced by the P response. However, we found the opposite effect, that the magnitude of change in cortisol response to stress mediated the change in P during the stress session. This pattern suggests that it is the strength of the cortisol response to stress that is influencing the P response to the same stressor. The influence of cortisol response on P response may be related to the magnitude of adrenal output relative to the robustness of a stressor. As the magnitude of a stressor increases, adrenal output of cortisol should also increase, and this increase in general adrenal output may also result in greater P output.

This stress-induced increase in P may serve multiple purposes, such as facilitating the negative feedback loop of the HPA axis via the P metabolite allopregnanolone (Patchev et al., 1994), reducing feelings of anxiety, tension, stress, or depression (Dennerstein et al., 1985), and/or inducing a slight sedative effect as indicated by feelings of increased fatigue, as measured via the Profile of Mood State (Freeman et al., 1992; Freeman et al., 1993; Söderpalm et al., 2004). Progesterone also has been shown to increase in response to manipulations of social closeness (Brown et al., 2009), an effect that might be beneficial during times of stress, as feelings of social support increase well-being in the face of stress (Cohen and Wills,

1985). Thus, increases in the hormone during times of stress might promote individuals to seek out beneficial social support systems (Wirth, 2011). This concept may also include seeking out intimate support from partners, as evidenced by the increase in sexual receptivity from adrenal P release resulting from both pharmacological and stress manipulations observed in female rodents (Feder and Ruf, 1969; Plas-Roser and Aron, 1981).

Our study does suffer from two limitations that should be mentioned. The first of these is the potential for lower than desired power given our smaller sample size, particularly with regard to the use of a median split. Although we do suffer from a relatively low sample size, we do observe moderate to large effect sizes for most tests conducted. However, this work would benefit from replication with a larger sample size. The second is related to menstrual cycle phase. Despite revealing a dual role for P in the stress response during the menstrual cycle, namely that higher baseline P levels are associated with higher baseline levels of bioavailable cortisol and larger cortisol response to stress, and that stress exposure also leads to rises in P, this study is limited in that it does not address P response to stress across the entire menstrual cycle, i.e., across follicular and luteal phases. Based on the work showing that high ovarian output of P is associated with suppressed adrenal P output (Holzbauer and Godden, 1974; Wirth et al., 2007), it may be that stress-induced adrenal P response would be diminished as a result of the higher ovarian P output during the luteal phase of the menstrual cycle. If, as suggested above, the role of stress-induced P release is to mitigate the feelings of anxiety and aid in the return to homeostasis, then the higher baseline P levels during the high-ovarian-output phases may be already serving the purpose that adrenal P release would typically serve in a low P environment by fulfilling these roles.

5. Conclusions

Together, our findings suggest both methodological and theoretical considerations for future studies examining the effects of menstrual cycle phase and/or hormone levels on the stress response. First, our finding that P increases in response to stress suggests that the methodological norm of averaging P values across multiple time points in menstrual cycle and stress studies (e.g., Nielsen et al., 2013a, 2014; Petersen et al., 2014) should be re-evaluated. Namely, time points post-stress should not be used in determining P values that are representative of a particular menstrual cycle phase. Second, involvement of P in the stress response should be regarded as multidimensional, whereby high P levels may possibly lead to higher baseline cortisol levels and greater cortisol response to stress, and also may be involved in the regulation of the negative feedback loop dictating HPA axis activity while also reducing feelings of anxiety and potentially leading individuals to seek out social support during recovery periods.

Acknowledgments

This work was supported by the National Institute on Aging (grant numbers R01AG038043 and R21AG048463). We would like to thank the laboratory of Dr. Pinchas Cohen for providing space and equipment to process the saliva samples.

References

Breier, A., Buchanan, R.W., 1992. The effects of metabolic stress on plasma progesterone in healthy volunteers and schizophrenic patients. Life Sci. 51, 1527–1534.
Brien, T., 1981. Human corticosteroid binding globulin. Clin. Endocrinol. 14, 193–212.
Brown, G., Courtney, G., Marotta, S., 1976a. A comparative study of adrenal progesterone secretion during the estrous cycles of hamsters and rats. Steroids 28, 283–294.
Brown, G., Courtney, G., Marotta, S., 1976b. Progesterone secretion by adrenal glands of hamsters and comparison of ACTH influence in rats and hamsters. Steroids 28, 275–282.
Brown, S.L., Fredrickson, B.L., Wirth, M.M., Poulin, M.J., Meier, E.A., Heaphy, E.D., Cohen, M.D., Schultheiss, O.C., 2009. Social closeness increases salivary progesterone in humans. Horm. Behav. 56, 108–111.
Childs, E., Dlugos, A., De Wit, H., 2010. Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. Psychophysiology 47, 550–559.
Cohen, S., Wills, T.A., 1985. Stress, social support, and the buffering hypothesis. Psychol. Bull. 98, 310.
Cooper, C., Evans, A., Cook, S., Rawlings, N., 1995. Cortisol, progesterone and β-endorphin response to stress in calves. Can. J. Animal Sci. 75, 197–201.
Deis, R., Leguizamon, E., Jahn, G., 1989. Feedback regulation by progesterone of stress-induced prolactin release in rats. J. Endocrinol. 120, 37–43.
Dennerstein, L., Spencer-Gardner, C., Gotts, G., Brown, J., Smith, M., Burrows, G., 1985. Progesterone and the premenstrual syndrome: a double blind crossover trial. Bmj 290, 1617–1621.
Duncan, G.E., Knapp, D.J., Carson, S.W., Breese, G.R., 1998. Differential effects of chronic antidepressant treatment on swim stress-and fluoxetine-induced secretion of corticosterone and progesterone. J. Pharmacol. Exp. Ther. 285, 579–587.
Elman, I., Breier, A., 1997. Effects of acute metabolic stress on plasma progesterone and testosterone in male subjects: relationship to pituitary-adrenocortical axis activation. Life Sci. 61, 1705–1712.
Fajer, A., Holzbauer, M., Newport, H.M., 1971. The contribution of the adrenal gland to the total amount of progesterone produced in the female rat. J. Physiol. 214, 115–126.
Feder, H., Ruf, K., 1969. Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents. Endocrinology 84, 171–174.
Freeman, E., Purdy, R., Coutifaris, C., Rickels, K., Paul, S., 1993. Anxiolytic metabolites of progesterone: correlation with mood and performance measures following oral progesterone administration to healthy female volunteers. Neuroendocrinology 58, 478–484.
Freeman, E.W., Weinstock, L., Rickels, K., Sondheimer, S.J., Coutifaris, C., 1992. A placebo-controlled study of effects of oral progesterone on performance and mood. Br. J. Clin. Pharmacol. 33, 293–298.
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. F1000Research 3.
Genazzani, A., Petraglia, F., Bernardi, F., Casarosa, E., Salvestroni, C., Tonetti, A., Nappi, R., Luisi, S., Palumbo, M., Purdy, R., 1998. Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. J. Clin. Endocrinol. Metab. 83, 2099–2103.
Greenblatt, R.B., Colle, M.L., Mahesh, V.B., 1976. Ovarian and adrenal steroid production in the postmenopausal woman. Obstet. Gynecol. 47, 383–387.
Hayes, A.F., 2013. Introduction to Mediation, Moderation, and Conditional Process Analysis: a Regression-based Approach. Guilford Press.
Holzbauer, M., Godden, U., 1974. Variation in the proesterone content of the rat adrenal gland during the oestrous cycle. J. Steroid Biochem. 5, 109–111.
Kirschbaum, C., Kudielka, B., 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. Psychosom. Med. 61, 154–162.
Kirschbaum, C., Wust, S., Hellhammer, D.H., 1992. Consistent sex differences in cortisol responses to psychological stress. Psychosom. Med. 54, 648–657.
Kudielka, B., Kirschbaum, C., 2005. Sex differences in HPA axis responses to stress: a review. Biol. Psychol. 69, 113–132.
Lighthall, N.R., Mather, M., Gorlick, M.A., 2009. Acute stress increases sex differences in risk seeking in the balloon analogue risk task. PLoS One 4.
Lighthall, N.R., Sakaki, M., Vasunilashorn, S., Nga, L., Somayajula, S., Chen, E.Y., Samii, N., Mather, M., 2012. Gender differences in reward-related decision processing under stress. Soc. Cognit. Affect. Neurosci. 7, 476–484.
Mann, D.R., Barraclough, C.A., 1973. Changes in peripheral plasma progesterone during the rat 4-day estrous cycle: an adrenal diurnal rhythm. Exp. Biol. Med. 142, 1226–1229.
Mather, M., Lighthall, N.R., Nga, L., Gorlick, M.A., 2010. Sex differences in how stress affects brain activity during face viewing. NeuroReport 21, 933–937.
Nielsen, S.E., Ahmed, I., Cahill, L., 2013a. Sex and menstrual cycle phase at encoding influence emotional memory for gist and detail. Neurobiol. Learn Mem. 106, 56–65.
Nielsen, S.E., Ahmed, I., Cahill, L., 2014. Postlearning stress differentially affects memory for emotional gist and detail in naturally cycling women and women on hormonal contraceptives. Behav. Neurosci. 128, 482–493.
Nielsen, S.E., Segal, S.K., Worden, I.V., Yim, I.S., Cahill, L., 2013b. Hormonal contraception use alters stress responses and emotional memory. Biol. Psychol. 92, 257–266.
Patchev, V., Shoaib, M., Holsboer, F., Almeida, O., 1994. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience 62, 265–271.
Petersen, N., Kilpatrick, L.A., Goharzad, A., Cahill, L., 2014. Oral contraceptive pill use and menstrual cycle phase are associated with altered resting state functional connectivity. NeuroImage 90, 24–32.

Piva, F., Gagliano, P., Motta, M., Martini, L., 1973. Adrenal progesterone: factors controlling its secretion 1. Endocrinology 93, 1178–1184.
Plas-Roser, S., Aron, C., 1981. Stress related effects in the control of sexual receptivity and in the secretion of progesterone by the adrenals in cyclic female rats. Physiol. Behav. 27, 261–264.
Resko, J.A., 1969. Endocrine control of adrenal progesterone secretion in the ovariectomized rat. Science 164, 70–71.
Roca, C.A., Schmidt, P.J., Altemus, M., Deuster, P., Danaceau, M.A., Putman, K., Rubinow, D.R., 2003. Differential menstrual cycle regulation of hypothalamic-pituitary-adrenal axis in women with premenstrual syndrome and controls. J. Clin. Endocrinol. Metab. 88, 3057–3063.
Romeo, R.D., Karatsoreos, I.N., McEwen, B.S., 2006. Pubertal maturation and time of day differentially affect behavioral and neuroendocrine responses following an acute stressor. Horm. Behav. 50, 463–468.
Romeo, R.D., Lee, S.J., McEwen, B.S., 2004. Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. Neuroendocrinology 80, 387–393.
Söderpalm, A.H., Lindsey, S., Purdy, R.H., Hauger, R., de Wit, H., 2004. Administration of progesterone produces mild sedative-like effects in men and women. Psychoneuroendocrinology 29, 339–354.
Tanabe, Y., Nakamura, T., Fujioka, K., Doi, O., 1979. Production and secretion of sex steroid hormones by the testes, the ovary, and the adrenal glands of embryonic and young chickens (Gallus domesticus). General Comp. Endocrinol. 39, 26–33.
Tunn, S., Mollmann, H., Barth, J., Derendorf, H., Krieg, M., 1992. Simultaneous measurement of cortisol in serum and saliva after different forms of cortisol administration. Clin. Chem. 38, 1491–1494.
Vining, R.F., McGinley, R.A., Symons, R.G., 1983. Hormones in saliva: mode of entry and consequent implications for clinical interpretation. Clin. Chem. 29, 1752–1756.
Wirth, M.M., 2011. Beyond the HPA axis: progesterone-derived neuroactive steroids in human stress and emotion. Front. Endocrinol. 2.
Wirth, M.M., Meier, E.A., Fredrickson, B.L., Schultheiss, O.C., 2007. Relationship between salivary cortisol and progesterone levels in humans. Biol. Psychol. 74, 104–107.