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Oxytocin, cortisol, and cognitive control during acute and naturalistic stress

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ABSTRACT

Although stress is a strong risk factor for poor health, especially for women, it remains unclear how stress affects the key neurohormones cortisol and oxytocin, which influence stress-related risk and resilience. Whereas cortisol mediates energy mobilization during stress, oxytocin has anti-inflammatory, anxiolytic, and analgesic effects that support social connection and survival across the lifespan. However, how these neurohormones interrelate and are associated with cognitive control of emotional information during stress remains unclear. To address these issues, we recruited 37 college-aged women ($M_{\text{age}} = 19.19$, $SD = 1.58$) and randomly assigned each to a one-hour experimental session consisting of either an acute stress (emotionally stressful video) or control (non-stressful video) condition in a cross-sectional manner across the semester. Salivary cortisol and oxytocin samples were collected at baseline and after the video, at which point participants also completed measures assessing affect and an emotional Stroop task. As hypothesized, the emotional stressor induced negative emotions that were associated with significant elevations in cortisol and faster Stroop reaction times. Moreover, higher baseline oxytocin predicted greater positive affect after the stressor and also better cognitive accuracy on the Stroop. Analyses examining the naturalistic stress effects revealed that basal oxytocin levels rose steeply three weeks before the semester's end, followed by rising cortisol levels one week later, with both neurohormones remaining elevated through the very stressful final exam period. Considered together, these data suggest that women's collective experiences of stress may be potentially buffered by a synchronous oxytocin surge that enhances cognitive accuracy and reduces stress "when the going gets tough".

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Introduction

Stress profoundly impacts human health and development (Slavich, 2016; Tost et al., 2015). Approximately half of Americans report experiencing stress daily, with Millennials and women exhibiting the greatest stress-related cardiovascular, emotional, and cognitive health problems (American Psychological Association, 2017, 2019; Slavich & Sacher, 2019). Although the pathways underlying these effects are still being documented, stress is known to activate the sympathetic nervous system, which releases catecholamines that initiate a coordinated biochemical response leading to increases in oxytocin, vasopressin, and corticotropin-releasing factor that facilitate adrenocorticotropin hormone bioavailability (Jezova et al., 1995; Slavich, 2020a). Stress also upregulates the hypothalamic–pituitary–adrenal (HPA) axis and inflammatory activity, as indexed by cortisol and several inflammatory biomarkers (Apter-Levi et al., 2016; Baumeister et al., 2016; Furman et al., 2019; Slavich & Auerbach, 2018).

Cortisol and inflammatory changes are particularly well documented in response to stress (Adam, 2006), with 2- to 3-fold cortisol increases for most individuals exposed to socially evaluative public speaking tasks (Frisch et al., 2015) and marked same-day increases for students taking exams (Verschoor & Markus, 2011). That said, researchers also have documented substantial variability in stress reactivity across individuals (Dickerson & Kemeny, 2004; Slavich & Irwin, 2014), especially in response to socially stressful and fearful conditions (Anderson et al., 2018; Carpenter et al., 2010). These differences suggest the presence of factors that modulate inter-individual stress reactivity.

One biological factor that may explain some of this variability is oxytocin. Oxytocin functions as a neurotransmitter and paracrine hormone. It is present across pregnancy/gestation, labor, delivery, nursing/caregiving, and social bonding (Feldman et al., 2007), and may mediate one of the proposed trifurcated stress responses of "fight or flight" versus "tend and befriend" (Taylor et al., 2000). Oxytocin has analgesic

(Goodin et al., 2015), anxiolytic, and anti-stress effects (Neumann & Landgraf, 2012). When administered directly, oxytocin can increase feelings of calm, well-being, and trust (Ishak et al., 2011), and enhance facial emotion recognition (Lischke et al., 2012). Higher plasma oxytocin has also been associated with active social coping and, paradoxically, higher anxiety, and personal and relationship distress (see Crespi, 2016; Woolley et al., 2014). Importantly, there is reciprocal activation between the oxytocin system and HPA axis (Feldman, 2012b), suggesting bidirectional regulation that is influenced in part by psychosocial factors (Dabrowska et al., 2011; Feldman & Bakermans-Kranenburg, 2017). Indeed, better partner support has been related to elevated oxytocin for both sexes and with lower systolic blood pressure for women, consistent with data suggesting that oxytocin may have cardio-protective effects (Grewen et al., 2005).

More broadly speaking, it is known that oxytocin has stress anxiolytic and anti-inflammatory effects, as well as analgesic and healing properties, which confer evolutionarily adaptive advantages across pregnancy/gestation, labor, delivery, nursing/caregiving, and social bonding (Feldman et al., 2007). Might oxytocin also buffer the negative effects of other types of challenges, including individual-level (Taylor et al., 2010) and collective stressors (Feldman, 2020; Taylor, 2006; Taylor et al., 2000)? Consistent with this possibility, we investigated how oxytocin and cortisol change and are inter-related in response to both acute and naturalistic life stress.

The significance of oxytocin *vis-a-vis* cortisol for women is particularly relevant from the perspective of the survival of the species, as proposed by Taylor et al. (2000) and expanded on by Steinman et al. (2019) who highlighted oxytocin's varied effects (see also Beery, 2015). Specifically, oxytocin's association with increased social attention and salience clarifies how oxytocin may help facilitate divergent responses to different situational factors that require either an appetitive approach or avoidant behaviors depending on whether the situation is safe or rewarding versus threatening or aversive. Indeed, modulating behavior based on these cues helps foster mother-child bonding and is critical for numerous collective behaviors that support the survival of the species (Atzil et al., 2014; Feldman, 2020).

Additionally, oxytocin may play a role in reducing women's risk for some stress-related illnesses. Despite women experiencing greater stress across the lifespan (Harkness et al., 2010), for example, women consistently exhibit greater longevity than men (Austad, 2006). Although oxytocin is present in both males and females, testosterone is known to suppress oxytocin and oxytocin receptor activity (Okabe et al., 2013). In light of oxytocin's known survival and health benefits (Grewen & Light, 2011; Gutkowska et al., 2014; Szeto et al., 2008), therefore, the role that this neurohormone plays in promoting stress resilience and the survival of the species warrants further investigation.

In terms of interactions between cortisol and oxytocin, greater oxytocin and social support have been associated with lower cortisol and perceived stress levels for men (Heinrichs et al., 2003). However, we know of no comparable studies with women. Additionally, no studies have examined how oxytocin and cortisol change and co-vary during acute

and naturally occurring stress, even though such research could help elucidate biological processes underpinning differences in stress-related risk and resilience.

Finally, even though cognitive control affects emotion regulation ability (Gross, 2015) and stress reactivity (Shields et al., 2017a; Slavich, 2020b), the role that cognitive control plays in influencing cortisol and oxytocin responses to stress remains poorly understood. Cognitive control involves the ability to focus on the most important aspects of a situation and direct attention to perform behaviors that are needed to achieve the desired goal (Diamond, 2011). Cognitive control is particularly important during times of stress when cognitive resources are increasingly taxed and adaptive responses are critical (Quinn et al., 2020). Moreover, cognitive control plays a crucial role in emotion regulation, which is important for adaptation, social behavior, and wellbeing (Gross, 2015). Although a small literature exists examining associations between oxytocin and various aspects of cognitive control (e.g. Striepens et al., 2016), few studies have investigated these associations in the context of stress and we know of none that have assessed both cortisol and oxytocin during stress. Given oxytocin's social cognitive performance-enhancing effects and the fact that cortisol mobilizes metabolic resources that are needed for quick decision making during stress, we hypothesized that higher oxytocin levels would be associated with better cognitive-emotional accuracy in the context of stress and, in addition, that cortisol levels would be related to quicker cognitive reaction times.

To test these hypotheses, we investigated how a laboratory-based emotional stressor affected participants' oxytocin and cortisol levels, and how these neurohormones related to performance on an emotional Stroop task following acute stress. Additionally, given research documenting that threat experiences activate "fight or flight" responses and that women exhibit elevated oxytocin levels under duress (Taylor et al., 2010), we systematically brought participants into the laboratory across the semester which enabled us to cross-sectionally examine how naturally occurring academic stress related to oxytocin and cortisol production as the semester progressed. Given the complex relation between these neurohormones in men, and our specific interest in the trifurcated stress response and in understanding the relatively greater stress-related burden tolerance for women (American Psychological Association, 2017; Graham & McGrew, 1980), all participants were female.

Method

Participants and design

Participants were 37 healthy undergraduate college women using no hormone medications (e.g. estrogen, progesterone) for 3 months prior to recruitment. Most participants were college freshmen (59%), 18–22 years old ($M_{\text{age}} = 19.19$, $SD = 1.58$), ranging in height from 5' to 5'11", and weighing between 100 and 220 pounds ($M = 137.2$, $SD = 28.7$) with an average body mass index (BMI) of 23.07 ($SD = 5.07$). More than half of the participants ($n = 21$) were in their menstrual cycle luteal phase when they came into the lab for the

experimental portion of the study (see [Supplemental Material, Table S1a](#)). A power analysis revealed that a sample size of 40 was required to achieve a 90% probability of detecting a moderate effect at the $\alpha=0.05$ level. Therefore, the study was powered to detect an approximately moderate effect. The study design utilized randomized block assignment to the experimental condition and the neurohormone analyses were double-blind. All procedures were approved by the Institutional Review Board.

Recruitment and procedure

Healthy females were recruited from psychology courses and flyers posted around the campus of a medium-sized, primarily undergraduate university. Emails were sent with scheduling information and specific instructions for dietary, beverage, food, and oral care behaviors for the day of the session (see [Supplemental Material, Participant Instructions](#)). One hour appointments were scheduled on sequential Fridays between 12:00pm and 6:00pm, beginning mid-semester (March 8) through the final week of classes (May 3). Upon arrival at the lab, participants consented and a pre-participation survey was completed (See [Supplemental Material, Photograph1](#)). Lab appointment time was associated with participants' menstrual cycle phase, BMI, and age ($ps < .05$). Therefore, we included these factors as covariates in analyses.

After consent and the surveys were completed, participants were shown how to take their own blood pressure while seated in front of the video monitor. Participants were then provided with a 100 ml BD collection tube (Franklin Lakes, NJ) for a passive drool saliva sample using standard procedures. Next, participants were randomly assigned to one of two experimental conditions. Participants watched either a negative emotionally evocative video (i.e. Emotional Stressor Condition) or a neutral video (i.e. Control Condition). Women in the Emotional Stressor Condition watched a 4-min video depicting a male surgeon circumcising a two-day-old crying male infant, which we have previously shown induces a negative mood state and upregulates inflammatory activity (Shields et al., 2016). In contrast, those in the Control Condition watched a 4-min emotionally neutral video showing a male tiling a shower. Both video narrators spoke in a similarly calm voice as they worked, which was matched for general effect, steadiness, word count, verbal pacing, and duration.

Naturalistic time of semester stress

Individual participants were systematically brought into the lab for hour-long appointments to complete the experimental portion of the study. Participants were run on each Friday of successive weeks during the academic semester, which enabled us to investigate their short-term acute stress responses and to cross-sectionally examine how oxytocin and cortisol levels changed on average between participants, week-by-week, as a result of changes in naturally occurring academic stress. The study began mid-semester when course demands were relatively low (i.e. few papers, labs, or exams)

and ran through the last weeks of the semester and just prior to finals, when course demands were greatest. Each Friday, we brought an average of five new participants into the clinic/lab (range: 4–7 participants), thus providing a window into academic stress-related changes in neurohormonal activity. Given known morning cortisol peaks, college-age students' known irregular sleep schedules (Verlander et al., 1999), night shift cortisol effects (Lindholm et al., 2012), and past oxytocin research methods, all participants were brought into the lab between 12:00pm and 6:00pm in the afternoon.

Cortisol and oxytocin assays

Baseline saliva samples were collected immediately before viewing the video (i.e. 8–12 min after entering the laboratory) and a second saliva sample was collected approximately 8–10 min after the start of video viewing. The experimenter avoided physical touch at all times during the visit. For saliva collection, the experimenter handed the participant a dry ice-lined thermal cup containing a 100 mL BD tube and instructed her to spit into the tube up to the 10 mL mark. Upon completion, the tube was capped and placed in a dry-ice lined thermal container and transported to a 80 C freezer at the end of the day's session. At the end of the study, all samples were removed from storage and air-shipped overnight to the University of North Carolina (UNC) at Chapel Hill Cytokine and Biomarker Analysis Facility where they were thawed, lyophilized, and split for immediate immunoassay processing. The cortisol assays were completed by this lab, and the oxytocin assays were transferred to and conducted by, the Karen Grewen lab at UNC (see below).

Cortisol was measured using ELISA assay kits manufactured by R&D Systems (Minneapolis, MN), which have a minimum detectable dose of 0.156 ng/mL. Consistent with standards, participants' baseline cortisol levels were $M=4.73$ (ng/mL), $SD=5.61$, $SE=0.922$. Saliva samples were split, and a portion of each sample was lyophilized and sent out for oxytocin analysis.

Oxytocin levels in extracted saliva were measured using the Oxytocin Enzyme Immunoassay kit and protocol from Enzo Life Sciences (Ann Arbor, MI, cat. #900-153) by the Karen Grewen lab at UNC. The endogenous oxytocin hormone competes with oxytocin linked to alkaline phosphatase for the oxytocin antibody binding sites. After overnight incubation at 4 C, the excess reagents were washed away and the bound oxytocin phosphatase was incubated with the substrate. After 1 h, this enzyme reaction that generates a yellow color is stopped. The optical density was then read on a Sunrise plate reader (Tecan, Research Triangle Park, NC) at 405 nm. The intensity of the color is inversely proportional to the concentration of oxytocin in the sample. The hormone content (pg/mL) was determined by plotting the optical density of each sample against a standard curve. The sensitivity of the assay is 11.6 pg/mL, with a standard range of 15–1000 pg/mL. The intra- and inter-assay variation was 4.8% and 8%, respectively. Enzo Life Sciences reports cross-reactivity for similar neuropeptides found in mammalian sera at less than 0.001.

Two participants were excluded because they either had insufficient saliva for the oxytocin baseline sample analysis ($n=1$) or because their baseline oxytocin levels were too low to be measured ($n=1$). Consistent with standards, the remaining participants' ($n=35$) baseline oxytocin level were $M=15.07$ pg/mL, $SD=10.10$, $SE=1.71$.

Cognitive control of emotional information

Cognitive control of emotional information was assessed using an emotional Stroop task. Based on theoretical (Taylor, 2006, 2011; Taylor et al., 2000) and methodological considerations, we selected 5 male faces for the stimulus slide backgrounds from the pictures of facial affect. Each photo depicted a single positive emotion associated with happiness or a negative emotion associated with sadness or anger. Stroop stimuli words were printed on the slide/face center. The stimuli emotional words were Happy (*happy, joyful, bliss, and merry*), Sad (*grieve, sorrow, mourn, and despair*), and Angry (*angry, wrath, livid, and furious*), all chosen for comparable length, familiarity, and theoretical distressing relevance. Blurred background faces were included as control stimuli and used as the baseline reference for background face emotional expression distraction (See [Supplemental Material, Photograph S2](#)).

Participants were instructed to ignore the background face and accurately categorize each foreground emotion word depicted as Happy, Sad, or Angry; the underlying male faces, in turn, variously featured congruent or incongruent emotions. Participants viewed 108 Ekman male happy, sad, or angry expression faces including 36 face/words plus 12 control blurred faces in 3 sets of block trials (48 trials per block). Using a Shears Goggle Viewing Device and 17" laptop computer raised to waist height, participants were instructed to position their face against the green goggles, rest the left index finger on "F" key, right index finger on "J" key, and thumbs on spacebar with 3 randomly assigned keys strokes designating "happy," "sad" or "anger" emotion word cued. After a training trial, participants began the actual trials. The inter-trial intervals varied from 845–2000 ms. One participant entered the same key in response to each slide and was thus excluded from analyses involving the Stroop.

Self-report questionnaires

After viewing the Emotional Stressor or Control video, participants completed several measures that provided a manipulation check for the experimental stressor. These measures included the Quantitative Affect Scale (QAS; Kuchenbecker, 1976), which asks "How did you feel while watching the video?" Responses are captured on a Likert scale, ranging from *Extremely Negative* (−5) through *Neutral* (0) to *Extremely Positive* (+5), thus indicating both direction and emotional intensity. As part of the QAS, participants also rated how strongly they felt several emotions – namely, *happy, excited, sad, angry, fearful, agitated, and distressed* – from 1 (*No Emotional Experience*) to 7 (*Strong Emotional Experience*). Lastly, participants completed the Positive and Negative

Affect Scale (PANAS; Watson et al., 1988) reporting on their current state affect. For the PANAS, participants rated 20 emotions from 1 (*very slightly or not at all*) to 5 (*extremely*), yielding two separate scores: one for the ten positive emotions (i.e. *attentive, active, alert, enthusiastic, excited, determined, inspired, interested, proud, and strong*) and another for the ten negative emotions (*afraid, ashamed, distressed, guilty, hostile, irritable, jittery, nervous, scared and upset*).

In addition to these manipulation check measures, online before watching their video, participants completed the Satisfaction with Life Survey (SWLS), which has a test–retest reliability of 0.82 (Diener et al., 1985), and the Perceived Stress Scale (PSS-4), which has a test–retest reliability of 0.55 (Cohen et al., 1983). These two commonly used, well-validated measures were used to quantify how participants' general wellbeing and perceived stress levels changed across the semester.

Results

Changes in self-reported emotions to the laboratory-based emotional stressor

We first examined whether watching the emotionally evocative video altered participants' self-reported emotions. According to the QAS, where a lower number indicates a more intensely negative emotional experience, participants in the Emotional Stressor Condition reported feeling significantly more emotionally negative ($M=3.6$, $SD=1.79$) than those in the Control Condition ($M=5.82$, $SD=1.29$), $t(35)=4.38$, $p<.001$. Likewise, participants in the Emotional Stressor Condition experienced several specific negative emotions more strongly than those in the Control Condition – namely (in decreasing order), *Negative-Distressed* ($M=3.5$, $SD=2.35$ vs. $M=1.35$, $SD=1.22$; Mean Rank 23.68 vs. 13.50; $U(37)=263.5$, $p=.004$), *Negative-Sad* ($M=3.37$, $SD=1.98$ vs. $M=1.18$, $SD=1.22$; Mean Rank 24.29 vs. 12.03; $U(36)=271.5$, $p=.0001$), *Negative-Agitated* ($M=2.90$, $SD=2.38$ vs. $M=1.06$, $SD=0.24$; Mean Rank 22.92 vs. 14.38; $U(37)=248.5$, $p=.015$), and *Negative-Angry* ($M=2.35$, $SD=1.87$ vs. $M=1.17$, $SD=0.33$; Mean Rank 22.18 vs. 15.26; $U(37)=233.5$, $p=.052$). The two groups did not differ significantly with respect to *Negative-Fearful* ($M=2.15$, $SD=1.69$ vs. $M=1.24$, $SD=0.75$; $p=.080$), *Positive-Happy* ($M=1.8$, $SD=1.15$ vs. $M=2.06$, $SD=1.43$; $p=.662$), or *Positive-Excited* ($M=1.70$, $SD=1.03$ vs. $M=1.59$, $SD=1.18$; $p=.619$).

Finally, with respect to emotion levels as assessed by the PANAS, as expected, participants in the Emotional Stressor Condition reported feeling significantly more negative emotions (PANAS-Negative, $M=18.42$, $SD=7.34$) as compared to those in the Control Condition ($M=12.59$, $SD=4.40$), $t(34)=-2.927$, $p=.0006$. Interestingly, participants in the Emotional Stressor Condition did report somewhat lower positive emotions (PANAS-Positive, $M=16.71$, $SD=3.89$) than those in the Control Condition ($M=17.50$, $SD=6.30$), but this difference was not significant, $t(31)=0.439$, $p=.664$. Therefore, the laboratory-based emotional stressor was successful in inducing a negative emotional state.

Emotional Stroop performance for participants in the acute stress vs. control condition

Emotional Stroop reaction time

Participants' reaction times to the emotional Stroop task administered post-video ranged from 525 to 2060 ms. As expected, participants in the Emotional Stressor Condition demonstrated cognitive-emotional distraction, as exhibited by significantly slower Stroop reaction times ($M = 1101.61$ ms, $SE = 49.003$, 95% CI [1001.06, 1202.16]) as compared to those in the Control Condition ($M = 865.01$ ms, $SE = 56.74$, 95% CI [748.60, 981.43]), $F(1, 34) = 9.41$, $p = .005$, while adjusting for all covariates (i.e. BMI, age, menstrual phase, and lab appointment time).

Stroop accuracy percent (%)

Participants in the Emotional Stressor Condition also exhibited somewhat lower Stroop % accuracy ($M = 86.8\%$, $SD = 0.19$, $SE = 0.043$, 95% CI [77.8%, 95.7%]) than those in the Control Condition ($M = 95\%$, $SD = 0.08$, $SE = 0.02$, 95% CI [90.1%, 98.9%]), but this difference was not significant, $F(1, 32) = 2.25$, $p = .146$, $\eta_p^2 < 0.303$.

Biological responses for participants in the acute stress vs. control condition

Baseline cortisol and oxytocin levels

Examining the baseline biological data revealed that there were no significant differences between participants' baseline cortisol or oxytocin levels for those randomly assigned to the Emotional Stressor vs. Control Condition, indicating successful random assignment.

Cortisol

Participants' post-video cortisol levels for those in the Emotional Stressor vs. Control Condition were not significantly different. Both groups exhibited small declines in cortisol following their respective videos (Post-video cortisol levels for the Emotional Stressor Condition: $M = 3.82$, $SD = 4.94$, $SE = 1.10$ vs. Control Condition: $M = 3.97$, $SD = 4.76$, $SE = 1.13$, $t(35) = 0.091$, $p = .93$).

Oxytocin

Participants' oxytocin levels post-Emotional Stressor video and post-Control video were very similar. Both groups demonstrated small oxytocin declines following their respective videos (Emotional Stressor Condition: $M = 12.58$, $SD = 7.46$ vs. Control Condition: $M = 13.26$, $SD = 7.43$, $t(37) = 0.28$, $p = .78$), unadjusted or while adjusting for all covariates in addition to baseline oxytocin levels. The post-video Estimated Marginal Means for oxytocin in the Emotional Stressor Condition were $M = 13.24$, $SE = 1.23$, 95% CI [10.71, 15.76], and for the Control Condition were $M = 12.53$, $SE = 1.48$, 95% CI [9.49, 15.57], $F(1, 33) = 0.130$, $p = .72$.

Interaction of basal neurohormone levels in acute stress vs. control condition

Given that natural neurohormone baseline levels serve as the platform for research investigating therapeutic and receptor antagonists' effects and that a participant's relative natural neurohormone level is the reference point in "dose dependent effects" used in almost all research on this topic, we next looked at neurohormone effects more closely by examining participants' baseline neurohormone levels. Consistent with Kragel et al. (2019) who employed a low versus high grouping when assessing autonomic control of visceromotor activity in cognitively demanding tasks, we took participants' first saliva sample and performed a baseline cortisol levels median split to designate a low basal cortisol group (1.17–2.50 ng/mL) and a high basal cortisol group (>2.61 ng/mL). Similarly, we performed a median split on participants' baseline oxytocin levels by grouping participants into a low basal oxytocin group (2.77–11.48 pg/mL) and a high basal oxytocin group (12.36–39.44 pg/mL).

Basal cortisol and oxytocin effects for participants in the acute stress vs. control condition

Participants entering the lab with higher baseline cortisol levels exhibited significantly quicker emotional Stroop reaction times ($M = 895.13$ ms, $SE = 53.56$, 95% CI [785.24, 1005.02]) across conditions as compared to those with low baseline cortisol levels ($M = 1071.50$ ms, $SE = 58.75$, 95% CI [950.95, 1192.04]), $F(1, 34) = 4.24$, $p = .049$, adjusting for all covariates, but there was no significant interaction of baseline cortisol by experimental condition on Stroop reaction time (Figure 1a). Participants with high baseline cortisol also displayed somewhat better cognitive accuracy performance on the Stroop task (Figure 1b), but this difference was not significant and there was no interaction between the baseline cortisol and experimental condition. Therefore, higher basal cortisol was associated with significantly faster reaction times for both the experimental and control condition. Higher basal cortisol was also associated with slightly (but not significantly) better Stroop cognitive accuracy in emotion word categorizing performance with no interaction between baseline cortisol and condition on either dependent measure.

Participants in the Emotional Stressor Condition exhibited slower emotional Stroop reaction times ($M = 1082.73$ ms, $SE = 54.36$, 95% CI [910.71, 1194.69]) as compared to those in the Control Condition ($M = 869.31$ ms; $SE = 68.19$, 95% CI [729.09, 1009.98]), $F(1, 32) = 5.752$, $p = .024$, but the interaction of Experimental Condition by baseline low vs. high oxytocin was not significant, $F(1, 32) = 0.294$, $p = .592$ (Figure 2a).

Basal oxytocin levels were associated with participants' ability to accurately label emotion words with distracting background faces (angry, sad, neutral) on the emotional Stroop task. Across conditions, high basal oxytocin participants exhibited 99% accuracy on the Stroop ($SD = 0.19$, $SE = 0.038$, 95% CI [91%, 100.1%]), whereas low oxytocin participants exhibited 85% accuracy ($M = 95\%$, $SE = 0.39$, 95% CI

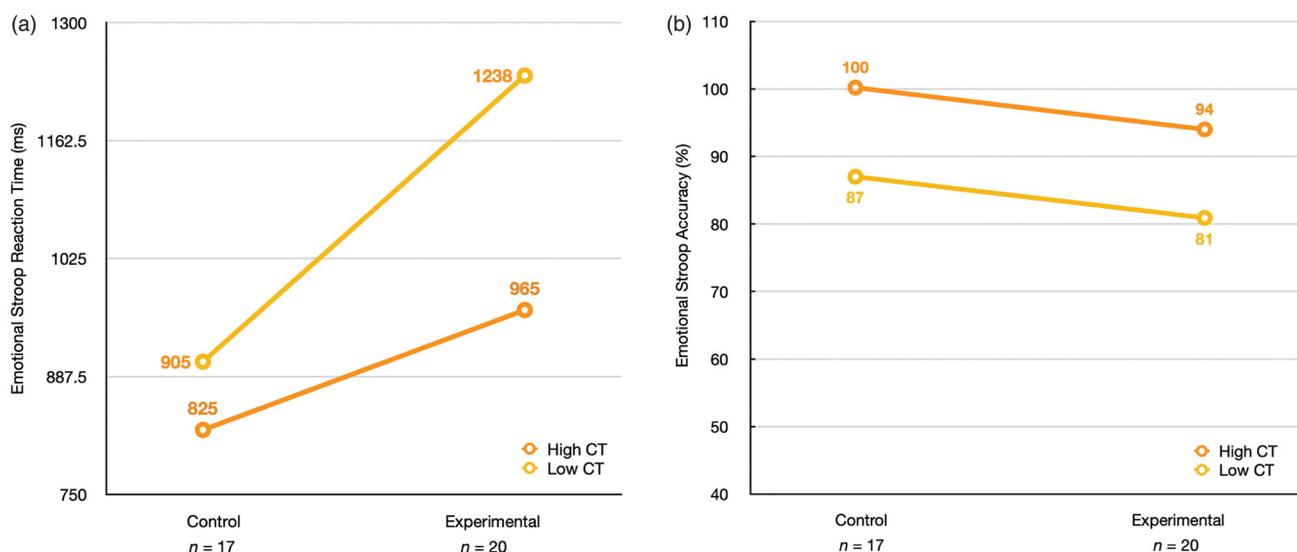


Figure 1. Emotional Stroop and basal cortisol levels across conditions. (a) Stroop reaction times in milliseconds (ms) and (b) Stroop % accuracy by basal cortisol (CT) mean levels (ng/mL) (Low, High) for participants randomly assigned to the Control versus Experimental condition. Covariates included body mass index, age, menstrual cycle phase (follicular vs. luteal), and lab appointment time. ($n = 37$)

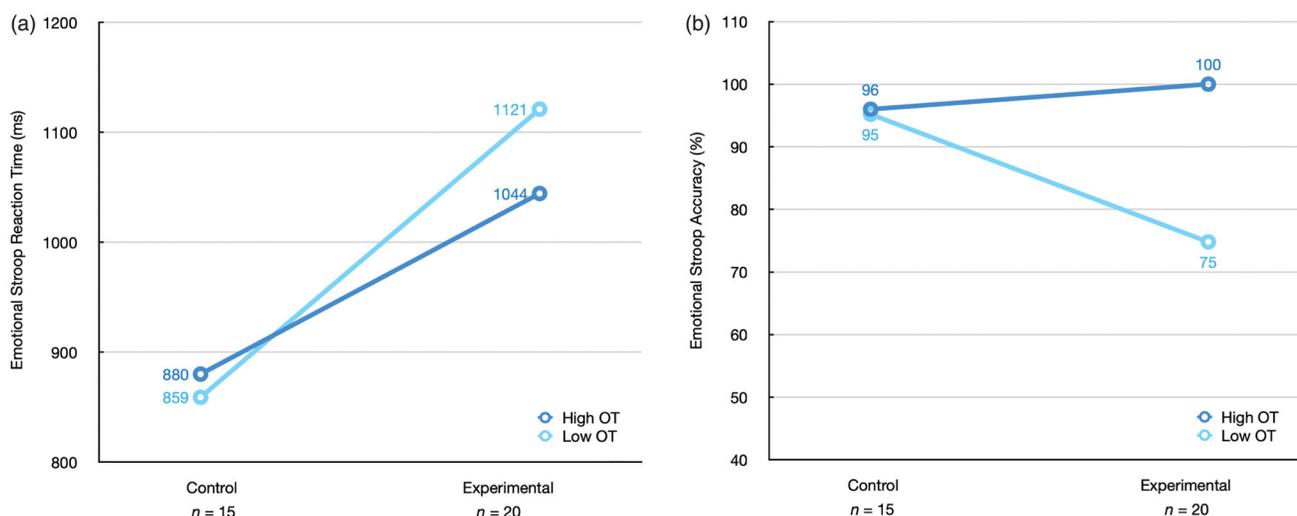


Figure 2. Emotional Stroop and basal oxytocin levels across conditions. (a) Stroop reaction times in milliseconds (ms) and (b) Stroop % accuracy by basal oxytocin (OT) mean levels (pg/mL) (Low, High) for participants randomly assigned to the Experimental versus Control condition. Covariates included body mass index, age, menstrual cycle phase (follicular vs. luteal), and lab appointment time. ($n = 35$)

[77%, 93%]), $F(1, 32) = 5.99, p = .022, \eta_p^2 < 0.653$, while adjusting for all covariates (Figure 2b).

Importantly, stress revealed a strong association between oxytocin and Stroop accuracy performance, as indicated by a basal oxytocin level by Condition interaction, $F(1, 32) = 4.813, p = .038, \eta_p^2 < 0.559$, adjusting for all covariates. Specifically, high basal oxytocin participants assigned to the Control Condition achieved 96% accuracy on the Stroop ($SE = 0.064, 95\% \text{ CI } [87.0\%, 113.3\%]$), whereas high oxytocin participants assigned to the Emotional Stressor condition achieved 100.1% accuracy ($SE = 0.054, 95\% \text{ CI } [82.8\%, 105.1\%]$).

In contrast, participants with low basal oxytocin levels assigned to the Control Condition achieved 95% accuracy on the emotional Stroop task ($SE = 0.069, 95\% \text{ CI } [72.8\%, 101.1\%]$). However, low basal oxytocin participants in the Emotional Stressor Condition achieved only 75% accuracy on the Stroop ($SE = 0.062, 95\% \text{ CI } [68.2\%, 93.6\%]$). Therefore, acute stress appears to be particularly damaging to the

cognitive-emotional accuracy of participants experiencing stress who have relatively low oxytocin levels (See Figure 2).

Examining these effects further revealed a positive association between basal oxytocin levels and positive affect for the young women randomly assigned to the Emotional Stressor Condition (PANAS-Positive – basal oxytocin level correlation, $r(17) = 0.50, p = .043$). Therefore, it is possible that high oxytocin levels both enhance cognitive-emotional accuracy (i.e. 100% Stroop accuracy) and promote positive affect, which may combine to buffer negative emotions and promote psychosocial resilience during times of stress.

Cortisol and oxytocin responses for participants in the acute stress vs. control condition

Participants' basal cortisol and oxytocin levels strongly predicted their post-video levels, and this was true for both

neurohormones. Specifically, significant pre- to post-video partial correlations were evident for both cortisol, $r(30) = 0.973$, $p < .0001$, and oxytocin, $r(28) = 0.716$, $p < .0001$ (Supplemental Material, Table S6).

Associations between oxytocin and cortisol within conditions

Notably, the associations between cortisol and oxytocin differed by Condition. Within the Control Condition, participants' baseline cortisol levels were strongly related to their post-video cortisol levels, $r(17) = 0.96$, $p < .001$, and also correlated with participants' basal oxytocin levels, $r(17) = 0.52$, $p = .045$, as well as their post-video oxytocin levels, $r(17) = 0.56$, $p = .024$. Therefore, the non-emotional (i.e. shower-tiling) control video apparently facilitated maintaining a status-quo relationship between cortisol and oxytocin.

However, a different pattern of results emerged for participants in the Emotional Stressor Condition. Here, baseline cortisol levels again were strongly related to participants' post-video cortisol levels, $r(17) = 0.98$, $p < .001$. Additionally, participants' oxytocin baseline levels still correlated from pre- to post-video, $r(18) = 0.77$, $p < .001$, but more modestly than what was observed for the Control group participants. Moreover, there was no significant association between cortisol and oxytocin levels from pre- to post-Emotional Stressor, $r(18) = -0.01$, $p = .69$, providing potential evidence of dynamic changes occurring for these two neurohormones as participants' responded to the content of the video.

Self-reported stress and well-being across the semester

Next, we examined the extent to which stress, well-being, and neurohormone levels differed as a function of when participants were assessed during the semester. This enabled us to investigate the question of how participants were affected by both the acute laboratory-based emotional stressor and also the naturalistic academic stress that they experienced, which we did by systematically bringing different students into the lab on successive Friday afternoons during the semester. Participants' satisfaction with life was somewhat higher mid-semester (Mdn = 28; Mean Rank = 21.65) than late-semester (Mdn = 25; Mean Rank = 15.88), although this difference was not significant, $U(37) = 117.0$, $p = .110$. Likewise, perceived stress levels were higher late-semester (Mdn = 5; Mean Rank = 22.79) than mid-semester (Mdn = 6; Mean Rank = 16.20), but, again, this association was only marginally significant, $U(37) = 226.0$, $p = .091$.

Basal cortisol levels across the semester

As expected from prior research documenting academic stress-related increases in cortisol (e.g. Verschoor & Markus, 2011), baseline cortisol levels were significantly elevated for all participants toward the end of the semester when students' scholastic demands were greatest. As shown in Figure 3, this cortisol spike occurred on April 26th ($M = 13.35$, $SE = 2.427$, 95% CI [18.35, 18.35]), with participants' levels remaining elevated through May 3rd ($M = 6.85$, $SE = 2.20$, 95% CI [2.32, 11.38]), $F(10, 25) = 2.73$, $p = .020$. These end-of-semester cortisol elevations for these two dates were 2 SDs

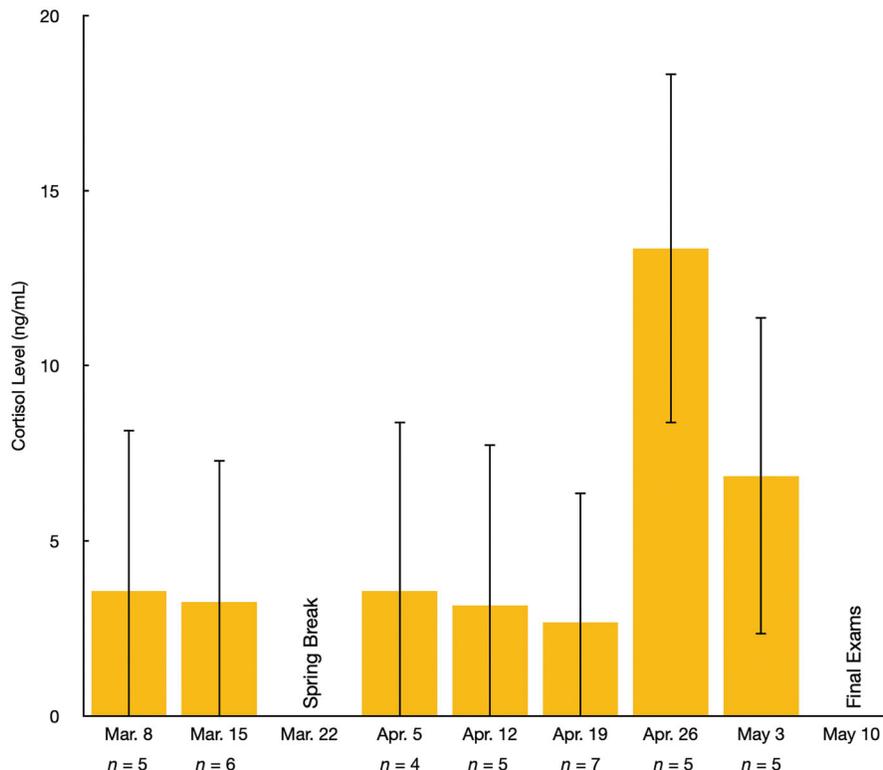


Figure 3. Basal cortisol (CT) levels (ng/mL) by time of semester. Covariates included body mass index, age, menstrual cycle phase (follicular vs. luteal), and lab appointment time. Error bars represent standard errors of the mean. ($n = 37$)

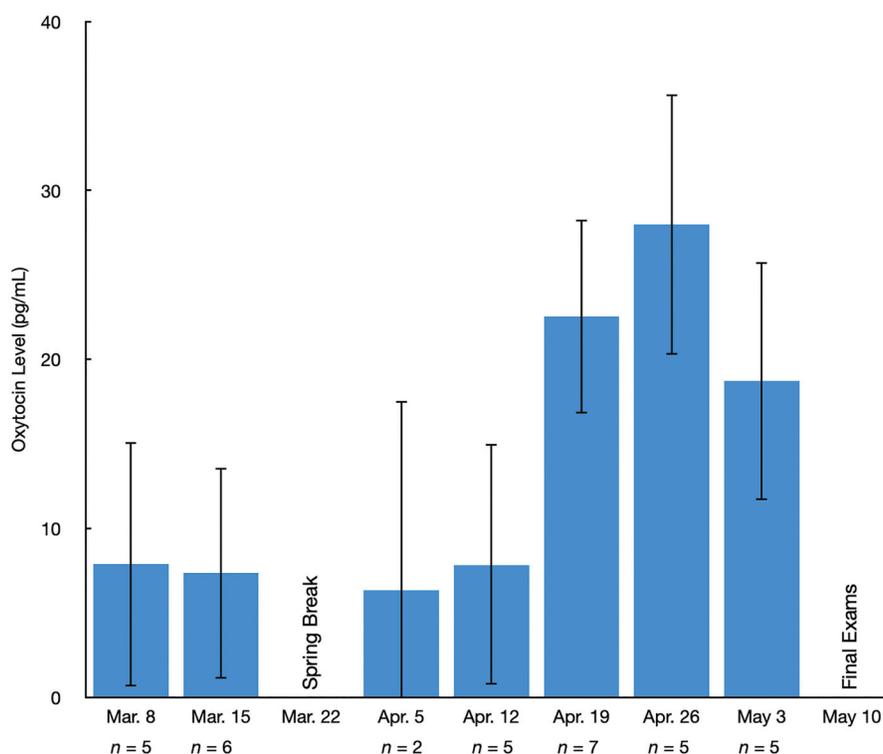


Figure 4. Basal oxytocin (OT) levels (pg/mL) by time of semester. Covariates included body mass index, age, menstrual cycle phase (follicular vs. luteal), and lab appointment time. Error bars represent standard errors of the mean. ($n = 35$)

higher than the cortisol levels that participants exhibited mid-semester. Indeed, pairwise comparisons revealed that participants' cortisol levels were significantly higher on April 26th (i.e. the second-to-last week of the semester) than on all of the other previous weeks of data collection from mid- to late-middle semester (see [Supplemental Material, Table S4 and S5](#)), demonstrating that end of semester stress was significantly associated with sustained increases in cortisol.

Basal oxytocin levels across the semester

Lastly, we examined how basal oxytocin levels changed across the semester in response to changing levels of academic stress. As shown in [Figure 4](#), participants' basal oxytocin levels were significantly higher later in the semester when scholastic demands are greatest. More specifically, participants' oxytocin levels abruptly increased for all participants coming into the lab on each of the three Fridays before the semester's end. Notably, this spike in oxytocin occurred one week prior to the increase in cortisol, which emerged the following week (see [Supplemental Material, Tables S2 and S3](#)).

This highly significant group-level increase in oxytocin was 2–3 *SDs* greater than all participants' mid- to late-middle semester oxytocin levels, with late-semester estimated marginal means being $M = 22.66$, $SD = 9.15$, $SE = 1.78$, 95% CI [19.01, 26.30], versus mid-semester means of $M = 7.58$, $SD = 3.52$, $SE = 1.67$, 95% CI [4.15, 11.01], $F(1, 28) = 36.444$, $p = .0001$. The mid-semester baseline oxytocin estimated marginal means were March 8, $M = 7.86$, $SE = 3.30$, 95% CI [.65, 15.12]; March 15, $M = 7.37$, $SE = 5.42$, 95% CI [1.12, 13.61]; April 5, $M = 6.34$, $SE = 5.42$, 95% CI [4.88, 17.56]; and April

12, $M = 7.85$, $SE = 3.43$, 95% CI [0.75, 14.94]. In comparison, the late semester estimated marginal means were: April 19, $M = 22.55$, $SE = 2.77$, 95% CI [16.81, 28.28]; April 25, $M = 27.99$, 95% $SE = 3.72$, 95% CI [20.28, 35.70]; and May 3, $M = 18.73$, $SE = 3.40$, 95% CI [11.70, 25.76]. This temporal stress-related effect on oxytocin was significant while adjusting for all covariates, $F(10, 23) = 4.207$, $p = .002$, demonstrating that this shared semester-end stress was associated with substantial elevations in oxytocin among these healthy young women.

Discussion

Despite substantial interest in how acute and chronic stress affect cortisol, oxytocin, and cognition, no studies have examined how these factors interrelate and change together in response to stress. We addressed this issue in the present study by assessing cortisol, oxytocin, and cognitive-emotional control levels in a sample of healthy young women who were randomly assigned to watch either an emotionally stressful or control video. As expected, young women randomly assigned to watch an emotionally distressing video reported experiencing greater increases in both general negative affectivity and specific negative emotions. Although this brief laboratory-based stressor did not significantly affect oxytocin or cortisol levels, there was a significant Emotional Stressor vs. Control Condition differential effect of low versus high baseline oxytocin and cortisol levels on participants' Stroop performance (see below).

By systematically running different participants on successive Friday afternoon sessions through the end of the semester, we were also able to cross-sectionally investigate how

cortisol and oxytocin levels changed on average over the semester as scholastic demands increased. Notably, every participant coming into the lab during the last three weeks of the semester exhibited significantly elevated basal oxytocin levels. Additionally, the following week, every participant presented with significantly elevated basal cortisol levels, presumably in response to the increase in assignments, deadlines, and final exams occurring during this time. Stress-induced increases in cortisol are well documented in general and have also been shown for college students while anticipating and taking multiple-choice exams (Nicolson, 1992; Verschoor & Markus, 2011). Relevantly, these results are consistent with data from Anderson et al. (2018), who found group-based collective increases in cortisol during shared threatening outdoor river-rafting experiences, thus suggesting possible social-environmental synchronization of neurohormonal processes (Atzil et al., 2014).

Elaborating a bit further, every woman entering the lab during the last three weeks of the semester revealed elevated basal oxytocin levels by 2–3 *SDs* over mid-semester oxytocin levels. The fact that oxytocin increases preceded semester stress-induced increases in cortisol provides important new insight into the naturalistic timing of oxytocin level increases vis-a-vis increases in cortisol in response to stress. One possibility is that such increases represent an anticipatory neurohormonal response that promotes a collective bio-behavioral reaction (e.g. “tend and befriend”) that helps protect women from the negative effects of stress (Barrett, 2017; Taylor et al., 2000). Consistent with this possibility, substantial theorizing has focused on characterizing highly coordinated interactions between interpersonal processes, social brain networks, and biological responses that help individuals better deal with impending social-environmental challenges (Atzin et al., 2018; Barrett, 2017; Feldman, 2012a, 2012b; Kleckner et al., 2017; Slavich, 2020b).

The ability to cognitively control emotional information has been described as a critical process regulating individuals’ biological response to stress (Diamond, 2011; Shields et al., 2017b). Therefore, we also assessed participants’ reaction times and accuracy in a post-video emotional Stroop task in which participants were instructed to categorize foreground emotion words as Happy, Sad, or Anger while ignoring the background angry, sad, happy, or blurred face. Consistent with a role for cortisol in enabling individuals to quickly and accurately perceive the environment, higher baseline cortisol levels were associated with faster emotional Stroop reaction times. Higher baseline oxytocin levels, in turn, were associated with greater cognitive-emotional accuracy on the Stroop. More specifically, women with high oxytocin levels randomly assigned to watch the emotionally stressful video performed at a striking 100% accuracy on the emotional Stroop after watching the video as compared to only 75% accuracy for those exhibiting low basal oxytocin levels in this experimental condition. In addition, higher basal and post-video viewing oxytocin levels were associated with greater positive affect following the emotionally stressful video. Therefore, it is possible that oxytocin plays a role in helping women accurately process emotional information

and maintain a more positive attitude during stress (Shiota et al., 2017).

More broadly speaking, the Stroop task that we used required women to ignore the background male face (distractor) while attending to and categorizing the emotion word (task). In real life, the ability to make a split-second accurate assessment of a potentially threatening (male) assailant may increase the likelihood of survival for both the woman and the child she is protecting. Though speculative, therefore, the present data are consistent with a potential role for oxytocin in enhancing cognitive processes that would help promote the survival of the species.

Despite chronic stress-related increases in both cortisol and oxytocin, women in this sample did not report significant decreases in well-being or increases in perceived stress during the most academically challenging times of the semester. Although the present data cannot address the mechanistic reason for these unexpected findings, one possibility is that the women’s elevated oxytocin at semester’s end buffered them from the negative emotions that often accompany stress. Consistent with this possibility, oxytocin is known to mediate positive affect and perceived event sociality (Isgett et al., 2017) and may provide chronic stress-buffering effects (Holt-Lunstad et al., 2008; Quirin et al., 2011). Associations between changes in the autonomic control of visceromotor activity, cognitive control of demanding tasks, allostasis (e.g. as found by Kragel et al., 2019), and oxytocin might be explored in future research. It is also possible that scales that assess processes that are more subject to change (e.g. weekly depressive symptoms, daily stress levels) could have done a better job of capturing some of the expected stress variability across the semester, but longitudinal, within-person sampling of cortisol, oxytocin, sociality, and perceived stress is needed to further investigate this possibility.

The association between oxytocin and positive affect that we found makes sense when viewed within the tend-and-befriend framework. Ultimately, oxytocin triggered positive affect *tending-and-befriending* caregivers would be more likely to manage stress and efficaciously perform support behaviors that are needed (Taylor, 2006) if fueled by positive emotions such as *alert*, *active*, *determined*, and *strong*. Indeed, the positive association seen between increased positive affect and oxytocin, and the group-level natural oxytocin neurohormone convergence evidenced here, both support oxytocin’s acute and chronic stress-buffering effects for women and may help explain some prior findings showing stress-buffering benefits of positive emotion (for a review, see Pressman et al., 2019).

Feldman (2017) points out that bio-behavioral synchrony is critical for survival. Moreover, the oxytocin system is known to help support social affiliation and group cohesion by increasing the salience of social cues and regulating stress in humans (Bartz et al., 2011; Taylor, 2011). Levy et al. (2016), in turn, demonstrated that oxytocin selectively modulates brain responses to stimuli probing social synchrony with increased oxytocin robustly affecting social processing. Contextual group, oxytocin receptors, and individual oxytocin effects on agency are relevant, particularly as related to caregiving and parenting (Bakermans-Kranenburg et al., 2012; Feldman et al., 2013). It is possible, therefore, that the present data showing

increases in oxytocin and, subsequently, cortisol among women at semester's end are partly representative of social-biological synchrony that helps individuals and groups better prepare for challenges that lie ahead. Oxytocin stress-buffering and positive affect within the *tend and befriend* and *survival of the species* models subserve the prioritization of offspring care. Increased cognitive acuity and positive emotions, as found here, would further be valuable for initiating and performing needed instrumental actions that help promote survival, particularly in stressful situations.

Limitations

Several limitations of this study should be noted. First, we sampled college-age women taking no hormones. Additional research exploring these effects among men, hormone-consuming women, and persons of other ages and social groups is thus necessary to explore issues of generalizability. Second, our sample size of 37 women was modest with only twenty in the Emotional Stressor and seventeen in the Control Condition. Additionally, women's natural basal neurohormone levels collected across the semester included only four to seven participants per week. Although we found significant between-group differences toward the semester's end, these cell sizes are indeed small. Given the limited sample size, it is possible that low power may partly explain marginal or non-significant findings in self-report and acute stressor measures. As such, these results should be regarded as preliminary until replication studies are conducted. Third, participants' cortisol and oxytocin levels were quantified in saliva. Evidence exists supporting the reliability and validity of salivary cytokine and cortisol measures (e.g. Slavish et al., 2015; Shields et al., 2019), but validity questions remain regarding salivary oxytocin (McCullough et al., 2013). Studies examining salivary oxytocin have provided evidence for its reliability (van Ijzendoorn et al., 2012) and have shown that salivary levels correlate strongly with plasma levels (e.g. $r=0.59$; Grewen et al., 2010). Moreover, similar results have been reported with samples obtained from saliva, urine, and plasma (Feldman et al., 2011; Holt-Lunstad et al., 2015). Nevertheless, we recognize that this is a concern and recommend that future studies consider using other sampling procedures, bio-assays, and mass spectrometry to address this issue.

Fourth, although we standardized the timing of the experimental lab sessions such that they all occurred on a Friday afternoon, oxytocin and cortisol both have known diurnal effects, and future research could benefit from using an even narrower sampling window. Finally, additional research is needed to examine different processes that might account for the strong association that we observed between individual and group-level changes in cortisol and oxytocin toward the end of the semester. Increased social interactions and sociality (Isgett et al., 2017), shared convergent emotional expressions that are known to occur during stress (Totterdell et al., 1998), and oxytocinergic mechanisms contributing to species survival (Holt-Lunstad et al., 2019) could have all contributed to these effects. However, pheromones, cyclical estrogen, and other neurophysiological processes

could also be relevant and should be explored in future research.

Given the significant associations between cortisol and oxytocin observed here, we also encourage researchers to examine neurohormone responses to stress during other challenging social group experiences, such as groups meeting important deadlines (e.g. NASA launch), athletic teams playing high-stakes games across the season, or emergency response teams or civilians working or living through natural disasters (e.g. earthquakes, floods, fires, pandemics). Likewise, diurnal cortisol effects are well known, but additional research is needed to examine possible diurnal oxytocin effects, as well as how cortisol and oxytocin change in concert across a day and across time within individuals and social groups. Additionally, given our finding that basal oxytocin and cortisol levels change significantly across the semester, future research should investigate time-of-semester effects or at least control for assessment timing when studying these neurohormones.

Conclusions

In conclusion, the present study examined psychological and biological responses to acute and longer-term naturalistic stress and revealed what we believe is new information about how healthy young women respond to such stress. More specifically, we found that as compared to women assigned to a no-stress control condition, those randomly assigned to experience an acute emotional stressor exhibited greater increases in negative emotionality but no significant changes in cortisol or oxytocin. Rather, it was participants' basal cortisol and oxytocin levels that yielded significant insights into how these neurohormones relate to cognitive-behavioral processes during stress. When separated into high vs. low median-split groups, while most control group emotional Stroop performances were similar, after viewing the Emotional Stressor video, higher basal cortisol levels were significantly associated with faster cognitive-emotional Stroop reaction times; higher basal oxytocin levels, in turn, were related to greater Stroop cognitive-emotional accuracy.

Finally, we found cross-sectional evidence for a consistent spike in participants' oxytocin levels three weeks before the semester's end, with cortisol levels increasing the week after. This cortisol finding is consistent with prior research on biological responses during group stress synchrony (e.g. Anderson et al., 2018) but extends this work to include both acute and chronic forms of stress and both neurohormones. More broadly, investigating the dynamic nature of oxytocin and cortisol may reveal a choreographed dance – a sort of do-si-do pattern – wherein increases in cortisol are associated with complementary increases in oxytocin. Nuanced and responsive to social-environmental situations, oxytocin's blanket framing as a *love hormone*, the *tend and befriend hormone*, or as a *pro- vs. anti-social hormone* is over-simplistic and misses the social attention and salience processes promoted by oxytocin, as well as the divergent, situation-specific effects that are associated with this neurohormone (Beery, 2015; Steinman et al., 2019). Perhaps a more inclusive view

of oxytocin *vis-a-vis* cortisol might be as protective wear: a sort of whole-body Kevlar vest that provides anticipatory protection (Barrett, 2017) against stressful situations in advance of them actually occurring, with the oxytocin response, and its effects, being shaped by the specific social features of the situation being experienced (Influs et al., 2019).

Finally, this study is the first that we know of to examine the cognitive correlates of these two neurohormones, though additional research is needed to more fully understand the mechanisms underlying these associations. It is possible, for example, that oxytocin provides women with an anxiolytic and affective boost that supports social synchrony and facilitates getting through stressful times together, as is suggested by affiliative neuroscience (Feldman, 2020), *survival of the species*, and *tend and befriend* (Taylor et al., 2000). However, additional studies are needed to understand the relevance of the present findings for collective well-being and behavior. Further research is also warranted to explore how endogenous systemic oxytocin and cortisol responses are interrelated during times of stress and how such effects are in turn associated with emotion regulation, cognitive control and allostasis, stress-buffering, and individual and collective positive affect and agency in ways that structure the stress response and promote psychosocial resilience “when the going gets tough.”

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