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Stress-Induced Oxytocin Reactivity as a Predictor of Daily Support Seeking After Stress

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by

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Submitted to the Graduate Faculty as partial fulfillment of the

requirements for the Master of Arts Degree in Psychology

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An Abstract of

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The current study examined whether stress-induced peripheral oxytocin (OT) was associated with support-seeking behaviors after stress in daily life. Healthy male and female participants (N=94) performed a standardized laboratory stressor, the Trier Social Stress Test (TSST), and then completed two weeks of daily assessments of support seeking after stress. In line with preregistered hypotheses, plasma OT reactivity to the TSST (indexed as the area under the curve with respect to increase; AUCi) was associated with more frequent support seeking behaviors following stress in daily life. Moreover, this association was stronger for individuals with higher levels of attachment anxiety, relative to those with lower levels of attachment anxiety, after controlling for attachment avoidance. While attachment avoidance did not moderate the effect of AUCi, our preregistered exploratory analyses revealed a significant moderation of attachment avoidance using another common index of neuroendocrine (i.e., area under the curve with respect to ground; AUCg). Taken together, these results provide empirical support for theoretical models implicating the OT system in affiliative behaviors following stress, as well as its interactions with adult attachment style.

Keywords: oxytocin, social support, attachment avoidance, attachment anxiety

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Stress-Induced Oxytocin Reactivity as a Predictor of Daily Support Seeking After Stress

Social relationships, and in particular perceptions of social support, play an important role in both mental and physical health (1). In fact, social support and feelings of social connectedness have been linked to a 50% reduction in mortality rates, an effect size that is comparable to well-established health-promoting behaviors such as quitting smoking and physical activity (2). In particular, having a social network (e.g., friends, family members) that is supportive and responsive to our needs may be especially protective during times of stress (3, 4). According to transactional models of stress, social support may be one way in which individuals increase their resources to meet the demands of stressful situations (5). Although the receipt of social support may not always lead to optimal outcomes, receiving support that is responsive and sensitive to our needs may have beneficial outcomes (6, 7), and seeking social support remains a common way by which individuals cope with stressful events (8). However, to date, little is known about the biological mechanisms underlying the tendency to seek support after stress.

The Role of Oxytocin

One possible mechanism may involve the neuropeptide oxytocin (OT), which, in addition to its role in many physiological processes, has garnered considerable attention as a modulator of social behaviors in non-human animals (9). Research in non-human animals has extensively implicated the OT system in attachment- and affiliation-related behaviors, from maternal (10, 11) and alloparental behavior (12, 13), to adult pair-bonding (14). Synthesized in the magnocellular and parvocellular neurons of the hypothalamus, OT is secreted both within the brain (i.e., centrally) and into peripheral circulation via the posterior pituitary (15). In humans, peripheral levels of OT can be measured via blood, urine or saliva samples (16–18), with the goal of

assessing either individual differences in basal levels of OT, or changes in OT levels in response to stimuli.

Studies assessing basal levels of OT, which comprise the majority of studies on peripheral OT in humans, have found positive associations between OT levels and attachment- and affiliation-related behaviors (19), much like those observed in non-human animals (for review; 20). Although relatively fewer studies have examined *changes* in peripheral OT levels, some evidence suggests that plasma OT levels increase following parent-infant interactions (21, 22) and in response to warm contact between romantic partners (23), though this effect is sometimes only observed in women (24). Similarly, a recent study by Papasteri and colleagues (2020) found that women's salivary OT levels significantly increased in response to receiving positive social feedback from an experimenter. However, this study also found that receiving negative social feedback from the experimenter resulted in increases in OT levels in women.

In fact, there is consistent evidence that peripheral OT levels increase in response to stressful situations in both non-human animals, as well as humans (26). For example, in human studies, lab-based paradigms that reliably induce psychosocial stress have been used to elicit significant increases in OT levels, both in plasma (27) and saliva samples (16, 28, 29), though inconsistent results have also been observed in some studies (30, 31). Moreover, stress-induced central and peripheral levels of oxytocin in non-human animals have been found to be positively correlated (32), suggesting that stress-induced changes in peripheral levels may be a proxy for changes in central levels. In sum, OT is secreted in response to stressful situations, and is also implicated in attachment and affiliative behaviors; in this way, the OT system could regulate affiliative behaviors (e.g., support seeking) in response to stressful events.

Theoretical Models and Potential Mechanisms of Stress-Induced OT in Affiliative Behavior

To date, several theoretical models have described the potential role of OT as a motivator of affiliative behavior following stress. According to the "tend-and-befriend" hypothesis (33), oxytocin release may serve an evolutionarily adaptive function, by enhancing the desire for social contact in response to stress, and therefore reducing one's vulnerability in dangerous or threatening situations, particularly in women. In line with this idea, Depue and Morrone-Strupinsky (2005) theorized that the OT system is a part of a broad emotional-motivational system, activated by social cues in the environment and geared towards facilitating the development and maintenance of close social bonds. Via interactions with the dopaminergic and endogenous opioid systems, the OT system has been proposed to modulate attentional processes involved in the motivation for affiliation (i.e., appetitive processes), as well as in the pleasurable experience of affiliation (i.e., consummatory processes), respectively (34). More recently, the "social salience hypothesis" expanded on the role of OT as a modulator of social attention in humans, suggesting that OT is involved in reorienting attention towards relevant social cues – whether aversive or rewarding – through interactions with the mesocorticolimbic dopamine system (35).

Previous research in non-human animals has found support for the role of OT in reward and motivational processes. For example, Xiao and colleagues (2017) demonstrated in mice that OT neurons in the paraventricular nucleus of the hypothalamus project directly to the ventral tegmental area (VTA), which is involved in processing and encoding emotionally salient stimuli of both positive and negative valence. The authors also found that endogenous release of OT from these neurons directly enhances activity of dopamine neurons in the VTA. Taken together, these findings suggest that that the OT system is capable of exerting direct control over the

activity of dopamine neurons in the VTA (36), which plays a critical role in encoding the salience of social (and non-social) stimuli (37). Accordingly, intranasal OT administration in humans has been shown to enhance activity in the VTA in response to both social reward and threat (38). Moreover, dopaminergic neurons in the VTA have been shown to mediate social approach behaviors in mice via downstream projections to other limbic structures (39). In this way, dopaminergic circuitry involving the VTA may be one mechanism by which OT could motivate social behaviors in response to threats in the environment.

Another potential neural region through which OT may modulate affiliative behavior following stress is the bed nucleus of the stria terminalis (BNST)—a component of the extended amygdala, which is highly sensitive to aversive contexts (40). In particular, in rodent models, OT-facilitated activity of neural circuits in the anterior divisions of the BNST modulate both social approach and avoidance behaviors in response to aversive social contexts (41). Moreover, studies have observed sex-specific effects of OT within the BNST that result in divergent patterns of social behaviors in response to stress (42, 43). These studies further underscore the importance of considering sex-specific effects when examining the role of OT in both stress reactivity and post-stress affiliative motivation.

Despite support for the aforementioned theoretical models of OT in studies of non-human animals, to our knowledge, no studies in humans have examined whether stress-induced OT reactivity is associated with support seeking behaviors after stress. Furthermore, individuals may differ in the extent to which their OT system is sensitive and reactive to social cues in the environment, and differences in OT reactivity may underlie variations in the way individuals respond to their social environment (44). In a study by Tabak et al. (2011), individuals who recently experienced an interpersonal transgression were asked to imagine and portray a

confrontation with their transgressor; in response to this task, individuals with greater plasma OT reactivity reported greater post-conflict anxiety and less forgiveness. In this way, individual differences in OT reactivity could represent a potential biomarker of individuals' sensitivity to their social relationships. Therefore, while stressors may increase OT levels across individuals, there may be individual differences in the magnitude of the OT response, which could be related to differences in support seeking behaviors following stress.

The Role of Attachment

The decision to seek support after stress may be affected by a multitude of characteristics, both at the individual and cultural level (46). At the individual level, differences in support seeking after stress may arise as a function of different expectations regarding the availability and responsiveness of close others to our needs. According to attachment theory (47), children develop internal working models of how attachment figures (i.e., supportive others, such as primary caregivers) are expected to behave and respond to their needs. For example, previous experiences with caregivers who are sensitive and responsive to a child's needs would lead to the development of attachment security (i.e., an internal working model that safety and support from attachment figures will be available when needed). By contrast, a history of experiences with caregivers who are inconsistently sensitive and responsive, or who lack these characteristics altogether, would result in attachment insecurity (i.e., an internal working model of attachment figures as unreliably supportive). These internal working models of attachment are theorized to persist throughout the lifespan, and to influence our thoughts and behaviors regarding support seeking in times of need (48).

During adolescence and adulthood, the construct of attachment styles (or orientations) is often parsed into two dimensions: attachment *avoidance* and *anxiety* (49). The dimension of

avoidance relates to individual differences in one's discomfort towards, and avoidance of, emotional closeness and intimacy in relationships with others, whereby individuals with higher levels of attachment avoidance tend to feel uncomfortable trusting others to meet their emotional needs (49). By contrast, the dimension of anxiety characterizes the extent to which individuals feel worried about the availability and responsiveness of others, and anxious regarding their value to others in relationships. In this way, individuals with higher levels of attachment anxiety tend to more frequently seek reassurance in their relationships than those with lower levels of attachment anxiety (49).

Importantly, these differences in attachment orientation influence support seeking behaviors. For example, Collins and Feeney (2000) found that individuals wither higher levels of attachment avoidance sought less support from their relationship partners when stressed, relative to those with lower levels of attachment avoidance. Similarly, in response to anxiety-provoking situations, avoidantly attached women tend to seek less support with increasing levels of anxiety (51), however this association was not replicated in men (52). Likewise, women's attachment avoidance has been associated with less proximity-seeking (e.g., physical contact) with romantic partners and greater avoidance behaviors (e.g., distraction) in anticipation of stressful experiences such as separation, whereas results were more mixed in men (53). By contrast, several studies have found no association between attachment anxiety and support seeking behaviors (50, 51, 53). To date, however, no study has examined the extent to which the OT system may interact with attachment tendencies within the context of support seeking. Given the OT system's involvement in both the stress response as well as post-stress affiliative behavior, individual differences in OT reactivity could potentially amplify attachment-related tendencies in response to stress.

Despite considerable work on the role of OT in attachment, however, few studies have examined whether adult attachment orientations are associated with differences in OT reactivity to stress. One study examined whether adult attachment styles – assessed categorically – were associated with OT reactivity to a lab stressor, among individuals with past trauma (54). Although the study found evidence of OT reactivity to stress, no differences in reactivity were observed between the four attachment classifications (i.e., autonomous/secure, dismissing, preoccupied, and unresolved), suggesting that stress-induced OT reactivity may not be associated with adult attachment. That said, individual differences in adult attachment styles are best represented by dimensional models of attachment, rather than categorical models, and the use of dimensional measures are said to more appropriately capture the construct of adult attachment (55). In addition to these psychometric considerations, it remains unclear whether the findings observed by Pierrehumbert et al. (2012) are specific to individuals who experienced trauma. More recently, Speck and colleagues (2019) examined whether adult attachment was associated with OT reactivity to films depicting loss of an attachment figure, among individuals with schizophrenia and healthy controls. Using a dimensional measure of attachment anxiety and avoidance (specifically developed for use among individuals with psychosis), their study found that neither of the attachment dimensions was associated with OT reactivity across both clinical and healthy samples. However, this measure of attachment has been shown to display questionable psychometric properties (57). Taken together, although prior studies have found no attachment-related differences in OT reactivity, it remains unclear whether this lack of association would be replicated in larger, non-clinical sample, using a dimensional measure of adult attachment with robust psychometric properties.

As mentioned previously, it is also possible that attachment orientations influence the association between OT reactivity and support seeking behaviors. While OT reactivity may be positively associated with support seeking after stress, this association may depend on an individual's attachment orientation (and related expectations regarding the availability of others). For instance, the association between OT reactivity and support seeking after stress may be weaker in individuals with greater attachment avoidance, given the desire for self-reliance and independence which characterizes attachment avoidance. By contrast, the association between OT reactivity and support seeking after stress may be stronger in individuals with greater attachment anxiety, given that reassurance seeking is predominantly featured within attachment anxiety.

The Present Investigation

In the current study, we experimentally induced OT reactivity in the lab, employing a well-validated psychosocial stressor. Plasma OT reactivity was measured across 5 repeated assessments, the timing of which was designed to appropriately capture the fast and pulsatile pattern of peripheral OT release (58). In this way, we first tested whether plasma OT levels changed in response to the stressor, and whether OT reactivity differed based on gender and attachment orientation (i.e., attachment anxiety and avoidance). We then used lab-based OT reactivity as a proxy for individuals' typical OT reactivity to psychosocial stress in daily life, and examined the association between OT reactivity and the tendency to seek support after stress in daily life, as assessed through two weeks of daily diaries. Lastly, we examined whether attachment orientation moderated the association between OT reactivity and daily support seeking after stress.

As preregistered (https://osf.io/mr6vg), we proposed the following hypotheses: 1) OT reactivity in response to a lab-based stressor will be positively associated with support seeking after stress in daily life, and this association will be of greater magnitude in women compared to men, and 2) this association will be moderated by attachment orientation, whereby attachment avoidance will reduce the strength of this association, and attachment anxiety will strengthen it.

Methods

Participants

Participants were recruited from Southern Methodist University (SMU) in Dallas, Texas. Participants were required to be over 18 years old, and fluent in English. Exclusion criteria for the study included: having a fear of needles/syringes, having a history of nausea or fainting during blood collection, previous difficulty experienced by nurses/phlebotomists finding veins for blood draws, current pregnancy, plans to become pregnant in the next month, current breastfeeding, current or previous medical illnesses (e.g., diabetes, hypertension, migraines), current or previous psychiatric diagnoses, current use of prescription medications (with the exception of hormonal contraceptives), regular use of non-prescription medication, regular use of smoking or vaping tobacco, working regular night shifts, atypical sleep-wake cycles, or unwillingness to abstain from caffeine/drugs/alcohol the night before and day of the in-person session.

Of the 115 participants who were recruited to participate in the study, 14 participants did not complete the study due to difficulty during blood draws (e.g., feeling lightheaded), and 7 participants did not complete the daily diary component of the study. This resulted in a sample of 94 participants (59 women: age range = 18-22, M_{age} = 19.66, SD = 1.27; 35 men: age range = 18-25, M_{age} = 19.86, SD = 1.38). Participants identified as White (68.1%), Asian (18.1%), Black or

African American (4.3%), “Other” (7.4%), Native Hawaiian or Other Pacific Islander (1.1%), Native American or Alaska Native (1.1%). Moreover, 18.5% of our sample self-identified as Hispanic or Latino/a. Information regarding menstrual cycle phase and use of hormonal contraceptives was assessed during the laboratory session; the current sample included 28 female participants (48.3%) taking hormonal contraceptives.

All participants provided written informed consent, and received course credit, monetary compensation, or a combination of both, for their participation in the study. The study was approved by the SMU Institutional Review Board.

Study Design

The current study was part of a broader data collection effort (for full procedures and methods used see: https://osf.io/fdexm). Following the completion of an online questionnaire to determine eligibility, eligible participants were then contacted by phone for a more comprehensive interview with a research assistant, who confirmed participants’ answers to the online questionnaires and sought clarification on particular items if needed. Participants who remained eligible for the study were scheduled for Part 1 of the study.

Part 1

The first part of the study comprised of a 2-hour in-person session, in which participants were asked to complete a set of online questionnaires and behavioral tasks. These questionnaires included a measure of attachment (described in the Self-Reported Adult Attachment section below). At the end of the session, participants were scheduled for their second in-person session, which took place between 1:00-5:00pm to minimize the effects of diurnal fluctuations in hormone levels (59). Moreover, participants were given additional instructions prior to the second session: participants were asked to abstain from drugs (including alcohol and caffeine)

for 24 hours prior to the session, as well as to refrain from physical exercise and from consuming any food or drinks (other than water) for 2 hours prior to the session.

Part 2

Upon arrival for the laboratory session, participants' eligibility was assessed (i.e., confirming that they followed all instructions in preparation for the session). If participants remained eligible to continue in the study, they were fitted with a small intravenous catheter into their non-dominant arm, and an initial blood sample was collected. Following this first blood draw, participants were asked to remain seated for 30 minutes, and were provided a magazine with neutral content to read; this period allowed for catheter habituation. Following this 30-minute habituation period, a baseline blood sample was collected (i.e., 1 minute prior to the start of the stress-induction task). Acute psychosocial stress was then induced using the Trier Social Stress Test (TSST; 60); details regarding TSST procedures can be found in the Stress Induction Task section below. The stress-induction task was then followed by 4 blood draws, spanning 30 minutes post-stress (see Blood Draw Protocol and Processing section for more details). At the end of the session, participants were provided instructions for the at-home daily diary component of the study.

Part 3

For the third part of the study, participants completed a daily 5-minute online questionnaire for two weeks. This questionnaire was distributed to them via Qualtrics at 8PM every day, over the 14 days following the second in-lab session. Every day, participants reported on their experience of negative affect and distress over the past day, as well as on their support seeking behaviors after stress (see Daily Diary Measures section for details).

Laboratory Measures

Stress Induction Task

The TSST is a highly standardized and well-validated psychosocial stressor, which has been shown to reliably and effectively induce stress across a variety of biological and psychological indicators (60, 61). Following protocol recommendations based meta-analytic data (62), the TSST consisted of an anticipatory period (3 minutes), followed by a mock job interview, in which participants were asked to deliver a speech (5 minutes), and then a mental arithmetic task (5 minutes), in front of an audience. During the anticipatory period of the TSST, participants were told that they will be performing a speech in front of a "panel of behavioral experts" (i.e., two student actors: one male and one female) who will be evaluating their performance. Specifically, participants were instructed to prepare a job interview-type speech which outlines their qualifications for their ideal job. Following the anticipatory period, participants completed their speech in front of the audience, and were then asked to perform a mental arithmetic task, in which they were asked to count backwards from 2023 to 0 in 17-step sequences.

Manipulation Check

To test whether the TSST induced changes in state anxiety in the current sample, we used a short, 6-item (63) State version of the State-Trait Anxiety Inventory (STAI) (64). This measure was administered to participants at six timepoints throughout the session: following IV placement (baseline), 1 minute before the start of the TSST (during the preparation period), and then +1, +6, +20 and +45 minutes post-TSST.

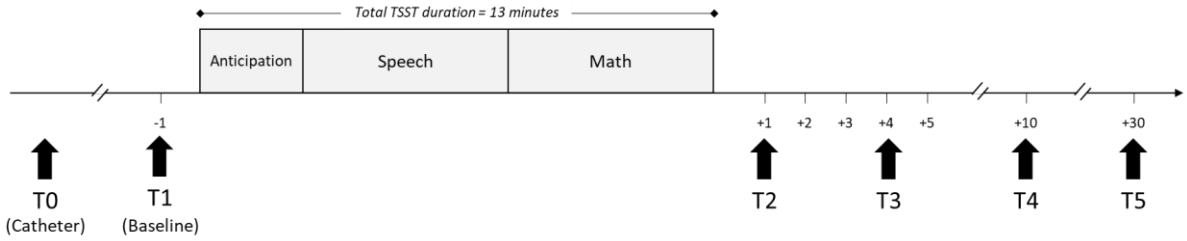
Blood Draw Protocol and Processing

Throughout the laboratory session, a total of six blood samples were drawn; immediately after catheter insertion, an initial 10ml blood sample was collected for purposes unrelated to the

present study, and the remaining samples were drawn into two 6-ml Vacutainer tubes with EDTA. A baseline sample of oxytocin was collected following a 30 minute habituation period (T1). In order to capture the relatively rapid and pulsatile release of OT (58), four blood draws were performed after completion of the TSST: these samples were collected at +1 minute (T2), +4 (T3), +10 (T4), and +30 minutes (T5) post-TSST. The experimenter manually added 0.38ml of Aprotinin agent to each tube immediately after blood samples were collected. Each tube was gently rocked, submerged into an ice bath, and then centrifuged at 1600 × *g* for 20 minutes at 4°C to separate the plasma, which was then pipetted into microtubes for storage at -80°C until shipment. All samples were shipped frozen on dry ice to the laboratory of Dr. Armando Mendez at the Diabetes Research Institute in the University of Miami Miller School of Medicine for OT extraction and assay.

Figure 1

Timeline of Protocol



Measurement of OT

To measure OT reactivity in blood plasma, we followed the recommended procedures outlined in Szeto et al. (2011). All samples were analyzed in duplicate. Solid phase extraction of samples was conducted using 200mg C18 Sep-Pak columns (Phenomenex, Torrance, CA). Extracts were reconstituted with 450 µl assay buffer and measured by radioimmunoassay (RIA) as previously described (58) using the oxytocin antibody from Woomera Therapeutics (Lebanon, NH). The limit of detection was 1.0 pg/tube, and intra- and inter-assay coefficients of variability

(CV) were 5% and 14%. Information regarding missing data, outliers and hemolyzed samples can be found in Table 1 below (moreover, details regarding related sensitivity analyses can be found in the statistical analysis section). In the present sample, 12.39% of all datapoints were below the level of detection; values for these "undetectable" samples were imputed at 0.01 pg/ml.

Table 1

Available Data Per Timepoint

	N	Non-Detectable	Missing	Hemolyzed	Outliers
T1	94	13	0	5	0
T2	92	12	2	2	1
T3	89	9	5	9	2
T4	93	11	1	5	2
T5	92	12	2	3	0

Indexing Individual Differences in OT Reactivity

Given this study's focus on measuring individual differences in OT *reactivity* to the TSST, we used the area under the curve with respect to increase (AUCi; 66) as our primary index of OT reactivity. AUCi is generally considered an index of the biological system's sensitivity, with a particular emphasis on changes in levels over time, and has been used previously in several studies examining oxytocin reactivity to the TSST (29, 67, 68).

Measurement of Progesterone and Estradiol

To account for menstrual cycle variation and use of hormonal contraception in women, we measured levels of progesterone and estradiol at baseline (T1) in female participants only. Baseline plasma levels of progesterone and estradiol were quantified using RIA, following the procedures described in Tabak et al. (2011). The limit of detection for progesterone was of 0.16 nmol/L (0.05 ng/ml), and the intra- and inter-assay coefficients of variability (CV) were <1.0%

and <6.8%. For estradiol, the limit of detection was of 18.4 pmol/L (5 pg/ml), and the intra- and inter-assay coefficients of variability (CV) were <1.8% and <4.5%.

Self-Reported Adult Attachment

To assess individual differences in attachment orientation, we used an 18-item version (69) of the Experience in Close Relationships – Revised (ECR-R) questionnaire (70). Items on the ECR-R assess the extent to which individuals worry that close others may reject them (i.e., in the attachment anxiety subscale), as well as the extent to which they feel uncomfortable with closeness and dependency in relationships (i.e., in the attachment avoidance subscale). Items were rated on a scale from 1 (*Strongly Disagree*) to 7 (*Strongly Agree*). Total attachment anxiety (α = .93) and attachment avoidance (α = .92) scores were obtained by averaging scores across all items in each subscale. There was no data missing on this measure.

Daily Diary Measures

Support Seeking after Stress

To assess the extent to which participants engage in support seeking when stressed, participants were asked to complete a 3-item self-report measure, which we designed to capture the various ways in which individuals may reach out to others. Instructions for this measure included the following prompt: “After feeling stressed today, I…”, to which participants indicated how often they “sought out a friend, family member, or co-worker to talk over text”, “on the phone”, or “to hang out and talk in person.” Participants rated how often they engaged in these behaviors following stress over the past day, using a scale with the following options: “None,” “Once,” “Twice,” and “Three or More.” For each day, a total count of support seeking behaviors was obtained by calculating the sum of the support-seeking items; this score indexed

the number of times participants either sought support through text, phone, or in person, throughout the day.

Negative Affect and Distress

The current study did not include a specific assessment of daily stress, however previous studies have noted moderate within-person correlations between the experience of daily negative affect and self-reported stress (71, 72). To characterize the day-to-day variability in participants' experience of stress, we therefore considered two daily diary measures as indices of daily experiences of negative affect and distress.

Negative Affect. As a measure of daily negative affect, we used a validated 10-item shortened version of the Positive and Negative Affect Schedule (73). Items were rated on a scale from 1 (*Not at all*) to 5 (*Extremely*). Scores on the five items assessing negative affect were averaged to create a daily Negative Affect subscale ($\alpha = .80$).

Fear and Distress Symptoms. We used Conway et al.'s (2014) measure of fear and distress symptoms as another index of daily stress levels. This measure included 11 items assessing daily variations in symptoms of fear (e.g., "My heart was racing or pounding") and distress (e.g., "I felt nervous"). Items were rated on a scale from 1 (*Not at all*) to 5 (*Extremely*). A total score was obtained by calculating the average of scores across these items ($\alpha = .88$).

Identifying "No Distress" Days. Scores on the negative affect and fear and distress symptoms measures were highly correlated ($r=0.80$) in the current sample. We used these measures to identify days in which participants reported experiencing no negative affect and distress (i.e., days on which participants obtained a mean score of 1.00 = "Not At All" on both the negative affect scale of the PANAS and our measure of daily fear and distress). This cutoff was used to characterize days in which participants experienced "no distress" throughout the day

(or days on which stress did not impact participants' negative affect and symptoms of distress). These "no distress" days represented 24.80% of our daily diary data.

Statistical Analyses

Psychological Stress Reactivity to the TSST

First, we examined whether the TSST successfully induced changes in state anxiety (via the STAI). Using multilevel modeling (MLM) to account for repeated assessments (i.e., 6 timepoints within subjects), we examined differences in mean levels of STAI scores across timepoints. The use of MLM with an unstructured error covariance matrix allows all variances and covariances to be freely estimated with no restrictions and allows for missing data on some of the repeated measures.

$$\begin{aligned} STAI_{ij} = {} & \gamma_{00} + \gamma_{10}*\text{T1-vs-T2}_{ij} + \gamma_{20}*\text{T1-vs-T3}_{ij} \\ & + \gamma_{30}*\text{T1-vs-T4}_{ij} + \gamma_{40}*\text{T1-vs-T5}_{ij} + \gamma_{50}*\text{T1-vs-T6}_{ij} \\ & + \varepsilon_{ij} \end{aligned}$$

OT Reactivity to the TSST

Similarly, MLM was used to test for differences in the mean levels of OT across the 5 timepoints (i.e., T1-T5), to verify that the TSST elicited the expected changes in OT levels throughout the study. We also examined the moderating effect of gender (0=Female, 1=Male) and attachment orientation (i.e., attachment avoidance and attachment anxiety) in these analyses.

$$\begin{aligned} OT_{ij} = {} & \gamma_{00} + \gamma_{01}*\text{Gender}_j + \gamma_{02}*\text{Avoidance}_j + \gamma_{03}*\text{Anxiety}_j \\ & + \gamma_{04}*\text{Avoidance}_j*\text{Gender}_j + \gamma_{05}*\text{Anxiety}_j*\text{Gender}_j \\ & + \gamma_{10}*\text{T1-vs-T2}_{ij} + \gamma_{20}*\text{T1-vs-T3}_{ij} + \gamma_{30}*\text{T1-vs-T4}_{ij} + \gamma_{40}*\text{T1-vs-T5}_{ij} \\ & + \gamma_{11}*\text{Gender}_j*\text{T1-vs-T2}_{ij} + \gamma_{21}*\text{Gender}_j*\text{T1-vs-T3}_{ij} \\ & + \gamma_{31}*\text{Gender}_j*\text{T1-vs-T4}_{ij} + \gamma_{41}*\text{Gender}_j*\text{T1-vs-T5}_{ij} \\ & + \gamma_{12}*\text{Avoidance}_j*\text{T1-vs-T2}_{ij} + \gamma_{22}*\text{Avoidance}_j*\text{T1-vs-T3}_{ij} \\ & + \gamma_{32}*\text{Avoidance}_j*\text{T1-vs-T4}_{ij} + \gamma_{42}*\text{Avoidance}_j*\text{T1-vs-T5}_{ij} \end{aligned}$$

$$+ \gamma_{13}*\text{Anxiety}_j*\text{T1-vs-T2}_{ij} + \gamma_{23}*\text{Anxiety}_j*\text{T1-vs-T3}_{ij}$$
$$+ \gamma_{33}*\text{Anxiety}_j*\text{T1-vs-T4}_{ij} + \gamma_{43}*\text{Anxiety}_j*\text{T1-vs-T5}_{ij}$$
$$+ \gamma_{14}*\text{Avoidance}_j*\text{Gender}_j*\text{T1-vs-T2}_{ij}$$
$$+ \gamma_{24}*\text{Avoidance}_j*\text{Gender}_j*\text{T1-vs-T3}_{ij}$$
$$+ \gamma_{34}*\text{Avoidance}_j*\text{Gender}_j*\text{T1-vs-T4}_{ij}$$
$$+ \gamma_{44}*\text{Avoidance}_j*\text{Gender}_j*\text{T1-vs-T5}_{ij}$$
$$+ \gamma_{15}*\text{Anxiety}_j*\text{Gender}_j*\text{T1-vs-T2}_{ij}$$
$$+ \gamma_{25}*\text{Anxiety}_j*\text{Gender}_j*\text{T1-vs-T3}_{ij}$$
$$+ \gamma_{35}*\text{Anxiety}_j*\text{Gender}_j*\text{T1-vs-T4}_{ij}$$
$$+ \gamma_{45}*\text{Anxiety}_j*\text{Gender}_j*\text{T1-vs-T5}_{ij}$$
$$+ \varepsilon_{ij}$$

In addition, a follow-up analysis was performed in the subsample of women, in order to statistically control for variability in progesterone and estradiol levels associated with the use of hormonal contraception and/or variations in menstrual cycle phase (i.e., during the laboratory session when OT reactivity was assessed).

$$\text{OT}_{ij} = \gamma_{00} + \gamma_{01}*\text{Estradiol}_j + \gamma_{02}*\text{Progesterone}_j$$
$$+ \gamma_{10}*\text{T1-vs-T2}_{ij} + \gamma_{20}*\text{T1-vs-T3}_{ij}$$
$$+ \gamma_{30}*\text{T1-vs-T4}_{ij} + \gamma_{40}*\text{T1-vs-T5}_{ij}$$
$$+ \varepsilon_{ij}$$

Primary Analyses

OT Reactivity and Support Seeking. To test whether OT reactivity was associated with support seeking after stress across the 14 daily diary assessments, we performed a linear mixed effects model with robust standard errors (to correct for violations of multivariate normality), using the GENLINMIXED procedure in SPSS. Multilevel modeling was used to account for repeated assessments (i.e., 14 daily diaries) within subjects, and to test the specificity of effects on days in which participants experienced distress vs. days in which they experienced no

distress. Therefore, as a Level 1 (within-person) predictor, we included a "Distress" dummy variable (0=No Distress, 1=Distress), distinguishing between days in which participants experienced "no distress", and days in which they experienced some distress. As Level 2 (between-person) predictors, we included gender (0=Female, 1=Male) and OT reactivity. We also included the cross-level interaction of gender and OT reactivity to test whether OT reactivity differentially predicted support seeking after stress for men and women.

$$\begin{aligned}\text{Support Seeking}_{ij} = {} & \gamma_{00} + \gamma_{01}*\text{OT}_j + \gamma_{02}*\text{Gender}_j + \gamma_{03}*\text{OT}_j*\text{Gender}_j \\ & + \gamma_{10}*\text{ Distress}_{ij} \\ & + \gamma_{11}*\text{OT}_j*\text{Distress}_{ij} + \gamma_{12}*\text{Gender}_j*\text{Distress}_{ij} \\ & + \gamma_{13}*\text{OT}_j*\text{Gender}_j*\text{Distress}_{ij} + \varepsilon_{ij}\end{aligned}$$

We also performed a follow-up analysis in the subsample of women, controlling for levels of progesterone and estradiol levels.

$$\begin{aligned}\text{Support Seeking}_{ij} = {} & \gamma_{00} + \gamma_{01}*\text{OT}_j + \gamma_{02}*\text{Estradiol}_j + \gamma_{03}*\text{Progesterone}_j \\ & + \gamma_{10}*\text{Distress}_{ij} \\ & + \gamma_{11}*\text{OT}_j*\text{Distress}_{ij} + \varepsilon_{ij}\end{aligned}$$

Attachment Orientation. To test the moderating effects of attachment orientation (i.e., avoidance and anxiety), two three-way interactions (i.e., OT×Stress×Avoidance and OT×Stress×Anxiety) were specified, along with all lower-level interaction terms in the same model. Additionally, a follow-up analysis was conducted to test for gender differences in these effects, by including two four-way interactions (i.e., OT×Stress×Avoidance×Gender and OT×Stress×Anxiety×Gender), and all lower-level interactions in the same model.

$$\begin{aligned}\text{Support Seeking}_{ij} = {} & \gamma_{00} + \gamma_{01}*\text{OT}_j + \gamma_{02}*\text{Avoidance}_j + \gamma_{03}*\text{Anxiety}_j \\ & + \gamma_{04}*\text{OT}_j*\text{Avoidance}_j + \gamma_{05}*\text{OT}_j*\text{Anxiety}_j \\ & + \gamma_{10}*\text{Distress}_{ij} \\ & + \gamma_{11}*\text{OT}_j*\text{Distress}_{ij} + \gamma_{12}*\text{Avoidance}_j*\text{Distress}_{ij} + \gamma_{13}*\text{Anxiety}_j*\text{Distress}_{ij}\end{aligned}$$

$$+ \gamma_{14}*OT_j*Avoidance_j*Distress_{ij} + \gamma_{15}*OT_j*Anxiety_j*Distress_{ij} + \varepsilon_{ij}$$

Lastly, a separate analysis was performed in the subsample of women, statistically controlling for levels of progesterone and estradiol.

$$\begin{aligned} Support\ Seeking_{ij} = {} & \gamma_{00} + \gamma_{01}*OT_j + \gamma_{02}*Avoidance_j + \gamma_{03}*Anxiety_j \\ & + \gamma_{04}*Estradiol_j + \gamma_{05}*Progesterone_j \\ & + \gamma_{06}*OT_j*Avoidance_j + \gamma_{07}*OT_j*Anxiety_j \\ & + \gamma_{10}*Distress_{ij} \\ & + \gamma_{11}*OT_j*Distress_{ij} + \gamma_{12}*Avoidance_j*Distress_{ij} + \gamma_{13}*Anxiety_j*Distress_{ij} \\ & + \gamma_{14}*OT_j*Avoidance_j*Distress_{ij} + \gamma_{15}*OT_j*Anxiety_j*Distress_{ij} + \varepsilon_{ij} \end{aligned}$$

Exploratory Analyses

Other Indices of OT Reactivity. Exploratory analysis were conducted to examine whether the results from our primary analyses changed meaningfully when utilizing other common indices of biological reactivity (75, 76). The maximum-minimum OT difference (MaxMin) was computed as the difference between the maximum and minimum concentrations of OT obtained throughout the study (76). MaxMin indexes the largest change in concentrations of OT throughout the study (76). The area under the curve with respect to ground (AUCg), which is often viewed as an index of total hormonal output, was computed using the formulas described in Pruessner et al. (2003).

Sensitivity Analyses

Primary and exploratory analyses were re-run after recalculating AUCi and AUCg following the removal outliers (i.e., data points that were more than 3 standard deviations from the sample's mean across timepoints); for the subsample of women, these analyses also excluded 3 participants with estradiol and progesterone levels that were considered outliers. Additionally, these analyses were re-run after recalculating AUCi and AUCg from a dataset in which hemolyzed samples were removed and subsequently imputed using the expectation maximization

(EM) algorithm; for baseline samples that were hemolyzed, estradiol and progesterone levels were likewise estimated using EM in the subsample of women. Lastly, analyses were re-run after accounting for individual differences in participants' tendency to experience "distress" days (vs. "no distress" days). This was accomplished by statistically controlling for the proportion of days in which participants reported experiencing distress (i.e., by calculating an average of the "distress" variable for each participant).

Statistical Power Considerations

Results from a recent study by Engert et al. (2016) showed a significant change in plasma OT following the TSST (Cohen's f =.286). With the current study's sample size of 94 participants, we had greater than .95 power to detect an effect of this magnitude, as well as gender-moderation and attachment-moderation effects. To assess statistical power for our analyses testing the association of OT reactivity and daily social experiences after stress (i.e., support seeking), post-hoc analyses were performed using the MLM power analysis program Power in Two-Level Models (PinT 2.12) (77). These analyses revealed that we had greater than .95 power to detect small effects (for both the main effect of OT reactivity, and the cross-level interaction of OT reactivity and Distress).

Results

Psychological Stress Reactivity to the TSST

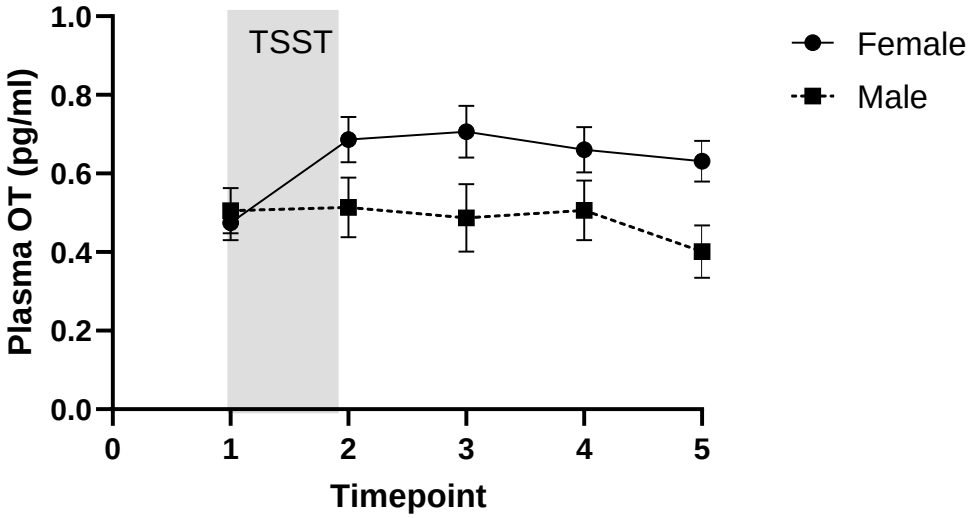
In examining whether the TSST successfully induced changes in state anxiety, results showed significant differences in STAI scores across the 6 timepoints, $F(5, 93.63) = 30.47$, $p<.001$. Pairwise comparisons between successive timepoints revealed that STAI scores increased across the first three timepoints, and then decreased across the latter three timepoints (p's <.001).

OT Reactivity to the TSST

Moreover, OT levels differed significantly across all timepoints, $F(4, 89.43) = 3.61$, $p = .009$. Pairwise comparisons revealed that levels of OT increased significantly between T1 and T2 ($p = .011$), with no significant changes between the other sequential timepoints (all p's > .05). In exploring whether gender moderated changes in OT levels across timepoints, there was a significant interaction between Gender and Timepoint, $F(4, 89.68) = 3.65$, $p = .008$, which is depicted in Figure 2 below. An analysis of the simple effects showed that the aforementioned increases in OT levels between T1 and T2 were observed in women ($b = 0.21$, $SE = 0.049$, $p < .001$, 95% CI = [0.11, 0.31]), but not in men ($b = 0.009$, $SE = 0.065$, $p = .89$, 95% CI = [-0.12, 0.14]). In the subsample of women, controlling for levels of progesterone and estradiol, the results remained the same as in the full sample.

Figure 2

Plasma OT Levels as a Function of Timepoint and Gender



In examining whether attachment orientation (i.e., attachment avoidance and anxiety) moderated changes in OT levels across the timepoints, results showed no significant interaction between Avoidance and Timepoint, $F(4, 88.96) = 0.73$, $p = .57$, nor between Anxiety and

Timepoint, $F(4, 90.00) = 2.15$, $p = .08$, suggesting that attachment orientation did not significantly influence changes in OT levels throughout the lab session. Of note, we also tested whether attachment orientation moderated the previously described gender interaction by testing the three-way interaction of Gender×Timepoint×Avoidance and Gender×Timepoint×Anxiety. Results showed that neither interaction was significant, $F(4, 89.60) = 1.16$, $p = .33$, and $F(4, 89.93) = 0.52$, $p = .72$, respectively, suggesting that attachment orientation did not significantly impact gender differences in OT reactivity throughout the study.

Correlations Between Predictors and Outcomes

Descriptive statistics and bivariate correlations can be found in Table 2 below. Indices of OT reactivity (i.e., AUCi, AUCg, and MaxMin) were not significantly associated with participants' average level of support seeking across "distress" days (all p's > .05). Similarly, neither attachment anxiety, nor attachment avoidance, was significantly associated with support seeking. Regarding the intercorrelations amongst indices of OT reactivity, AUCi was moderately correlated with AUCg, $r(92) = .43$, $p < .001$, as well as with MaxMin, $r(92) = .59$, $p < .001$. Likewise, AUCg was moderately correlated with MaxMin, $r(92) = .47$, $p < .001$. Moreover, there was a strong positive correlation between baseline levels of OT (i.e., T1) and AUCg, $r(92) = .71$, $p < .001$, and a moderate negative association between baseline levels and AUCi, $r(92) = -.33$, $p = .002$.

Table 2

Correlations Between Predictor and Outcome Variables

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. Baseline OT	–							
2. AUCi	-.33**	–						
3. AUCg	.71**	.43**	–					
4. MaxMin	.026	.59**	.47**	–				
5. Anxious Attachment	-.081	-.004	-.090	.045	–			
6. Avoidant Attachment	.081	-.084	.011	.12	.22*	–		

7. Social Support (Distress)	.12	.33**	.35**	.19	.12	.14	–	
8. Social Support (No Distress)	.041	-.019	.014	-.022	.039	.08	.55**	–
N	94	94	94	94	94	94	92	65
Mean	.49	3.92	25.18	0.57	3.79	3.05	1.58	0.66
SD	.35	11.82	16.07	0.44	1.52	1.43	1.56	1.33

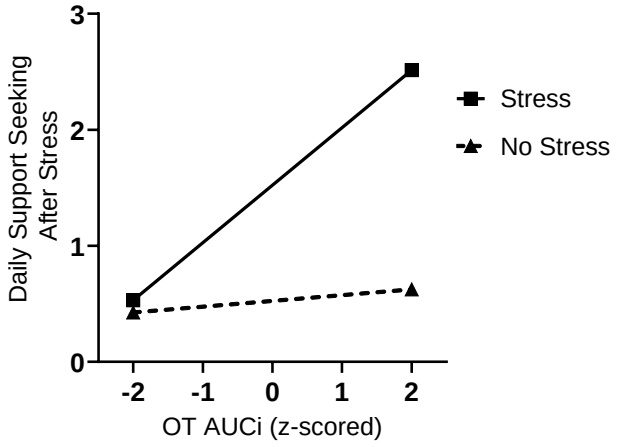
Note. AUCi = area under the curve with respect to increase; AUCg = area under the curve with respect to ground; MaxMin = maximum-minimum OT difference; Social Support (Distress) = mean of participants' average support seeking scores across "distress" days; Social Support (No Distress) = mean of participants' average support seeking scores across "no distress" days, *p<.05, **p<.01

OT Reactivity and Support Seeking

In examining whether lab-based OT reactivity predicted daily support seeking behaviors after stress, results revealed a significant interaction of OT reactivity and the Distress dummy-coded variable, $F(1, 1097) = 6.68$, $p = .01$, suggesting that the association between OT reactivity and support seeking after stress differed based on participants' experience of distress (vs. no distress) during the day. An examination of the simple slopes (depicted below in Figure 3) revealed that, on days in which participants experienced "no distress," OT reactivity was not significantly associated with support seeking after stress, $b = 0.059$, $SE = 0.11$, $p = .66$, 95% CI = [-0.17, 0.27]. By contrast, OT reactivity was positively associated with support seeking after stress on days in which participants experienced distress, $b = 0.50$, $SE = 0.17$, $p = .004$, 95% CI = [0.16, 0.83]. These results were maintained across all sensitivity analyses.

Figure 3

Daily Support Seeking After Stress as a Function of Stress-Induced OT Reactivity (AUCi)



A follow-up analysis showed no evidence of gender differences in the aforementioned effect; that is, there was no significant interaction between Gender, OT reactivity, and Distress (vs. No Distress), b = -0.092, SE = 0.44, p = .84, 95% CI = [-0.96, 0.78]. However, to statistically control for differences in progesterone and estradiol levels among women, an additional analysis was performed in the subsample of women only. This analysis in the subsample of women, after controlling for lab-based progesterone and estradiol, yielded the same results to those described in the full sample. Specifically, there was a significant OT×Distress interaction in the subsample of women, F(1, 675) = 5.52, p = .019, which was characterized by a significant slope of AUCi on days in which participants reported experiencing distress, b = 0.50, SE = 0.21, p = .019, 95% CI = [0.083, 0.92], but not on "no distress" days, b = 0.056, SE = 0.17, p = .75, 95% CI = [-0.29, 0.40]. Of note, however, the OT×Distress interaction became non-significant after removing outliers on progesterone and estradiol levels (and recalculating AUCi with OT outliers removed), F(1, 636) = 3.06, p = .081. That said, results regarding the simple slope of AUCi were maintained in these analyses (i.e., AUCi on "distress" days: b = 0.49, SE = 0.21, p = .02, 95% CI = [0.077, 0.91]; AUCi on "no distress" days: b = 0.091, SE = 0.17, p = .66, 95% CI = [-0.37, 0.50]). Moreover, results were maintained across all other sensitivity analyses.

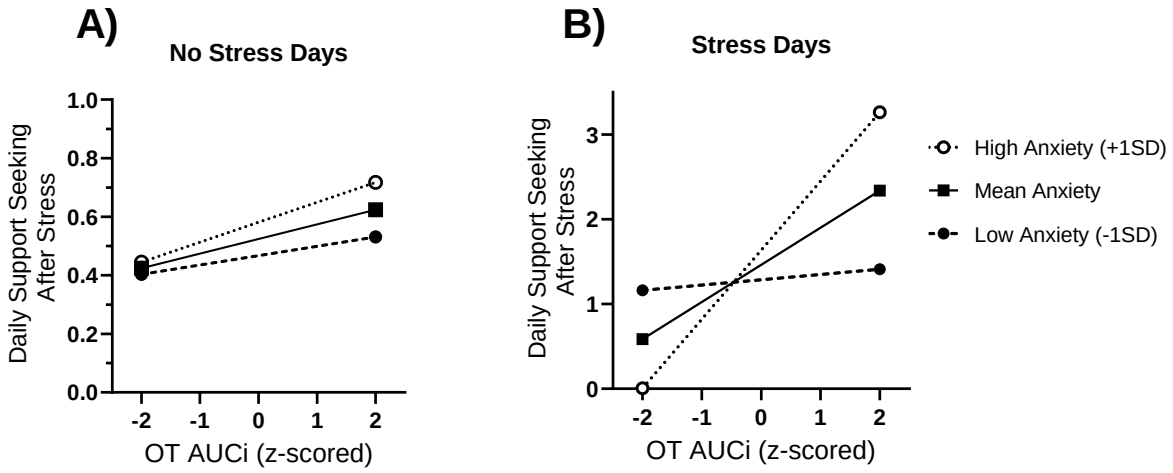
Moderation by Attachment Orientation

Results revealed a significant three-way interaction of OT reactivity, Distress, and attachment anxiety (b = -0.36, SE = 0.17, p = .033, 95% CI = [-0.69, -0.028]), but not for the three-way interaction with attachment avoidance (b = -0.34, SE = 0.19, p = .074, 95% CI = [-0.72, 0.033]). Further, the two-way OTxAnxiety was significant on days in which participants experienced distress (but not on "no distress" days), suggesting that the association between OT and support seeking was moderated by attachment anxiety on these "distress" days, b = 0.38, SE = 0.17, p = .029, 95% CI = [0.039, 0.71]. To further explore these interactions, follow-up analyses were conducted to test the simple slopes of OT reactivity for high (+1SD), average, and low (-1SD) levels of attachment anxiety, after controlling for attachment avoidance. As depicted in Figure 4a, on days in which participants experienced "no distress," OT reactivity was not significantly associated with support seeking after stress, regardless of participants' levels of attachment anxiety.

By contrast, on days in which participants experienced distress (Figure 4b), attachment anxiety was associated with an increase in the magnitude of the association between OT reactivity and support seeking after stress. Specifically, for individuals with low levels of attachment anxiety (-1SD), OT reactivity was not significantly associated with support seeking after stress, b = 0.062, SE = 0.16, p = .71, 95% CI = [-0.26, 0.38]. For individuals with the mean level of attachment anxiety, OT was positively associated with support seeking after stress, b = 0.44, SE = 0.14, p = .002, 95% CI = [0.16, 0.72]. Furthermore, this association was stronger for individuals with high levels of attachment anxiety (+1SD), b = 0.82, SE = 0.27, p = .003, 95% CI = [0.29, 1.34].

Figure 4

Daily Support Seeking After Stress as a Function of Stress-Induced OT Reactivity (AUCi) and Attachment Anxiety



In the sensitivity analysis using EM-estimated OT values for hemolyzed samples, the three-way OT×Distress×Anxiety interaction remained significant (b = -0.30, SE = 0.15, p = .042, 95% CI = [-0.59, -0.011]), however the lower-level OTxAnxiety interaction on "distress" days became marginally significant, b = 0.29, SE = 0.16, p = .064, 95% CI = [-0.017, 0.60]. That said, all results regarding simple slopes were maintained in these sensitivity analyses.

In examining gender differences in these effects, neither four-way interaction of OT×Distress×Avoidance×Gender, b = 0.14, SE = 0.40, p = .72, 95% CI = [-0.64, 0.93], or OT×Distress×Anxiety×Gender, b = -0.52, SE = 0.34, p = .12, 95% CI = [-1.19, 0.14], was significant, suggesting that the previously described moderating effect of attachment anxiety did not differ by gender. Moreover, the follow-up analysis in the subsample of women showed that results did not differ meaningfully from those described in the full sample, after controlling for levels of progesterone and estradiol. As with sensitivity analyses in the full sample, the three-way OT×Distress×Anxiety interaction remained significant in the sensitivity analysis using EM-

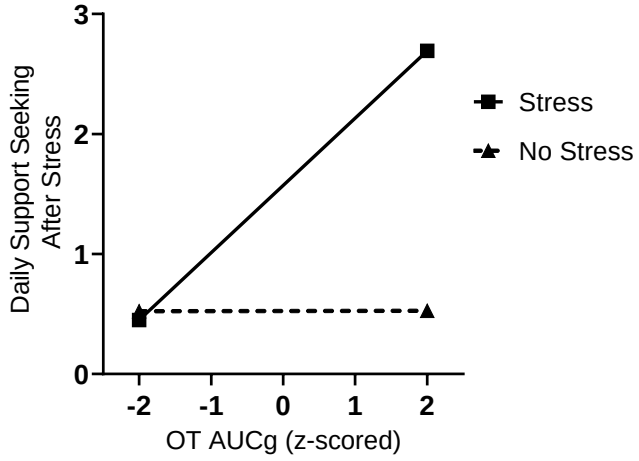
estimated values for OT, progesterone and estradiol levels (in hemolyzed samples); however, results in the subsample of women revealed a non-significant two-way interaction between OTxAnxiety, on days in which participants experienced distress, $b = 0.28$, $SE = 0.22$, $p = .22$, 95% CI = [-0.16, 0.71].

Exploratory Analyses

AUCg. Using AUCg, there was significant interaction of OT reactivity and Distress, $F(1, 1097) = 9.49$, $p = .002$, replicating the findings from analyses using AUCi. An examination of the simple slopes (depicted in Figure 5) revealed that, on days in which participants experienced "no distress," lab-based OT reactivity was not significantly associated with support seeking after stress, $b = 0.001$, $SE = 0.089$, $p = .99$, 95% CI = [-0.17, 0.18]. By contrast, OT reactivity was positively associated with support seeking after stress on days in which participants experienced distress, $b = 0.56$, $SE = 0.18$, $p = .002$, 95% CI = [0.21, 0.92]. As with analyses using AUCi, there was no gender moderation of this effect, $b = -0.069$, $SE = 0.41$, $p = .87$, 95% CI = [-0.74, 0.88]. These results were maintained across all sensitivity analyses.

Figure 5

Daily Support Seeking After Stress as a Function of Stress-Induced OT Reactivity (AUCg)



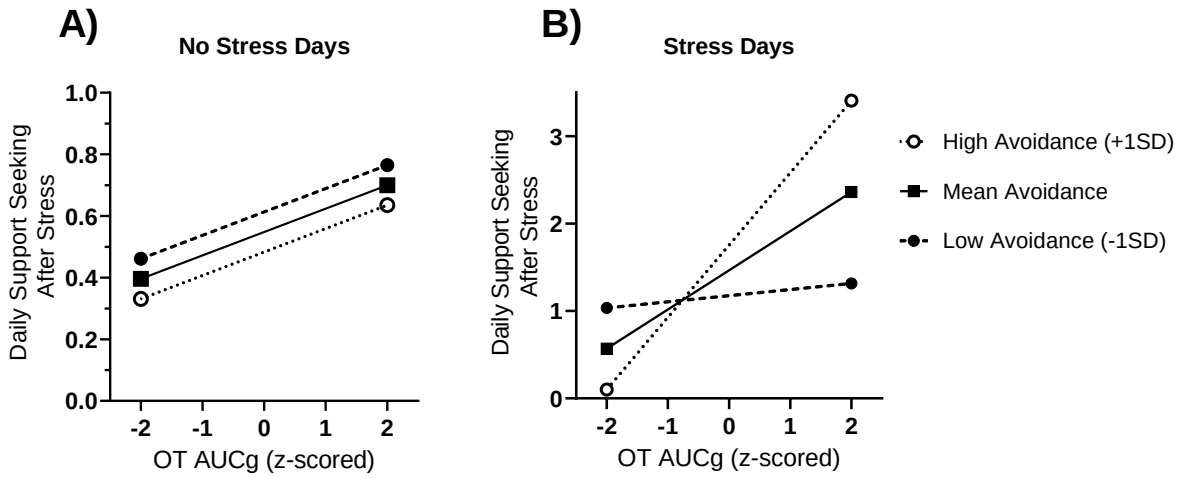
For analyses examining the moderating role of attachment orientation (i.e., avoidance and anxiety), a different pattern of results emerged using AUCg. There was a significant three-way interaction of OT reactivity, Distress, and attachment *avoidance* (*b* = -0.38, *SE* = 0.14, *p* = .009, 95% CI = [-0.66, -0.094]), but not for the three-way interaction with attachment *anxiety* (*b* = -0.18, *SE* = 0.15, *p* = .24, 95% CI = [-0.47, 0.12]). Follow-up analyses were conducted to test the simple slopes of OT reactivity for high (+1SD), average, and low (-1SD) levels of attachment avoidance, after controlling for attachment anxiety. On days in which participants experienced "no distress" (Figure 6a), attachment avoidance did not significantly moderate the association between OT reactivity and support seeking (i.e., the OT×Avoidance interaction was not significant, *b* = 0.00, *SE* = 0.13, *p* = .99, 95% CI = [-0.24, 0.25]). Similarly, the simple slopes of OT reactivity were not significant for individuals with low (*b* = 0.076, *SE* = 0.19, *p* = .69, 95% CI = [-0.30, 0.45]), average (*b* = 0.077, *SE* = 0.18, *p* = .66, 95% CI = [-0.27, 0.42]), and high (*b* = 0.077, *SE* = 0.24, *p* = .75, 95% CI = [-0.39, 0.55]) levels attachment avoidance.

By contrast, on days in which participants experienced distress (Figure 6b), attachment avoidance was associated with an increase in the magnitude of the association between OT reactivity and support seeking after stress (i.e., the OT×Avoidance interaction was significant, *b* = 0.38, *SE* = 0.14, *p* = .007, 95% CI = [0.11, 0.65]). Specifically, for individuals with low levels of attachment avoidance (-1SD), OT reactivity was not significantly associated with support seeking after stress, *b* = 0.070, *SE* = 0.20, *p* = .72, 95% CI = [-0.32, 0.46]. For individuals with average levels of attachment avoidance, OT reactivity was positively associated with support seeking after stress, *b* = 0.45, *SE* = 0.13, *p* < .001, 95% CI = [0.20, 0.69]. For individuals with high (+1SD) levels of attachment avoidance, OT reactivity was positively associated with support seeking after stress, and this effect was larger relative to lower levels of avoidance, *b* =

0.83, *SE* = 0.18, $p < .001$, 95% CI = [0.48, 1.17]. Results were maintained across all sensitivity analyses.

Figure 6

Daily Support Seeking After Stress as a Function of Stress-Induced OT Reactivity (AUCg) and Attachment Avoidance



MaxMin. Using MaxMin, there was no interaction of OT reactivity and Distress, $F(1, 1097) = 3.50$, $p = .062$, suggesting that there was no difference in the association of OT reactivity and support seeking after stress, on days in which participants experienced distress (relative to "no distress" days). Moreover, there was no significant main effect of MaxMin on support seeking after stress, after dropping the interaction term, $F(1, 1098) = 1.64$, $p = .20$. Additionally, there was no significant gender moderation, $b = 0.11$, $SE = 0.42$, $p = .79$, 95% CI = [-0.71, 0.93], nor was there a moderation effect of attachment anxiety and avoidance ($b = 0.025$, $SE = 0.19$, $p = .90$, 95% CI = [-0.35, 0.39], and $b = 0.099$, $SE = 0.18$, $p = .58$, 95% CI = [-0.25, 0.45], respectively).

Discussion

The aim of the present investigation was to empirically test prior theories, largely based on research in non-human animals, implicating the OT system in affiliative behaviors following stress. In the current study, we found that levels of plasma OT increased following a psychosocial stress induction task in the laboratory, particularly in women. This finding replicates previous studies showing that peripheral OT levels increase in response to stress (16, 27–29). Furthermore, in line with our hypotheses, we found that individuals with greater stress-induced OT reactivity reported engaging in more frequent support seeking following stress in daily life, relative to those with lower levels of stress-induced OT reactivity. Although we hypothesized that this association would be stronger for women, relative to men, we found no evidence of gender-moderation for this effect.

The link between stress-induced OT reactivity and affiliative behaviors has been previously theorized by others (33, 44, 78). However, to our knowledge, this is the first human study to empirically test whether stress-induced OT reactivity in the lab is associated with support-seeking behaviors following stress in daily life. Still, our findings build upon theories emphasizing OT's role in modulating attention and reward processes within social environments (34, 35). According to the "social salience hypothesis", the OT system regulates attention towards social cues in the environment, enhancing the salience of social information in a given context (35). Within socially threatening situations (e.g., in which one is at risk of being ostracized by others), the OT system is involved in reorienting our attention towards cues signaling threat, facilitating an adaptive response. By contrast, in the absence of threatening cues, OT activation may orient our attention towards cues signaling safety among familiar others, upregulating feelings of social reward and connectedness, and reinforcing relationship-enhancing

behaviors. In this way, OT reactivity is not specific to threatening social situations, but may instead reflect an underlying sensitivity to salient social information in general.

Based on the social salience theory, one possibility is that individuals with greater OT reactivity to the TSST may also display higher levels of OT reactivity in response to rewarding social interactions (e.g., after the receipt of social support). As a result, greater OT reactivity to both stressors and social support could potentially reinforce relationship-enhancing behaviors such as support seeking in response to stress. This possibility could be explored in future research by first examining within-person associations between OT reactivity to aversive and rewarding social interactions, and then linking OT reactivity to individual differences in social reward processes (e.g., via neuroimaging) and affiliative behavior. Another possibility is that increased salience of threatening information could potentially lead individuals to perceive stressors as more stressful. From a stress-coping perspective, this could lead individuals to seek additional resources (via social support) in order to meet the demands of the situation.

Moderation by Attachment

In addition to showing that stress-induced OT is associated with support seeking after stress in daily life, we provide initial evidence that attachment orientation may act as a moderator of this association. Consistent with our hypotheses, attachment anxiety amplified the association between OT reactivity and support seeking after stress. Specifically, on days in which individuals reported experiencing distress, OT reactivity was associated with more frequent support seeking behaviors among individuals with higher levels of attachment anxiety, relative to those with lower levels of attachment anxiety. This finding fits well within attachment theory (47), which posits that anxiously attached individuals tend to engage in excessive reassurance seeking in their close relationships.

Although attachment theory does not specifically address the role of OT in behavioral responses to stress, studies using intranasal OT administration have previously reported that OT enhances communal (other-oriented) tendencies among insecurely attached individuals (79). As a consequence of heightened focus on others, OT may exacerbate interpersonal insecurities for anxiously attached individuals, who are chronically preoccupied with their close relationships (79). Similarly, our findings may suggest that a highly reactive OT system can heighten preexisting social tendencies in response to stress, leading to an amplification of support seeking behaviors among anxiously attached individuals. On the other hand, contrary to our hypotheses, attachment avoidance did not significantly moderate the association between OT reactivity and support seeking after stress.

In interpreting these attachment-related results, it is important to consider the different ways in which attachment anxiety may contribute to support seeking behaviors in response to stress. As noted previously, one possibility is that higher levels of OT reactivity simply exacerbate feelings of distress in response to daily stressors among anxiously attached individuals, which would lead to greater support seeking. Alternatively, greater OT reactivity may also exaggerate affiliative behaviors among anxiously attached individuals, due to increased motivation to affiliate with close others. At the same time, attachment anxiety and avoidance have been shown to correlate negatively with perceived social support (80). Therefore, another possibility is that greater sensitivity to social interactions (via a more reactive OT system) may increase anxiously attached individuals' tendency to feel unsupported in their interactions after stress. As a result, these individuals may seek out additional support from others to deal with stressors throughout the day. Importantly, there may also be partner effects underlying our observed effects. Although not assessed in the current study, characteristics of partners as well as

relationship dynamics may be related to behavioral responses to stress among insecurely attached individuals (81, 82). Therefore, it remains difficult to disentangle whether the aforementioned attachment-related findings are attributed to the characteristics of the participants in the current study, to those with whom they sought support, or to both actor and partner effects.

Adding to the limited research examining whether OT reactivity to stress is associated with individual differences in attachment orientations, we found that neither attachment anxiety nor attachment avoidance was associated with OT reactivity to the TSST. The lack of association between OT reactivity and attachment orientation replicates previous findings from studies using the TSST to induce stress among individuals who experienced trauma (54), or using videos of attachment and loss to induce OT reactivity among individuals with schizophrenia and healthy controls (56). The current study extends prior research in this area by including a dimensional assessment of adult attachment, which is preferred to categorical assessments (55), and by testing this association within a non-clinical young adult sample.

Results from Exploratory Analyses Involving AUCg and MaxMin

Additionally, our study aimed to address a potential barrier to research on OT reactivity: the lack of consistency in the measurement of OT reactivity – namely in the use of different indices of biological reactivity, such as AUCi, AUCg, and MaxMin. Despite theoretical distinctions (75, 76), these indices are often used interchangeably as a measure of OT reactivity, hindering comparisons between studies. In the current study, we attempted to address this issue by preregistering our hypotheses with a primary index of reactivity (i.e., AUCi), and then exploring whether our results were maintained using other commonly cited indices of reactivity (i.e., AUCg and MaxMin). Given our study's specific focus on stress-induced *changes* in OT reactivity, we selected AUCi as our primary index. In contrast to AUCi, basal levels of OT are

not removed as part of the calculation of AUCg. In this way, AUCg is said to reflect total hormonal output, and takes into account variability due to levels of OT present at baseline (i.e., the first timepoint in the study). In fact, our analyses showed that AUCg was strongly correlated with basal levels of OT (i.e., r=0.70), which suggests that AUCg may be more indicative of basal levels than reactivity per se. Using AUCg, we replicated the association between OT and support-seeking after stress. However, we found a divergent pattern of results with regards to the moderating effect of attachment avoidance and anxiety.

In particular, we found that attachment avoidance moderated the effects of AUCg, but attachment anxiety did not. Given the theoretical distinctions between AUCi and AUCg, this suggests that, while attachment anxiety may be more sensitive to OT *reactivity* in response to stress, attachment avoidance may be more sensitive to *basal* OT levels. It is important to note, however, that single assessments of baseline levels with extracted plasma have shown poor reliability (at least in an all-male sample; 83). Thus, our results related to baseline concentrations of OT should be viewed with caution. In addition, the moderating effect of attachment avoidance was in the opposite direction of what we had originally hypothesized. Instead of diminishing the association between OT and support seeking after stress, our results showed that attachment avoidance increased the magnitude of this association. In this way, amongst avoidantly attached individuals, basal levels of OT may represent a protective factor against the tendency to withdraw from others in response to stress. In fact, in our sample, avoidantly attached individuals seemed to benefit the most from higher basal levels of OT, with greater AUCg predicting the highest levels of support seeking behaviors amongst these individuals. Indeed, studies using intranasal OT administration have previously found that OT selectively enhances prosocial

behaviors in individuals who possess lower motivation to affiliate with others, such as those with higher levels of avoidant attachment (79).

The MaxMin index of OT reactivity was not significantly associated with support seeking after stress. MaxMin is calculated as the range of OT levels throughout the study (i.e., by subtracting the minimum OT level from the maximum OT level). Thus, MaxMin theoretically represents the largest change in OT levels in response to the TSST. However, this is only the case when the minimum level of OT is observed prior to the TSST. In the case where OT levels drop below baseline levels after stress (e.g., during the recovery period), MaxMin may actually measure recovery rather than reactivity. Therefore, one potential confound with the use of MaxMin is that it may be indexing two different constructs (i.e., reactivity and recovery) across individuals in a sample. This lack of reliability in the measurement of OT reactivity may have attenuated the association between OT reactivity (as measured using MaxMin) and support seeking following stress in daily life. Future research should therefore take into consideration the potential limitations of MaxMin when choosing between indices of biological reactivity.

Study Strengths

One key strength of the present study is the measurement of OT reactivity. Indeed, our study aimed to address several methodological issues commonly observed in the measurement of plasma OT. For example, in order to appropriately capture OT reactivity in blood plasma, the timing of blood draws should reflect the dynamics of OT in the periphery; in particular, endogenous OT release into peripheral circulation is fast and pulsatile, displaying a relatively short half-life in the periphery (58, 68). In this way, the use of multiple timepoints with short intervals may be more suited to capture the temporal dynamics of peripheral OT, compared to sampling times with longer intervals. Furthermore, in quantifying levels of OT in blood plasma,

our study followed the recommended procedures described in Szeto et al. (2011), which demonstrated the importance of sample extraction in obtaining valid results. In preparing blood samples, the extraction step removes potentially interfering molecules, which may otherwise erroneously inflate estimates of OT levels in blood plasma.

Limitations Regarding Measurement of Support Seeking Behaviors

One limitation of the current study involves our daily diary assessment of participants' support seeking behaviors after stress, which has not been validated as a measure of support seeking. We designed this measure to capture the different ways in which participants might seek support from others after experiencing stress in daily life (e.g., by text, over the phone, and in person), asking participants to indicate the frequency with which they engaged in these behaviors after stress over the past day. In particular, the range of responses for each item was limited to "three or more" instances of support seeking, which may have restricted the range of support seeking in our sample. For example, individuals who may have called their friends more than three times throughout the day would obtain the same score as those who called their friends only three times. In this way, our analyses were not sensitive to more frequent support seeking behaviors. As a result, individuals who utilized multiple mediums of support seeking throughout the day (e.g., using text, phone calls, and meeting in-person) were weighted more heavily than those who relief on just one method of support seeking (e.g., individuals who only use text to reach out to others). Thus, it is difficult to disentangle whether high scores on this measure reflect more frequent support seeking behaviors, or a greater variety of support seeking methods.

Lastly, it is difficult to know whether participants interpreted response options in the same way. Regarding support seeking via text messages in particular, individuals may have different definitions of what constitutes an instance of support seeking (e.g., sending one text

message vs. a conversation). Therefore, it will be important for future research to examine the association between stress-induced OT reactivity and support seeking after stress, using well-validated measures of support seeking.

Limitations of Using Lab-Based OT Reactivity as a Proxy for Daily OT Reactivity to Stress

Another important limitation of the current study involves our use of lab-based OT reactivity as a proxy for individual differences in OT reactivity to daily stressors. Although the current study was designed to improve replicability, following standardized protocols for both in-lab stress induction and peripheral OT measurement, it remains unclear whether participants experienced similar levels of OT reactivity to daily stressors across the two weeks of daily diary assessments. Indeed, the stability of OT reactivity to stress over time has yet to be tested empirically, although a recent study found that other hormonal markers of stress remained relatively stable across assessments four months apart (84). Thus, more work is needed to establish the stability of OT reactivity to stress. Future research could also examine whether individual differences in OT reactivity to the TSST are associated with support seeking behaviors after the lab stressor. Lastly, future studies may also consider assessing whether OT reactivity to daily stressors is associated with subsequent support seeking behaviors in daily life.

Cultural Considerations

Research from cross-cultural psychology emphasizes important variability across ethnocultural groups in terms of the norms and expectations governing social relationships. One cultural dimension that may be particularly relevant to this discussion is collectivism, a set of values and norms characterized by an emphasis on the interdependence of individuals within a social group, and a prioritization of group goals over individual/personal goals (85, 86). In particular, collectivism has been proposed as a core component in organizing social behaviors

among Asian individuals, with distinct facets of collectivism potentially motivating different social behaviors (87). According to Lui and Rollock (2018), among Asian individuals, collectivism may be expressed/enacted through one's motivation to promote positive social environments, to maintain harmony within one's social group, and to maintain self-worth (and not lose face). It stands to reason that collectivistic values emphasizing prosociality, harmonious relationships, and concerns for face, may be influential in one's decision to seek out support when stressed, particularly among Asian individuals.

In fact, studies have noted differences in the use and effectiveness of social support in coping with stress. For example, Taylor et al. (2004) found that, compared to European Americans, Asians and Asian Americans tended to rely less on social support to cope with their stress. Additionally, compared to U.S.-born Asians, Asian immigrants and Asian nationals were significantly less likely to seek emotional support from others, whereas differences in support seeking between U.S.-born Asians and European Americans were minimal. Together, these findings suggest that differences in support seeking behaviors after stress may be culturally-rooted. Consistent with conceptualizations of collectivism among Asian cultures (87), Taylor et al. (2004) found that Asians and Asian Americans, relative to their European American counterparts, endorsed greater concern that support seeking would 1) disrupt the harmony of their social group, 2) make things worse by making others concerned, 3) result in criticism or poor evaluation by others, and 4) embarrassment or a loss of face, as well as 5) greater endorsement in the belief that each person is responsible to solve their own problems. Moreover, the extent to which individuals endorsed these concerns was negatively associated with their willingness to seek social support in dealing with life stressors. Of note, similar findings have been observed within the context of seeking support following cancer diagnoses, with research

highlighting the central influence of cultural values and attitudes towards support seeking in one's decision to seek support from close others after being diagnosed with cancer (89).

In this way, although the present research's findings suggest that an association between stress-induced OT reactivity and support seeking behaviors after stress, these findings may not generalize to cultures with more collectivist beliefs and norms regarding support seeking behaviors. In particular, culturally-rooted beliefs and concerns regarding the negative interpersonal consequences of support seeking may deter individuals from seeking support from others when stressed.

Conclusion

In sum, we found that individual differences in stress-induced peripheral OT reactivity were associated with the tendency to seek social support after stress in daily life. These findings provide empirical support for theoretical models implicating the human OT system in motivating affiliative behaviors following stress (33, 78), which to date have mainly been studied in non-human animals. In addition, the current study highlights the importance of considering individual differences in attachment orientations when investigating the effects of OT within the context of affiliative behaviors such as support seeking. Lastly, our research underscores the importance of carefully selecting (and preregistering) the most appropriate index of OT reactivity for a given research question, as our findings suggest that these indices may capture different aspects of the OT response.

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