Estradiol and Daily Affective Experiences in Trauma-Exposed Women

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ESTRADIOL AND DAILY AFFECTIVE EXPERIENCES
IN TRAUMA-EXPOSED WOMEN

by

JENNA K. RIEDER

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Jenna K. Rieder

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT
Estradiol and Daily Affective Experiences in Trauma-Exposed Women
by
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People who experience trauma can develop enduring trauma-related symptoms. In daily life, post-trauma symptoms (e.g., elevated physiological arousal) can be triggered by affectively salient cues in the environment, especially by cues that act as trauma reminders. Trauma exposure is associated with enduring changes in two biological stress systems: the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis. In women, activity in both systems is additionally modulated by fluctuations in levels of sex hormones (e.g., estradiol), which could influence physiological responses to trauma reminders. Additionally, previous work has linked the sex hormone estradiol with affect, suggesting that menstrual cycle might influence trauma-related symptoms or daily affect more broadly within the context of trauma exposure. However, we do not yet have a clear understanding of how estradiol influences affective experiences post-trauma.

We used a multi-method approach to examine the influence of estradiol on daily affective experiences in a non-clinical, trauma-exposed sample of 40 naturally cycling premenopausal women. The first specific goal of this study was to test the hypothesis that low estradiol would be related to trauma symptoms, including an asymmetrical profile of SNS and HPA axis stress reactivity to a naturalistic trauma reminder. Lower estradiol was related to greater number and severity of PTSD symptoms, and participants in low versus high estradiol menstrual cycle phases
showed higher SNS and reduced HPA axis reactivity to a trauma reminder. These results suggest that lower estradiol is associated with a less adaptive profile of stress system reactivity and increased PTSD symptom expression.

The second specific goal of this study was to test the influence of menstrual cycle phase on daily affect in a subset of 30 participants. We assessed affective experience over the course of a 10-day ecological momentary assessment (EMA) period, which included the early follicular (low estradiol) and late follicular (high estradiol) phases. We selected these menstrual cycle phases to capture a portion of the cycle where estradiol increased, whereas progesterone remained low, allowing us to test the effects of estradiol without the confound of progesterone. Participants reported more frequent aversive affective experiences, defined as negatively valenced, high arousal states, including PTSD symptoms, during the early versus late follicular phase. During the early versus late follicular phase, participants also reported greater negative and positive affect and showed greater variability in affective ratings. These results suggest that lower estradiol menstrual cycle phases are characterized by more frequent aversive affective experiences, greater affective lability and increased PTSD symptom severity.

Together, these results have potential implications for clinical assessment, as menstrual cycle phase at the time of assessment could influence diagnosis of PTSD or symptom severity. Additionally, clinicians working with women with PTSD might anticipate greater affective lability and increased symptom severity during low estradiol phases of the menstrual cycle.
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1. Introduction

In the U.S., more than half of the population will experience a traumatic event at some point in their lives (e.g., Kessler et al., 2005), and some will develop enduring symptoms of post-traumatic stress disorder (PTSD). Trauma is an event in which a person experiences a threat or perceived threat to his/her life or physical integrity or that of another person, especially a close other (American Psychiatric Association, 2013). Hallmark PTSD symptoms include re-experiencing of the traumatic event through intrusive thoughts and memories, nightmares or flashbacks. In addition to re-experiencing symptoms, there are three other DSM-5 symptom clusters: avoidance, negative alterations in cognitions and affect and arousal symptoms. Avoidance symptoms include active cognitive avoidance of trauma-related memories or thoughts and behavioral avoidance of people, places or situations associated with the event. Negative alterations in cognition and affect include negative cognitions (e.g., inappropriate self-blame, strongly negative beliefs about oneself, other people or the world), increased negative affect and reduced positive affect. Finally, arousal symptoms include heightened physiological arousal, tonically and in response to stressors, irritability or angry outbursts, risky behaviors and hypervigilance for threat even in safe environments. All of these PTSD symptoms can lead to considerable subjective distress and functional impairment for trauma-exposed people.

In daily life, PTSD symptoms can be triggered by exposure to affectively salient cues in the environment, especially by cues that act as trauma reminders. In women, stress reactivity and negative affect vary according to menstrual cycle phase, and fluctuations in female sex hormones (e.g., estradiol) might influence trauma-related symptoms. However, despite these potential effects of hormone fluctuations on PTSD symptoms, we do not yet have a clear understanding of the relation
between steroid sex hormones and daily affective experiences within the context of trauma exposure. This knowledge could potentially inform more effective treatments for trauma-exposed women.

Most prior PTSD research has focused on men, and most studies that included women have not controlled for hormonal status or menstrual cycle phase. Compounding the imbalance, animal models of PTSD have often excluded females to avoid the potential confounds posed by estrous cycle variation. Thus, the role of fluctuations of female sex hormones on PTSD symptoms is poorly understood. It is important to address this disparity, given that women are twice as likely to develop PTSD following trauma than men (e.g., Breslau, 2002; Haskell et al., 2010; Perrin et al., 2014) and typically experience more severe (e.g., Seedat, Stein, & Carey, 2005) and persistent (e.g., Holbrook, Hoyt, Stein, & Sieber, 2002) symptoms. Although men and women differ in rates of exposure to different types of trauma, women’s increased risk for PTSD persists in studies controlling for trauma type (Tolin & Foa, 2006) and has been reported across cultures (Altemus, 2006), suggesting that societal factors alone cannot explain increased risk. Learning more about physiological processes unique to women that might contribute to differential vulnerability for post-trauma symptoms could enhance preventative therapeutic strategies for trauma-exposed women. Research in this area could also inform treatment tailoring for women with PTSD through the identification of hormone changes associated with greater vulnerability for clinical symptoms.

One potential risk factor for increased symptoms in women is fluctuations in gonadal hormones. In particular, estradiol (17β-estradiol), the most prevalent and biologically potent estrogen in non-pregnant premenopausal women, is broadly associated with affective responses, and estradiol fluctuations might increase risk for aversive affective experiences. Throughout the lifespan, dramatic changes in circulating estradiol levels are associated with changes in affect (e.g., Hickey, Bryant, & Judd, 2012), and risk for affective disorders increases when estradiol steeply declines
during the perimenopause, menopause and postpartum periods (e.g., Callegari et al., 2007; Freeman, Sammel, Lin, & Nelson, 2006; Vesga-Lopez et al., 2008). Among women treated for clinical symptoms of affective disorders during these hormonal transitions, pharmacological treatment with estrogens has been shown to augment treatment outcomes (e.g., Sichel, Cohen, Robertson, Ruttenberg, & Rosenbaum, 1995; Soares, Almeida, Joffe, & Cohen, 2001).

In naturally cycling, premenopausal women, circulating estradiol fluctuates more gradually across the menstrual cycle, and these fluctuations might predict patterns of daily affect, including risk for affective symptoms. The menstrual cycle consists of two main phases: the follicular phase, which spans menses to ovulation, and the luteal phase, which begins after ovulation and lasts until the end of the cycle. The follicular phase is characterized by changes in estradiol, whereas the luteal phase is characterized by changes in estradiol and progesterone.

Fluctuations in gonadal hormones over the course of a typical menstrual cycle are shown in Figure 1. At the beginning of the menstrual cycle, during the early follicular phase, levels of estradiol and progesterone are low. During the late follicular phase, estradiol rises and comes to a peak, then declines following ovulation. Estradiol levels again increase from the early to mid-luteal phase, when they reach a secondary peak, and then decline during the late luteal phase. However, the luteal phase is also characterized by changes in progesterone. Progesterone starts to increase during the early luteal phase, peaks during the mid-luteal phase and declines during the late luteal phase. Menstrual cycle variation has been associated with changes in affect, but we do not yet have a clear understanding of how gonadal hormone fluctuations influence post-trauma symptoms.
Estradiol impacts activation of neural affective circuitry

Among premenopausal women, normative individual differences in estradiol are broadly associated with affect. For example, lower estradiol is associated with higher trait emotional reactivity (e.g., Ziomkiewicz, Wichary, Bochenek, Pawlowski, & Jasienska, 2012). Consistent with self-report measures, lower estradiol is associated with heightened activation in affective brain areas (e.g., amygdala) and reduced activation in prefrontal regulatory areas during affective processing (e.g., Zeidan et al., 2011), which together fit a characteristic neural profile of elevated stress and anxiety states. In addition to individual differences, premenopausal women also experience fluctuations in affect across the menstrual cycle, with greater negative affect (e.g., anxiety, depressed mood) generally reported during lower estradiol menstrual cycle phases (e.g., Gonda et al., 2008).

Maladaptive stress reactivity is one aspect of affective responding that might increase distress during low estradiol phases of the menstrual cycle. Previous studies have demonstrated menstrual cycle variation in functional activation of the stress response circuitry that underlies the neuroendocrine stress response. Compared to women in the late follicular phase (high estradiol), women in the early follicular phase (low estradiol) show greater recruitment of the stress response circuitry (e.g., central amygdala, paraventricular and ventromedial hypothalamic nuclei,

![Figure 1. Cyclic variation in steroid sex](image-url)
brainstem nuclei, orbitofrontal cortex) during aversive image processing (e.g., Goldstein et al., 2005; Jacobs et al., 2014). Additionally, in one study, women in the early follicular versus late follicular phase show greater hippocampal deactivation in response to a psychological stressor along with greater self-reported distress (e.g., Albert, Pruessner, & Newhouse, 2015). Sustained hippocampal activity is thought to reflect intact regulation of the stress response (e.g., Pruessner et al., 2010), whereas greater stress-related hippocampal deactivation during lower estradiol states might index greater vulnerability to stress-related symptoms.

**Two distinct systems contribute to the stress response**

Stressful events or situations elicit activity in two physiological stress systems: the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis, which contribute to different components of the stress response (see Figure 2). SNS activity underlies immediate responses to stressors, commonly referred to as the fight or flight response. The SNS response is initiated by increased activity of the norepinephrine-producing neurons of the locus coeruleus in the brainstem, and increased norepinephrine signaling spreads to affective brain areas (e.g., amygdala) (Pickel, Segal, & Bloom, 1974). Some of these projections exit the central nervous system through spinal nerves and affect a variety of organ systems via postganglionic sympathetic nerve fibers. SNS activity generates rapid physiological changes (e.g., increased heart rate, faster breathing, release of norepinephrine and epinephrine) that enable an organism to cope with an imminent stressor (Chrousos & Gold, 1992, for review). Following the SNS, a second response is initiated by the HPA axis, which underlies a slower, but more sustained response to stressors. Activity along the HPA axis is initiated by the hypothalamus, which communicates with the pituitary gland, enacting a hormonal cascade that ultimately triggers the release of the well-known stress
hormone cortisol from the adrenal glands. In response to a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH) onto the anterior pituitary gland, which in turn releases adrenocorticotropic hormone (ACTH). ACTH released into the bloodstream travels to the adrenal glands, which release cortisol (e.g., Tsigos & Chrousos, 1994). In contrast to norepinephrine, whose levels peak within a few minutes of stressor onset, cortisol levels peak approximately 30 minutes post-stressor onset (Kirschbaum & Hellhammer, 2000).

Successful coordination of sympathetic and HPA axis activity is adaptive in the face of actual threats, whereas heightened or inappropriate activation of these systems can become maladaptive. Measurement of activity in these stress systems using peripheral biomarkers has allowed researchers to learn more about individual variation in stress reactivity and its relation to psychological functioning. Activity in both systems can be measured non-invasively and cost-effectively in saliva samples using commercially available immunoassay kits. Measurement has to date mostly included salivary cortisol, a well-established index of HPA axis function. More recently, the digestive enzyme salivary alpha amylase (AA) has emerged as a surrogate marker of SNS activity, as salivary glands increase production of AA in response to increased noradrenergic signaling (e.g., Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996). AA levels reliably increase in response to a variety of laboratory psychological stressors (e.g., Nater & Rohleder, 2009; Schumacher, Kirschbaum, Fydrich, & Ströhle, 2013).
Figure 2. Two physiological systems underlie the stress response (adapted from Eisenberger & Cole, 2002). The sympathetic nervous system (SNS; right) provides a nearly immediate response to stressors. SNS activation is followed by activation of the hypothalamic-pituitary-adrenal (HPA) axis (left).

Previous studies of SNS and HPA axis function have shown that activity in these systems is influenced by sex and by trauma history, although the interaction of these factors is not yet understood. Women show lower HPA reactivity to laboratory psychosocial stressors than men (e.g., Kajantie & Phillips, 2006; Uhart, Chong, Oswald, Lin, & Wand, 2006), although this effect varies by menstrual cycle phase, with relatively high HPA responses (equivalent to those of men) observed in women during the mid-luteal (higher estradiol) phase (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). The few studies of sex differences in AA reactivity to psychological or physiological stress tasks (e.g., Schoofs, Hartmann, & Wolf, 2008; van Stegeren, Wolf, & Kindt, 2008) suggest no overall differences in reactivity, although estrogens are known to increase parasympathetic tone, resulting in lower SNS activity at rest in
premenopausal women (e.g., Conte, 2003). In general, however, stress reactivity also varies as a function of gonadal hormone status and menstrual cycle phase.

Stress reactivity differs not only by sex, but also by trauma history. Trauma exposure is usually associated with heightened sympathetic reactivity to aversive or trauma-related stimuli (e.g., Ali & Pruessner, 2012; McTeague et al., 2010). Trauma is also associated with changes in HPA axis reactivity to laboratory stressors, although the results are mixed, with heightened reactivity reported in some studies (e.g., Bremner et al., 2003; Inslicht et al., 2006) and blunted reactivity in other studies (e.g., Carpenter, Shattuck, Tyrka, Geracioti, & Price, 2011; Elzinga et al., 2008). The inconsistencies in the evidence suggest that additional variables might influence the relationship between trauma and stress reactivity. Steroid sex hormones such as estradiol might be one such factor, especially given that blunting of the HPA axis is more frequently observed in women (e.g., Meewisse, Reitsma, De Vries, Gersons, & Olff, 2007). Additionally, there are overlapping adrenal and gonadal steroid hormone receptors in areas of the stress response circuitry (e.g., paraventricular nucleus, amygdala, bed nucleus of the stria terminalis; Handa & Weiser, 2014), suggesting that estradiol could directly influence stress system function.

**Estradiol impacts stress reactivity**

Estradiol is associated with reactivity to laboratory stressors in the SNS and HPA axis systems. For example, women in low estradiol menstrual cycle phases (e.g., early follicular) show lower HPA axis reactivity compared to women in high estradiol phases (e.g., mid-luteal; Kirschbaum et al., 1999) show lower HPA axis reactivity. Similarly, women using hormonal contraceptives that interfere with endogenous estrogen production show lower HPA responses compared to naturally cycling women (e.g., Nielsen, Segal, Worden, Yim, & Cahill, 2013; Roche,
Conversely, most evidence suggests that estradiol attenuates sympathetic reactivity. For example, sympathetic reactivity increases when estradiol levels drop at menopause (e.g., Lindheim et al., 1992), which is a change that underlies some of the well-known, common menopausal symptoms (e.g., hot flashes), whereas estrogen treatment reduces this effect (e.g., Komesaroff, Esler, & Sudhir, 1999). Naturally cycling women show increased sympathetic activity during low estradiol versus high estradiol phases (e.g., McFetridge & Sherwood, 2000), along with reduced parasympathetic activity and greater self-reported negative affect (e.g., Kanojia et al., 2013).

Given the above evidence for the effects of estradiol on the SNS and HPA axis, estradiol fluctuations might influence coordination of the two systems. For example, suppression of the HPA axis is associated with heightened sympathetic reactivity (e.g., Andrews, D’Aguiar, & Pruessner, 2012), suggesting that menstrual cycle variation in one stress system might drive or exacerbate changes in the other. This is an important consideration, as the ratio of sympathetic to HPA axis activity predicts stress and depression in people who have experienced adverse life events (e.g., Ali & Pruessner, 2012). Thus, a combination of higher sympathetic and lower HPA axis reactivity associated with low estradiol states might increase risk for post-trauma symptoms, especially symptoms related to elevated physiological arousal.

**Estradiol impacts fear and anxiety responses**

In addition to elevated neural and neuroendocrine reactivity to stressors, lower estradiol has also been linked with behavioral indices of maladaptive fear and anxiety in the absence of actual threat. For example, in both women and female rodents, estrogens facilitate fear extinction, a learning process in which an organism is presented with a previously aversively conditioned cue in
the absence of any aversive event (e.g., Graham & Milad, 2013). Successful extinction learning is indexed by diminished conditioned fear responses (e.g., skin conductance response), whereas extinction impairments are indexed by persistent fear responses to stimuli that no longer signal threat (Maren, 2001, for review).

During trauma exposure, people can acquire associations between the traumatic event and innocuous stimuli in the environment. Post-trauma, persistent responses to these cues can lead to distressing PTSD symptoms, and a robust literature supports impaired fear extinction as a candidate mechanism underlying vulnerability for anxiety and stress-related disorders (e.g., Hermann, Ziegler, Birbaumer, & Flor, 2002; Michael, Blechert, Vriends, Margraf, & Wilhelm, 2007), including PTSD (e.g., Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Milad et al., 2008; Norrholm et al., 2011). Additionally, given that extinction learning is the basis of exposure therapy, extinction deficits can confer resistance to treatment (e.g., Rothbaum et al., 2014; Sijbrandij). Thus, extinction deficits associated with hormone events could increase symptom severity and hinder treatment in trauma-exposed women.

Converging evidence from studies with women and animal models suggests that low estradiol states are associated with impaired extinction. In both women and female rodents, estrogen-blocking hormonal contraceptives hinder fear extinction, whereas estrogen treatment reverses this effect (e.g., Graham & Milad, 2013; Lonsdorf et al., 2015). In rodents, natural estrous cycle variation in estrogen has also been associated with extinction learning, with the most successful extinction observed during the proestrus (higher estrogen phase of the estrous cycle; e.g., Milad, Igoe, Lebron-Milad, & Novales, 2009). In women, individual variation in estradiol is also associated with extinction learning, such that lower levels are associated with greater fear following extinction (e.g., Hwang et al., 2015; Milad et al., 2010; Milligan-Saville & Graham, 2016; White & Graham, 2016;
Zeidan et al., 2013) and women in the late follicular phase (high estradiol) show better extinction learning relative to those in early follicular phase (e.g., Antov & Stockhorst, 2014). Further, women taking hormonal contraceptives, which generally reduce levels of endogenous estradiol, show impaired extinction learning compared to naturally cycling women (e.g., Lonsdorf et al., 2015; Merz et al., 2012). Associations between extinction and estradiol or menstrual cycle phase have been observed in healthy women (e.g., Milad et al., 2010) and women with PTSD (e.g., Glover et al., 2012) and other affective disorders (e.g., specific phobia; Li & Graham, 2016). Some evidence, however, suggests that the direction of these associations might differ between healthy women and women with PTSD. For example, in one study, healthy trauma-exposed women showed better extinction learning during the mid-luteal phase, in which progesterone levels peak, compared to the early follicular phase, whereas this relationship is opposite in women with PTSD (Pineles et al., 2016).

In addition to fear extinction, lower estradiol has also been associated with other models of maladaptive fear and anxiety. For example, in rodents, behavioral indices of anxiety on standard laboratory paradigms (e.g., the elevated plus maze) are greater during the diestrum (lower estrogen) versus proestrus (higher estrogen) phase of the estrous cycle (e.g., Mora, Dussaubat, & Díaz-Véliz, 1996). In women, lower estradiol has been linked with impaired inhibition of fear in the presence of safety cues (e.g., Glover et al., 2013).

There is also some evidence that lower estradiol is associated with intrusive memories following exposure to highly arousing unpleasant information in the context of laboratory stressors, although the results are somewhat mixed. Women in the luteal versus follicular phase reported greater intrusive memories following exposure to an aversive film clip (Ferree, Kamat, & Cahill, 2011). However, this result is difficult to interpret with regard to the role of estradiol,
given considerable fluctuations in both estradiol and progesterone that occur across the luteal and follicular phases. Additionally, some evidence suggests interactive effects of estradiol and progesterone on intrusive memories. For example, women in the early luteal phase (low estradiol and increasing progesterone) reported greater intrusive memories compared to women in the late luteal (low estradiol and progesterone) or mid-follicular phases (increasing estradiol and low progesterone) (e.g., Soni, Curran, & Kamboj, 2013). Increased intrusive memories during the early luteal phase were driven by the interaction of estradiol and progesterone, with no effect of estradiol alone.

In contrast, results from studies that control for the effects of progesterone, and thereby focus on the effects of estradiol alone, suggest that estradiol protects against intrusive memories. For example, lower estradiol has been linked with self-reported intrusive memories, with no association between progesterone and intrusive memories (e.g., Wegerer, Kerschbaum, Blechert, & Wilhelm, 2014). Further, during processing of aversive laboratory stimuli, estradiol has also been associated with neural activity in affective brain areas. In naturally cycling women, higher estradiol has been associated with increased activity in the ventromedial prefrontal cortex, which has been implicated in the downregulation of negative affect in response to unpleasant stimuli (Miedl, Wegerer, Kerschbaum, Blechert, & Wilhelm, 2018). Conversely, women in lower estradiol states, such as women in the late luteal versus late follicular phase (Ossewaarde et al., 2010), and women using hormonal contraceptives (Miedl et al., 2018), show increased activity in limbic (i.e., amygdala, insula) and cingulate areas (e.g., dorsal anterior cingulate cortex), which are thought to amplify fear and stress-related responses (e.g., Milad et al., 2007).

Finally, under conditions of elevated SNS activity upon exposure to affective information, lower estradiol has been linked with biased memory for negatively valenced
compared to positively valenced affective information (Nielsen, Barber, Chai, Clewett, & Mather, 2015). Given that people experience high SNS activity when confronted with a traumatic event, estradiol could affect the consolidation of traumatic memories and therefore influence later symptoms. Together, these results suggest that low estradiol states are associated with increased vulnerability to negative affect and intrusive memories following exposure to distressing content.

In sum, associations between lower estradiol and affective responses including fear and anxiety have been observed in healthy women and women with PTSD, suggesting typical increases in risk for persistent or exaggerated fear responses during low estradiol phases of the cycle. In trauma-exposed women, low estradiol phases might additionally be associated with risk for PTSD symptoms. For example, failure to inhibit responses to cues associated with a traumatic event and vulnerability to intrusive memories following exposure to distressing content are two mechanisms that could directly relate to PTSD re-experiencing symptoms.

Consistent with the notion that estradiol protects against PTSD symptoms, women who were administered emergency contraception (EC) shortly following sexual trauma reported reduced symptom severity (Ferree, Wheeler, & Cahill, 2012). Unlike standard hormonal contraceptives, which deliver low doses of synthetic hormones over a period of time and reduce endogenous ovarian hormone production, EC delivers a large single dose of synthetic hormones. Among women admitted to the emergency room within hours following sexual trauma, women who received synthetic estrogen- and progestin-based EC had lower PTSD symptom severity at 6-month follow-up relative to women who received progestin only EC, or women who declined EC (Ferree et al., 2012).

Initial prospective evidence also suggests that endogenous estradiol is protective against PTSD symptoms. Among women in the emergency room, lower estradiol, measured within a
few hours post-trauma, was associated with greater PTSD symptom severity at 1 and 3-month follow-ups (Jovanovic, 2017). In addition to individual differences in risk for symptoms associated with estradiol, natural fluctuations in estradiol across the menstrual cycle might also predict patterns of vulnerability for symptoms.

**Menstrual cycle variation and psychological symptoms**

Some work has documented normative menstrual cycle variation in a variety of psychological symptoms. Most evidence suggests that estradiol protects against negative affect (e.g., anxiety, depressed mood, neuroticism). For example, greater self-reported psychological symptoms have been observed in women during the late luteal phase, when estradiol levels are declining to baseline, relative to the mid to late follicular phases, when estradiol levels are rising (e.g., Gonda et al., 2008; Kanjolia et al., 2013). Reduced self-reported psychological symptoms have also been observed during the mid-luteal phase, which is characterized by relatively high estradiol, but also high progesterone (e.g., Walder, Statucka, Daly, Axen, & Haber, 2012).

In addition to psychological symptoms, menstrual cycle phase has been associated with lifestyle and health behaviors that could influence mental health. For example, women show increased binge eating during the mid and late luteal phase, when ovarian hormones decline, versus the late follicular phase, when estradiol increases (Klump, Keel, Culbert, & Edler, 2008). Female smokers show increased cigarette consumption, along with greater self-reported depressed mood during the early follicular and luteal phases compared to the late follicular phase (e.g., Sakai & Ohashi, 2013). Further, among female smokers attempting to quit, greater withdrawal symptoms have been observed during the mid to late luteal phase versus mid to late follicular phase (e.g., Allen, Allen, Lunos, & Hatsukami, 2009).
In normative populations, lower estradiol phases have been linked with increased risk for clinical symptoms, particularly in women with trait vulnerabilities. For example, among women with higher anxiety sensitivity, greater cognitive panic symptoms related to physical changes (i.e., CO₂ challenge) were reported by women in the late luteal (lower estradiol) compared with the late follicular (higher estradiol) phase (Nillni, Rohan, & Zvolensky, 2012). Similarly, in women with borderline personality features, symptoms of borderline personality disorder varied across the menstrual cycle, such that lower estradiol predicted increased symptoms, but only when progesterone levels were high (Eisenlohr-Moul, DeWall, Girdler, & Segerstrom, 2015).

Menstrual cycle variation in symptoms has also been observed in a variety of clinical conditions. For example, women with premenstrual dysphoric disorder (PMDD) demonstrate a well-defined pattern of fluctuations in symptoms across the menstrual cycle. PMDD is characterized by a constellation of severe cognitive, affective and physical symptoms that are confined to the late luteal phase and the early follicular phase during menses (APA, 2013), which represent a low estradiol portion of the menstrual cycle. For a diagnosis of PMDD, these symptoms must remit during the mid follicular phase when estradiol levels increase.

In addition to fluctuations in self-reported symptoms, women with PMDD show measurable increases in physiological reactivity (e.g., exaggerated startle; Epperson et al., 2007), increased panic responses to biological challenges (e.g., C02 challenge; Nillni, Pineles, Rohan, Zvolensky, & Rasmusson, 2017) and impaired performance on cognitive tasks (e.g., Reed, Levin, & Evans, 2008) during menses or the late luteal (lower estradiol) phases relative to the mid or late follicular (higher estradiol) phases. Consistent with negative affect and elevated arousal, women with PMDD show greater limbic and reduced prefrontal regulatory activity during
affective processing relative to healthy controls, but only during the late luteal phase (Protopopescu et al., 2008).

Menstrual cycle phase variation has also been reported in other clinical conditions, such as eating disorders. For example, women with bulimia show increased binge eating during lower estradiol phases of the cycle (e.g., Edler, Lipson, & Keel, 2007), although some of these effects are potentially mediated by progesterone. The early follicular period (low estradiol) is also associated with the greatest risk for suicide attempts (e.g., Sein, Chodorowski, Ciechanowicz, Wiśniewski, & Pankiewicz, 2005).

Some initial evidence suggests that trauma-exposed women experience greater general psychological distress during low estradiol menstrual cycle phases (e.g., Nillni et al., 2015), but less is known about how cyclic variation in estradiol contributes to trauma-related symptoms. Additionally, some of the evidence for differences in negative affect or stress-related symptoms by menstrual cycle phase is based on comparisons between women in the entire follicular and luteal phases, despite the variability in ovarian hormones within these broad phases. Other evidence is based on comparisons between women in the early follicular and mid-luteal phases. However, given that the luteal phase is characterized by increases in both estradiol and progesterone, it is difficult to disentangle the unique contributions of the two hormones. For example, greater intrusive memories in trauma survivors have been reported during the mid-luteal (higher estradiol) phase of the cycle (e.g., Bryant et al., 2011), but this result could also reflect high progesterone, rather than estradiol. Finally, many previous studies have relied on data gathered from single laboratory sessions or a small number of sessions. These studies cannot capture patterns of daily affective experiences over the course of the menstrual cycle.
To acquire a more nuanced understanding of how daily affect is influenced by fluctuations in estradiol, it is important to track affective experiences at different points in the menstrual cycle. In particular, it is important to assess participant affect and symptoms during high and low menstrual cycles phases, while controlling for progesterone levels. The transition from the early to late follicular phase captures a portion of the cycle marked by estradiol change, as levels increase from the cycle baseline and approach the pre-ovulation peak. Additionally, by using an experience sampling method, rather than relying on single lab visits during each phase, researchers can more accurately capture daily affective experiences across low and high estradiol phases.

**Ecological momentary assessment for capturing daily affect**

Traditional assessments of affect and clinical symptoms generally take place in a laboratory setting and require participants to report on symptoms over a defined period of time. For example, some PTSD assessments ask participants to report on symptoms experienced over the past month (First, Spitzer, Gibbon & Williams, 1996; Weathers, Blake, Schnurr, Kaloupek, Marx & Keane, 2013a). These retrospective assessments are limited by biases in reporting, as self-report can be influenced by momentary affect (Mill, Realo, & Allik, 2016). Ecological Momentary Assessment (EMA) offers an alternative to single timepoint assessments and allows researchers to track phenomena of interest over a period of time (e.g., Moskowitz & Young, 2006; Ebner-Priemer & Trull, 2009). EMA methods entail prompting participants to respond to questionnaires assessing momentary affect, cognitions or behaviors using handheld devices. These methods enable more accurate reporting of affect and symptoms over time, and also capture experiences within naturalistic settings (i.e., participants’ daily life settings).
Given previous evidence linking estradiol with affect, menstrual cycle phase could influence trauma symptoms, as well as daily affective experiences more broadly. Affect refers to a person’s subjective experience at a given moment and varies along two well-established dimensions of valence (unpleasant to pleasant) and arousal (low to high activation) (e.g., Barrett, 2006; Posner, Russell, & Peterson, 2005; see Figure 3). Estradiol could influence patterns of daily affect along either or both dimensions. For example, given the evidence for greater fear and anxiety responses associated with lower estradiol, menstrual cycle phases characterized by low estradiol (e.g., early follicular phase) might be associated with more frequent aversive daily affective experiences. In particular, lower estradiol might increase risk for negatively valenced high arousal states, including PTSD symptoms.

![Figure 3. Circumplex model of affect which includes the dimensions of valence and arousal. Aversive affective experiences, defined as negatively valenced (unpleasant) high arousal states, are located within the upper left quadrant.](image)

**Aims and hypotheses**

The first aim of this study was to test the relation between estradiol and stress reactivity to a naturalistic trauma reminder. The second aim was to test the relation between estradiol and daily affective experiences, including PTSD symptoms. To address our specific aims, we used a multi-method approach that included both cross-sectional and experience sampling methods. We measured physiological stress reactivity to a naturalistic trauma reminder and assessed trauma-
related symptoms during a structured clinical interview and over the course of a 10-day ecological momentary assessment phase that included low and high estradiol menstrual phases. We hypothesized that in trauma-exposed women, lower estradiol would be associated with greater severity of trauma-related symptoms (Hypothesis 1) and a stress response profile of greater sympathetic and lower HPA axis reactivity to a trauma reminder (Hypothesis 2). We also hypothesized that women would report more frequent aversive affective experiences (i.e., unpleasant high arousal states), including PTSD symptoms, during a low versus high estradiol phase of the menstrual cycle (Hypothesis 3). These hypotheses were generated in accordance with previous studies that observed increased psychological symptoms during low estradiol phases of the menstrual cycle and with the literature on estradiol and its relation to stress system function.

Finally, as exploratory analyses, we examined associations between estradiol and affective variability across the early and late follicular phases. These analyses allowed us to test the potential effect of within-person variability in estradiol on affect and PTSD symptoms.

2. Method

2.1. Participants

We recruited 40 naturally cycling premenopausal trauma-exposed women who were undergraduate students fulfilling credit requirements for introductory psychology at an urban university in the northeastern US. Prior to the study, we conducted a power analysis and determined that a sample of approximately 30 participants would provide adequate power to detect differences in affect and symptoms by menstrual cycle phase. Given subject attrition during the EMA portion of the study, we recruited 10 additional women.
We recruited participants according to self-reported exposure to potentially traumatic events on a standard laboratory prescreen, and trauma exposure was subsequently confirmed during the clinical interview.

During both recruitment and a clinical interview, we verified that participants did not meet any exclusion criteria for the study, which included: (1) current use of hormonal contraceptives or use of hormonal contraceptives in the past 6 months, (2) pregnancy or lactation within the past year (3) use of psychotropic or steroid medications known to influence physiological arousal or stress system function (e.g., betablockers, anxiolytic drugs, corticosteroids), (4) habitual cigarette smoking, which is known to influence salivary flow rate and salivary stress biomarkers (e.g., Granger et al., 2007), (5) meeting criteria for current Axis I disorders other than PTSD and (6) younger or older than the 18-35 age range.

2.2. Recruitment

Recruitment consisted of two steps. First, we selected potential participants for invitation to the study according to self-reported potential trauma exposure on an introductory psychology prescreen, and then participants completed a phone screen assessing menstrual cycle-related inclusion criteria. We recruited participants from the subject pool according to responses on the Life Events Check List (LEC; Weathers et al., 2013a), which is a 17-item self-report screen for exposure to potentially traumatic life events (see Appendix). The respondents checked either “Happened to me”, “Witnessed it”, “Learned about it”, “Not sure” or “Doesn’t apply to me” for each event. We recruited people who responded with “Happened to me” for one or more traumatic event items on the LEC.

Potential participants received an email invitation to the study with instructions to contact the laboratory for a phone screen. We used the phone screen to assess menstrual cycle regularity.
and use of medication and other substances that would render participants ineligible to participate. Participants were asked to report on the date of their most recent menstrual cycles and approximate average cycle length. Inclusion criteria for the study included menstrual cycle regularity, defined as an average cycle length between 25 and 36 days and no missed periods within the past six months. We also asked participants about use of hormonal contraception and cigarette smoking. We only recruited participants who were non-smokers and who were not currently using hormonal contraceptives and had not used hormonal contraceptives within the past 6 months.
2.3. Procedure

The study included two parts: a laboratory visit and a 10-day ecological momentary assessment (EMA) period spanning the early and late follicular menstrual cycle phases. All study procedures were approved by Institutional Review Board and were conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.3.1. Laboratory visit

The laboratory visit consisted of informed consent, a clinical interview, and training in the use of a handheld device for the EMA portion of the study. During the laboratory visit, participants provided saliva samples in the post-consent resting period, which served as a study baseline, and following a discussion of a traumatic event.

Clinical interview. All participants completed a structured clinical interview to verify the presence of trauma exposure and to assess symptoms of PTSD and other Axis I disorders. The interview included the Structured Clinical Interview for DSM-IV Disorders (SCID; First, Spitzer, Gibbon & Williams, 1996) and the Clinician-Administered PTSD Scale (CAPS; Weathers et al., 2013).

The SCID is a structured diagnostic assessment for DSM-IV Axis I disorders (e.g., mood, anxiety, substance use and eating disorders, psychotic symptoms). We conducted all modules of the SCID to exclude participants who met criteria for current disorders with the exception of PTSD. We verified trauma exposure using Criterion A of the PTSD module: (1) the person experienced, witnessed or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of the self or others; and (2) the person’s response involved intense fear, helplessness or horror. Participants briefly described the traumatic event, and this discussion also served as a trauma reminder.
After the participant described the traumatic event, we assessed PTSD symptoms over the past month using the Clinician-Administered PTSD Scale (CAPS). Unlike other PTSD measures, such as the SCID, which yield only binary symptom reports (by asking participants whether they experienced a given symptom over the past month), the CAPS assess both symptom count and severity. The severity score is based on both the frequency and intensity of reported symptoms.

In addition to psychiatric symptoms, we also assessed general health, medication and substance use and recent menstrual cycle history during the interview session. Participants provided the dates of their previous two menstrual cycles and approximate average cycle length. The EMA portion of the study started on day 2 (early follicular phase) of the next menstrual cycle. We calculated mean cycle length using the dates of previous menstrual cycles and the date of the cycle that started during the EMA portion of the study.

*Saliva collection.* Participants provided saliva samples following informed consent, which served as a study baseline, immediately following trauma description during the interview and 20 minutes later (see Figure 4). At each timepoint, participants provided samples using the passive drool method via Salimetrics saliva collection aids (Salimetrics, LLC). We instructed participants to allow saliva to pool naturally in the mouth and to release the saliva through the saliva collection aid into a cryovial. The passive drool method was preferred over a salivette-based method, as the latter has been shown to distort gonadal hormone readings (Shirtcliff, Granger, Schwartz, & Curran, 2001). All participants had been awake for a minimum of one hour prior to the study session, ensuring that our timepoints did not overlap with the cortisol awakening response. Participants had refrained from eating or drinking, except for water, for one hour prior to the study session.
The first saliva sample (T1) was collected in the resting period following informed consent at approximately 10:05 AM. We collected the first sample at the same time for all participants to control for diurnal variation in levels of cortisol, alpha amylase and estradiol (e.g., Bao et al., 2003; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). This post-consent sample served as a study baseline. Although not a true baseline in the sense that participants’ stress systems might have been elevated relative to a typical day (resting at home versus in the laboratory), the study baseline served as a point of comparison to the post-stressor samples.

The second sample (T2) was collected immediately following description of the traumatic event during the interview. The second sample allowed us to capture peak AA, as this enzyme increases almost immediately (within 2-5 minutes) post-stressor onset (Nater et al., 2005; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). The third sample (T3) was collected exactly 20 minutes after the second.

Levels of salivary alpha amylase at each timepoint indexed sympathetic activity at that timepoint. Given that cortisol peaks approximately 20 minutes following a stressor (Kirschbaum & Hellhammer, 2000), cortisol levels at each timepoint indexed HPA axis activity approximately 20 minutes prior to collection. The samples were aliquoted into cryovials for later assays and stored in a -20 C freezer until the time of analysis.

Figure 4. Saliva collection during the laboratory visit. Participants provided saliva samples at baseline, during and after discussion of a traumatic event.
2.3.2. Ecological momentary assessment phase

*Ecological momentary assessment procedure.* The ecological momentary (EMA) phase comprised a period of ten days spanning the early follicular (low estradiol) and late follicular (higher estradiol) phases. During the ten days, participants completed questionnaires at five daily timepoints, for a total of fifty assessment timepoints. The timing and length of our EMA design allowed us to capture the scope of daily affective experiences across low and high estradiol portions of the cycle, while also being within the window after which EMA compliance has been shown to decrease (Broderick, Schwartz, Shiffman, Hufford, & Stone, 2003).

We provided each participant with a handheld device (i.e., android phone) for the duration of the ten-day EMA phase. During the EMA phase, participants completed questionnaires at five daily assessment points: after waking, before bed and at three variable daytime assessment points. The three daytime assessment points were programmed to occur semi-randomly at approximately three-hour intervals within the participant’s typical waking hours. At each daytime assessment point, participants received a text message instructing them to complete a questionnaire. Participants were asked to complete daytime assessments within 30 minutes of receiving a prompt to the extent that this was possible.

The EMA phase began on day 2 following the onset of menstruation and went through day 11 of the cycle. The early follicular phase, which is characterized by low estradiol and progesterone, was operationalized as menstrual cycle days 2-6. The late follicular phase, which is characterized by higher estradiol and low progesterone, was operationalized as days 7-11. On the first day of her menstrual cycle, each participant contacted an experimenter to report that her cycle had started, and the EMA prompts were programmed to start the following day. All
completed assessments were automatically time-stamped, and EMA data were uploaded automatically to the laboratory server.

We confirmed that participants were in the expected menstrual cycle phases during the EMA portion of the study by measuring levels of gonadal hormones in two at-home saliva samples. Participants collected at-home saliva samples on the first and last days of the EMA phase, which correspond to Day 2 (early follicular phase) and Day 11 (late follicular phase of the cycle). We instructed participants to collect the saliva samples via the passive drool method immediately upon waking, prior to moving around, brushing their teeth, or consuming food or beverages (other than water). Participants were accustomed to the passive drool method and were able to practice under the guidance of an experimenter during the laboratory visit. At-home saliva samples were stored in participants’ home freezers. After the EMA portion of the study, participants transported the samples to the lab in insulated lunch bags. To reduce overall transit time for the samples, participants were asked to schedule transport on days when they could travel directly to lab from home. Once they arrived at the lab, the samples were stored in a -20 C freezer and prevented from undergoing any additional freeze-thaw cycles prior to estradiol assay.

Ecological momentary assessment measures. An overview of the daily questionnaire items is presented in Table 1. The EMA questionnaires included measures for affect and trauma-related symptoms and also assessed lifestyle behaviors.

At all assessment points, participants rated current affect (i.e., valence, unpleasant to pleasant; arousal, low to high). Valence was rated on a scale ranging from 1-9, where “1” was “extremely unpleasant”, “5” was “neutral” and “9” was “extremely pleasant”. Arousal was also rated on a scale ranging from 1-9, where “1” was “extremely non-stimulated or activated”, “5” was “moderately stimulated or activated” and “9” was “extremely stimulated or activated”. We
operationalized unpleasant high arousal states as assessment points where participants rated valence below “5” and arousal above “5”.

The morning and evening questionnaires also included the Positive and Negative Affect Schedule (PANAS-SF; Watson, Clark, & Tellegen, 1988). The PANAS-SF is a 10-item self-report measure of current positive and negative affective states (e.g., upset, inspired). For each item, the participant indicated the extent to which she was currently feeling that affective state on a scale ranging from “1” (very slightly or not at all) to “5” (extremely).

We assessed trauma-related symptoms once each day on the evening questionnaires using the PTSD Checklist for DSM-5 (PCL-5; Weathers et al., 2013c). The PCL-5 is a 20-item measure that assesses DSM-5 symptoms of PTSD. For each symptom, the participant reported the extent to which she was bothered by the symptom that day on a scale ranging from 0 (“not at all”) to 4 (“extremely”).

The daily morning and evening questionnaires also included lifestyle items. On the morning questionnaires, participants reported on their sleep quality the night before, specifically, the number of hours that they slept and any nightmares or interruptions to their sleep. On the evening questionnaires, participants reported daily consumption of alcohol and other substances and exercise.
2.2.3. Salivary biomarker assays

Salivary biomarker assays were conducted in-house by lab personnel and all samples were processed in duplicate. We assayed the three samples collected during the laboratory visit for levels of the stress biomarker cortisol and the surrogate stress biomarker alpha amylase.

We conducted alpha amylase assays using Salimetrics kinetic reaction assay kits (Salimetrics, LLC), which utilize a chromogenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. The amount of alpha amylase present in each sample is directly proportional to the increase in absorbance, measured spectrophotometrically by a standard plate reader at 405 nm. The intra- and inter-assay coefficients of variation for these kits are less than 7.5% and 6%, respectively.

We conducted cortisol assays using Salimetrics enzyme immunoassay kits (Salimetrics, LLC), which use a microtiter plate coated with monoclonal anti-cortisol antibodies. Cortisol in
samples and standards competes with cortisol conjugated with peroxidase for the antibody binding sites. The amount of cortisol enzyme conjugate detected is inversely proportional to the amount of cortisol present in the sample, measured spectrophotometrically by a standard plate reader at 450 nm. The intra- and inter-assay coefficients of variation for these kits are less than 7% and 10%, respectively.

We measured levels of estradiol present on the morning of the laboratory visit (T1). To confirm appropriate cycle phase during the EMA phase, we also measured levels of estradiol and progesterone in the at-home saliva samples. We conducted estradiol assays using Salimetrics enzyme immunoassay kits (Salimetrics, LLC). The kit utilizes a microtiter plate coated with rabbit anti-estradiol antibodies. Estradiol in samples and standards competes with estradiol linked to horseradish peroxidase for the antibody binding sites. The amount of estradiol linked to horseradish peroxidase detected is inversely proportional to the amount of estradiol present in the sample, measured spectrophotometrically by a standard plate reader at 450 nm. The intra- and inter-assay coefficients of variation for these kits are less than 8.5 and 9%, respectively.

We measured levels of progesterone in at-home saliva samples to ensure that participants remained in the follicular phase for the duration of the EMA protocol. We conducted progesterone assays using Salimetrics enzyme immunoassay kits (Salimetrics, LLC), which utilize a microtiter plate coated with rabbit anti-progesterone antibodies. Progesterone in samples and standards competes with progesterone conjugated to horseradish peroxidase for the antibody binding sites. The amount of progesterone conjugate detected is inversely proportional to the amount of progesterone present in the sample, measured spectrophotometrically by a standard plate reader at 450 nm. The intra- and inter-assay coefficients of variation for these kits are less than 8.5% and 10%, respectively.
2.4. Data analyses

2.4.1. Estradiol and PTSD symptoms

We used Pearson product-moment correlations to test the hypothesized associations between estradiol and PTSD symptom count and severity as reported during the clinical interview.

2.4.2. Estradiol and stress reactivity

We used Pearson product-moment correlations to test the hypothesized associations between estradiol and sympathetic and HPA axis reactivity to the trauma reminder. Sympathetic nervous system activity at each timepoint was indexed by salivary alpha amylase (AA) at that timepoint and the difference between levels of AA at the first and second timepoints (T2-T1) indexed sympathetic reactivity. Conversely, given that salivary cortisol levels at each timepoint index HPA axis activity approximately 20 minutes prior to collection, we calculated HPA axis reactivity as the difference in cortisol between the second and third timepoints (T3-T2).

2.4.3. Menstrual cycle phase and stress reactivity

In addition to testing associations between estradiol level and stress reactivity, we also tested the association between menstrual cycle phase on the day of the laboratory visit and stress reactivity. We determined participants’ menstrual cycle phase during the laboratory visit using the dates of their three most recent menstrual cycles and average cycle length. We categorized participants into two groups: low and high estradiol phase. The low estradiol phase group comprised participants in the early follicular, early luteal and late luteal phases, whereas the high estradiol phase group comprised participants in the late follicular/peri-ovulatory and mid-luteal
phases. We used Wilcoxon rank-sum tests to test group differences in alpha amylase and cortisol reactivity.

2.4.4. Menstrual cycle phase and daily affect

Ecological Momentary Assessment data was analyzed by collapsing across the early follicular (days 2 to 6) and late follicular (days 7 to 11) phases. To test differences in daily affective experiences by menstrual cycle phase, we used paired samples $t$-tests to test differences in self-reported valence, arousal and PTSD symptoms during the early and late follicular phases. Additionally, to test the relation between changes in estradiol and daily affect, we calculated estradiol increase for each participant as the difference between estradiol detected in the late follicular and early follicular at-home saliva samples. We used Pearson product-moment correlations to test the association between estradiol increase and daily affect measures (i.e., valence, arousal and PTSD symptoms).

3. Results

3.1. Descriptive data

We recruited 40 naturally cycling premenopausal trauma-exposed women ($M$ age $= 21.9$, $SD = 4.2$, range: 18-33). Participant characteristics are reported in Table 2. Consistent with the local metropolitan area, our population was diverse. Participants had regular menstrual cycles with average cycle length varying between 26 and 35 days ($M = 30.5$, $SD = 3.4$).

All participants reported experiencing at least one traumatic event. Trauma type varied, although for the majority of our sample, the index trauma (i.e., traumatic event that affected them
the most) was of an interpersonal nature, and the most frequently reported index trauma was sexual assault (see Table 2).

Hormone levels over the course of the study are shown in Table 3. Four participants had estradiol or progesterone values in the at-home samples that were inconsistent with the expected menstrual cycle phase and were therefore excluded from all analyses with EMA data.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, $M (SD)$, range</td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity, $n$ (%)</td>
<td></td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>8 (20.0)</td>
</tr>
<tr>
<td>Black, non-Hispanic</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10 (25.0)</td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>11 (27.5)</td>
</tr>
<tr>
<td>Multiple</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Other race</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>Average cycle length (days), $M (SD)$, range</td>
<td>30.5 (3.4), 26-35</td>
</tr>
<tr>
<td>Trauma type, $n$ (%)</td>
<td></td>
</tr>
<tr>
<td>Interpersonal</td>
<td>33 (82.5)</td>
</tr>
<tr>
<td>Non-interpersonal</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Index trauma, $n$ (%)</td>
<td></td>
</tr>
<tr>
<td>Sexual assault</td>
<td>20 (50.0)</td>
</tr>
<tr>
<td>Physical assault</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Transportation accident</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td>Other unwanted sexual experience</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>Assault with a weapon</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>Chronic bullying</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Warzone</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Fire</td>
<td>1 (2.5)</td>
</tr>
</tbody>
</table>
Table 3. Stress and gonadal hormone levels over the course of the study

<table>
<thead>
<tr>
<th>Analyte</th>
<th>M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory visit</td>
<td></td>
</tr>
<tr>
<td>Morning estradiol (pg/ml)</td>
<td>1.21 (0.49)</td>
</tr>
<tr>
<td>Low estradiol phase group estradiol (n = 23)</td>
<td>1.09 (0.43)</td>
</tr>
<tr>
<td>Early follicular phase estradiol (n = 13)</td>
<td>1.09 (0.55)</td>
</tr>
<tr>
<td>Early luteal phase estradiol (n = 3)</td>
<td>1.29 (0.18)</td>
</tr>
<tr>
<td>Late luteal phase estradiol (n = 7)</td>
<td>1.01 (0.23)</td>
</tr>
<tr>
<td>High estradiol phase group estradiol (n = 17)</td>
<td>1.36 (0.54)</td>
</tr>
<tr>
<td>Late follicular phase estradiol (n = 10)</td>
<td>1.38 (0.58)</td>
</tr>
<tr>
<td>Mid-luteal phase estradiol (n = 7)</td>
<td>1.33 (0.52)</td>
</tr>
<tr>
<td>Baseline alpha amylase (U/ml)</td>
<td>43.80 (35.19)</td>
</tr>
<tr>
<td>Post-stressor alpha amylase (U/ml)</td>
<td>56.62 (45.13)</td>
</tr>
<tr>
<td>Baseline cortisol (µg/dl)</td>
<td>0.17 (0.09)</td>
</tr>
<tr>
<td>Post-stressor cortisol (µg/dl)</td>
<td>0.15 (0.07)</td>
</tr>
<tr>
<td>EMA phase</td>
<td></td>
</tr>
<tr>
<td>Early follicular phase estradiol (pg/ml)</td>
<td>0.96 (0.30)</td>
</tr>
<tr>
<td>Late follicular phase estradiol (pg/ml)</td>
<td>1.66 (1.10)</td>
</tr>
<tr>
<td>Late follicular phase progesterone (pg/ml)</td>
<td>70.0 (30.8)</td>
</tr>
</tbody>
</table>

Note: The range of expected progesterone is 28-82 pg/ml for the follicular phase and 127-446 pg/ml for the luteal phase (Soni et al., 2013). Our observed ~70% increase in estradiol from the early follicular to late follicular phase is consistent with other studies with healthy naturally-cycling women (e.g., Colzato et al., 2010).

3.1. Trauma-related symptoms

Trauma symptoms reported during the clinical interview are summarized in Table 4.

Eight participants met criteria for a provisional PTSD diagnosis. However, consistent with our
high-functioning undergraduate sample, participants scored at the lower end of the symptom severity range.

Table 4. Trauma symptoms

<table>
<thead>
<tr>
<th>Variable (possible range)</th>
<th>M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re-experiencing symptom severity (0-20)</td>
<td>2.4 (2.5)</td>
</tr>
<tr>
<td>Re-experiencing symptom number (0-5)</td>
<td>0.7 (1.1)</td>
</tr>
<tr>
<td>Avoidance symptom severity</td>
<td>1.8 (1.8)</td>
</tr>
<tr>
<td>Avoidance symptom number (0-5)</td>
<td>0.7 (0.8)</td>
</tr>
<tr>
<td>Neg cognitions &amp; affect symptom severity (0-28)</td>
<td>4.4 (4.2)</td>
</tr>
<tr>
<td>Neg cognitions &amp; affect symptom number (0-7)</td>
<td>1.5 (1.8)</td>
</tr>
<tr>
<td>Arousal symptom severity (0-24)</td>
<td>3.0 (3.1)</td>
</tr>
<tr>
<td>Arousal symptom number (0-6)</td>
<td>1.0 (1.2)</td>
</tr>
<tr>
<td>Total symptom severity (0-80)</td>
<td>11.6 (9.9), range 0-32</td>
</tr>
<tr>
<td>Total symptom number (0-20)</td>
<td>3.9 (4.1), range: 0-12</td>
</tr>
<tr>
<td>Provisional current PTSD diagnosis, n (%)</td>
<td>8 (20%)</td>
</tr>
</tbody>
</table>

3.2. Estradiol and PTSD symptoms

Because estradiol levels were not normally distributed, the values were square root transformed. Associations between estradiol on the morning of the laboratory visit and PTSD symptom are shown in Table 5. Lower estradiol was associated with greater total number, $r = -0.35$, $p = 0.025$, and severity of trauma-related symptoms, $r = -0.32$, $p = 0.041$, as reported during the clinical interview. In addition to overall symptom count and severity, we also tested correlations between estradiol and PTSD symptom clusters. Lower estradiol was associated with greater re-experiencing (B cluster) symptom number: $r = -0.34$, $p = 0.034$, but not re-
experiencing symptom severity, \( r = -0.20, p = 0.222 \). Lower estradiol was also associated with greater avoidance symptom (C cluster) number, \( r = -0.36, p = 0.022 \) and severity, \( r = -0.36, p = 0.024 \), as well as greater arousal (E cluster) symptom number, \( r = -0.35, p = 0.025 \) and severity, \( r = -0.35, p = 0.028 \). Estradiol was not associated with negative alterations in cognitions and mood (D cluster) symptom number, \( r = -0.21, p = 0.195 \), or severity, \( r = -0.24, p = 0.133 \) (see Figure 5).

Table 5. Correlations between estradiol on the morning of the laboratory visit and PTSD symptoms

<table>
<thead>
<tr>
<th></th>
<th>Morning estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PTSD symptom number</td>
<td>( r = -0.35^* )</td>
</tr>
<tr>
<td>Total PTSD symptom severity</td>
<td>( r = -0.32^* )</td>
</tr>
<tr>
<td>Re-experiencing symptom number</td>
<td>( r = -0.34^* )</td>
</tr>
<tr>
<td>Re-experiencing symptom severity</td>
<td>( r = -0.20 )</td>
</tr>
<tr>
<td>Avoidance symptom number</td>
<td>( r = -0.36^* )</td>
</tr>
<tr>
<td>Avoidance symptom severity</td>
<td>( r = -0.36^* )</td>
</tr>
<tr>
<td>Neg cognitions &amp; affect symptom number</td>
<td>( r = -0.21 )</td>
</tr>
<tr>
<td>Neg cognitions &amp; affect symptom severity</td>
<td>( r = -0.24 )</td>
</tr>
<tr>
<td>Arousal symptom number</td>
<td>( r = -0.35^* )</td>
</tr>
<tr>
<td>Arousal symptom severity</td>
<td>( r = -0.35^* )</td>
</tr>
</tbody>
</table>

Note: * \( p < 0.05 \)
Figure 5. Relation between estradiol and PTSD symptoms. Lower estradiol on the morning of the lab visit was associated with greater total PTSD symptom count and severity, greater re-experiencing (B cluster) symptom count, avoidance (C cluster) symptom count and severity, and arousal (E cluster) symptom count and severity. Estradiol was not associated with negative alterations in cognitions and mood (D cluster symptoms).
3.3. Estradiol and stress reactivity

Levels of the salivary stress biomarkers alpha amylase and cortisol across the laboratory visit are presented in Table 3. Pre-stressor (study baseline) cortisol ($M = 0.17$, $SD = 0.09$, range: 0.06 - 0.49) and alpha amylase ($M = 43.80$, $SD = 35.19$, range: 6.72-159.99) values were normally distributed with no outliers. We conducted non-parametric paired-samples tests (Wilcoxon signed-rank test) to test differences in alpha amylase and cortisol from the study baseline to the trauma reminder. Overall, participants showed an increase (Mean percent change = 55.97% ($SD = 112.92$), range: -65.92% to 470.38%) in alpha amylase from the study baseline to the trauma reminder, $Z = 2.65$, $p = 0.008$, $r = 0.41$. Overall, cortisol levels did not increase in response to the trauma reminder (Mean percent change = -4.08% ($SD = 42.52$), range: -73.42% to 165.08%), but rather decreased, $Z = -2.07$, $p = 0.038$, $r = -0.33$ (see Figure 6).

![Figure 6. Stress reactivity to the trauma reminder. Participants showed an increase in alpha amylase (AA) from the study baseline (T1) to the trauma reminder. Conversely, cortisol levels decreased from the study baseline (T2) to the trauma reminder. Error bars represent standard errors.](image-url)
We used Pearson product-moment correlations to test associations between estradiol levels on the morning of the laboratory visit and reactivity in the two stress systems in the entire sample. Estradiol was not associated with alpha amylase, $r = 0.038$, $p = 0.814$, or cortisol reactivity, $r = -0.026$, $p = 0.872$, to the trauma reminder.

Due to variation in menstrual cycle phase during the laboratory visit, we also tested sympathetic and HPA axis reactivity by cycle phase. We separated the data into two group categories based on lab visit day cycle phase: low estradiol phases (early follicular, early luteal, late luteal) or high estradiol phases (late follicular/peri-ovulatory, mid-luteal). Wilcoxon rank-sum tests comparing participants in low versus high estradiol phases of the cycle indicated group differences in stress reactivity. Compared with participants in high estradiol phases ($n = 23$), participants in low estradiol phases ($n = 17$) had greater alpha amylase reactivity, $Z = -2.05$, $p = 0.040$, $r = -0.32$, but lower cortisol reactivity, $Z = 2.74$, $p = 0.006$, $r = 0.43$, to the trauma reminder (see Figure 7).

*Figure 7.* Stress reactivity by menstrual cycle phase. Participants in low versus high estradiol (E2) phases of the menstrual cycle showed greater SNS reactivity, indexed via percent change in salivary alpha amylase (AA), and reduced HPA axis reactivity, indexed via percent change in salivary cortisol, to the trauma reminder. Error bars represent standard errors.
3.4. Ecological momentary assessment protocol compliance

Participant compliance with the ecological momentary assessment (EMA) protocol is outlined in Table 6. Six participants had missing or insufficient EMA questionnaire data (more than 50% of day forms missing or completed at times inconsistent with the prompts) and were therefore excluded from these analyses. The remaining sample showed high compliance with the protocols.

Table 6. Participant compliance with EMA protocol

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<tr>
<td>Morning forms</td>
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<tr>
<td>Daytime forms</td>
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<tr>
<td>Daytime forms within 30 mins</td>
<td>71.4%</td>
</tr>
<tr>
<td>Evening forms</td>
<td>96.7%</td>
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</tbody>
</table>

3.5. Phase differences in affect

We conducted paired samples t-tests to test differences in self-reported affect during the EMA portion of the study by menstrual cycle phase. Overall valence ratings, \( t(29) = -0.22, p = 0.824, d = -0.03 \), and arousal ratings, \( t(29) = 0.27, p = 0.792, d = 0.03 \), did not differ by menstrual cycle phase. However, the variability of affect ratings, indexed via standard deviations, varied by phase, such that standard deviations for valence ratings, \( t(29) = 3.11, p = 0.004, d = 0.49 \), and arousal ratings, \( t(29) = 2.41, p = 0.022, d = 0.35 \), were higher during the early versus late follicular phase. Additionally, the strongest effect of cycle phase was on the percentage of timepoints in which participants reported experiencing unpleasant high arousal states (defined as valence <5 and arousal >5). The percentage of self-reported aversive affective experiences was higher during the early versus late follicular phase, \( t(29) = 3.20, p = 0.003, d = 0.82 \) (see Figure 8).
Figure 8. Affect by cycle phase. Mean valence and arousal ratings did not differ by menstrual cycle phase (Panel A). However, participants showed greater variability in valence and arousal ratings during the early versus late follicular phase (Panel B). Additionally, during the early versus late follicular phase, participants reported greater number of unpleasant (negatively valenced) high arousal affective states (Panel C). Error bars represent standard errors.
Positive and negative affective states, indexed by scores on the Positive and Negative Affect Schedule (PANAS), also differed by cycle phase (see Figure 9), but only on evening assessments. Positive and negative affect reported on the morning assessments did not differ by cycle phase (Morning positive affect scores: $t(29) = 1.64, p = 0.112, d = 0.25$; Morning negative affect scores: $t(29) = -0.14, p = 0.890, d = -0.019$). On the evening assessments, participants reported greater negative affect during the early versus late follicular phase at the trend level, $t(29) = 2.02, p = 0.052, d = 0.28$. Participants also reported greater positive affect on the evening assessments, $t(29) = 2.51, p = 0.018, d = 0.32$. 
3.6. Phase differences in trauma-related symptoms

We conducted non-parametric paired-samples tests (Wilcoxon signed-rank tests) to test differences in self-reported daily PTSD symptoms by cycle phase. Participants reported greater overall symptom severity during the early versus late follicular phase, $Z = 2.28$, $p = 0.023$, $r = 0.30$. Additionally, participants reported greater severity of negative alterations in cognitions and mood (D cluster) symptoms, $Z = 2.40$, $p = 0.016$, $r = 0.31$ and arousal (E cluster) symptoms, $Z = 2.40$, $p = 0.016$, $r = 0.31$. 

Figure 9. Positive and negative affective states by cycle phase. Self-reported positive and negative affective reported on morning assessments did not differ by cycle phase. However, on evening assessments, participants reported greater negative affect at the trend level and greater positive affect during the early versus late follicular phase. Error bars represent standard errors.
1.86, $p = 0.063$, $r = 0.24$, during the early versus late follicular phase. Self-reported re-experiencing (B cluster), $Z = 1.54, p = 0.125$, $r = 0.20$, and avoidance (C cluster), $Z = 0.60, p = 0.547$, $r = 0.08$, symptom severity did not differ by cycle phase (see Figure 10).

**Figure 10.** PTSD symptoms by cycle phase. During the early versus late follicular phase, participants reported greater total severity of PTSD symptoms, as well as greater severity of negative alterations in cognitions and mood (D cluster) symptoms and a trend toward greater arousal (E cluster) symptoms. Error bars represent standard errors.

3.7. Hormone fluctuations and daily affect

We tested Pearson correlations between the magnitude of estradiol increase from the early to late follicular phase (over the course of the 10-day EMA period) and self-reported affect for the duration of the EMA protocol and within each cycle phase. Increase in estradiol was not associated with overall valence, $r = 0.18, p = 0.332$, or arousal ratings, $r = 0.05, p = 0.796$, across the EMA period. However, increase in estradiol was associated at the trend level with greater variability in arousal, as indexed by standard deviation of arousal ratings, $r = 0.34, p = 0.063$. When we tested the association between estradiol increase and valence ratings within each cycle
phase, the strongest association between estradiol increase and standard deviation of arousal ratings was during the early follicular phase, $r = 0.36, p = 0.048$. Estradiol increase was also associated at the trend level with standard deviation of arousal ratings during the late follicular phase, $r = 0.32, p = 0.086$. Estradiol increase was not associated with self-reported PTSD symptoms during the EMA period (all $p$-values $> 0.05$).

3.8. Lifestyle factors by cycle phase

In addition to daily affect, participants also reported on sleep quality, exercise and substance use across the ten day EMA phase. Participant report of hours slept per night did not differ by menstrual cycle phase, $t(29) = 1.45, p = 0.158$. Participants reported relatively little exercise during the ten days, averaging less than one session during both the early follicular and late follicular phases. Exercise did not differ by cycle phase, $t(29) = 1.22, p = 0.234$. Our participants reported extremely low rates of alcohol and other substance abuse, with most participants reporting no consumption at any time, precluding a comparison of substance use by cycle phase.

4. Discussion

The overall goal of this dissertation was to test the effect of estradiol on daily affective experiences, including trauma-related symptoms. We addressed this goal using a multi-method approach, including cross-sectional and ecological momentary assessment (EMA) techniques, to examine stress reactivity in response to a trauma reminder and daily affect across low and high estradiol phases of the menstrual cycle. Despite well-established increased risk for severe and
persistent symptoms following trauma in women compared to men (e.g., Breslau et al., 2002; Haskell et al., 2010; Seedat et al., 2005) and well-established differences in affective and stress-relevant neural circuitry (e.g., Mareckova et al., 2016; Kajante & Phillips, 2006; Wrase et al., 2003), most of our current knowledge of PTSD has come from studies examining men or male animals. A better understanding of hormone fluctuations unique to women could explain increased symptom vulnerability and could inform treatment tailoring.

The previous literature on this topic is mostly divided into two main areas: research on menstrual cycle variability in stress and affect, and research on the effects of estradiol on indices of maladaptive fear, such as impaired fear extinction and intrusive memories, that are associated with risk for PTSD symptoms. Given the reported associations between low estradiol and mechanisms underlying increased risk for PTSD symptoms (e.g., impaired extinction; Graham & Milad, 2013), it follows that trauma-exposed women might be more prone to symptoms at low estradiol phases of the cycle. To date, however, very few studies (e.g., Nilni et al., 2015) have tested the effect of estradiol or menstrual cycle phase on affective experiences in trauma-exposed populations. Additionally, most previous studies of menstrual cycle variability in stress or affect have relied on data from a small number of laboratory sessions, or focused on comparisons between the early follicular and mid-luteal phases. However, high levels of both estradiol and progesterone characterize the mid-luteal phase, and progesterone is known to influence cognition, affect and psychological symptoms (e.g., Ertman, Andreano, & Cahill; Klump et al., 2013). Given this potential confound, previous research precludes definitive conclusions regarding the unique effects of estradiol on affective experiences. To address these gaps in the literature, we examined PTSD symptoms during a laboratory visit and over the course of a ten-day EMA procedure that spanned the early follicular and late follicular phases. This EMA design
allowed us to examine a portion of the menstrual cycle characterized by increases in estradiol, with minimal influence of progesterone. We verified appropriate menstrual cycle phase by measuring gonadal hormone levels, and confirmed that all participants’ progesterone levels remained within the normative range for the follicular phase. During the laboratory visit, we also tested associations between estradiol and physiological stress reactivity to a naturalistic trauma reminder.

Consistent with our first hypothesis, estradiol levels during the laboratory visit were associated with greater severity of PTSD symptoms reported during the clinical interview. This association was driven by re-experiencing, avoidance and arousal symptoms, with no association between estradiol and negative alterations in cognitions and mood. Increased report of symptoms associated with lower estradiol adds to the existing evidence for a protective effect of estradiol on intrusive thoughts and memories (re-experiencing symptoms) (e.g., Soni et al., 2013; Wegerer et al., 2014). Our data also suggest that during lower estradiol phases of the menstrual cycle, trauma-exposed women are more prone to cognitive and behavioral avoidance of trauma-related information and arousal symptoms, such as irritability, difficulty sleeping, exaggerated startle response, risky behaviors and hypervigilance for threat.

We also demonstrate differences in stress reactivity by menstrual cycle phase. Consistent with previous work in trauma-exposed populations (e.g., Carpenter et al., 2011), our sample as a whole showed SNS reactivity, but blunted HPA axis reactivity, in response to a trauma reminder. Inconsistent with our second hypothesis, however, individual variation in estradiol was not associated with physiological stress reactivity to a trauma reminder. This result was somewhat surprising, given the association between estradiol and self-reported arousal symptoms. However, when we divided the sample into two groups according to menstrual cycle phase
during the interview, group differences emerged, such that participants in lower estradiol phases (i.e., early follicular, early luteal, late luteal) had greater sympathetic and lower HPA axis stress reactivity compared to those in higher estradiol phases (i.e., late follicular/mid-cycle, mid-luteal). These data are consistent with evidence linking the stress response profile of higher SNS and lower HPA axis activity with greater affective symptoms in trauma-exposed populations (e.g., Ali & Pruessner, 2012).

The association between estradiol and arousal symptoms along with the absence of an association between estradiol and stress reactivity underscore the non-redundancy of self-report and physiological measures. Self-reported distress associated with lower estradiol was not explained by objective physiological differences in stress system function. Instead, estradiol levels at the time of assessment might influence current affective state, coloring perception of trauma-related symptoms and related distress over the past month. This interpretation is consistent with evidence for biased retrospective reports of affect (e.g., Ben-Zeev, Young, & Madsen, 2009; Mill et al., 2016) and physical symptoms (e.g., Brown & Moskowitz, 1997) in healthy people and clinical populations. Alternatively, differential stress reactivity in high compared to low estradiol menstrual cycle phases might speak to the importance of within-person estradiol fluctuations, in addition to individual differences. Previous evidence for the influence of estradiol on stress reactivity (e.g., Kajante & Phillips, 2006; Kirschbaum et al., 1999) could have been due to the absolute magnitude of estradiol levels, which differ by phase, or due to within-person increases and decreases in estradiol across the menstrual cycle. Our results provide stronger evidence for the latter in a non-clinical sample of naturally cycling young women with regular menstrual cycles.
Consistent with an effect of estradiol fluctuations on affect, and in line with our third hypothesis, daily affective experiences differed by menstrual cycle phase. Self-reported affect was more variable, indicating greater affective lability, during the early follicular (low estradiol) compared to late follicular (high estradiol) phase. In addition, during the early follicular phase, participants reported more frequent aversive affective experiences, falling within the unpleasant, high arousal quadrant of the affective circumplex. This quadrant includes affective states that can be highly distressing and potentially interfere with daily life function. These data suggest that affective lability during low estradiol phases might contribute to greater subjective distress, which is consistent with evidence demonstrating that affective variability contributes to negative affect (e.g., Wichers et al., 2010).

In addition to self-reported affect, daily total PTSD symptom severity was also greater during the early versus late follicular phase. This effect was driven by two symptom clusters: arousal symptoms and negative alterations in cognitions and mood. The arousal symptom cluster encompasses heightened physiological arousal, both tonically and in response to external stimuli (e.g., exaggerated startle response), whereas negative alterations in cognitions and mood encompasses persistent negative affect as well as negative cognitions, such as inappropriate self-blame and exaggerated negative beliefs (e.g., about the self, other people or the world). These data are consistent with evidence for poorer psychological well-being (e.g., Kanojia et al., 2013; Nillni et al., 2015) and increased physiological arousal (e.g., Kanojia et al., 2013) during low estradiol phases of the cycle. To our knowledge, this is the first study to demonstrate greater negative cognitions in trauma-exposed women during a low compared to high estradiol phase.

Finally, during the early follicular phase, participants more strongly endorsed both negative and positive affective states, although this effect only applied to evening reports.
Evening reporting of greater positive and negative affect provides additional evidence for more variable, rather than strictly negative, affect during low estradiol states. Morning reports of affect did not differ by cycle phase, underscoring the influence of time of day on self-reported affective experiences. Compared to morning reporting, evening reporting of daily affect was more sensitive to differences by menstrual cycle phase.

Our exploratory analysis of hormone fluctuations and daily affect revealed that greater estradiol increase from the early to late follicular phase was associated with greater variability in self-reported arousal across the EMA phase of the study. This association was strongest during the early follicular (low estradiol) phase. We speculate that greater variability in estradiol across the menstrual cycle might be associated with increased risk for affective instability during low estradiol cycle phases.

Overall, our data speak to the importance of estradiol fluctuations on daily affective experiences in trauma-exposed women. Further, given that we controlled for progesterone, we demonstrate unique effects of estradiol on daily affect. Our data suggest that estradiol buffers against negative affect and stress-related symptoms and is associated with more stable affect. Variation in daily affect associated with estradiol might reflect an evolutionary advantage. Greater symptoms of negative affect during lower estradiol portions of the cycle, particularly the late luteal phase, might have rendered women more withdrawn and cautious, potentially adaptive behaviors when a woman has become pregnant. Conversely, high estradiol levels and the associated mood buffer might have encouraged women to seek reproductive opportunities during the most fertile portion of the menstrual cycle. In a modern context, however, menstrual variation in mood might render women more susceptible to stress-related symptoms.
Fluctuations in daily affect could have particular implications for clinical populations. Client report of more trauma-related symptoms during low compared to high estradiol phases could influence diagnostic status or PTSD severity. Clinicians might therefore improve diagnostic accuracy by accounting for client menstrual cycle phase at the time of assessment. To maximize sensitivity to clients’ symptoms, clinicians might schedule assessments during low estradiol phases of the cycle.

Greater knowledge of menstrual cycle variability in affect can also inform treatment. Clinicians who work with PTSD populations could gather information about clients’ recent menstrual cycles to predict when clients will experience more frequent aversive daily experiences, including symptoms. Clinicians can also potentially tailor treatments and schedule interventions at specific points in the menstrual cycle.

Finally, the relation between lower estradiol and increased report of re-experiencing, avoidance and arousal symptoms suggests that individual differences in estradiol might influence risk for symptoms post-trauma. Identifying women at increased risk for symptoms due to low estradiol levels could enhance preventative interventions post-trauma.

5. Limitations and future directions

We demonstrate a profile of increased sympathetic and blunted HPA axis reactivity to a naturalistic trauma reminder during a low versus high estradiol phase. Differential stress reactivity by cycle phase suggests that estradiol fluctuations influence the coordination of stress system function. We interpret the absence of a relationship between estradiol and physiological stress reactivity as evidence for a stronger effect of estradiol fluctuations, rather than absolute estradiol levels, on stress reactivity in trauma-exposed women. However our interpretation is
somewhat limited by the fact that we were not able to directly test the effect of cycle phase on stress reactivity since we did not pre-assign lab visit timing based on cycle phase. To better address this issue, researchers might assess all participants during a specific cycle phase and subsequently test associations between estradiol and stress reactivity.

Additionally, all of our participants had gonadal hormone levels within normative ranges. The relation between estradiol and stress reactivity might be different in women with abnormally high or low hormone levels, menstrual cycle irregularities or endocrine disorders. Our results should be replicated in these populations. In these populations, the absolute magnitude of estradiol might have a stronger influence on stress reactivity.

Our data suggest more frequent aversive affective experiences and increased PTSD symptom severity during low compared to high estradiol menstrual cycle phases. We also report greater affective lability, indexed by the standard deviation of participant arousal ratings across the 50 daily timepoints, during the early follicular phase. However, our data analyses cannot provide information about specific patterns of affective instability. For example, we cannot determine whether increased variability was driven by greater within-day fluctuations or by greater day to day differences in affect across the early follicular phase. A more nuanced understanding of the patterns of affective variability would require replication of our study with a much larger sample, which would provide adequate statistical power for multilevel modeling analyses.

In addition, our ecological momentary assessment results should be replicated in clinical populations with PTSD. Our sample consisted of high-functioning trauma-exposed women, most of whom did not meet criteria for PTSD. A subset of participants met criteria for a provisional diagnosis of PTSD, but scored on the low end of the severity range. We speculate that our
observed differences in affect by menstrual cycle phase, which were detectable in high-functioning trauma-exposed women, would be more evident in clinical populations. However, it is possible that women with PTSD experience symptoms more consistently across the cycle, with no alleviation of symptoms during high estradiol phases.

Our results should also be replicated in trauma-exposed samples with specific trauma types, as the relation between estradiol and symptoms could differ by trauma type. Survivors of interpersonal compared to non-interpersonal trauma tend to report more PTSD symptoms (e.g., Luthra et al., 2009), especially negative cognitions (Kirkpatrick et al., 1989), which differed by menstrual cycle phase in our sample. Thus, the protective effect of estradiol on these particular symptoms might therefore be especially relevant for survivors of interpersonal trauma or for survivors of specific index traumas (e.g., sexual trauma). Due to our small numbers of participants reporting non-interpersonal trauma, and the heterogeneity in exposure to specific index traumas, we did not have adequate statistical power to test differences in the relation between estradiol and stress reactivity or affect by trauma type.

In addition to trauma type, age at trauma is another potential moderator of the relations among estradiol, stress reactivity, and symptoms. Existing evidence suggests that stress system function following trauma and its relation with symptoms varies as function of the time since trauma, such that blunted activity as (e.g., Weems & Carrion, 2007). Further, developmental stage at the time of trauma exposure can influence subsequent effects on brain development and vulnerability for symptoms (e.g., Andersen et al., 2008; Bick & Nelson, 2016; Sullivan et al., 2006). In our sample, time since trauma was not associated with cortisol ($r = -0.84$, $p = 0.61$) or alpha amylase ($r = 0.18$, $p = 0.27$) reactivity to the naturalistic stressor. However, larger sample
sizes would provide sufficient power to test trauma timing as a mediator or moderator of our reported effects.

Finally, although we interpret our data as evidence for greater negative affect and affective lability during low estradiol phases, differences between the early and late follicular phases could reflect biases in self-report as a function of time. There could be potential order effects, given that we could not counterbalance the early follicular phase (first five EMA days) and late follicular phase (last five EMA days). Recent evidence also suggests an “attenuation effect” in studies with experience sampling methods, whereby participants show initial elevations in reports of affective experiences, especially negative affective states, at the beginning of an assessment period (Shrout et al., 2018). The attenuation effect is generally strongest for initial assessment timepoints, with smaller effects for later timepoints on the initial day of assessment and no effects past the initial assessment day (Shrout et al., 2018). Given that our EMA data collapses across the entire early and late follicular phases (five days each), we suggest that any attenuation effects would have little impact on our results. Additionally, our results highlight greater affective lability, rather than simply increased reporting of negative affective states, during the early compared to late follicular phase.

Conclusions

The current dissertation makes a novel contribution to the literature on estradiol and affect by tracking momentary affect and daily symptoms across low and high estradiol menstrual cycle phases, rather than relying on single-timepoint retrospective reports, and by controlling for the influence of progesterone. Additionally, by demonstrating increased sympathetic and decreased stress reactivity during low versus high estradiol phases, the current study underscores
the importance of estradiol and menstrual cycle phase for research on stress reactivity in trauma-exposed women.

Our data contribute to our understanding of how fluctuations in sex hormones potentially contribute to risk for post-trauma symptoms. We demonstrate differential stress reactivity by menstrual cycle phase, whereby trauma-exposed women showed a profile of greater sympathetic but lower HPA axis activity to trauma reminders during a low compared to high estradiol phase. We propose that this profile of asymmetrical stress system response is a potential mechanism underlying greater risk for symptoms during low estradiol phases.

Our data add to the existing evidence for menstrual cycle variability in psychological symptoms (e.g., Edler et al., 2007; Gonda et al., 2008; Kanojia et al., 2013; Walder et al., 2012). We additionally address a gap in the literature and demonstrate that daily affective experiences differ between low and high estradiol phases, while progesterone remains low. We demonstrate more frequent reporting of aversive affective experiences and greater affective lability during a low compared to high estradiol phase. We suggest that trauma exposed women are more likely to experience PTSD symptoms during lower estradiol phases and speculate that this effect might be more evident in clinical PTSD populations. These data contribute to our understanding of changes in hormones that influence patterns of symptoms in women, and have the potential to inform treatment tailoring.
References


