EFFECTS OF ACUTE STRESS ON EMOTION RECOGNITION
IN THE MENSTRUAL CYCLE

A Thesis
Submitted to the Department of Psychology
In Partial Fulfillment of the Requirements
For the Degree of

Bachelor of Science Honours
in
Psychology
University of Regina

By
Michaela M. Flaman
Regina, Saskatchewan
April 2020

©Copyright 2020: M. Flaman
Abstract

The early follicular menstrual cycle phase is associated with enhanced female emotion recognition performance compared to the midluteal phase, likely due to differing estradiol and progesterone levels. Acute stress appears to also influence emotion recognition in females, but the direction of the effect is inconclusive. To fill in the gap in the literature, the present study examined the effects of acute stress on emotion recognition in specific phases of the menstrual cycle. Nineteen female psychology students between the ages of 18-29 years with regular menstrual cycles (i.e. 26-31 days in length) were recruited from the University of Regina via the Psychology Participant Pool. Participants performed a high or low stress task in the early follicular phase (low estradiol and progesterone levels) or midluteal phase (high estradiol and progesterone levels) before completing facial and auditory emotion recognition tasks. Results for auditory emotion recognition accuracy show the high stress condition outperformed the low stress condition for those in the early follicular phase group, but the opposite occurred in the midluteal phase group. Similar trends emerged for auditory emotion recognition reaction time and facial emotion recognition accuracy and reaction time. The present study suggests an interaction between sex hormones and the HPA axis on emotion recognition, but it is limited by its small sample size and estimation of sex hormone levels based on menstrual phase. Future studies could consider recruiting male participants, investigating oral contraceptive use, and administrating hormones to further examine the interaction between sex hormone levels and stress on emotion recognition performance.
Acknowledgements

I would like to thank my supervisor and role model, Dr. Laurie Sykes Tottenham, for her immense dedication, detailed feedback, and advanced expertise throughout the past year. I am grateful for the opportunity to work under such a hard-working, passionate woman. Additionally, I would like to thank Alexander Cameron for creating the Eprime versions of the emotion recognition tasks, Alexandra Ennis for creating the general reaction time tasks, and both of these individuals for previously collecting some of the data that I was able to use in my analyses. For donating their time to act as committee members for the Trier Social Stress Test, I would like to thank my volunteers, Daria Chernova, Gladys Orobosa, Minhal Mussawar, Suhana Patel, and Thomas Flicek. I would also like to thank my cohort, particularly Bethany Sander, Emilio Filomeno, Louise Castillo, Tenielle Workman, Asia Libke, and Eddye Kirk for always taking the time to listen to and empathize with me throughout the past year. To the staff at Campion College, thank you for the support, guidance, and opportunities you have given me over the past four years.
Dedication

I would like to dedicate my thesis to my family and friends, particularly my parents, Michael and Shauna Flaman; my sister, Jessica Flaman; my dog, Usher Flaman; and my dearest friend, Carly Hill, for their continuous love and support throughout the past year and my entire degree.
Table of Contents

Abstract.........................................................................................................................ii
Acknowledgments.........................................................................................................iii
Dedication.......................................................................................................................iv
Table of Contents............................................................................................................v
List of Tables.................................................................................................................vi
List of Illustrations.........................................................................................................vii

CHAPTER 1: Introduction.................................................................................................1
  1.1 Sex Differences in Emotion Processing and Recognition .................................2
  1.2 Emotion Recognition and Processing in the Menstrual Cycle............................2
  1.3 Emotion Recognition and Processing and the Stress Response.........................4
  1.4 Sex Hormones and the Stress Response...............................................................6
  1.5 Present Study.........................................................................................................7

CHAPTER 2: Method........................................................................................................9
  2.1 Participants............................................................................................................9
      2.1.1 Exclusion criteria...............................................................................................9
  2.2 Stress Manipulation..............................................................................................10
      2.2.1 High stress task...............................................................................................11
      2.2.2 Low stress task...............................................................................................11
  2.3 Measures and Materials......................................................................................11
      2.3.1 Emotion recognition tasks...............................................................................11
          2.3.1.1 Auditory emotion recognition task............................................................12
          2.3.1.2 Facial emotion recognition task.................................................................12
2.3.2 Acute stress measures…………………………………………………13
   2.3.2.1 State anxiety questionnaire……………………………………13
   2.3.2.2 Heart rate……………………………………………………………13
   2.3.2.3 Salivary cortisol levels……………………………………………14
2.3.3 Demographics and salivary screening questionnaire…………………14
2.3.4 Depressive symptoms questionnaire…………………………………14
2.3.5 Trait anxiety questionnaire……………………………………………15
2.3.6 General reaction time tasks……………………………………………15
   2.3.6.1 Auditory reaction time task………………………………………16
   2.3.6.2 Visual reaction time task…………………………………………16
2.4  Procedure……………………………………………………………16
   2.4.1 Participant recruitment………………………………………………16
   2.4.2 Phone screen session………………………………………………17
   2.4.3 Lab session…………………………………………………………17

CHAPTER 3:  Results……………………………………………………………..18
3.1  Preliminary Analyses………………………………………………………..19
   3.1.1 Independent samples t-test…………………………………………19
3.2  Main Analyses………………………………………………………………22
   3.2.1 Auditory emotion recognition reaction time………………………..22
   3.2.2 Auditory emotion recognition accuracy…………………………….22
   3.2.3 Facial emotion recognition reaction time……………………………28
   3.2.4 Facial emotion recognition accuracy………………………………..32
   3.2.5 Subjective measures…………………………………………………..32
3.2.6 Correlations

CHAPTER 4: Discussion

4.1 Limitations

4.2 Future Directions

REFERENCES

APPENDIX A: Ethics Approval
List of Tables

Table 1. Independent samples t tests comparing high and low stress conditions on acute stress measure change scores for salivary cortisol, STAI-state, and heart rate……………………………………20

Table 2. Pearson’s r correlations between emotion recognition performance and acute stress measure change scores for salivary cortisol, heart rate, and STAI-state…………………………36
List of Figures

Figure 1. Change in heart rate in low and high stress conditions.................................21
Figure 2. Change in state anxiety in low and high stress conditions.................................23
Figure 3. Change in salivary cortisol level for the second sample in low and high stress conditions.................................................................24
Figure 4. Change in salivary cortisol level for the third sample in low and high stress conditions.................................................................................25
Figure 5. Change in salivary cortisol level for the peak sample in low and high stress conditions.........................................................................................26
Figure 6. Nonsignificant four-way interaction for mean recognition reaction time of auditory intonations in voices........................................................................27
Figure 7. Four-way interaction for mean recognition accuracy of auditory intonations in voices.................................................................................................29
Figure 8. Mean recognition accuracy of angry nonsense sentences.................................30
Figure 9. Mean recognition accuracy of neutral sense sentences................................31
Figure 10. Nonsignificant three-way interaction for mean recognition reaction time of emotional facial expressions........................................................................33
Figure 11. Nonsignificant three-way interaction for mean recognition accuracy of emotional facial expressions.................................................................34
**Effects of Acute Stress on Emotion Recognition in the Menstrual Cycle**

Independent lines of research have shown that emotion recognition is influenced by sex (Hampson, van Anders, & Mullin, 2006; McClure et al., 2004; Scholten, Aleman, Montagne, & Kahn, 2005; Scholten, Aleman, & Kahn, 2008; Thayer & Johnsen, 2000; Williams et al., 2009), menstrual cycle phase (Derntl, Hack, Kryspin-Exner, & Habel, 2013; Derntl, Kryspin-Exner, Fernbach, Moser, & Habel, 2008a; Derntl et al., 2008b; Marečková et al., 2014; Pearson & Lewis, 2005; Rubin et al., 2011), and by the stress response (Duesenberg et al., 2016; Ennis, 2017; Feeney, Gaffney, & O’Mara, 2012; Quintana, Guastella, Outhred, Hickie, & Kemp, 2012; Smeets, Dziobek, & Wolf, 2009). Evidence suggests that these influences are largely due to sex hormones, such as estradiol, progesterone, and testosterone, and stress hormones (Derntk et al., 2008a; Derntl et al., 2008b; Derntl et al., 2013; Duesenberg et al., 2016; Ennis, 2017; Feeney et al., 2012; Goldstein et al., 2001; Guapo et al., 2009; Herman, Mcklveen, Solomon, Carvalho-Netto, & Myers, 2012; Kamboj, Krol, & Curran, 2015; Marečková et al., 2014; Pearson & Lewis, 2005; Reul & Kloet, 1985; Rubin et al., 2011; Smeets et al., 2009; Whittle, Yücel, Yap, & Allen, 2011; Witte, Savli, Holik, Kasper, & Lanzenberger, 2010). Although studies have investigated sex differences in the effects of the stress response on emotion recognition (Duesenberg et al., 2016; Ennis, 2017; Smeets et al., 2009), the research literature regarding the effects of stress on emotion recognition between menstrual cycle phases is lacking. It is important to examine the effects of stress on emotion recognition between menstrual cycle phases to gain insight into how acute stress may influence female individuals’ social interaction as hormone levels vary throughout their menstrual cycle, because emotion recognition is a critical component of interpersonal relationships. The present study sought to contribute to the
literature by observing the effects of an acute stressor on emotion recognition in specific menstrual cycle phases.

1.1 Sex Differences in Emotion Processing and Recognition

Studies suggest that circulating sex hormones, such as estradiol, progesterone, and testosterone, influence human emotion processing and recognition (Goldstein et al., 2001; Hampson et al., 2006; McClure et al., 2004; Scholten et al., 2005; Scholten et al., 2008; Thayer & Johnsen, 2000; Williams et al., 2009; Witte et al., 2010). There are high concentrations of sex hormone receptors in brain regions involved in emotion processing, such as the hippocampus and amygdala (Goldstein et al., 2001), and circulating sex hormone levels have been associated with structural brain changes in young adult brains (Witte et al., 2010), likely influencing brain function as well. The influence of sex hormones on emotion-processing brain regions is a likely explanation for the sex differences in emotion recognition, with females demonstrating better facial emotion recognition accuracy and decreased reaction time than males, especially for negative or threatening emotions (i.e., anger, sadness, and fear; Hampson et al., 2006; McClure et al., 2004; Scholten et al., 2005; Thayer & Johnsen, 2000; Williams et al., 2009). Females also demonstrate better accuracy in identifying emotion in voices for happy, sad, angry, and anxious intonations (Scholten et al., 2008). Sex differences in the neural activation of specific brain regions during emotion-processing have also been demonstrated, likely influenced by circulating sex hormones (see Whittle et al., 2011 for review).

1.2 Emotion Recognition and Processing in the Menstrual Cycle

Facial emotion recognition performance has been found to fluctuate across the natural female menstrual cycle (Derntl et al., 2013; Derntl et al., 2008a; Derntl et al., 2008b; Pearson & Lewis, 2005; Rubin et al., 2011), suggesting that estradiol and progesterone influence emotion
processing and recognition not only between sexes, but also within females. Females in the early follicular phase (low estradiol and progesterone levels) of their menstrual cycle have demonstrated greater facial recognition accuracy for sad, happy, fearful, angry, disgustful, and neutral expressions compared to females in the midluteal phase (high estradiol and progesterone levels) of their menstrual cycle (Derntl et al., 2013; Rubin et al., 2011). Similarly, females in the follicular phase (i.e. when progesterone levels are low, and estradiol levels are low but slowly rising and significantly high at the end) have demonstrated greater facial recognition accuracy for fearful, happy, angry, disgustful, sad, and neutral expressions compared to females in the luteal phase (i.e. generally high estradiol and progesterone levels); analyses confirmed that the mean estradiol and progesterone levels were lower in the follicular phase group than the luteal phase group, suggesting that increased estradiol and progesterone levels impair emotion recognition processing in females (Derntl et al., 2008a; Derntl et al., 2008b). Additionally, across naturally cycling females, blood progesterone levels have been negatively correlated with accuracy scores in identifying faces expressing anger, disgust, fear, joy, sadness, and neutral (Derntl et al., 2008a), and salivary progesterone levels have been positively correlated with reaction times in identifying faces expressing anger, happiness, sadness, and neutral (Kamboj et al., 2015). In naturally cycling females, the group of females tested in their early to midfollicular phase (low or rising estradiol levels; low progesterone levels) had worse facial emotion recognition accuracy of fear compared to the group of females tested prior to ovulation (high estradiol levels; low progesterone levels), indicating that high estradiol levels may enhance the facial recognition of fear (Pearson & Lewis, 2005). In contrast, in naturally cycling females, blood estradiol levels have been negatively correlated with accuracy in facial emotion.
recognition of anger (Guapo et al., 2009), and salivary estradiol levels have been negatively correlated with accuracy in the facial emotion recognition of disgust (Kamboj et al., 2015).

Menstrual phase differences have also been associated with altered neural activation while processing emotional facial expressions. While processing angry facial expressions, stronger amygdala activation in the follicular phase compared to the luteal phase has been found (Derntl et al., 2008b), and increased activation has been demonstrated in the fusiform face area in females mid-cycle compared to females during menstruation (Marečková et al., 2014). Although differences in auditory emotion recognition at specific time points in the female menstrual cycle have not been explored, sex differences in auditory emotion recognition suggest that sex hormones such as estradiol and progesterone are likely involved in auditory emotion recognition.

1.3 Emotion Processing and the Stress Response

Acute stress has been found to be associated with facial emotion recognition (Duesenberg et al., 2016; Ennis, 2017; Feeney et al., 2012; Quintana et al., 2012; Smeets et al., 2009). The human body has two types of stress responses: a fast-acting neural response via the activation of the sympathetic nervous system and suppression of the parasympathetic nervous system, and a slower response via hormones (Chrousos & Gold, 1992). The fact-acting neural stress response leads to a wide variety of responses, including an increased heart rate (Sztajzel, 2004) and increased blood pressure in certain regions (Osborn, 1997). Resting heart rate variability, a measure of parasympathetic nervous system activation (and hence, sympathetic nervous system suppression), is positively associated with facial emotion recognition (Quintana et al., 2012).

The slower stress response is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, in which a physical or psychological stressor leads to the hypothalamus releasing corticotropin-releasing hormone (CRH) into the anterior pituitary gland through small blood vessels, triggering
the release of adrenocorticotropic hormone (ACTH) into the bloodstream (Herman et al., 2012). Once ACTH reaches the adrenal cortex, glucocorticoids are released into the bloodstream to cause a wide range of effects throughout the body. Cortisol, the prominent glucocorticoid released by the human body, is able to cross the blood-brain barrier (Pardridge & Mietus, 1979), and animal studies show that there are high densities of glucocorticoid receptors in emotion-processing brain regions, especially the hippocampus and amygdala (Reul & Kloet, 1985); therefore, cortisol levels are able to influence emotion-processing (Herman et al., 2012).

Numerous studies have demonstrated the association between acute cortisol levels and emotion recognition (Duesenberg et al., 2016; Feeney et al., 2012; Smeets et al., 2009). Salivary cortisol levels in a sample of males and females has been negatively correlated with reaction times for recognizing joyful and angry facial expressions, and negatively correlated with accurate recognition of neutral facial expressions (Feeney et al., 2012). Regarding auditory emotion recognition, both male and female participants with increased salivary cortisol levels in response to social stress demonstrated higher accuracy and lower reaction times for anger, fear, and disgust compared to those not demonstrating a cortisol increase (Ennis, 2017).

Additionally, sex differences have been found in how salivary cortisol levels after social stress are associated with emotion recognition (Ennis, 2017; Smeets et al., 2009). Male high cortisol responders have been found to be more accurate at recognizing emotional states of people in a video compared to male low cortisol responders, whereas female low cortisol responders have been found to be more accurate at recognizing emotional states of people in a video (which displayed both auditory and facial emotions) compared to both controls and female high cortisol responders. Regarding facial emotion recognition reaction times, after participants experienced a social stressor, females who demonstrated a salivary cortisol stress response were
slower than female non-responders, whereas male responders were faster than male non-responders (Ennis, 2017). Females also exhibited faster facial emotion recognition reaction times than males after completing a low stress task, but slower reaction times than males after completing a high stress task (Ennis, 2017). In contrast, the oral administration of the exogenous version of cortisol was not associated with a sex difference in facial emotion recognition – females even had higher accuracy in recognizing angry facial expressions than men (Duesenberg et al., 2016). A likely explanation for these contrasting results is the lack of control of estradiol and progesterone levels in the female samples. Although Ennis (2017) and Smeets and colleagues (2009) excluded females on oral contraceptives in their studies, they did not examine the menstrual cycle phase or estradiol and progesterone levels of their female participants. In contrast, most of the females in Duesenberg and colleagues’ study (2016) were either on oral contraceptives, in which estradiol and progesterone levels are subnormal, or in the luteal phase, during which estradiol and progesterone levels vary, initially increasing and then decreasing. The inclusion of females with subnormal estradiol and progesterone levels in the Duesenberg study may have prevented a sex difference from being demonstrated for all emotions other than anger, while also masking potential menstrual phase effects.

1.4 Sex Hormones and the Stress Response

There is a bidirectional interaction between the HPA axis and the female hypothalamic-pituitary-gonadal (HPG) axis (Acevedo-Rodriguez et al., 2018; Chrousos, Torpy, & Gold, 1998; Toufexis, Rivarola, Lara, & Viau, 2014), which could explain inconsistent findings between studies that do not account for estradiol and progesterone levels when examining the impact of stress and sex on emotion recognition. The hypothalamus mediates the release of estradiol and progesterone via the HPG axis; the hypothalamus releases gonadotrophin-releasing hormone
(GnRH) into the anterior pituitary through small blood vessels, causing the anterior pituitary to release gonadotrophins into the bloodstream (Acevedo-Rodriguez et al., 2018). In females, these gonadotrophins reach the ovaries and lead to the release of estradiol and progesterone. Estradiol and progesterone have been shown to influence glucocorticoid levels (Altemus, Roca, Galliven, Romanos, & Deuster, 2001; Viau & Meaney, 1991). For example, the injection of estradiol or both estradiol and progesterone in female rats post-stressor led to longer lasting post-stressor elevations of corticosterone compared to controls (Viau & Meaney, 1991). In humans, increased levels of ACTH post-exercise have been found in females in the midluteal phase compared to the early follicular phase (Altemus et al., 2001). Estradiol’s and progesterone’s apparent enhancement of the stress response may be responsible for females’ poorer performance on emotion recognition after a stressor. Hormones involved in the HPA axis have been shown to inhibit all levels of the HPG axis (Chrousos, Torpy, & Gold, 1998). CRH has been shown to inhibit the hypothalamic secretions of GnRH, and glucocorticoids have been shown to decrease the release of gonadotrophins, estradiol, and progesterone, as well as desensitize tissues to estradiol. This indicates that acute stress is important to measure when considering the effect of menstrual cycle phase or estradiol and progesterone levels on emotion recognition performance because acute stress could influence the circulating levels of estradiol and progesterone, and, as a result, influence emotion recognition processes.

1.5 Present Study

The present study contributes to the research literature by being the first to look at the effects of acute stress on emotion recognition during specific phases of the menstrual cycle. Because the research literature on emotion recognition at specific time points in the menstrual cycle and the literature on stress effects on emotion recognition often neglect exploring auditory
emotion recognition, both auditory and facial emotion recognition tasks were included in the present study. A between-subjects design was used to compare the auditory and facial emotion recognition accuracy and reaction time for various emotions after either a high or low stress task in the midluteal phase (associated with high estradiol and progesterone levels) and the early follicular phase (associated with low estradiol and progesterone levels). The comparison of stress effects on participants’ emotion recognition performance at these specific menstrual cycle phases allows for inferences to be made about interactive effects of sex and stress hormones in women.

Based on the previous research discussed above, it was expected that the early follicular phase would be associated with higher accuracy and lower reaction times on the emotion recognition tasks compared to the midluteal phase. It was also expected that the low stress group would demonstrate higher accuracy and lower reaction times compared to the high stress group. Because participants are exposed to a stressor, the effect of emotion type may differ for threatening emotions, such as anger, fear, and disgust; however, due to the opposing associations between estradiol and progesterone with the recognition of anger, the results are difficult to predict. Moreover, because high estradiol and progesterone levels appear to increase the cortisol stress response (Altemus et al., 2001; Viau & Meaney, 1991), it was hypothesized that lowest accuracy and highest reaction times on both the auditory and facial emotion recognition tasks would be found in the midluteal phase group after the high stress task, and the highest accuracy and lowest reaction times on both the auditory and facial emotion recognition tasks would be found in the early follicular phase group after the low stress task. At the individual level, it was also hypothesized that the size of the stress response demonstrated would be negatively associated with facial and auditory emotion recognition accuracy, and positively associated with facial and auditory emotion recognition reaction time, showing that women who experience
larger responses to stress have poorer emotion recognition in both the early follicular phase and midluteal phase, especially in the midluteal phase.

**Method**

**2.1 Participants**

Nineteen women with regular menstrual cycles (i.e., 26-31 days in length) between 18-29 years of age were recruited to participate in this study. Thirteen of the participants were recruited in winter 2020, and the rest were recruited between winter 2016 and winter 2017. Participants were enrolled in a 100-level or 200-level psychology course at the University of Regina at the time of data collection and were recruited to participate in this study via the Department of Psychology Participant Pool. Participants were compensated with an additional two percent toward their final mark in a 100-level or 200-level psychology course they were registered in at the time of participation.

**2.1.1 Exclusion criteria**

Prospective participants were excluded from participating in this study if they met specific criteria that could lead to confounding or extraneous variables. Participants who habitually smoked or used nicotine, were currently pregnant or lactating, or were currently taking or recently took (in the past 3 months) hormonal medications (including hormonal contraceptives) were excluded because these factors are associated with altered hormones, stress responses, and cognitive performance (al’Absi, Amunrud, & Wittmers, 2002; Badrick, Kirschbaum, & Kumari, 2007; Conway et al., 2007; Derntl et al., 2008a; Kamboj et al., 2015; Kassel, Sita, & Miller 1996; Michnovicz, Hershcopf, Naganuma, Bradlow, & Fisherman, 1987; Steptoe & Ussher, 2006; Stroud, & Paronis, 2003). Participants with an uncorrected visual or auditory impairment were excluded, to avoid an untoward influence on the emotion recognition
tasks. Participants were excluded if they put anything other than water in their mouth in the hour prior to participating (e.g. food, drinks, gum, toothbrush/floss, etc.), to limit salivary sample contamination. Participants with oral injuries or disease that could cause bleeding or high levels of bacteria were also excluded to avoid spiked cortisol concentrations, and to limit salivary sample contamination and researchers’ exposure to pathogens. Participants were also excluded if they consumed alcohol within 12 hours or caffeine within 3 hours before the lab session because acute alcohol and caffeine consumption is associated with changes in stress measures (Charney, Heninger, & Jatlow, 1985; Nickell & Uhde, 1994; Romanowicz, Schmidt, Bostwick, Mrazek, & Karpyak, 2011), hormone levels (Charney et al., 1985; Nickell & Uhde, 1994; Sarkola, Mäkisalo, Fukunaga, & Eriksson, 1999), and cognitive and motor functioning (Hernández, Vogel-Sprott, Huchín-Ramirez, & Aké-Estrada, 2006; Peterson, Rothfleisch, Zelazo, & Pihl, 1990; Rogers et al., 2005). Additionally, participants were excluded if they engaged in vigorous exercise within 90 minutes or woke up within 3 hours prior to the lab session to limit the influence of these factors on cortisol levels (Edwards, Clow, Evans, & Hucklebridge, 2001; Kudielka & Kirschbaum, 2003; Rudolph & McAuley, 1995). Participants with a psychiatric illness or major medical condition were also excluded to avoid inducing stress in at-risk populations and to avoid recruiting participants with altered cortisol levels that could influence the cortisol in the salivary samples collected (Wolkowitz, Reus, & Mellon, 2011).

2.2 Stress Manipulation

One of two stress conditions was assigned to each participant. This assignment was pseudo-random, as it was determined by the availability of the research volunteers; one research volunteer was needed to be available for the high stress task to occur, so if no research volunteers were available, the participant experienced the low stress task.
2.2.1 High stress task

The Trier Social Stress Test (TSST) was used in the high stress condition in attempt to induce a stress response in participants (Kirschbaum, Pirke, & Hellhammer, 1993). The task consisted of participants taking part in a two-minute speech preparation, five-minute speech performance, and five-minute arithmetic task in front of a panel of two judges with neutral demeanors. Participants were asked to take on the role of a job applicant, to discuss their strengths and weaknesses in regard to their ideal job. Notes were taken by participants during the speech preparation period, but the notes were not allowed to be used during the speech performance. The arithmetic task consisted of participants counting backwards from 2000 by seven, with the judges asking participants to restart whenever an error was made. This task has been shown to increase heart rate and cortisol levels (Kirschbaum et al., 1993).

2.2.2 Low stress task

Similar to the TSST, participants in the low stress condition were asked to take part in a two-minutes speech preparation, five-minute speech performance, and a five-minute arithmetic task, except participants partook in these tasks by themselves, without the panel of judges. Additionally, the speech topic was participants’ favourite movie or book, and the arithmetic task was counting upward by ones starting at zero. This task was modeled after the TSST placebo task introduced by Het, Rohleder, Schoofs, Kirschbaum, and Wolf (2009).

2.3 Measures and Materials

2.3.1 Emotion recognition tasks

Two emotion recognition tasks were administered via Eprime: one auditory and one facial. The order the trials were presented in each task were randomized for each participant.
2.3.1.1 Auditory emotion recognition task. Green’s Emotion Perception Task was used to assess auditory emotion recognition (Green, Flaro, & Allen, 1999). Each trial consists of a recording of a sentence spoken by the same woman, which the participants heard via headphones, with either fear, anger, joy, sadness, or neutral intonations expressed in her voice. Ninety trials occurred for each participant: 45 sense sentences (complete sentences with meaning), and 45 nonsense sentences (sentences made with nonmeaningful combined syllables). A total of six different sentences occurred: three sense, and three nonsense. Each trial combination of intonation and sentence type was repeated three times for each participant. This task has been shown to have high internal reliability and high test-retest reliability. After each sentence, for each trial, the five emotions were displayed in words on a monitor, with corresponding numbers representing the five intonations that participants may hear (1 for happy, 2 for sad, 3 for anger, 4 for fear, and 5 for neutral). Participants were asked to press the corresponding number on the keyboard as quickly and as accurately as possible for each trial, so both auditory emotion recognition accuracy and reaction time (in ms) could be recorded.

2.3.1.2 Facial emotion recognition task. The face set produced by Matsumoto and Ekman (1989) was used in the facial emotion recognition task. The faces in this set have been confirmed to have facial muscle positions that match the emotion they are intended to convey according to the Facial Action Coding System (Ekman & Friesen, 1978), without extraneous muscle activation, and emotion intensity is generally constant with moderate to high intensity for each face. Twenty-four trials occurred for each participant, with one trial for each of the possible combinations of faces including two ethnicities (Japanese or Caucasian), two sexes (male or female), and six emotional expressions (fear, anger, joy, sadness, surprise, or disgust) presented on a monitor. Each face only appeared once and for each trial, on the monitor, the face began
displaying a neutral facial expression and morphed to a specific emotional expression in two seconds, which was done using FantaMorph software. After the stimulus was shown, for each trial, the six emotion types were be displayed in words on a monitor, with corresponding numbers representing the six facial expressions that participants may have seen (1 for happy, 2 for sad, 3 for angry, 4 for fear, 5 for disgust, and 6 for surprise). Participants were asked to press the corresponding number on the keyboard as quickly and as accurately as possible for each trial; both facial emotion recognition accuracy and reaction time (in ms) were recorded.

2.3.2 Acute stress measures

Several measures of acute stress were taken throughout the lab session to measure the change in participants’ stress responses throughout the study.

2.3.2.1 State anxiety questionnaire. Spielberger’s State-Trait Anxiety Inventory Form Y-1 (STAI state) was used to measure participants’ subjective stress (Spielberger et al., 1970). The STAI state is a self-report, 20-item measure of state anxiety levels with internal consistency reliability coefficients ranging from .65 to .96, with a median of .92, and a larger range in the test-retest reliability coefficient because it measures transient anxiety levels, with coefficients ranging from .34 to .96, with a median of .68 (Barnes et al., 2002). Each item consists of an anxiety symptom that participants can indicate on a 4-point Likert scale how they feel right now, at the moment (1 indicating not at all, 2 indicating somewhat, 3 indicating moderately so, and 4 indicating very much so). Scores for each item were summed to get a total STAI state score, with higher scores indicating more state anxiety.

2.3.2.2 Heart rate. A wireless blood pressure cuff made by iHealth Lab was used to measure the participants’ heart rate as an index of sympathetic nervous system activity. Heart
rate was measured at five specific time points in the lab session via an iHealth application on a smartphone, and participants wore the cuff around their non-dominant wrist.

2.3.2.3 Salivary cortisol levels. Salivary samples used to assess cortisol were collected from participants at three time points in the lab session: at the beginning of the lab session, approximately 20 minutes after the start of the stressor, and approximately 30 minutes after the start of the stressor. The last two time points have been shown to demonstrate peak salivary cortisol levels after the TSST (Kirschbaum et al., 1993). Saliva was collected by participants depositing it through a short straw into microcentrifuge tube. During collection, participants were in a room alone for privacy, with photos of various foods to help induce salivation. Salivary samples were immediately placed in a -40°C freezer, where they were stored for up to six weeks before being analyzed. Commercially available Salimetrics cortisol kits were used for the salivary assays and the intraassay cv was 6.94.

2.3.3 Demographics and salivary screening questionnaire

A questionnaire was administered to participants at the start of the lab session to collect demographic information, brief menstrual cycle and medical information, and recent activities participants may have engaged in that could influence their salivary cortisol levels, the integrity of the samples, or their performance on the laboratory tasks.

2.3.4 Depressive symptoms questionnaire

The Center for Epidemiological Studies Depression Scale (CES-D) was used to measure participants’ depressive symptoms (Radloff, 1977), which have been shown to be related to salivary cortisol levels (Gallagher-Thompson et al., 2006). The CES-D is a self-report, 20-item scale that has been shown to have high internal consistency reliability for measuring depressive symptoms with coefficients between .85 and .90, with a coefficient of 0.87 for post-secondary
psychology students, and a moderate test-retest reliability with a correlation between .45 and .70 (Radloff, 1977, 1991). Each item consists of a depressive symptom that participants’ rate the frequency of having experienced in the past week, indicated on a four-point Likert scale (0 indicating rarely or none of the time (less than one day), 1 indicating some or a little of the time (one to two days), 2 indicating occasionally or a moderate amount of time (three to four days), and 3 indication most or all of the time (five to seven days)). The scores for each item were summed to get a total CES-D score, with higher scores indicating more depressive symptoms.

2.3.5 Trait anxiety questionnaire

Spielberger’s State-Trait Anxiety Inventory Form Y-2 (STAI trait) was used to measure participants’ trait anxiety levels (Spielberger, Gorsuch, & Lushene, 1970) because chronic anxiety has been associated with altered cortisol levels. The STAI-trait is a self-report, 20-item measure of trait anxiety levels with internal consistency reliability coefficients ranging from .72 to .96, with a median of .90, and test-retest reliability coefficients ranging from .82 to .94, with a median of .88 (Barnes, Harp, & Jung, 2002). Each item consists of an anxiety symptom that participants indicated on a 4-point Likert scale how often they generally experience each symptom (1 indicating almost never, 2 indicating sometimes, 3 indicating often, and 4 indicating almost always). Scores for each item were summed to get a total STAI-trait score, with higher scores indicating more trait anxiety.

2.3.6 General reaction time tasks

General auditory and visual reaction time tasks were also administered via Eprime so that individual differences in general response speed could be accounted for as covariates in analyses. The order in which the trials were presented in each task was randomized for each participant.
2.3.6.1 Auditory reaction time task. Each of the trials consisted of an auditory recording of a colour word, which the participant heard via headphones. After the stimulus was presented, for each trial, the five colour types were displayed in words on a monitor, with corresponding numbers representing the five colour words that participants may have heard (blue, green, red, yellow, or orange). Participants were asked to press the corresponding number on the keyboard as quickly and as accurately as possible for each trial; both recognition accuracy and reaction time (in ms) were recorded.

2.3.6.2 Visual reaction time task. Each of the 24 trials consisted of a visual presentation of a coloured oval on a monitor. After the stimulus was shown, for each trial, the six colour types were displayed in words on the monitor, with corresponding numbers representing the six colours that participants may have seen (blue, green, red, yellow, purple, and orange). Participants were asked to press the corresponding number on the keyboard as quickly and as accurately as possible for each trial; both recognition accuracy and reaction time (in ms) were recorded.

2.4 Procedure

2.4.1 Participant recruitment

After approval was granted by the University of Regina Ethics Board (see Appendix A) to carry out the study, an advertisement containing details about the study (i.e., purpose, questionnaires, tasks, exclusion criteria, location, duration, participant compensation) was posted on the University or Regina’s Participant Pool website. If prospective participants were interested, they were asked to contact the researcher to set up a time and date for a phone screen session.
2.4.2 Phone screen session

The phone screen session repeated the details posted in the advertisement, and additionally ask prospective participants about factors that may influence their hormone levels or performance on the tasks, including brief demographic information and medical history. For privacy reasons, only each participant’s first name, phone number, and email address were documented and attached to their phone screen questionnaire. These documents were immediately placed in a locked room, in a locked cabinet. Prospective participants were also asked details about their menstrual cycle, so that a lab session could be scheduled at the soonest available date either during their early follicular phase (between day two to six, when estradiol and progesterone levels are low) or their midluteal phase (between day 18-24, when estradiol and progesterone levels are high) of their menstrual cycle. Prospective participants were given a list of activities to avoid before the lab session, to limit the influence of factors on their hormone levels or performance, and had the opportunity to ask any questions about their participation in the study before the lab session.

2.4.3 Lab session

Lab sessions were scheduled between 12:00pm to 6:00pm to limit the effects of diurnal hormone fluctuations (Kirschbaum & Hellhammer, 1989), and were held in RIC 408.3. At the beginning of the lab session, prospective participants were given the consent form to carefully look over, ask questions about, and sign if they agreed to participate. It was emphasized that participants could withdraw from the study at any time without any consequences, prior to the session ending. Once informed consent was obtained, participants began the lab session by being taken to the saliva collection for an oral rinse in attempt to limit contamination of the saliva samples. Participants then completed the demographics and salivary screening questionnaire,
followed by the CES-D, and then the STAI-state and -trait. All participants indicated that they followed the guidelines, and avoided the activities listed that could possibly influence their hormone levels or performance. Next, participants’ baseline heart rate was measured, and then they were taken to the saliva collection room to collect their baseline saliva sample. After the saliva collection, participants began the stress task, with their heart rate being measured at approximately two minutes into both the speech performance and arithmetic task. Two participants ended the arithmetic task in the high stress task early, so a recording was not taken. Next, the emotion recognition tasks took place, with the auditory emotion recognition task and facial emotion recognition task being counterbalanced to limit order effects, and heart rate was measured two minutes into the auditory emotion recognition task, and 30 seconds into the facial emotion recognition task. In some cases, there were issues with the blood pressure cuff, so the timing of the measurements was not exact. Participants then completed a second saliva sample collection (approximately 10 minutes post-end of stressor), a second STAI-state, and then the final saliva sample collection (approximately 20 minutes post-end of stressor). Lastly, the general reaction time tasks were administered, with the auditory and visual tasks being counterbalanced to limit order effects. At the end, participants were provided with an educational debriefing and given the phone number for counselling services, in case they want to discuss the stressors in their lives.

**Results**

A total of 19 females participated in the study, with 8 being in the early follicular phase of their menstrual cycle (4 in the low stress condition) and 11 being in the midluteal phase of their menstrual cycle (4 in the low stress condition).
3.1 Preliminary Analyses

3.1.1 Independent samples t-test

To begin statistical analyses, independent samples t-test were completed as a manipulation check. The change in salivary cortisol levels, change in heart rate, and change in state anxiety, were compared between the high stress condition and low stress condition to determine if there was a difference in the change in stress response between the two groups. Change in salivary cortisol levels was determined for the second and third samples separately, by subtracting participants’ baseline salivary cortisol level from the salivary cortisol level of their second and third samples. The change in peak salivary cortisol levels was also determined by subtracting participants’ baseline salivary cortisol levels from the highest value of their second and third samples. Change in heart rate was determined by subtracting participants’ baseline value from the mean heart rate during the stress task. For two of the participants, only one heart rate measurement was able to be taken during the stress task, so only the one value obtained was used in computing the change score, instead of the mean of the two values. Change in state anxiety was determined by subtracting participants’ score on their baseline STAI state from the second STAI state.

According to Levene’s test for equality of variances, equal variances were assumed for all of the analyses (ps > .05). As displayed in Table 1, change in heart rate was significantly different for the high stress condition and the low stress condition (see Figure 1), indicating that the high stress condition experienced a greater increase in heart rate (acute stress) during the stress task compared to the low stress condition. Change in STAI-state and change in salivary cortisol levels for the second, third, and peak samples were not significant between the high stress condition and the low stress condition. This indicates that there were no significant
Table 1

Independent samples t tests comparing high and low stress conditions on acute stress measure change scores for salivary cortisol, STAI-state, and heart rate.

<table>
<thead>
<tr>
<th>Measure</th>
<th>T</th>
<th>Df</th>
<th>p</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAIchange</td>
<td>-1.682</td>
<td>17</td>
<td>.056</td>
<td>4.640</td>
</tr>
<tr>
<td>Cort21</td>
<td>-1.501</td>
<td>17</td>
<td>.076</td>
<td>.062</td>
</tr>
<tr>
<td>Cort31</td>
<td>-1.515</td>
<td>17</td>
<td>.074</td>
<td>.057</td>
</tr>
<tr>
<td>PeakCortChange</td>
<td>-1.427</td>
<td>17</td>
<td>.084</td>
<td>.062</td>
</tr>
<tr>
<td>HRchange</td>
<td>-2.176</td>
<td>17</td>
<td>.022</td>
<td>5.216</td>
</tr>
</tbody>
</table>

*Note. P values are 1-tailed.*
Figure 1

Change in heart rate in low and high stress conditions.

Note. Error bars are mean +/- 1 SEM.
differences between the high stress condition and low stress conditioning regarding change in state anxiety and salivary cortisol levels during the session (see Figure 2-5); however, trends are in the expected direction such that the high stress condition had a larger increase in state anxiety and cortisol levels during the session compared to the low stress condition. As a result, the stress manipulation was considered relatively successful.

3.2 Main Analyses

3.2.1 Auditory emotion recognition reaction time

A 2 (menstrual phase: early follicular, midluteal) by 2 (stress condition: high stress or low stress) by 2 (sentence type: sense, nonsense) by 5 (auditory emotion type: fear, anger, joy, sadness, neutral) mixed-design ANOVA was conducted for auditory emotion recognition reaction time. The dependent variable was the mean in ms of the time taken to respond after the end of the stimulus sentence presentation for correct responses only. The mean score from the general auditory reaction time task was used as a covariate. There were no significant main effects of menstrual phase or stress group, nor were there any significant interactions involving these variables (all $p$s > .18). See Figure 6 for the nonsignificant 4-way interaction.

3.2.2 Auditory emotion recognition accuracy

A 2 (menstrual phase: early follicular, midluteal) by 2 (stress condition: high stress or low stress) by 2 (sentence type: sense, nonsense) 5 (auditory emotion type: fear, anger, joy, sadness, neutral) mixed-design ANOVA was done for auditory emotion recognition accuracy (percentage of correct responses). The interaction between menstrual phase and stress group approached significance $F(1,15) = 3.81, p = .07, \eta^2_p = .20$, as did the interaction between emotion, menstrual phase, and stress group, $F(4,60) = 2.44, p = .06, \eta^2_p = .14$. These interactions, however, were superseded by a significant 4-way interaction between sentence type, emotion
Figure 2

Change in state anxiety in low and high stress conditions.

Note. Error bars are mean +/- 1 SEM.
Figure 3

*Change in salivary cortisol level for the second sample in low and high stress conditions.*

Note. Error bars are means +/- SEM.
Figure 4

*Change in salivary cortisol level for the third sample in low and high stress conditions.*

Note. Error bars are mean +/- SEM.
Figure 5

*Change in salivary cortisol level for the peak sample in low and high stress conditions.*

Note. Error bars are mean +/- SEM.
Figure 6

Nonsignificant four-way interaction for mean recognition reaction time of auditory intonations in voices.

Note. Error bars are mean +/- SEM.
type, stress group, and menstrual phase, $F(4,60) = 2.87, p = .03, \eta_p^2 = .16$ (see Figure 7). No other main effects or interactions involving menstrual phase or stress group were significant (all $ps > .28$).

To decompose the 4-way interaction, each possible combination of emotion type and sentence type were analyzed separately in a series of 2 (menstrual cycle phase: early follicular, midluteal) by 2 (stress condition: low, high) between-subjects ANOVAs. For nonsense sad sentences, there was a trend toward a main effect of stress group, $F(1,15) = 3.29, p = .09, \eta_p^2 = .18$, indicating that the low stress condition trends toward a greater accuracy than the high stress condition (low stress: $M = .99, SD = .04$; high stress: $M = .90, SD = .11$). Regarding the recognition accuracy of nonsense angry sentences, there was a significant interaction between menstrual cycle phase and stress condition, $F(1,15) = 11.22, p = .004, \eta_p^2 = .43$ (see Figure 8). For the recognition accuracy of sense sad sentences, a significant main effect was found for stress condition, $F(1,15) = 8.68, p = .01, \eta_p^2 = .37$, indicating that the low stress condition had greater accuracy than the high stress condition (low stress: $M = .94, SD = .09$; high stress: $M = .74, SD = .16$). There was also a significant interaction between menstrual cycle phase and stress condition on the recognition accuracy of sense neutral sentences $F(1,15) = 10.74, p = .005, \eta_p^2 = .42$ (see Figure 9). No other significant main effects or interactions were found (all $ps > .12$).

3.2.3 Facial emotion recognition reaction time

A 2 (menstrual phase: early follicular, midluteal) by 2 (stress condition: high stress or low stress) by 6 (facial emotion type: fear, anger, joy, sadness, surprise, or disgust) mixed-design ANOVA was conducted for facial emotion recognition reaction time. The dependent variable was the mean in ms of the time taken to respond after the end of the stimulus sentence presentation for correct responses only. The mean score from the general visual reaction time
**Figure 7**

*Four-way interaction for mean recognition accuracy of auditory intonations in voices.*

![Graph showing four-way interaction for mean recognition accuracy of auditory intonations in voices.](image)

Note. Error bars are mean +/- SEM.
Figure 8

Mean recognition accuracy of angry nonsense sentences. Demonstrates an interaction between menstrual cycle phase and stress condition. During the early follicular phase, accuracy of angry nonsense sentences is greater in the high stress condition compared to the low stress condition, but in the midluteal phase, accuracy is greater in the low stress condition compared to the high stress condition.

Note: Error bars are mean +/- SEM.
Figure 9

Mean recognition accuracy of neutral sense sentences. Demonstrates an interaction between menstrual cycle phase and stress condition. During the early follicular phase, accuracy of neutral sense sentences is greater in the high stress condition compared to the low stress condition, but in the midluteal phase, accuracy is greater in the low stress condition compared to the high stress condition.
task used as a covariate. There were no significant main effects of menstrual phase or stress group, nor were there any significant interactions involving these variables (all $p$s $>.13$). See Figure 10 for the nonsignificant 3-way interaction.

### 3.2.4 Facial emotion recognition accuracy

A 2 (menstrual phase: early follicular, midluteal) by 2 (stress condition: high stress or low stress) by 6 (facial emotion type: fear, anger, joy, sadness, surprise, or disgust) mixed-design ANOVA was conducted for facial emotion recognition accuracy. There were no significant main effects of menstrual cycle phase or stress group, nor were there any significant interactions involving these variables (all $p$s $>.23$). See Figure 11 for the nonsignificant 3-way interaction.

### 3.2.5 Subjective measures

A 2 (menstrual phase: early follicular, midluteal) by 2 (stress condition: high stress or low stress) between-subjects ANOVA was conducted for depressive symptoms. The dependent variable was the total CES-D score. There were no significant main effects of menstrual cycle phase or stress group, nor were there any significant interactions involving these variables (all $p$s $>.22$).

Similarly, a 2 (menstrual phase: early follicular, midluteal) by 2 (stress condition: high stress or low stress) between-subjects ANOVA was conducted for trait anxiety. The dependent variable was the total STAI-trait score. There were no significant main effects of menstrual cycle phase or stress group, nor were there any significant interactions involving these variables (all $p$s $>.32$).

### 3.2.6 Correlations

Pearson’s $r$ correlations were performed to examine whether the change in salivary cortisol levels (peak – baseline, second sample – baseline, third sample – baseline), change in heart rate, and change in state anxiety scores were related to the facial and auditory emotion recognition accuracy and reaction time scores for each of the menstrual cycle phase groups.
Figure 10

Nonsignificant three-way interaction for mean recognition reaction time of emotional facial expressions.

Note. Error bars are mean +/- SEM.
Figure 11

Nonsignificant three-way interaction for mean recognition accuracy of emotional facial expressions.

Note. Error bars are mean +/- SEM.
separately. This was done to examine whether individual differences in stress reactivity were related to emotion processing scores, independent of assigned stress group.

Regarding the early follicular phase, all measures of cortisol change and the change in state anxiety were significantly negatively correlated with the recognition accuracy of happy and angry facial expressions, but change in heart rate was not (see Table 2). This indicates that a greater change in cortisol levels and state anxiety was associated with poorer accuracy of happy and angry facial expressions in the early follicular phase. In the early follicular phase, change in heart rate was positively correlated with the recognition reaction time for surprise facial expression and negatively correlated with the recognition accuracy of sad sense sentences (see Table 2). This demonstrates that while in the early follicular phase, a greater change in heart rate was associated with slow reaction times in recognizing the facial expression surprise and decreased accuracy of recognizing sad sense sentences. There were no other significant correlations in the early follicular phase (all $p > .08$).

In the midluteal phase, the only significant correlations were found for the reaction time in recognizing fearful faces with the change in cortisol levels at the second sample and the peak change in cortisol levels (see Table 2). This indicates while in the midluteal phase, the greater the increase in cortisol levels, the slower the recognition of fearful faces. There were no other significant correlations in the midluteal phase (all $p > .05$).

**Discussion**

The present study was conducted to examine effects of acute stress on emotion recognition during specific phases of the menstrual cycle A manipulation check was done to determine whether the group that experienced the high stress task had a greater change in stress response than the group that experienced the low stress task. Although only the increase in
Table 2

Pearson’s r correlations between emotion recognition performance and acute stress measure change scores for salivary cortisol heart rate, and STAI-state.

<table>
<thead>
<tr>
<th></th>
<th>Happy Face Accuracy</th>
<th>Angry Face Accuracy</th>
<th>Surprise Face RT</th>
<th>Fearful Face RT</th>
<th>Sad Sense Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early follicular phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second sample</td>
<td>-.83*</td>
<td>-.83*</td>
<td>.17</td>
<td>-.15</td>
<td>-.23</td>
</tr>
<tr>
<td>Third sample</td>
<td>-.89**</td>
<td>-.89**</td>
<td>.12</td>
<td>-.09</td>
<td>-.32</td>
</tr>
<tr>
<td>Peak sample</td>
<td>-.84*</td>
<td>-.84*</td>
<td>.16</td>
<td>-.14</td>
<td>-.23</td>
</tr>
<tr>
<td>HR change</td>
<td>-.26</td>
<td>-.26</td>
<td>.84*</td>
<td>.49</td>
<td>-.71*</td>
</tr>
<tr>
<td>STAI-state change</td>
<td>-.89**</td>
<td>-.89**</td>
<td>.22</td>
<td>.08</td>
<td>-.60</td>
</tr>
<tr>
<td>Midluteal phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second sample</td>
<td>-.08</td>
<td>.26</td>
<td>.66*</td>
<td>-.01</td>
<td></td>
</tr>
<tr>
<td>Peak sample</td>
<td>-.07</td>
<td>.29</td>
<td>.68*</td>
<td>.01</td>
<td></td>
</tr>
</tbody>
</table>

Note. *p < .05 **p < .01.
heart rate was significantly greater in the high stress condition than the low stress conditions, trends indicate that the high stress condition also experienced greater increases in cortisol levels and state anxiety than the low stress condition, which is relatively consistent with previous literature (Kirschbaum et al., 1993). Considering the small sample size included in this study, these results indicate that the stress induction was relatively successful.

The hypothesis that the early follicular phase group would have higher accuracy and lower reaction times compared to the midluteal phase group was not supported because no main effects of menstrual cycle phase on facial or auditory recognition accuracy or reaction time was found. This contrasts to previous research, in which females in the early follicular phase or with lower estradiol and progesterone levels generally perform better than females in the midluteal phase or than those with higher estradiol and progesterone levels (Derntl et al., 2008b; Derntl et al., 2013; Guapo et al., 2009; Kamboj et al., 2015; Rubin et al., 2011). It appears the main effect of menstrual cycle phase on emotion recognition was masked by the interaction between menstrual cycle phase and stress condition on emotion recognition, discussed below.

The hypothesis that the low stress group would have higher accuracy and lower reaction times compared to the high stress group also was not supported, which is inconsistent with previous findings that stress is associated with enhanced emotion recognition (Duesenberg et al., 2016; Ennis, 2017; Feeney et al., 2012; Quintana et al., 2012). Only trends were found for main effects of stress condition on auditory accuracy for both sense and nonsense sad sentences, indicating that there was greater accuracy in the low stress condition compared to the high stress condition. Although no main effects of stress condition were found, this is not surprising because it was expected that stress condition would interact with menstrual cycle phase.
For the main hypothesis of this study—that lowest accuracy and highest reaction times on both the auditory and facial emotion recognition tasks would be associated with the midluteal phase after the high stress task, and the highest accuracy and lowest reaction times on both the auditory and facial emotion recognition tasks would be associated with the early follicular phase after the low stress task—the results provide only partial support. The results unexpectedly show that in the early follicular phase, the high stress condition has better auditory intonation recognition accuracy, but, unexpectedly, nonsignificant patterns suggest the low stress condition has slower auditory intonation reaction time compared to the high stress condition. The nonsignificant interactions between menstrual cycle phase and stress group for the other dependent variables examined do not allow for conclusions to be drawn for the reaction time aspects of the hypothesis.

For the recognition accuracy of auditory intonations, a significant four-way interaction between emotion type, sentence type, menstrual cycle phase, and stress group was found. To decompose this interaction, each emotion and sentence type was examined separately. These analyses showed a significant interaction between menstrual cycle phase and stress group for the recognition of neutral sense and angry nonsense sentences, unexpectedly indicating that during the early follicular phase accuracy of angry nonsense sentences and neutral sense sentences was greater in the high stress condition compared to the low stress condition, but in the midluteal phase accuracy was lower in the high stress condition compared to the low stress condition, as predicted. Although the interaction between menstrual cycle phase and stress group was nonsignificant for the rest of the auditory intonation recognition accuracy, it appears in the early follicular phase accuracy was better in the low stress condition for recognizing happy voices, sad voices, and fearful nonsense voices compared to the high stress condition, but in the midluteal
phase, accuracy was poorer for all emotional intonations in the high stress condition compared to the low stress condition in line with the hypothesis.

Regarding auditory intonation recognition reaction time, no significant main effects of menstrual phase or stress groups were found, nor were there any interactions involving these variables; however, in line with the hypothesis the overall pattern shows that during the early follicular phase, auditory intonation recognition reaction time was slower in the high stress condition compared to the low stress condition except for angry nonsense sentences and sad sense sentences. However, in the midluteal phase, reaction time was faster in the high stress condition compared to the low stress condition for all trial types except for fearful nonsense sentences, opposite to what was predicted.

Similarly, no significant menstrual phase by stress group interactions were found for facial emotion recognition accuracy or reaction time. Although a pattern appears for the midluteal phase, suggesting a faster reaction time and lower accuracy in the high stress condition compared to the low stress condition (except for the reaction time of fearful faces), as was also observed for the auditory task, which provides only partial support for the hypothesis. For the early follicular phase, no pattern appeared, as results differed depending on the type of facial emotion.

This phase-related difference in stress effects on emotion recognition is likely due to the interaction between the stress response and the female HPG axis. Because of the bidirectional interaction between the HPA axis and the female HPG axis (Acevedo-Rodriguez et al., 2018; Chrousos, Torpy, & Gold, 1998; Toufexis, Rivarola, Lara, & Viau, 2014), the activation of the stress response (and hence, cortisol levels) from a high stress task likely influenced the female HPG axis, as well as the circulating estradiol and progesterone levels likely also influenced the
HPA axis. Increased estradiol and progesterone levels is associated with enhanced and prolonged activation of the HPA axis (Altemus et al., 2001; Viau & Meaney, 1991). Additionally, the hormones involved in the HPA axis have been shown to inhibit all levels of the HPG axis (Chrousos, Torpy, & Gold, 1998), including glucocorticoids, decreasing the release of gonadotrophins, estradiol, and progesterone, as well as desensitizing tissues to estradiol. As a result, the interaction between the HPA and female HPG axes could lead to substantial differences in emotion processing and recognition. Because cortisol is able to cross the blood-brain-barrier (Pardridge & Mietus, 1979) to reach the high density receptors for glucocorticoids in emotion-processing regions, such as the hippocampus and amygdala (Reul & Kloet, 1985), any changes to the axis that regulates cortisol levels could influence emotion processing and recognition. Similarly, high density sex hormone receptors are found on these emotion-processing regions (Goldstein et al., 2001), so any changes to the female HPG axis likely influences emotion processing and recognition as well.

It is possible that moderate increases in cortisol facilitate emotion recognition. It may be that in the early follicular phase, when estradiol and progesterone levels are low, after an acute stressor, cortisol levels enhance emotion recognition performance, but when estradiol and progesterone levels are high in the midluteal phase, after an acute stressor, these sex hormones further enhance the cortisol levels to such an extent that emotion recognition performance in impaired.

This tendency can explain some of the mixed findings from studies regarding stress and sex differences in emotion recognition. For example, Ennis (2017) found that females exhibited faster facial emotion recognition reaction times than males after completing a low stress task, but slower reaction times than males after completing a high stress task. Because naturally cycling
females are generally at time points in their menstrual cycle in which estradiol and/or progesterone levels are increased, after an acute stressor, the sex hormones could further increase the activation of the HPA axis to the point at which cortisol levels are high enough to inhibit emotion recognition.

Similarly, this finding could explain why Smeets et al. (2009) found a reversal in sex differences, depending on cortisol response. Male high cortisol responders were found to have higher accuracy of emotional states compared to male low cortisol responders, whereas naturally cycling females who were low cortisol responders had higher accuracy of emotional states compared to both controls and high cortisol responders. For females with high estradiol and/or progesterone levels, these sex hormones could have enhanced HPA axis activity, perhaps resulting in high enough cortisol levels to adversely impact emotion recognition. In contrast, the females with low estradiol and progesterone levels may have demonstrated a level of cortisol response that enhanced emotion recognition because the low levels of estradiol and progesterone would have led to a smaller increase in HPA axis activity than high levels of estradiol and progesterone. However, the same effect was likely not seen in males because males have much lower levels of estradiol and progesterone; therefore, these sex hormones likely did not increase male cortisol levels to an extent that emotion recognition would be impacted, so the male high cortisol responders performed better than the low cortisol responders.

In contrast, Duesenberg et al. (2016) found that the oral administration of the exogenous version of cortisol did not demonstrate the emotion recognition sex difference of males outperforming females after increased cortisol levels. In fact, females more accurately recognized angry facial expressions than males. This is likely due to the fact that the majority of the females that participated in the study were either unspecified, on oral contraceptives (in
which estradiol and progesterone levels are subnormal), or in the luteal phase, in which estradiol and progesterone levels are increased. The females on oral contraceptives may have performed the opposite of females in the luteal phase due to the different sex hormone levels interacting with the HPA axis, so the averaged results did not lead to a sex difference.

The hypothesis that the size of the stress response would be negatively associated with facial and auditory emotion recognition accuracy, and positively associated with facial and auditory emotion recognition reaction time in both the early follicular phase and midluteal phase of the menstrual cycle was only somewhat supported by the results. For participants in the early follicular phase, change in cortisol levels and state anxiety were strongly negatively correlated with the recognition accuracy of happy and angry facial expressions, and change in heart rate was strongly positively correlated with the recognition reaction time for surprised facial expressions and strongly negatively correlated with the recognition of auditory sad sense sentences. For participants in the midluteal phase the reaction time for the recognition of fearful faces was positively correlated with change in cortisol from levels 20 minutes post-stressor and peak cortisol levels compared to baseline. Although the correlations that were found were in the predicted direction, no other correlations were found in either menstrual cycle phase, indicating that majority of the accuracy and reaction times for recognition of facial and auditory emotions are not associated with acute stress. The results suggest that acute stress is associated with poorer recognition accuracy of happy and angry faces, poorer recognition of sad intonations in voices, and slower recognition of surprise facial expressions when females are in the early follicular phase, which contrasts with the findings that emotion recognition accuracy is enhanced after an acute stressor. The results also suggest that acute stress is associated with slower recognition of
fearful faces when females are in the midluteal phase, which is consistent with nonsignificant patterns previously discussed.

Measures of parasympathetic activation have been positively associated with facial emotion recognition (Quintana et al., 2012); therefore, the sympathetic activation (change in heart rate) having a negative impact on emotion recognition is realistic. These findings contrast with Feeney and colleagues’ (2012) findings that high salivary cortisol levels in males and females were associated with faster reaction times for recognizing joyful, angry, and neutral facial expressions. This may be due to the fact that the association is dependent on the type of emotion being recognized – increased salivary cortisol levels may slow the recognition of fearful and surprised facial expressions, but lead to faster recognition of joyful, angry, and neutral facial expressions. Similar to Ennis (2017), it appears that acute stress increases the recognition accuracy of auditory emotions, but Ennis found these results for anger, fear, and disgust, not sadness. Ennis (2017) also found that males’ and females’ recognition reaction time of the vocal intonations for anger, fear, and disgust was faster after acute stress, unlike the present study, which found slower recognition reaction times for surprised faces in the early follicular phase and slower reaction recognition times for fearful faces in the midluteal phase. The lack of findings in the present study is likely due to the small sample size and the dissimilar findings compared to other studies may also be because both Smeets and colleagues (2009) and Ennis (2017) did not account for menstrual cycle phase or sex hormone levels. The different menstrual phases and associated differences in estradiol and progesterone levels could have influenced the effects of stress on emotion recognition performance because of the bidirectional interaction between the HPA axis and the female HPG axis (Acevedo-Rodriguez et al., 2018; Chrousos, Torpy, & Gold, 1998; Toufexis, Rivarola, Lara, & Viau, 2014). Additionally, the differences
could be because the levels of stress and cortisol differ between studies, and only a certain amount of increased stress and cortisol levels enhance performance – too much or too little increase could decrease performance.

4.1 Limitations

The major limitation of this study was the small sample size. Because of the small sample size, what would have been significant findings in a larger sample may have been nonsignificant in the present study. Additionally, the significant results that were found may not generalize to the rest of the population, because the results are only from a small number of females and strict exclusion criteria were used in this study. The ecological validity of this study is also limited because it occurred in a laboratory setting; however, speech and arithmetic tasks do occur in real-life settings, in which people may need to recognize emotions if they interact with emotional people shortly after. Although the facial emotion recognition task was created by morphing still images together, which may decrease ecological validity, the dynamic emotional expressions increased the ecological validity.

Another limitation of this study is that estradiol and progesterone levels were not analyzed. Although only females who met specific criteria regarding their menstrual cycles participated, so that estradiol and progesterone levels could be estimated by menstrual cycle phase as accurately as possible, the sex hormone levels were not analyzed to confirm the levels of estradiol and progesterone at the time of participation in the study; therefore, it cannot be confirmed that estradiol and progesterone levels were as predicted.

4.2 Future Directions

Future studies could continue to examine the interaction between menstrual cycle phases and different levels of stressors on both facial and auditory emotion recognition. Additionally,
the effects of stress on emotion recognition performance for females on oral contraceptives could be compared with females in specific phases of the menstrual cycle, to further examine the influence of varying estradiol and progesterone levels on emotion recognition performance. Similarly, females at specific time points in their cycle could be separately compared to males regarding their emotion recognition performance after stressors to examine if the varying sex differences after a stressor are influenced by estradiol and progesterone levels. To ensure estradiol and progesterone levels are estimated correctly, the concentrations of these sex hormones could also be analyzed. Administration of cortisol and/or estradiol and progesterone could also be done in a similar study in the future, to have more control on whether or not cortisol or estradiol and progesterone are the cause of the findings; however, ecological validity would be compromised in this approach. Because the only significant interaction between menstrual cycle phase and stress conditions was for auditory emotion recognition accuracy, it is important that future studies focus on both auditory and facial emotion recognition, not just facial emotion recognition.

Thus, the present study suggests that the interaction between menstrual cycle phase and stress levels influences emotion recognition. In the early follicular phase of the menstrual cycle, females tend to have better auditory emotion recognition accuracy after a high stress task compared to a low stress task, whereas, in the midluteal phase of the menstrual cycle, females tend to have better auditory emotion recognition accuracy after a low stress task compared to a high stress task. Findings suggest that low levels of estradiol and progesterone in females enhances the activity of the HPA axis after an acute stressor to the extent that emotion recognition performance is enhanced. In contrast, it appears that high levels of estradiol and progesterone may enhance the activity of the HPA axis after an acute stressor to the extent that
emotion recognition performance is adversely affected. It is valuable for individuals to know which specific time points female emotion recognition performance is enhanced or worsened because emotion recognition is vital to social interactions.
References


doi:10.1016/j.psyneuen.2009.02.007


doi:10.1007/s00213-005-0237-7


Scholten, M., Aleman, A., & Kahn, R. (2008). The processing of emotional prosody and semantics in schizophrenia: Relationship to gender and IQ. *Psychological Medicine, 38*(6), 887-898. doi:10.1017/S0033291707001742


Appendix A

Ethics Approval
**PRINCIPAL INVESTIGATOR:** Dr. Laurie Sykes Tottenham  
**DEPARTMENT:** Department of Psychology  
**REB#:** 2015-165

### TITLE:
The Effects of Stress on Emotion Recognition Across the Menstrual Cycle

### AMENDMENT APPROVAL OF:

- **Addition of three recruitment strategies including email and poster.**
  - 1. Poster distribution on campus at the University of Regina
  - 2. U of R Psychology Student Association to post to their Facebook.
  - 3. Email including poster to psychology professors and instructors currently teaching 100- or 200-level classes that are registered in the pool.

  **NEXT RENEWAL DATE:** November 10, 2020  
  **AMENDMENT APPROVAL DATE:** February 7, 2020

### AMENDMENT CERTIFICATION

The University of Regina Research Ethics Board has reviewed the changes to the above-named research project as outlined in your memo dated December 2, 2019, and they are approved.

### ONGOING REVIEW REQUIREMENTS

In order to receive annual renewal, a status report must be submitted to the REB Chair for Board consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for the renewal and closure forms:

[https://www.uregina.ca/research/for-faculty-staff/ethics-compliance/human/ethicsforms.html](https://www.uregina.ca/research/for-faculty-staff/ethics-compliance/human/ethicsforms.html)

---

**Ara Steininger**  
Research Ethics Board

---

Please send all correspondence to: Research Office  
University of Regina  
Research and Innovation Centre 109  
Regina, SK S4S 0A2  
Telephone: (306) 585-4775  
Fax: (306) 585-4893  
research.ethics@uregina.ca