2021

How HPA stress response relates to anxiety: Sex differences and estradiol

Carrie Shea
University of Northern Iowa

HOW HPA STRESS RESPONSE RELATES TO ANXIETY: SEX DIFFERENCES AND ESTRADIOL

An Abstract of a Thesis

Submitted

in Partial Fulfillment

of the Requirements for the Degree

Master of Arts

Carrie Shea

University of Northern Iowa

July 2021

ABSTRACT

Cortisol is a stress hormone secreted during activation of the hypothalamic-pituitary-adrenal (HPA) axis, a system within the body that shows increased activation in response to stress. Cortisol is often assumed to be related to anxiety. However, previous research on the relationship between cortisol and anxiety is mixed. The present research examined the moderating effects of sex differences and estrogen on the relationship between cortisol reactivity and anxiety. Participants (n = 54) completed the Trier Social Stress Test (TSST) and completed measures of anxiety. Consistent with previous research, it was hypothesized that men will exhibit increased cortisol reactivity compared to women. It was also hypothesized that sex will moderate the relationship between cortisol reactivity and anxiety. It was further hypothesized that cortisol reactivity will relate to trait anxiety in women. Finally, it was hypothesized that estrogen will moderate this effect in women. As hypothesized, men exhibited greater cortisol reactivity in response to the TSST than women. The relationships between state and trait anxiety measures and cortisol reactivity were marginally significant in women, but no relationships were seen in men. Sex significantly moderated the relationship between state anxiety and cortisol reactivity, but estradiol did not. These results suggest that men and women may react differently to stress physiologically, but it is still unclear if this is due to social or biological factors. Implications for further research are discussed.

HOW HPA STRESS RESPONSE RELATES TO ANXIETY: SEX DIFFERENCES AND ESTRADIOL

A Thesis

Submitted

in Partial Fulfillment

of the Requirements for the Degree

Master of Arts

Carrie Shea

University of Northern Iowa

July 2021

This Study by: Carrie Shea

Entitled: How HPA Stress Response Relates to Anxiety: Sex Differences and Estradiol

has been approved as meeting the thesis requirement for the

Degree of Master of Arts

Date	Dr. Catherine DeSoto, Chair, Thesis Committee
Date	Dr. Robert Hitlan, Thesis Committee Member
Date	Dr. Nicholas Schwab, Thesis Committee Member
Date	Dr. Jennifer Waldron, Dean, Graduate College

DEDICATION

I would like to thank my parents who have been unbelievably supportive and have taught me to be the hard-working, dedicated person I am today.

ACKNOWLEDGEMENTS

I would like to thank Dr. Catherine DeSoto who mentored me throughout this project. I would also like to thank my committee members, Dr. Robert Hitlan and Dr. Nicholas Schwab, for assisting me in this process as well. I would like to thank Dr. Hitlan for the hours that he put in helping me run the saliva samples for this project. I would also like to thank the organizations that offered funding for this project.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

CHAPTER 1

INTRODUCTION

Cortisol and the HPA Axis

Cortisol is a stress hormone secreted during activation of the hypothalamic-pituitary-adrenal (HPA) axis, a system within the body that shows increased activation in response to stress. When a human experiences stress, corticotropin-releasing hormone (CRH) is secreted by the hypothalamus. CRH travels to the pituitary gland and binds to receptors, which cause the release adrenocorticotropic hormone (ACTH). ACTH travels via the bloodstream to the adrenal cortex, the outermost portion of the adrenal glands, which sit atop the kidneys. The adrenal cortex then secretes glucocorticoids, including cortisol, into the bloodstream. Most of the cortisol released is bound to binding proteins in the blood and the rest of the cortisol is “free” (or unbound) cortisol and is active in the body.

Cortisol travels throughout the body to affect a number of systems, including the metabolism, immune system, cardiovascular system, and brain. When cortisol travels to the brain and is detected by the hypothalamus, it inhibits the secretion of CRH from the hypothalamus. The decline in CRH in turn decreases the secretion of ACTH. Without ACTH traveling to the adrenal cortex, cortisol is no longer released (Blascovich et al., 2011). The process of cortisol inhibiting further secretion of cortisol from the adrenal cortex is referred to as a negative feedback loop (Kudielka & Kirschbaum, 2005) and prevents excess cortisol release. Cortisol release in response to stress, which is sometimes referred to as cortisol reactivity, may relate to anxiety, either state or trait, but research

regarding this relationship thus far has been unclear (McEwen, 2008; Van den Bos et al., 2017; Yoon & Joormann, 2012). Clarity on the relationship between participants' reports of anxiety, which can be defined as feelings of worry, stress, and fear that can manifest physically through activation of the autonomic nervous system (Spielberger & Rickman, 1990), and the experience of stress as measured by cortisol reactivity, is needed.

Measuring Cortisol

As a hormone, cortisol circulates throughout the body to affect a number of different systems and can be measured in blood or saliva. Blood samples represent total cortisol levels because blood contains the cortisol bound to binding proteins as well as free active cortisol, as discussed above. However, blood samples require a trained phlebotomist. Researchers began measuring cortisol in saliva in the 1980's, and saliva samples have since become a popular method of measuring cortisol in research (Clements, 2012). Saliva and blood samples are highly correlated ($r > .90$), thus salivary samples are good representations of circulating cortisol and are easier to obtain than blood samples (Blascovich et al., 2011). Cortisol changes are not immediately observed in saliva. Cortisol levels in the saliva peak around 20–40 minutes after the onset of a psychosocial stressor (Dickerson & Kemeny, 2004).

A number of different stressors have been used to stimulate HPA axis activity. Physical stress such as submerging one's hand in ice cold water (Cold Pressor Test) as well as psychological stress both reliably result in increased cortisol (McRae et al., 2006). Stressors that involve participants performing difficult cognitive tasks as well as tasks

involving pressured verbal interactions (e.g. public speaking, speaking about relationship difficulties) have elicited significant cortisol reactivity (Dickerson & Kemeny, 2004).

An effective task in eliciting cortisol reactivity that involves a combination of performance and pressured verbal responding is the Trier Social Stress Test (TSST). Kirschbaum and his colleagues (1993) created the TSST to elicit increased cortisol levels. Previous research (Berger et al., 1987) had found inconsistent and moderate increases in cortisol as a result of various performance tasks, so the TSST was created to combine two of these performance tasks. It includes a preparatory period in which participants prepare a speech, as well as a speech and a mathematics task. The TSST has been found to elicit greater cortisol reactivity compared to performance tasks alone and was more consistent than other tasks in eliciting cortisol responses. In four studies examining the effectiveness of the TSST in eliciting various stress responses, it was found that cortisol, as well as other stress hormones, were consistently elevated in response to the TSST (Kirschbaum et al., 1993). In these studies, heart rate was also elevated in response to the TSST (Kirschbaum et al., 1993). Subsequent research has shown the TSST elicits greater cortisol reactivity than physical stressors such as the Cold Presser Test, a test in which participants immerse their hands in cold water. Such research has established the TSST as a valid tool to investigate individual level stress reactions (McRae et al., 2006.) Overall, these results suggest that the TSST is an effective task in consistently eliciting stress responses, including cortisol reactivity.

Sex Differences in Cortisol Reactivity

Larger increases in cortisol as a result of various stressors are observed in men compared to women (Kudielka & Kirschbaum, 2005). Men have shown a greater difference in cortisol response to the TSST than women despite similar changes in vocal stress cues. In a study (Pisanski et al., 2018) examining voice measures along with hormone levels in saliva, researchers recorded voice pitch, vocal stress cues, polygraph, and saliva samples before, during, and after participants completed the TSST. The TSST was associated with similar changes in voice pitch and skin conductance, as well as cortisol increases in both men and women, but men exhibited increased cortisol response to the TSST compared to women. These findings show similarities between men and women in regards to some physiological changes in response to stress, and sex differences in cortisol reactivity in response to stress. These findings suggest that differences in cortisol reactivity may go beyond simple sex differences in stress response.

Similar sex differences have been observed in other types of stressors beyond the TSST, including a task in which men and women introduced themselves to potential romantic partners. In this study, participants were asked to prepare a video introducing themselves and told that it would be shown to potential romantic partners. As in studies conducted using the TSST, men exhibited greater cortisol reactivity than women in response to the stress of sharing the video with potential romantic partners (Jaremka & Collins, 2017). However, research on cortisol response to exclusion employing a virtual Cyberball game where the participant is deliberately left out of a ball tossing game, showed nonsignificant sex differences or lower responses in males. In these studies, men

also reported less distress as a result of the task than women, indicating that exclusion may simply be less distressing for men than women, leading to lower cortisol reactivity in men (Helpman et al., 2017; Weik et al., 2010).

These and other studies suggest that type of stressor may influence sex differences in stress response. In a study employing different types of tasks including timed arithmetic, passage memorization, and social exclusion, greater cortisol reactivity was observed in women as a result of the social exclusion task and in men as a result of the arithmetic and memorization tasks. These results suggest that women may exhibit greater stress responses to social rejection, while men may exhibit greater stress responses to achievement tasks (Stroud et al., 2002). The reasons for this, which could be physiological, socialized, or some combination, are not understood. It could be due to the way that men and women appraise stressful situations differently, or it may be due to methodological differences between tasks. Consistent with previous research, in the present study, it was hypothesized that men would exhibit greater cortisol reactivity in response to the TSST.

Estrogen and Cortisol Reactivity

Other hormones, including sex hormones, have been found to be associated with individual differences in cortisol reactivity. Kirschbaum and colleagues (1999) found that sex and menstrual cycle related to cortisol in a study using the TSST. Men were compared with women using oral contraceptives, as well as women in the luteal or follicular phase of their menstrual cycle. Different phases of the menstrual cycle are associated with different estrogen levels, such that women in different phases of their

menstrual cycles may exhibit differences in cortisol response due to differences in estrogen levels. Estrogen fluctuates throughout the menstrual cycle, with estrogen levels starting low early in the follicular phase and increasing near ovulation, drop sharply, then increase again during the throughout most of the luteal phase (Knudtson & McLaughlin, 2019). Therefore, estrogen is typically low during most of the follicular phase, and high during luteal phase. Participants completed the TSST while their heart rate was continuously recorded. Participants provided saliva samples before and after the TSST and rated the stressfulness of the TSST after completion. Significant increases were found in all groups for cortisol levels, heart rate, and reported stress in response to the TSST, but men exhibited higher cortisol reactivity when compared to women in the follicular phase. Men did not exhibit higher cortisol reactivity when compared to women in the luteal phase. Women in the luteal phase showed significantly higher cortisol reactivity than women in the follicular phase (Kirschbaum et al., 1999). Although firm conclusions were not made, the results suggest the possibility that estrogen levels may be important when considering sex differences in cortisol reactivity to stress.

Kirschbaum and colleagues (1999) also examined cortisol increases as a result of injections of synthetic ACTH, the hormone that directly causes cortisol release from the adrenal cortex. Injection of ACTH showed similar results such that women in the luteal phase again showed the highest cortisol reactivity, followed by men, with the least increase from women in the follicular phase (Kirschbaum et al., 1999). These results indicate that there are differences between men and women as well as between women in

different menstrual phases with regards to cortisol reactivity in response to stressors as well as injections of ACTH.

The findings regarding estrogen's role are mixed, however, and clarity on estrogen's role in cortisol reactivity is not clear. Estradiol has been associated with increases and decreases in cortisol response, depending upon the study (Allen et al., 2017). For example, estradiol levels, as well as ACTH and cortisol, increased after participation in the TSST in one study (Lennartsson et al., 2012). Others concluded that cortisol reactivity was unrelated to differences in hormone levels due to menstrual cycle phase (Herbison et al., 2016). In this study, women were divided into three groups by menstrual phase: early follicular, ovulatory, and late luteal. Herbison and colleagues grouped women based on reported day of menstrual cycle, as Kirschbaum and colleagues (1999) did. Kirschbaum and colleagues, however also verified menstrual cycle phase using post hoc analysis of estradiol and progesterone levels, while Herbison and colleages did not. Both studies also grouped participants differently, as Herbison and colleagues separated females into follicular phase, luteal phase, and ovulatory phases, while Kirschbaum and colleagues only grouped females into luteal and follicular menstrual phases. The manner in which estrogen relates to cortisol reactivity after stress is still unclear.

Despite unanswered questions about estrogen and the stress response, the role of estrogen in negative feedback has been well studied. Estrogen has been found to influence the negative feedback loop that regulates cortisol levels. Sharma and colleagues (2014) conducted a study investigating the possible influence of estrogen and testosterone

on cortisol feedback, participants, who were all men or postmenopausal women, were injected with set amounts of cortisol at regular intervals. To control the amount of sex hormones secreted, participants were injected with leuprolide, which decreases estrogen levels in women and testosterone levels in men. After leuprolide injections, sex hormone levels were increased in some participants using regular doses of hormone-stimulating medications. Other participants took placebos. Participants then took a placebo or ketoconazole to inhibit cortisol production before injections of either a placebo solution or cortisol to induce feedback. Researchers then monitored ACTH levels and found that women had lower ACTH levels than men, and women treated with estrogen after leuprolide had lower ACTH levels than women treated with placebo after leuprolide. These results indicate that estrogen increases negative feedback by enhancing inhibition of ACTH secretion. This enhanced negative feedback was not seen in men treated with testosterone (Sharma et al., 2014), suggesting that estrogen alone enhances negative feedback. Because of estrogen's role in cortisol reactivity, it is possible that it plays a role in the relationship between experienced anxiety and cortisol reactivity.

Of interest recently, there has been research linking estrogen receptor genes and cortisol reactivity in that repeated sequences on estrogen receptor genes are related to cortisol activity in women. Researchers examined the sequences of genes that code for estrogen receptors and found that increased repeats of cytosine-adenine sequences in the estrogen receptor beta (*ESR2*) gene were associated with increased cortisol reactions to stress in female subjects (Hastings et al., 2018). These differences in gene sequences may influence the availability of estrogen receptors and therefore the ability of estrogen to

affect cortisol in the negative feedback loop. These findings further support estrogen's role in cortisol reactivity. Because of the role of estrogen in cortisol reactivity, it was hypothesized that estrogen would moderate the relationship between cortisol reactivity and anxiety.

Measuring Anxiety

Anxiety is a term used to describe current changes in tension and distress based on the situation and is also used to describe a tendency to feel stress across situations (Spielberger et al., 1971). Spielberger and colleagues (1971) defined two different types of anxiety, state and trait anxiety. Current anxiety that is situation-based is referred to as state anxiety, or how anxious an individual feels at the time, while anxiety across situations, or a tendency to show anxiety, is referred to as trait anxiety, or how anxious an individual feels generally. Changes in state anxiety are thought to be due to an interaction between the situation and trait anxiety (Endler & Kocovski, 2001).

Cortisol and Anxiety Disorders

Cortisol Reactivity and Anxiety Disorders

Research suggests that cortisol reactivity is related to anxiety disorders, but the exact relationship is unclear and likely nuanced. For example, according to a review of the research regarding the relationship between HPA axis activity and anxiety disorders, increased activity of the HPA axis throughout the day has been found to be positively associated with symptoms of generalized anxiety disorder and social anxiety disorder in some studies, while in others they were not associated (Faravelli et al., 2012). Furthermore, individuals with social anxiety disorder have shown greater cortisol

reactivity in response to a stressor involving a speech and working memory tasks than healthy controls (Yoon & Joormann, 2012).

However, it seems cortisol response may change over time in individuals with anxiety and some studies have shown decreases in cortisol reactivity associated with anxiety. Children with internalizing behaviors, such as those associated with depression and anxiety, showed decreases in general HPA axis activity from childhood to adolescence, indicating there may be a continued decrease in cortisol activation over time in these individuals (Ruttle et al., 2011). Psychological distress in general may be related to decreases in cortisol response over time. Adolescents with social anxiety tested for cortisol response to a public speaking stressor before and after the onset of puberty had decreased cortisol responses to the stressor after a year of pubertal development (Van den Bos et al., 2017). These findings suggest puberty may have an effect, indicating sexual maturity and sex hormones may affect these changes over time. Changes in hormone levels influence the relationship between experienced anxiety and cortisol reactivity in response to a stressor.

Thus, the relationship between cortisol reactivity and anxiety are not consistent, with some studies showing increases in cortisol reactivity associated with anxiety (McEwen, 2008; Yoon & Joormann, 2012) and others reporting a negative association (Van den Bos et al., 2017). Of interest, children who showed higher levels of daily cortisol and increased anxiety symptoms also showed increased cortisol reactivity in response to the TSST-C, a version of the TSST designed for children (Laurent et al., 2015). Trait anxiety has also failed to predict salivary cortisol response to the TSST after

controlling for age and gender, even though trait anxiety predicted other stress responses, including heart rate response (Huini et al., 2018). These findings suggest that age and gender may affect the relationship between trait anxiety and cortisol response. It is possible that this relationship changes over time. Regardless, these conflicting results raise the question of how cortisol responses to stress are related to anxiety.

Cortisol Reactivity and Repeated Stress

It has been posited that repeated stress may decrease HPA axis activity, resulting in a decrease in overall cortisol levels (Fries et al., 2005; Heims et al., 1998). Chronic stress is associated with changes several systems, including the gastrointestinal, cardiovascular, and immune systems. For example, sustained cortisol levels from stress increase the production of insulin to compensate for decreased insulin sensitivity brought on by cortisol. These changes in system functioning are the consequences that repeated stress has on the body, and are referred to as allostatic load (McEwen & Stellar, 1993).

Research has suggested that the HPA axis is one of the systems affected by allostatic load. A study investigating the effects of chronic stress on stress response found that workers of various professions who had higher allostatic load, measured by assessing fifteen different biomarkers from neuroendocrine, immune, metabolic, and cardiovascular systems, reported a higher number of chronic stress and burnout symptoms and lower cortisol reactivity in response to the TSST (Juster et al., 2011). These results suggest that allostatic load is associated with decreases in cortisol reactivity. These decreases in HPA axis activity have been associated with various stress-related disorders, as well, including post-traumatic stress disorder (Fries et al., 2005). These findings suggest that chronic

stress may influence the functioning of the hypothalamic-pituitary-adrenal axis. Chronic stress due to a tendency to respond to stressful situations with anxiety, or trait anxiety, could potentially influence HPA axis functioning.

Cortisol Reactivity and Personality Traits

Previous research has suggested that personality may relate to cortisol reactivity in different ways for men and women. For example, neuroticism is a measure of emotional instability related to both anxiety and depression, and sex differences have been found in the relationship between cortisol and neuroticism, such that women who reported higher neuroticism exhibited lower average cortisol levels, while men with higher neuroticism exhibited higher average cortisol levels (DeSoto & Salinas, 2015). Overall, the underlying reason for these sex differences in cortisol reactivity remains unclear. Estrogen, socialization, or both could be a factor in these sex differences. In order to further investigate these sex differences and estrogen's role, the present study examined the moderating effect of sex and estrogen on the relationship between cortisol reactivity and anxiety.

The Present Study

Consistent with previous research (Childs et al., 2010; DeSantis et al., 2011; Kirschbaum et al., 1999; Pisanski et al., 2018), it was hypothesized that men would exhibit increased cortisol reactivity in response to the TSST compared to women. It was further hypothesized that cortisol reactivity would relate to anxiety. Further, it was expected that this relationship would be different in men and women, so it was hypothesized that sex will moderate the relationship between cortisol reactivity and

anxiety. Estrogen's role in cortisol reactivity could be linked to this sex difference. Therefore, it was hypothesized that estradiol would also moderate the relationship between cortisol reactivity and anxiety in women.

CHAPTER 2

METHODS

Participants

Participants (24 men, 31 women) were recruited from Psychology 1001 courses at the University of Northern Iowa through an online research participation system. Participants had an option to select the current study among a number of different study options to receive course credit. Fifty-four participants ages 18 to 26 years (M = 19, SD = 1.60; 81.5% White) were included in analyses. This study was approved by the university Institutional Review Board, and the methods met COVID safety guidelines.

Questionnaires

The Spielberger State–Trait Anxiety Inventory (STAI) has scales for both state and trait anxiety (Marteau & Bekker, 1992). Scores on the state scale of the STAI tend to increase or decrease as a function of stressful situations, while scores on the trait scale remain relatively stable over time (Spielberger et al., 1971). The participant reads descriptive statements, such as "I feel tense," and rates the statements as to how well each one describes their feelings on a four-point Likert scale ranging from 1 (*Not at all*) to 4 (*Very much so*). The STAI is a longer questionnaire, consisting of 20 items for each scale. The present study used a six-item shortened version of the state scale of the STAI (Appendix E), which has been found to have a Cronbach's alpha of .82 and produces a similar mean score to the full 20-item scale (r = .95) (Marteau & Bekker, 1992). In the present study, the STAI state scale had a Cronbach's alpha of .81.

In order to measure trait anxiety, the trait scale was also used (Spielberger et al., 1983; See Appendix F). This scale measures more general anxiety over a longer period of time and included the entire 20-item scale. It is measured on the same four-point Likert scale as the state anxiety scale and includes statements similar to those included in the state anxiety scale (e.g., "I feel nervous and restless") but instead asks participants to rate how well the statements describe them generally instead of currently. In the present study, the STAI trait scale had a Cronbach's alpha of .90.

The STAI was designed to assess normal ranges of anxiety in non-clinical populations, although it has gained popularity in research with psychiatric populations as well. However, other scales have been designed to assess the more severe diagnostic symptoms of anxiety disorders specifically in a clinical setting, including the Symptom Checklist-90 (SCL-90; Derogatis & Lazarus, 1994).

The Symptom Checklist 90-R (SCL-90) is a 90-item self-report inventory that measures participants' psychological distress (See Appendix D). Participants rate statements as to how much they feel each one describes them on a five-point Likert scale ranging from 0 (*Not at all*) to 4 (*Extremely*). The SCL-90 includes nine symptom dimensions, including Somatization, Obsessive-Compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation, and Psychoticism. The reliability and validity of the SCL-90 has been established (Derogatis & Lazarus, 1994). The subscales in the SCL-90 have been found to have moderate to high internal consistencies, with Cronbach's alpha values ranging from .65 for the Hostility subscale to .89 for the Depression subscale (Derogatis & Lazarus, 1994). The anxiety scale was also

used as a second measure of trait anxiety. This scale is designed to incorporate a greater number of symptoms of anxiety disorders and includes more severe items (e.g. “Spells of terror or panic”), as well as more items assessing physical symptoms of anxiety (e.g. “Heart pounding or racing”) than the STAI scales. In the present study, the SCL Anxiety scale had a Cronbach’s alpha of .83.

Saliva Collection

Estrogen and cortisol were measured using salivary assays. The participants passively drooled into a cryovial through a polypropylene funnel. Samples were frozen at -60 degrees Fahrenheit within an hour of being collected until they could be assessed for cortisol and estrogen levels. Samples were analyzed at the Psychoneuroendocrinology (PNE) Lab at the University of Northern Iowa. Expanded range high sensitivity immunoassay ELISA test kits were used to analyze cortisol and estrogen as per Salimetrics procedures (Granger et al., 1999). The mean intra-assay coefficients were less than 10% and the mean inter-assay coefficients were less than 15%, indicating acceptable reliability of assay data (Schultheiss & Stanton, 2009). The assay protocol requires 25 µl of saliva per determination for cortisol and 100 µl of saliva per determination for estradiol.

Procedure

Cortisol levels spike 30 minutes after waking and dramatically decrease throughout the morning, steadying in the afternoon and evening (Blascovich et al., 2011). Because of this pattern, all participants were run in the early and midafternoon. Before

attending the session, participants were asked not to consume food, chew gum, or drink anything other than water within one hour of arriving.

Upon arrival, participants washed their hands or used hand sanitizer and rinsed their mouths with water at a drinking fountain. Participants then read and agreed to the informed consent and completed the pre-TSST questionnaire, which included demographics questions (See Appendix B) and questions about behaviors that may influence saliva samples (See Appendices A and C) as well as the shortened STAI State scale, the STAI Trait scale, and the SCL-90.

Upon completion of the pre-TSST questionnaire, participants provided the first saliva sample in order to measure baseline cortisol levels upon arrival. Participants were given up to 4 minutes and passively drooled into a cryovial using a saliva collection aid. After participants finished giving the saliva sample, the experimenter weighed and stored the sample at -60°C.

Next, participants completed the TSST. Participants started by preparing a 5-minute speech outlining why they would be a good candidate for their ideal job. The experimenter told participants that their speech would be videotaped and evaluated and that they could take notes but would not be allowed to use them during the speech. After 10 minutes, the experimenter returned and observed the speech. If participants stopped the speech before five minutes, the experimenter encouraged them to continue the speech until the time was completed. The participant then completed a 5-minute mathematics task in which the participant verbally subtracted the number 13 from 1022 repeatedly. Participants were told to start over from 1022 if a mistake was made.

Upon completion of the TSST, participants completed the post-TSST questionnaire, which included the STAI state scale shortened version, and questions regarding eating disorders (not part of the current study). The questionnaire also included questions regarding what participants had heard about the study and queried the participants regarding what they thought the study was about (See Appendix G).

After participants completed the post-TSST questionnaire, the experimenter returned and participants completed the second saliva sample in order to measure cortisol levels after completion of the TSST. Afterwards, the experimenter read the debriefing script and the participant left. The experimenter then weighed and stored the second sample at -60°C and sanitized the surfaces and keyboard in the laboratory.

Table 1 *Trier Social Stress Test Procedure*

Task	Time	Description
Questionnaire 1	10 minutes	Participants completed a questionnaire including saliva questions, demographics, SCL-90 items, and STAI items
Sample 1	5 minutes	Participants passively drooled into a cryovial for up to 4 minutes
Speech preparation	10 minutes	Participants prepare a speech describing their qualifications for their ideal job
Speech	5 minutes	Participants give their speech for 5 minutes in front of a camera that is not recording
Mathematics task	5 minutes	Participants repeatedly subtract 13 from 1022
Questionnaire 2	10 minutes	Participants completed a questionnaire including the STAI State scale items, eating behavior items unrelated to the present study, and crosstalk questions
Sample 2	5 minutes	Participants passively drooled into a cryovial for up to 4 minutes

CHAPTER 3

RESULTS

Preliminary Analyses

All variables were examined for normality and outliers. One participant had a cortisol level that was located more than 3 standard deviations from the mean and reported waking less than one hour before participation in the study. Due to the cortisol awakening response, waking so close to the time of the study would be expected to have a large influence on cortisol levels (Blascovich et al., 2011) and to override the effect of the TSST. No other participant reported awakening within three hours of participation, thus the participant was excluded from analyses. Additionally, both pre-TSST (skew = 1.46, $z = 4.49$, $p < .001$) and post-TSST (skew = 2.08, $z = 6.40$, $p = .002$) cortisol levels, as well as average scores on the SCL-90 Anxiety scale (skew = 2.18, $z = 6.70$, $p < .001$), were found to be significantly positively skewed. These variables were transformed using logarithmic transformation in accordance with the recommendations of Tabachnick and Fidell (2019). Skewness values for these variables decreased to .34 ($z = 1.04$, $p = .20$), .33 ($z = 1.02$, $p = .18$), and 1.06 ($z = 3.26$, $p = .06$), respectively. No other variables contained outliers or showed deviations from normality that required transformations.

The inter- and intra-assay coefficients of variability are measures of methodological reliability of assay data. The inter-assay coefficient of variability and measures how consistent measurements are across plates and uses control values, and the intra-assay coefficient of variability is a measure of how consistent measurements are for each sample. Generally, an inter-assay coefficient of variability of less than 15 percent

and an intra-assay coefficient of variability of less than 10 percent are acceptable (Schultheiss & Stanton, 2009). The inter-assay coefficient of variability for the cortisol assays was 13.9 percent. The intra-assay coefficient of variability for the cortisol assays was 6.3 percent. The intra-assay coefficient of variability for estradiol was 7.7 percent. These results indicate that the precision of assays was acceptable.

T-Test for Sex Differences in Cortisol Reactivity

It was hypothesized that men would exhibit greater cortisol reactivity in response to the TSST than women. This hypothesis was tested using a one-tailed t-test to compare the difference between post-TSST and pre-TSST cortisol levels (i.e., cortisol reactivity) in men and women. Cortisol reactivity was calculated by subtracting the pre-TSST cortisol levels from post-TSST cortisol levels. The hypothesis was supported regarding cortisol reactivity, such that men, $M = .08$, $SD = .15$, exhibited greater cortisol reactivity than women, $M = .01$, $SD = .12$, $t(52) = 3.19$, $p = .001$, one-tailed.

Regressions for Relationship Between Anxiety and Cortisol Reactivity

It was hypothesized that cortisol reactivity would relate to measures of anxiety in both men and women. Linear regressions were used to examine the relationship between cortisol reactivity and scores on the STAI Trait scale, STAI State scale, and SCL-90 Anxiety scale for men and women. In men, cortisol reactivity did not significantly predict scores on any of the anxiety measures. In women, while cortisol reactivity did not significantly predict scores on the SCL-90 Anxiety scale, the relationship between cortisol reactivity and scores on the STAI State Anxiety scale was marginally significant,

$r(29) = .33$, $p = .07$, and the relationship between cortisol reactivity and scores on the STAI Trait Anxiety scale was also marginally significant, $r(29) = .34$, $p = .07$.

Sex as a Moderator in the Relationship Between Cortisol Reactivity and Anxiety

It was hypothesized that sex would moderate the relationship between cortisol reactivity and measures of anxiety. Three moderation analyses were run using Hayes' PROCESS macro (PROCESS; Hayes, 2017) examining sex as a moderator in the relationships between cortisol reactivity and scores on the STAI State Anxiety, STAI Trait Anxiety, and SCL-90 Anxiety scales. For state anxiety associated with the TSST task, the post-TSST STAI State Anxiety scale scores were used, and pre-TSST STAI State Anxiety scale scores were used as a covariate in order to account for differences in pre-TSST anxiety levels. All continuous predictors were mean centered prior to creating product terms. The overall model was significant for State Anxiety, $F(4, 49) = 3.03$, $p = .03$, $R^2 = .20$ (See Table 2). The two-way interaction, $b = -.95$, 95% CI [-1.74, -.16], $t(49) = -2.41$, $p = .02$, indicated that the effect of cortisol reactivity on state anxiety was not the same for men and women. For men, cortisol reactivity trended as a negative correlation with state anxiety, $r(21) = -.29$, $p = .18$. Whereas, for women, cortisol reactivity showed a marginal positive association with state anxiety, $r(29) = .33$, $p = .07$. Thus, the relationship between state anxiety and cortisol reactivity in response to the TSST was different for men and women, as hypothesized. Simple slope analysis indicated that for women, cortisol reactivity was significantly positively associated with post-TSST state anxiety, such that as women's cortisol reactivity increased, their scores on the post-TSST STAI State Anxiety scale increased as well, $b = 1.75$, $t = 1.97$, $p = .05$. For men, cortisol

reactivity was negatively associated with post-TSST state anxiety, though the relationship was not significant. As men's cortisol reactivity increased, their scores on the post-TSST STAI State Anxiety scale decreased, b = -.91, t = -1.81, p = .28. As shown in Figure 1, men reported more anxiety when cortisol reactivity was low. When cortisol reactivity was higher, the women reported more anxiety compared to men. This indicates that as cortisol increased, state anxiety scores trended in opposite directions for men and women.

Trait anxiety measures were not as clear cut. Although the two-way interaction between cortisol reactivity, scores on the STAI Trait Anxiety scale, and sex was marginally significant, b = -.61, 95% CI [-1.24, .01], $t(50)$ = -1.96, p = .06, the overall model was not significant, F (3, 50) = 2.05, p = .12, R^2 = .11. This indicated that the relationship between cortisol reactivity and trait anxiety may not be the same for men and women, but the hypothesis is not fully supported. Finally, the overall model was not significant for SCL-90 Anxiety, F (3, 50) = .40, p = .75, R^2 = .02, and accounted for only 2% of the anxiety variance. This indicates that while the relationships between cortisol reactivity and STAI state and trait anxiety tended to be different for males and females, the relationship between cortisol reactivity and scores on the SCL-90 R anxiety scale did not tend to vary between males and females.

Estradiol as a Moderator in the Relationship Between Cortisol Reactivity and Anxiety

It was hypothesized that estradiol would moderate the relationship between cortisol reactivity and measures of anxiety in women. Three moderation analyses were run with estradiol as a moderator in the relationship between women's cortisol reactivity and scores on the STAI State Anxiety, STAI Trait Anxiety, and SCL-90 Anxiety scales.

Post-TSST STAI State Anxiety scale scores were used, and pre-TSST STAI State Anxiety scale scores were used as a covariate. All continuous predictors were mean centered prior to creating product terms. The overall model was significant for State Anxiety, F (4, 26) = 2.79, p = .047, R^2 = .30. However, the interaction between cortisol reactivity, scores on the STAI State Anxiety scale, and estradiol showed a weak trend in support of the hypothesis, but was not significant, contrary to predictions. However, the interaction did account for an additional three percent of the variance, R^2 = .03, F (1, 26) = 1.25, p = .27, indicating that estradiol did explain a portion of the variance in the relationship between cortisol reactivity and state anxiety in women. The overall model was not significant for STAI Trait anxiety, F (3, 27) = 1.42, p = .26, R^2 = .14. The overall model was not significant for SCL-90 Anxiety either, F (3, 27) = 0.50, p = .68, R^2 = .05. Overall, and contrary to hypotheses, estradiol did not significantly moderate the relationship between cortisol reactivity and any of the anxiety measures. This finding indicates that the relationship between cortisol reactivity and scores on anxiety measures did not vary significantly across levels of estradiol in women, which indicates that estradiol may not be a factor in explaining the sex differences in the relationship between cortisol reactivity and anxiety. However, further research is needed into this relationship.

Table 2 *The moderating effects of sex on the relation between cortisol reactivity and state anxiety*

Model	*b* [95% CI]	SE	*t*	*p*
Cortisol Reactivity	2.13 [.26, 4.00]	.93	2.29	.03
Sex	.01 [-.16, .18]	.09	.13	.90
State (Pre-TSST)	.35 [.06, .64]	.14	2.46	.02
Cortisol Reactivity x Sex	-.95 [-1.74, -.16]	.39	-2.41	.02
R^2 = .45, *MSE* = .32, *F* (4, 49) = 3.03, *p* = .03				

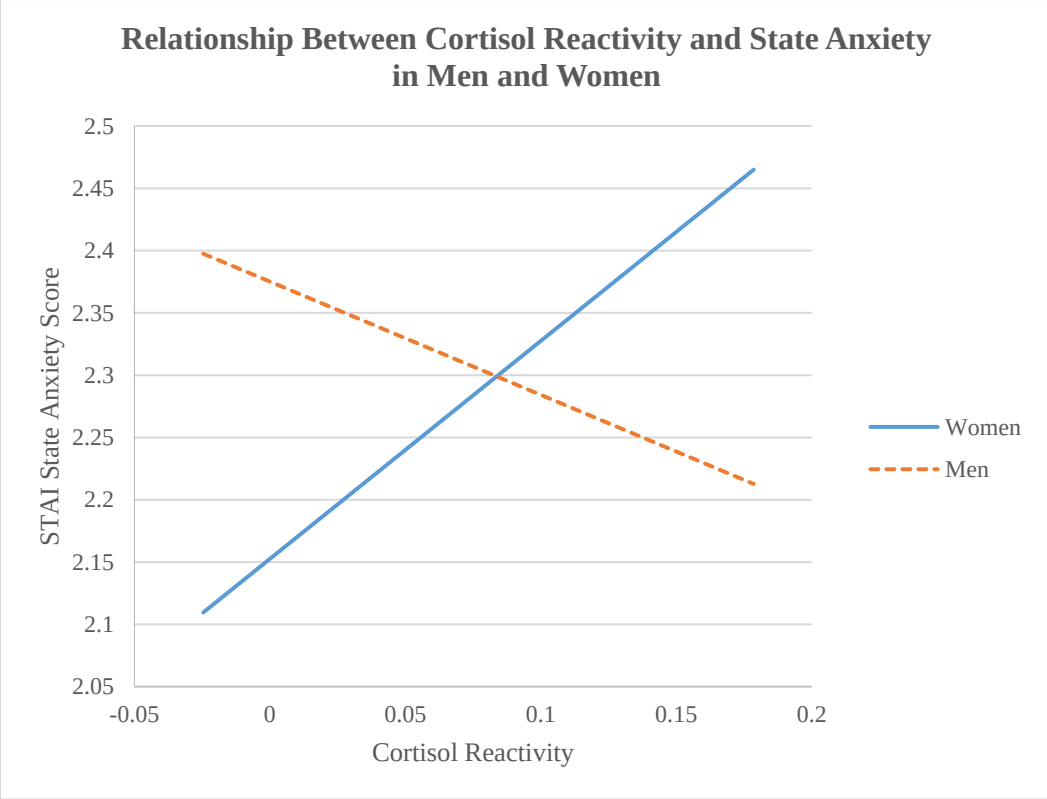

Figure 1 *The moderating effect of sex on the relationship between cortisol reactivity and post-TSST state anxiety scores*

Table 3 *Anxiety scale item mean and standard deviation by sex*

	Anxiety Scale	Mean [Min, Max]	Standard Deviation
Men	SCL-90 Anxiety	0.40 [0, 4]	0.32
	STAI Trait Anxiety	1.88 [1, 4]	0.36
	STAI State Anxiety (Post)	2.23 [1, 4]	0.60
Women	SCL-90 Anxiety	0.56 [0, 4]	0.57
	STAI Trait Anxiety	2.03 [1, 4]	0.52
	STAI State Anxiety (Post)	2.25 [1, 4]	0.63

CHAPTER 4

DISCUSSION

Sex Differences in Cortisol Reactivity

Consistent with previous research, men exhibited greater cortisol reactivity than women in response to the TSST, as hypothesized. Although these sex differences have been consistently found in research (Kudielka & Kirschbaum, 2005; Pisanski et al., 2018), it has been unclear exactly why these differences in cortisol reactivity in response to the TSST occur. The current research was undertaken to investigate this further, as well as how this relationship may relate to general anxiety. The current research investigated how reported anxiety and estrogen may be related to cortisol reactivity and sex differences in the relationship between anxiety and cortisol reactivity.

Relationships Between Cortisol Reactivity and Anxiety Measures

It was hypothesized that cortisol reactivity would relate to all three anxiety measures. While none of the measures of anxiety were significantly related to cortisol reactivity in men, both STAI anxiety measures were related to cortisol reactivity in women, and these relationships were marginally significant. Unfortunately, there were a limited number of females who participated in the present study, and as such, the power to detect effects was low, as discussed in detail below.

Scores on the SCL-90 Anxiety Scale were not related to cortisol reactivity in men or women. It is possible that this lack of relationships was due to the low scores on this scale. The SCL-90 Anxiety scale was significantly positively skewed, and the average item score was low for both men and women (see Table 3 for means and standard

deviations). Scores on the STAI scales were generally higher. This difference in scores is likely because the SCL-90 measures more severe symptoms of anxiety, such as having "spells of terror or panic" and "thoughts and images of a frightening nature" (Derogatis & Lazarus, 1994). The STAI Anxiety scales include milder and more common symptoms of anxiety, such as worrying too much over something that really doesn't matter and having troubles overcoming difficulties (Spielberger et al., 1983). Additionally, participants had slightly lower variance in scores on the SCL-90 Anxiety scale than on the STAI Anxiety scales. The participants included in this study were all college students, not a clinical population. This could explain why there is a lack of scores in the higher range of the scale and a lack of variability.

Sex as a Moderator

Both trait (STAI trait, and SCL) and current state (STAI state) were investigated in this study. Regarding state anxiety, as hypothesized, sex was shown to significantly moderate the relationship between cortisol reactivity and current anxiety. For men, the relationship between cortisol reactivity and state anxiety trended in a negative direction, while for females, the relationship between cortisol reactivity and state anxiety trended in a positive direction. In other words, in women, as cortisol reactivity increased, their scores on the post-TSST STAI state scale tended to increase, but in men, as cortisol reactivity increased, scores on the post-TSST STAI state scale tended to decrease. These findings indicate that the relationship between physiological response and reported anxiety as a result of the TSST differs in men and women.

On the other hand, the effect using anxiety as an individual trait was less clear cut. The moderating effect of sex on the relationship between cortisol reactivity and STAI Trait Anxiety was marginally significant, which partially supported the hypothesis. Relationships with the SCL-90 did not emerge. As discussed above, these results could be because the SCL-90 is a clinical scale and had low scores, which was not surprising given the participants were from a non-clinical college population. SCL-90 Anxiety scores for normative US samples tend to be around 0.30, so these scores were slightly higher, but they were not unusual for a non-clinical population (Derogatis & Lazarus, 1994).

The way cortisol reactivity predicts different measures of anxiety among men and women is of interest. Understanding how cortisol reactivity relates to experienced and reported anxiety can enhance the understanding of how men and women experience anxious situations physiologically and how the tendency to respond to situations with worry and stress may impact this physiological response. Although results did not reach significance, the relationship between cortisol reactivity and STAI measures of anxiety approached significance in women. In men, no such trend was found. It is possible that men's and women's bodies react differently to anxiety and to certain types of stress. Men tend to exhibit greater cortisol reactivity in response to stress than women (Kudielka & Kirschbaum, 2005; Pisanski et al., 2018). In the present study, men exhibited greater cortisol reactivity in response to the TSST than women. This sex difference could be due to differences in physiological responses to stress.

As discussed previously, this sex difference tends to vary by stressor, with men exhibiting higher cortisol reactivity in response to performance tasks, and women exhibiting higher cortisol reactivity in response to social exclusion (Stroud et al., 2002). However, the TSST is a mixture of social pressure from a speech task and performance on a mathematics task, which indicates that sex differences are not necessarily due to type of stressor.

Sex moderated the relationship between cortisol reactivity and state anxiety, but the moderation failed to reach significance for trait anxiety. Previous research has suggested that age and gender may both be factors for the relationship between anxiety and cortisol reactivity (Huini et al., 2018). It is possible that age is a factor, and because participants were young (average age of 19), changes that occur in the relationship between trait anxiety and cortisol reactivity have not yet become pronounced. It may take time for chronic stress to affect HPA axis activity. Research that has supported a relationship between chronic stress and HPA axis activity has shown HPA axis activity changes over years (Ruttle et al., 2011; Van den Bos et al., 2017).

Estradiol as a Moderator

It was hypothesized that a possible factor in the sex differences in the relationship between cortisol reactivity and anxiety could be estradiol. However, in this study, estradiol did not significantly moderate the relationship between cortisol reactivity and any of the anxiety measures. The overall model was significant for state anxiety, but the interaction was not significant. However, the interaction did account for an additional three percent of the variance. This indicates that estradiol explained some of the variation

in the relationship between cortisol reactivity and state anxiety. The overall model was not significant for trait anxiety. With a greater number of female participants, it is possible that the interaction would be significant for both state and trait anxiety. Estrogen is known to increase negative feedback (Sharma et al., 2014). Given what is known about estrogen's effect on the HPA axis, the small sample size, and the trend in the hypothesized direction, it is worth further investigating the impact of estradiol in the relationship between cortisol reactivity and reported anxiety.

Estrogen has an effect on the negative feedback loop that regulates HPA axis activity and therefore cortisol secretion (Sharma et al., 2014). It is possible that the number of women in the study was simply too low, decreasing power to detect effects. Studying a greater number of women could lead to a significant result.

Additionally, it is possible that additional unmeasured hormones play a role in the sex differences in the relationship between cortisol reactivity and anxiety. In one study, researchers found that testosterone and progesterone were both negatively associated with cortisol reactivity in response to the TSST, suggesting several different sex hormones are associated with inhibition of HPA axis response to stress (Stephens et al., 2016). In different studies, testosterone, progesterone, as well as estradiol have been associated with increases or decreases in cortisol response (Allen et al., 2017). It is possible that sex hormones play a role in cortisol reactivity but that the relationship is a complex one.

It is important to consider that the differences observed between men and women with regards to anxiety and stress response could be due to differences in environmental factors relating to social gender roles. It has been posited that differences in anxiety may

be due to differences in expression of anxiety due to differences in how acceptable it is for men and women to express fear and anxiety. Fear is less consistent with the male gender role, and some argue that this causes men to report fewer symptoms of anxiety (McLean & Anderson, 2009). In this study, although men scored lower than women on the SCL-90 Anxiety scale and both STAI scales (See Table 3), it did not reach statistical significance, that is, men's and women's scores on anxiety measures were not significantly different. Despite lower scores on anxiety measures, men exhibited greater cortisol reactivity than women, suggesting greater physiological stress response. It is possible that men experience similar or greater levels of anxiety but underreport anxiety due to gender expectations. Some research has found differences between men and women in explicit anxiety, or anxiety that individuals are consciously aware of and report, and implicit anxiety, or anxiety that individuals are not consciously aware of (Egloff & Schmukle, 2004; Weik et al., 2010). In a study comparing explicit conscious self-reported anxiety using scores on the Spielberger State–Trait Anxiety Inventory (STAI) trait scale with implicit measures of anxiety, including the Implicit Association Test and the Emotional Stroop Task, women exhibited higher levels of implicit and explicit anxiety (Egloff & Schmukle, 2004). However, the difference between women and men was much greater for explicit or reported anxiety than it was for implicit anxiety. These findings suggest that although women may report greater anxiety than men, there may be a smaller difference in actual levels of anxiety. If true, then this could explain the way in which sex moderates the relationship between physiological stress

response and current anxiety. Men may have simply reported lower anxiety, while their physiological response indicated their true anxiety levels.

Research has indicated that gender socialization may be a factor in differences in reported anxiety. Zalta and Chambless (2012) found that men tended to experience greater instrumentality and mastery than women, meaning that men tend to focus more on tasks and be more competitive and tend to have a greater belief that they can complete tasks effectively. They found that these gender differences mediated the relationship between gender and reported anxiety. Men could generally report less anxiety based on these socialized factors. These gender differences could make a difference in reported anxiety, specifically, such that men report less anxiety because they feel more confident and are more competitive.

Additionally, the way that participants appraised the task could affect the amount of anxiety they reported on the STAI State Anxiety scale after the TSST, as well as their cortisol reactivity in response to the TSST. According to Lazarus (1991), individuals in stressful situations experience challenge and threat. When a situation is appraised as a challenge, the individual perceives the situation as an opportunity to show their skills or gain from the situation. When perceived as a threat, individuals perceive the situation as one that could bring potential negative consequences (Lazarus, 1991). Challenge and threat appraisals activate physiological systems differently, such that a system called the sympathetic-adrenal-medullary axis activates more generally, and the HPA axis responds more specifically to threat (Jamieson, 2017). Participants who viewed the TSST as more of a challenge would exhibit lower post-TSST state anxiety and likely lower cortisol

levels as well. If men perceived the TSST as more of a challenge, this could explain some of the lower scores on the post-TSST state anxiety measures, but men also exhibited higher cortisol reactivity compared to women, which indicates that they perceived the TSST as a threat, which activated the HPA axis.

Limitations

The power to detect effects in this study was lower than desired due to the number of participants able to be recruited. A post-hoc power analysis using the effect size found using estradiol as a moderator in the relationship between cortisol reactivity and STAI state anxiety scores (f^2 = .42) determined that the present study had a 75% chance of finding an effect. Originally when proposed, a power analysis was conducted using G*Power to determine an optimum sample size to have an 80% chance of finding an effect if one exists. Sample size was based on the moderated regression analysis using estradiol as a moderator, as this analysis (which uses only women) requires the largest number of participants to obtain sufficient power to detect results. Although previous research has not assessed the moderating effects of estradiol levels or sex on the relationship between cortisol reactivity and anxiety, the effect size was estimated using effect sizes from sex differences in cortisol reactivity, which tend to be medium to large (Kudielka & Kirschbaum, 2005). An effect size closer to a medium effect size was used (f^2 = .20), and, as proposed, 59 females were needed for .80 power. The COVID-19 outbreak limited the amount of time available to run participants, as participants were college students and participated in person. Because of the pandemic, students were required to leave campus early during the first semester in which participants were run,

and in the following semester, fewer students were taking in-person classes, as there were online options. Additionally, enhanced COVID safety measures and associated additional IRB approvals were needed, delaying data collection. Finally, participants may have been less willing to participate due to COVID-19 as participation was in-person and involved saliva samples, and these details were stated clearly in the description posted to potential participants.

Future research should focus on doing another study examining these effects with a greater sample size. An additional power analysis was conducted based on the effect size found for the moderated regression analysis using estradiol as a moderator in the relationship between cortisol reactivity and STAI state anxiety scores. The effect size found was $f^2 = .42$. If the effect size for estradiol is correct, then a sample size of 42 women would be needed to detect the effect at conventional levels of significance. It is possible that with a greater number of participants, the non-significant results could have reached significance.

Due to the COVID-19 pandemic, additional safety measures had to be taken to ensure the safety of participants, including limiting the number of observers watching participants' speeches during the TSST, as the number of people in the same room had to be limited due to social distancing procedures. The TSST is often run with a greater number of observers, usually three (Kirschbaum et al., 1993). In order to increase the pressure on participants, future research should examine these effects with a greater number of observers. Cortisol reactivity was, lower than in previous studies, particularly in women. Kirschbaum and colleagues (1993) found that in multiple studies utilizing the

TSST, most participants were above .09 µg/dL. In the present study, men had an average cortisol reactivity of .08 µg/dL, and women had an average cortisol reactivity of .01 µg/dL. The mean for the sample was .04 µg/dL. However, there were still differences in cortisol levels between pre-TSST saliva samples and post-TSST saliva samples (up to .48 µg/dL), suggesting the modified TSST protocol worked as intended.

This study found the hypothesized effect for post task state anxiety. The simultaneous lack of an effect for trait anxiety was interesting. Future research may also look at participants of older ages in order to examine the relationship between cortisol reactivity and trait anxiety specifically. If cortisol responses to stress over time affect general anxiety levels, older participants might show a greater effect. There is some evidence that repeated stress over time can influence anxiety as individuals grow older (Ruttle et al., 2011). Repeated stress has been associated with decreases in HPA axis activity (Juster et al., 2011), which has been associated with stress-related disorders (Fries et al., 2005).

Older adults may exhibit a greater association between anxiety and cortisol reactivity due to increased stress decreasing cortisol reactivity. According to the theory of allostasis, repeated stress has consequences for the body, referred to as allostatic load. The body adjusts to this allostatic load by adjusting the way the body responds to stress. The adjustments the body makes to these systems is termed allostasis (McEwen & Stellar, 1993). Repeated stress has been associated with decreases in cortisol reactivity. Further research with a wider range of age may be able to investigate the affects of age

and how reacting to stressors with anxiety may relate to physiological stress response over time.

Future research should further investigate the relationship between cortisol reactivity and anxiety and the role sex and estradiol play in this relationship. The relationship is still unclear and likely nuanced. Future research could also examine how social factors relating to social roles by including measures of masculinity and femininity. This could help make clear whether the relationship between cortisol reactivity and anxiety measures differs more as a result of biological sex differences or social gender differences. Additionally, future research should include a wider range of age groups in order to examine whether age is a factor in the relationship between cortisol reactivity and trait anxiety.

The present research has implications for sex differences in cortisol response to the TSST. Sex moderates the relationship between cortisol reactivity and current anxiety, such that although men experienced lower reported anxiety during the TSST, they exhibited greater cortisol reactivity. Further research into these sex differences may help explain sex differences in cortisol reactivity in response to the TSST and may also help explain inconsistencies in the relationship between stress response and anxiety. This research indicates that sex may be a major factor in cortisol reactivity and its relationship with current anxiety. The relationship between cortisol stress response and current anxiety was different for men and women. The present research also indicated possible differences between men and women regarding the relationship between stress response and general anxiety. These findings have potential implications for sex differences in

anxiety and how men's and women's stress responses may change with anxiety levels. The results further highlight the difference between men's reported anxiety and their physiological stress response. Additionally, estrogen did not significantly moderate the relationship, which suggests that differences in estrogen levels may not be the reason for these sex differences and suggests that perhaps the differences are due to socialized gender differences. However, the relationship did trend in the hypothesized direction, so further research into the moderating effects of estradiol is needed.

Women and men experience stress and anxiety differently (McLean & Anderson, 2009), and understanding why has important implications for understanding the effects of anxiety on physiological systems. Experiencing anxiety and responding to situations with stress, particularly when the HPA axis is activated, may lead to changes in body systems over time. Chronic stress is associated with various problems, such as diabetes and cardiovascular problems (McEwen & Stellar, 1993). The relationship between HPA stress response and reported anxiety may also relate to trait anxiety as well, as the way individuals physiologically and psychologically respond to stressful situations has an effect on their general experience of anxiety.

REFERENCES

Allen, A. P., Kennedy, P. J., Dockray, S., Cryan, J. F., Dinan, T. G., & Clarke, G. (2017). The Trier Social Stress Test: Principles and practice. *Neurobiology of Stress*, *6*, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001

Berger, M., Bossert, S., Krieg, J.-C., Dirlich, G., Ettmeier, W., Schreiber, W., & von Zerssen, D. (1987). Interindividual differences in the susceptibility of the cortisol system: An important factor for the degree of hypercortisolism in stress situations?. *Biological Psychiatry*, *22*(11), 1327–1339. https://doi.org/10.1016/0006-3223(87)90067-9

Blascovich, J., Vanman, E. J., Berry Mendes, W., & Dickerson, S. (2011). *Social psychophysiology for social and personality psychology*. Sage Publications, Inc.

Childs, E., Dlugos, A., & De Wit, H. (2010). Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. *Psychophysiology*, *47*(3), 550–559. https://doi.org/10.1111/j.1469-8986.2009.00961.x

Clements, A. D. (2012). Salivary cortisol measurement in developmental research: Where do we go from here?. *Developmental Psychobiology*, *55*(3), 205–220. https://doi.org/10.1002/dev.21025

Derogatis, L. R., & Lazarus, L. (1994). SCL-90—R, Brief Symptom Inventory, and matching clinical rating scales. In M. E. Maruish (Ed.), *The use of psychological testing for treatment planning and outcome assessment* (pp. 217–248). Lawrence Erlbaum Associates, Inc.

DeSantis, S. M., Baker, N. L., Back, S. E., Spratt, E., Ciolino, J. D., Maria, M. M.-S., Dipankar, B., & Brady, K. T. (2011). Gender differences in the effect of early life trauma on hypothalamic-pituitary-adrenal axis functioning. *Depression and Anxiety*, *28*(5), 382–392. https://doi.org/10.1002/da.20795

DeSoto, C., & Salinas, M. (2015). Neuroticism and cortisol: The importance of checking for sex differences. *Psychoneuroendocrinology*, *62*, 174–179. https://doi.org/10.1016/j.psyneuen.2015.07.608

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*(3), 355–391. https://doi.org/10.1037/0033-2909.130.3.355

Egloff, B., & Schmukle, S. C. (2004). Gender differences in implicit and explicit anxiety measures. *Personality and Individual Differences*, *36*(8), 1807–1815. https://doi.org/10.1016/j.paid.2003.07.002

Endler, N. S., & Kocovski, N. L. (2001). State and trait anxiety revisited. *Journal of Anxiety Disorders*, *15*(3), 231–245. https://doi.org/10.1016/S0887-6185(01)00060-3

Faravelli, C., Sauro, C. L., Lelli, L., Pietrini, F., Lazzeretti, L., Godini, L., Benni, L., Fioravanti, G., Talamba, G. A., Castellini, G., & Ricca, V. (2012). The role of life events and hpa axis in anxiety disorders: A review. *Current Pharmaceutical Design*, *18*(35), 5663–5674. https://doi.org/10.2174/138161212803530907

Fries, E., Hesse, J., Hellhammer, J., & Hellhammer, D. H. (2005). A new view of hypocortisolism. *Psychoneuroendocrinology*, *30*(10), 1010–1016. https://doi.org/10.1016/j.psyneuen.2005.04.006

Granger, D. A., Shwartz, E. B., Booth, A., Curran, M., & Zakaria, D. (1999). Assessing dehydroepiadrosterone in saliva: A simple radioimmunoassay for use in studies of children, adolescents and adults. *Psychoneuroendocrinology*, *24*(5), 567–579. https://doi.org/10.1016/S0306-4530(99)00013-X

Hastings, W. J., Chang, A. M., Ebstein, R. P., & Shalev, I. (2018). Neuroendocrine stress response is moderated by sex and sex hormone receptor polymorphisms. *Hormones and Behavior*, *106*, 74–80. https://doi.org/10.1016/j.yhbeh.2018.10.002

Hayes, A. F. (2017). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach* (2nd ed.). Guilford Press.

Heims, C., Ehlert, U., Hanker, J. P., & Hellhammer, D. H. (1998). Abuse-related posttraumatic stress disorder and alterations of the hypothalamic-pituitary-adrenal axis in women with chronic pelvic pain. *Psychosomatic Medicine*, *60*(3), 309–318. https://doi.org/10.1097/00006842-199805000-00017

Helpman, L., Penso, J., Zagoory-Sharon, O., Feldman, R., & Gilboa-Schechtman, E. (2017). Endocrine and emotional response to exclusion among women and men; cortisol, salivary alpha amylase, and mood. *Anxiety Stress and Coping*, *30*(3), 253–263. https://doi.org/10.1080/10615806.2016.1269323

Herbison, C. E., Henley, D., Marsh, J., Atkinson, H., Newnham, J. P., Matthews, S. G., Lye, S. J., & Pennell, C. E. (2016). Characterization and novel analyses of acute stress response patterns in a population-based cohort of young adults: Influence of gender, smoking, and BMI. *The International Journal on the Biology of Stress*, *19*(2), 139–150. https://doi.org/10.3109/10253890.2016.1146672

Huini, P., Jianhui, W., Xiaofang, S., Qing, G., & Yuejia, L. (2018). Trait anxiety predicts the response to acute psychological stress. *Acta Psychologica Sinica*, *50*(9), 997–1006. https://doi.org/10.3724/SP.J.1041.2018.00997

Jamieson, J. P. (2017). Challenge and threat appraisals. In A. J. Elliot, C. S. Dweck, & D. S. Yeager (Eds.), *Handbook of competence and motivation: Theory and application* (2nd Ed., pp. 175–191). Guilford Publications.

Jaremka, L. M., & Collins, N. L. (2017). Cortisol increases in response to brief social exchanges with opposite sex partners. *Biological Psychology*, *124*, 39–46. https://doi.org/10.1016/j.biopsycho.2017.01.001

Juster, R.-P., Sindi, S., Marin, M.-F., Perna, A., Hashemi, A., Pruessner, J. C., & Lupien, S. J. (2011). A clinical allostatic load index is associated with burnout symptoms and hypocortisolemic profiles in healthy workers. *Psychoneuroendocrinology*, *36*(6), 797–805. https://doi.org/10.1016/j.psyneuen.2010.11.001

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Helhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine*, *61*(2), 154–162. https://doi.org/10.1097/00006842-199903000-00006

Kirschbaum, C., Pirke, K.-M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'—A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1–2), 76–81. https://doi.org/10.1159/000119004

Knudtson, J., & McLaughlin, J. E. (2019). Female reproductive endocrinology. In *Merck manual online*. Retrieved from https://www.merckmanuals.com/professional/gynecology-and-obstetrics/female-reproductive-endocrinology/female-reproductive-endocrinology

Kudielka, B. M., & Kirschbaum, C. (2005). Sex diference in HPA axis responses to stress: A review. *Biological Psychology*, *69*(1), 113–132. https://doi.org/10.1016/j.biopsycho.2004.11.009

Laurent, H. K., Gilliam, K. S., & Wright, D. B. (2015). Child anxiety symptoms related to longitudinal cortisol trajectories and acute stress responses: Evidence of development stress sensitization. *Journal of Abnormal Psychology*, *124*(1), 68–79. https://doi.org/10.1037/abn0000009

Lazarus, R. S. (1991). Progress on a cognitive-motivational-relational theory of emotion. *American Psychologist*, *46*(8), 819–834. http://dx.doi.org.proxy.lib.uni.edu/10.1037/0003-066X.46.8.819

Lennartsson, A. K., Kushnir, M. M., Bergquist, J., Billig, H., & Jonsdottir, I. H. (2012). Sex steroid levels temporarily increase in response to acute psychosocial stress in healthy men and women. *International Journal of Psychophysiology*, *84*(3), 246–253. https://doi.org/10.1016/j.ijpsycho.2012.03.001

Marteau, T. M., & Bekker, H. (1992). The development of a six-item short-form of the state scale of the Spielberger State–Trait Anxiety Inventory (STAI). *British Journal of Clinical Psychology*, *31*(3), 301–306. https://doi.org/10.1111/j.2044-8260.1992.tb00997.x

McEwen, B. S. (2008). Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *European Journal of Pharmacology*, *583*(2–3), 174–185. https://doi.org/10.1016/j.ejphar.2007.11.071

McEwen, B. S., & Stellar, E. (1993). Stress and the individual: Mechanisms leading to disease. *Archives of International Medicine*, *153*(18), 2093–2101. https://doi.org/10.1001/archinte.1993.00410180039004

McLean, C. P., & Anderson, E. R. (2009). Brave men and timid women? A review of the gender differences in fear and anxiety. *Clinical Psychology Review*, *29*(6), 496–505. https://doi.org/10.1016/j.cpr.2009.05.003

McRae, A. L., Saladin, M. E., Brady, K. T., Upadhyaya, H., Back, S. E., & Timmerman, M. A. (2006). Stress reactivity: Biological and subjective responses to the cold pressor and Trier Social stressors. *Human Psychopharmacology: Clinical and Experimental*, *21*(6), 377–385. https://doi.org/10.1002/hup.778

Pisanski, K., Kobylarek, A., Jakubowska, L., Nowak, J., Walter, A., Blaszczynski, K., Kasprzyk, M., Lysenko, K., Sukiennik, I., Piatek, K., Frackowiak, T., & Sorokowski, P. (2018). Multimodal stress detection: Testing for covariation in vocal, hormonal and physiological response to the Trier Social Stress Test. *Hormones and Behavior*, *106*, 52–61. https://doi.org/10.1016/j.yhbeh.2018.08.014

Ruttle, P. L., Shirtcliff, E. A., Serbin, L. A., Fisher, D., B.-D., Stack, D. M., & Schwartzman, A. E. (2011). Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviors in youth: Longitudinal and concurrent associations with cortisol. *Hormones and Behavior*, *59*(1), 123–132. https://doi.org/10.1016/j.yhbeh.2010.10.015

Schultheiss, O. C., & Stanton, S. J. (2009). Assessment of salivary hormones. In E. Harmon-Jones & J. S. Beer (Eds.), *Methods in Social Neuroscience* (pp. 17–44). Guilford Press.

Sharma, A. N., Aoun, P., Wigham, J. R., Weist S. M., & Veldhuis, J. D. (2014). Estradiol, but not testosterone heightens cortisol feedback on pulsatile ACTH secretion and ACTH approximate entropy in unstressed older men and women. *American Journal of Physiology*, *306*, 627–635. https://doi.org/10.1152/ajpregu.00551.2013

Spielberger, C. D., Gonzalez-Reigosa, F., & Martinez-Urrutia, A. (1971). Development of the Spanish edition of the State-Trait Anxiety Inventory. *Interamerican Journal of Psychology*, *5*(3–4), 145–158. https://doi.org/10.1002/9780470479216.corpsy0943

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Consulting Psychologists Press.

Spielberger, C. D., & Rickman, R. L. (1990). Assessment of state and trait anxiety. In N. Sartorius, V. Andreoli, G. Cassano, L. Eisenberg, P. Kielholz, P. Pancheri, & G. Racagni. (Eds.), *Anxiety: Psychobiological and clinical perspectives* (pp. 69–84). Taylor & Francis.

Stephens, M. A. C., Mahon, P. B., McCaul, M. E., & Wand, G. S. (2016). Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones. *Psychoneuroendocrinology*, *66*, 47–55. https://doi.org/10.1016/j.psyneuen.2015.12.021

Stroud, L. R., Salovey, P., & Epel, E. S. (2002). Sex differences in stress responses: Social rejection versus achievement stress. *Biological Psychiatry*, *52*(4), 318–327. https://doi.org/10.1016/S0006-3223(02)01333-1

Tabachnick, B. G., & Fidell, L. S. (2019). *Using multivariate statistics* (7th ed.). Pearson.

Van den Bos, E., Tops, M., & Westernberg, P. M. (2017). Social anxiety and the cortisol response to social evaluation in children and adolescents. *Psychoneuroendocrinology*, *78*, 159–167. https://doi.org/10.1016/j.psyneuen.2017.02.003

Weik, U., Maroof, P., Zöller, C., & Deinzer, R. (2010). Pre-experience of social exclusion suppresses cortisol response to psychosocial stress in women but not in men. *Hormones and Behavior*, *58*(5), 891–897. https://doi.org/10.1016/j.yhbeh.2010.08.018

Yoon, K. L., & Joormann, J. (2012). Stress reactivity in social anxiety disorder with and without comorbid depression. *Journal of Abnormal Psychology*, *121*(1), 250–253. https://doi.org/10.1037/a0025079

Zalta, A. K., & Chambless, D. L. (2012). Understanding gender differences in anxiety: The mediating effects of instrumentality and mastery. *Psychology of Women Quarterly*, *36*(4), 488–499. https://doi.org/10.1177/0361684312450004

APPENDIX A

SALIVA SAMPLE SCREENING QUESTIONS

1. Did you consume alcohol last night or today?
    a. Yes
    b. No
2. Have you eaten a major meal within the previous 60 minutes?
    a. Yes
    b. No
3. Have you consumed any dairy products within the past 20 minutes?
    a. Yes
    b. No
4. Have you consumed any high sugar foods within the past 20 minutes?
    a. Yes
    b. No
5. Have you consumed any foods high in acidity (e.g., lemons) within the past 20 minutes?
    a. Yes
    b. No
6. Did you exercise last night or today?
    a. Yes
    b. No

7. Have you smoked within the past two hours?
    a. Yes
    b. No
8. Are you experiencing any oral diseases or problems?
    a. Yes
    b. No
9. Approximately how long ago did you wake up? _____

APPENDIX B

DEMOGRAPHIC QUESTIONS

1. Please indicate your age _____
2. Please indicate your gender identity:
    1. Female
    2. Gender non-binary
    3. Gender queer
    4. Male
    5. Transgender male
    6. Transgender female
    7. Not listed (please specify) _____
3. Please indicate your biological sex:
    a. Female
    b. Intersex
    c. Male
4. Please indicate your ethnicity:
    1. African American/Black
    2. Asian/Pacific Islander
    3. Caucasian/White
    4. Hispanic/Latinx
    5. Native American
    6. Multiracial (please specify)

7. Not listed (please specify)
8. Prefer not to answer

5. Think of the ladder below as representing where people stand in the United States. At the top of the ladder are people who are best off, those who have the most money, the most education, and the most respected jobs. At the bottom are the people who are the worst off, who have the least money, least education, and the least respected jobs or no job. Please select the option below that corresponds with the numbered rung that represents where you think you stand on the ladder at this time in your life, relative to other people in the United States.
    a. 1
    b. 2
    c. 3
    d. 4
    e. 5
    f. 6
    g. 7
    h. 8
    i. 9
    j. 10


10
9
8
7
6
5
4
3
2
1

APPENDIX C

SYNTHETIC HORMONE USE QUESTIONS

The following questions are important for the proper assessment of participant biological markers as measured via saliva. Please answer each question with as much detailed information as possible.

1. Are you currently on a form of birth control?
    1. Yes, oral contraceptives (“the pill”)
    2. Yes, an IUD
    3. Yes, a shot
    4. Yes, coitus interruptus (“withdrawal method”)
    5. Yes, abstinence
    6. Yes, other _____
    7. No
2. Sex steroids are prescribed for any number of reasons. However, such steroids can alter the baseline concentrations of various analytes in saliva. Are you currently receiving any form of sex steroids (e.g., testosterone, estrogen, etc.)
    a. Yes
    b. No
3. If you answered “Yes” to the sex steroid question above, please list sex steroids you are currently taking on a regular basis._____

APPENDIX D

SCL-90-R

Trait Anxiety (SCL-90-R) - Below is a list of problems and complaints people sometimes have. Please read each one carefully and select the number that best describes how much you were bothered by that problem in the past week. (Likert scale: 0 = Not at all, 1 = A Little Bit, 2 = Moderately, 3 = Quite a Bit, 4 = Extremely)

1. Headaches
2. Nervousness or shakiness inside
3. Repeated unpleasant thoughts that won't leave your mind
4. Faintness or dizziness
5. Loss of sexual interest or pleasure
6. Feeling critical of others
7. The idea that someone else can control your thoughts
8. Feeling that others are to blame for most of your troubles
9. Trouble remembering things
10. Worried about sloppiness or carelessness
11. Feeling easily annoyed or irritated
12. Pains in heart or chest
13. Feeling afraid in open spaces or on the streets
14. Feeling low in energy or slowed down
15. Thoughts of ending your life
16. Hearing voices that others do not hear

17. Trembling

18. Feeling that other people cannot be trusted

19. Poor appetite

20. Crying easily

21. Feeling shy or uneasy with the opposite sex

22. Feelings of being trapped or caught

23. Suddenly scared for no reason

24. Temper outbursts that you could not control

25. Feeling afraid to go out of your house alone

26. Blaming yourself for things

27. Pains in lower back

28. Feeling blocked in getting things done

29. Feeling lonely

30. Feeling blue

31. Worrying too much about things

32. Feeling no interest in things

33. Feeling fearful

34. Your feelings being easily hurt

35. Other people being aware of your private thoughts

36. Feeling others do not understand you or are unsympathetic

37. Feeling that people are unfriendly or dislike you

38. Having to do things slowly to insure correctness

39. Heart pounding or racing

40. Nausea or upset stomach

41. Feeling inferior to others

42. Soreness of your muscles

43. Feeling that you are watched or talked about by others

44. Trouble falling asleep

45. Having to check and double-check what you do

46. Difficulty making decisions

47. Feeling afraid to travel on buses, subways, or trains

48. Trouble getting your breath

49. Hot or cold spells

50. Having to avoid certain things, places, or activities because they frighten you

51. Your mind going blank

52. Numbness or tingling in parts of your body

53. A lump in your throat

54. Feeling hopeless about the future

55. Trouble concentrating

56. Feeling weak in parts of your body

57. Feeling tense or keyed up

58. Heavy feelings in arms or legs

59. Thoughts of death or dying

60. Overeating

61. Feeling uneasy when people are watching or talking about you

62. Having thoughts that are not your own

63. Having urges to beat, injure, or harm someone

64. A wakening in the early morning

65. Having to repeat the same actions such as touching, counting, or washing

66. Sleep that is restless or disturbed

67. Having urges to break or smash things

68. Having ideas or beliefs that others do not share

69. Feeling very self-conscious of others

70. Feeling uneasy in crowds, such as shopping or a movie

71. Feeling everything is an effort

72. Spells of terror or panic

73. Feeling uncomfortable about eating or drinking in public

74. Getting into frequent arguments

75. Feeling nervous when you are left alone

76. Others not giving you proper credit for your achievements

77. Feeling lonely even if you are with people

78. Feeling so restless you couldn’t sit still

79. Feelings of worthlessness

80. The feeling that something bad is going to happen to you

81. Shouting or throwing things

82. Feeling afraid you will faint in public

83. Feeling that people will take advantage of you if you let them

84. Having thoughts about sex that bother you a lot

85. The idea that you should be punished for your sins

86. Thoughts or images of a frightening nature

87. The idea that something serious is wrong with your body

88. Never feeling close to another person

89. Feelings of guilt

90. The idea that something is wrong with your mind

APPENDIX E

STATE TRAIT ANXIETY INVENTORY STATE SCALE

Please rate the following as far as how you feel right now. (1 = not at all, 2 = somewhat, 3 = moderately so, 4 = very much so)

1. I feel calm
2. I am tense
3. I feel upset
4. I am relaxed
5. I feel content
6. I am worried

APPENDIX F

STATE TRAIT ANXIETY INVENTORY TRAIT SCALE

Please rate the following as far as how you generally feel. (1 = not at all, 2 = somewhat, 3 = moderately so, 4 = very much so)

1. I feel pleasant.
2. I feel nervous and restless.
3. I feel satisfied with myself.
4. I wish I could be as happy as others seem to be.
5. I feel like a failure.
6. I feel rested.
7. I am calm, cool, and collected.
8. I feel difficulties are piling up so that I cannot overcome them.
9. I worry too much over something that really doesn't matter.
10. I am happy.
11. I am inclined to take things hard.
12. I lack self-confidence.
13. I have disturbing thoughts.
14. I make decisions easily.
15. I feel inadequate.
16. I am content.
17. Some unimportant though runs through my mind and bothers me.
18. I have disappointments so keenly that I can't seem to put them out of my mind.

19. I am a steady person.
20. I get in a state of tension or turmoil as I think over my recent concerns and interests.

APPENDIX G

CROSS-TALK CHECK QUESTIONS

1. What did you think this study was about?
2. What did you hear about this study prior to participation?
3. If you have any comments about the study, please type them below.