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The Neuroendocrine and Performance Correlates of Posttraumatic Stress Disorder in Males and Females

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THE NEUROENDOCRINE AND PERFORMANCE CORRELATES OF

POSTTRAUMATIC STRESS DISORDER IN MALES AND FEMALES

By

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2009

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Abstract

Distinct gender differences exist in the incidence of posttraumatic stress disorder in the United States population. Currently, the direct biological mechanisms involved in the development of PTSD have not been elucidated. However, many studies indicate a dysregulation of the hypothalamic-pituitary-adrenal axis (HPA-axis) as a contributing factor in the development of PTSD. This study investigated performance and endocrine correlates of PTSD in 38 females without PTSD, 14 females with PTSD, 32 males without PTSD and 5 males with PTSD. We examined the differences between basal cortisol concentrations, as well as cortisol reactivity to the Trier Social Stress Test (TSST). In addition, participant performance was measured during the TSST. We administered the Liebowitz Social Anxiety Scale and the Abbreviated Math Anxiety Scale to investigate the relationship between math and performance anxiety and performance. We did not find significant differences in cortisol concentrations between groups. We found a significant effect of time when investigating subjective stress levels. We also found a significant effect of sex and PTSD status on the AMAS and an effect of PTSD on the LSAS. Our data suggest that the anxiety experienced in individuals with PTSD generalizes across domains but does affect performance. It may be that individuals without PTSD symptoms provide anxiety ratings that better predict their performance on the TSST tasks, either because their pre-existing levels of anxiety in these domains directly affect their performance or because they more accurately assess their performance and rate their anxiety in these domains accordingly.

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Chapter 1: Introduction

Current epidemiological studies indicate that females have a substantially higher incidence of developing posttraumatic stress disorder (PTSD) than males. The lifetime prevalence of PTSD for females is 9.7% compared to 3.6% for males in the general United States population (APA, 2000). At present, the direct biological mechanisms involved in the development of PTSD have not been elucidated. Many studies point towards a dysregulation of the hypothalamic-pituitary-adrenal axis (HPA-axis) as a contributing factor in the development of PTSD (Boscarino, 1996). Most studies examine HPA-axis function in males with PTSD. Few studies compare male and female HPA-axis function, and even fewer examine HPA-axis function in females with PTSD. This study will examine the neuroendocrine and performance correlates of the HPA-axis' involvement with PTSD symptoms.

Posttraumatic stress disorder occurs when an individual is exposed to a life-threatening situation. Any situation that involves threatened death or serious injury, which causes an individual to feel horrified, terrified, or helpless, can result in the development of PTSD (APA, 2000). Events that most commonly result in PTSD are combat, physical and sexual assault, and natural and man-made disasters. PTSD symptoms intensify with physical or emotional proximity to the event. Symptoms will be more intense if an individual experiences the triggering event first hand. Individuals usually develop symptoms within three months of exposure to the traumatic event. PTSD can be broken down into subtypes dependent on the duration and onset of symptoms. These subtypes are specified as being acute, chronic, or delayed onset. The specifier "acute" is used if the symptoms last less than three months and "chronic" is used when

symptoms last for three months or longer. "Delayed onset" is a term used when symptoms do not appear for six months or longer after the triggering event.

Additionally, there are three categories of symptom expression: re-experiencing, emotional numbing and avoidance, and hyperarousal (APA, 2000). Re-experiencing symptoms can be manifested in nightmares, unwanted intrusive thoughts, or a dissociative state in which the individual relives the traumatic experience. The dissociative state is what is commonly known as a "flash-back" and can last from seconds to hours. Triggers, physical or symbolic reminders of the traumatic event, usually cause re-experiencing symptoms.

Emotional numbing and avoidance occur when an individual feels detached from others and their own emotions (APA, 2000). Individuals may avoid situations that are reminiscent of the trauma, such as talking to people who experienced the trauma with them, talking about the trauma, and specific or symbolic stimuli relating to the trauma. Peritraumatic dissociation is a form of avoidance that consists of symptoms like depersonalization or derealization (Fullerton et al., 2001). Individuals who experience more peritraumatic dissociation symptoms are at a higher risk for developing chronic PTSD (Ursano et al., 1999). Emotional numbing may present itself as a lack of planning for the future, dissociation, feelings of hopelessness and inappropriate affect.

The last criterion, hyperarousal, is marked by physiological experiences of anxiety (APA, 2000). Individuals will experience symptoms such as persistent anxiety and depression. These symptoms are typically expressed as difficulty sleeping, hypervigilance, and difficulty concentrating. Hyperarousal may be expressed as an exaggerated response to noise or sudden movement. Hyperarousal may result in

gastrointestinal problems, suppressed immune function and chronic pain.

Gender Differences in PTSD

PTSD has a lifetime prevalence of around 8% in the United States population (APA, 2000). The disorder affects males and females at different rates. Community studies indicate that females have a lifetime prevalence of PTSD of 9.7% and males of 3.6%. Differences in prevalence may stem from type of trauma experienced and perception of the trauma (Pereira, 2002; Ennis, Kelly & Lambert, 2001; Irish et al., 2011). For example, males are more likely to experience assault, however, females are more likely to develop PTSD following assault (Breslau, Chilcoat, Kessler, Peterson & Lucia, 1999). This difference may stem from perception of the female's ability to defend herself against the perpetrator (Pereria, 2002).

Although females have a greater lifetime prevalence of PTSD, they are often under-diagnosed (Pereira, 2002). Females experience comorbid depression more often than males do (Haskell, et al., 2010). Symptoms of depression and PTSD overlap, which may result in more diagnoses of depression than PTSD. For example, symptoms like emotional numbing mimic depressive symptoms. Depression is marked by feelings of hopelessness and disconnection with others (APA, 2000). Furthermore, individuals with depression may exhibit signs of hypervigilence. Individuals with depression may have trouble sleeping, problems with diet, and increased anxiety. Moreover, in a military setting, women are more likely to be diagnosed with depression rather than PTSD based on gender-typical military job duties.

Life stress and traumatic stressors differ for males and females. Females face a variety of stressors unique to their gender. For example, females are often the sole

caretakers in their families and work full-time. Females also face significantly more sexual harassment in the work place (Street, Gradus, Stafford, & Kelly, 2007). Additionally, females are more likely to experience sexual trauma than males (Kimerling et al., 2003). Sexual trauma is the greatest predictor of PTSD in both males and females (Seedat et al., 2005). Pregnancy, domestic violence, and stress from single parenting are also more likely to affect females than males.

Biological reaction to stress varies in males and females (Kirschbaum et al., 1999; Matthews et al., 2001). This may lead to a difference in the physical and psychological experience of stress. The differences in stress response may be due to cyclic hormonal variations. Studies have shown females respond differently to stress during the different phases of the menstrual cycle (Childs, Dlugos, De Wit, 2010; Ennis, Kelly & Lambert, 2010; Kudielka & Kirschbaum, 2003). In conclusion, gender differences in PTSD may be related to the type of trauma experienced, comorbid psychopathologies, severity and perception of trauma, biological alterations and/or additional life stressors.

Cortisol and the Stress Response

The HPA-axis is a main component in regulating the body's response to stress (Lightman, 2008). When a person encounters a stressor, the sympathetic nervous system's fight or flight response activates by releasing adrenaline. The HPA-axis acts in response to the stressor following the release of adrenaline. When an individual experiences stress, the hypothalamus releases corticotrophin-releasing hormone (CRH), which then activates the anterior pituitary to release adrenocorticotrophin (ACTH). ACTH acts on the adrenal glands to prompt the release of cortisol into the bloodstream. Cortisol, a glucocorticoid, binds to glucocorticoid receptors in the body, blood, and brain.

Glucocorticoid receptors are heavily concentrated in the hippocampus and hypothalamus. Once cortisol binds to glucocorticoid receptors in the hypothalamus and pituitary, CRH and ACTH secretion are stopped, thus completing a negative feedback loop. Cortisol release follows a circadian rhythm as well as being released during a stress response (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). Cortisol measurements are highest thirty minutes after waking and steadily decrease throughout the day (Kudielka & Kirschbaum, 2003).

The body works to maintain homeostasis during a stressful event (Johnson, Kamilaris, Chrousos, & Gold, 1992). Cortisol release causes an increase in alertness and a decrease in the sensory threshold, suppressed hunger, digestion, reproduction and immune function. While these physiological alterations are adaptive in the context of short-term stress, prolonged exposure to stress can have detrimental effects on physical and psychological health. Constant exposure to a stressor can cause a dysregulation in the HPA-axis, leading to negative side effects like hypertension, diabetes, and immune suppression.

When an individual is exposed to a stressor, cortisol can be measured to assess the HPA-axis function in response to stress (Kircshbaum & Hellhammer, 1993). HPA-axis function can be investigated by measuring cortisol in blood plasma, cerebral spinal fluid (CSF), urine, and saliva (Lundberg, 2005). When cortisol is measured through saliva or urine, it is considered a free hormone fraction because it is not bound to proteins, as it is in blood plasma (Mendel, 1989). When cortisol is free from bound proteins, it can bind to glucocorticoid receptors. Thus, measuring the free fraction allows a more accurate measure of HPA-axis function.

Many studies have found distinct gender differences in endocrine stress reactivity. Studies that measure salivary cortisol release in response to laboratory stressors found that males have an increased response to stress when compared to females in the follicular phase of the menstrual cycle, but not with females in the luteal phase (Zimmer et al., 2003; Childs, Dlugos, & De Wit, 2010). The follicular phase of the menstrual cycle is marked by increased concentrations of estrogen and ends with ovulation. The luteal phase is marked by increased concentrations of progesterone and ends with menses. These studies also found that males recover from stress more quickly than females, as evidenced by rapidly decreasing cortisol concentrations. Females in both phases of the menstrual cycle recover from stress slowly, as evidenced by slowly decreasing cortisol concentrations. Gender differences in stress recovery have been linked to specific stress-related diseases that have different prevalence rates in males and females. Males are more prone to heart disease, which may be related to the large amounts of cortisol released during stress and fast fluctuations in recovery. Females, on the other hand, are more prone to autoimmune and inflammatory diseases, which may be associated with sustained levels of cortisol during the slow recovery phase (Kudielka & Kirschbaum, 2005).

Cortisol and Posttraumatic Stress Disorder

There are currently two theories regarding cortisol secretion in PTSD: hypercortisolism (increased cortisol concentrations) and hypocortisolism (blunted cortisol concentrations; Mason et al., 2001). Distinct neuroendocrine changes have been found in individuals with PTSD (Lauc, Zvonar, Vuksic-Mihaljevic & Flogel, 2004; Baker, et al., 2005). It is not known whether the changes occur as a result of trauma or are present prior to symptom onset and predispose the individual for developing PTSD. Previous research

has represented conflicting results in cortisol concentrations related to posttraumatic stress disorder. Some studies have found evidence of hypercortisolism in individuals with PTSD, while some studies found evidence of hypocortisolism.

Some studies found an increase in cortisol concentrations in individuals with PTSD. Lindley, Carlson and Benoit (2004), found elevated awakening cortisol concentrations in males and females diagnosed with PTSD resulting from childhood abuse. The researchers collected saliva at 8:00am, 4:00pm, and 10:00pm on two days. Participants were given a dose of dexamethasone to test HPA-axis negative feedback at 10:00pm. No significant differences were found in pre- or post-dexamethasone cortisol concentrations. Additionally, no significant differences were found between groups in response to dexamethasone. This study only examined pre- and post- dexamethasone cortisol concentrations. Although both sexes were examined, the researchers did not analyze sex differences. Additionally, Baker and colleagues (2005) found higher concentrations of basal cortisol in CSF of combat veterans with PTSD compared to healthy controls. This study compared samples of cortisol from urine and CSF collected over six hours from eight male combat veterans and eight age-matched healthy controls. No difference was found in urinary free cortisol. This group may have found higher concentrations of cortisol in CSF but not urine because the cortisol contained in urine is a free fraction, whereas CSF contains both free and bound cortisol.

Very few studies have examined HPA-axis function with regard to PTSD in a female population. Some studies that have investigated female populations have found that females with PTSD have blunted cortisol concentrations compared to controls. Most of the studies conducted focus on intimate partner violence and childhood abuse. Young,

Tolman, Witkowski, and Kaplan (2004), examined salivary cortisol in low-income females with recent PTSD, past PTSD and healthy controls. This study collected cortisol at awakening and bedtime as well as during participants' visit to the research site. The researchers found that individuals with recent trauma had higher awakening cortisol concentrations, however, they did not find a significant difference between individuals with a lifetime diagnosis of PTSD and controls. Inslicht et al. (2006) examined salivary cortisol in females with intimate partner violence (IPV)-related PTSD and abused females with no history of PTSD. They found that females with PTSD had higher concentrations of basal cortisol than females without. Samples were collected at four time points throughout the day. Lemieux and Coe (1995) examined samples of urinary cortisol collected over a 24-hour time period in females diagnosed with PTSD from childhood sexual abuse, those who experienced sexual abuse but did not develop PTSD, and healthy controls. Females with PTSD exhibited higher cortisol concentrations compared to females without PTSD and healthy controls.

Hypercortisolism can be a result of comorbid depression, particularly in studies using cortisol samples of plasma or CSF. Individuals with depression have higher concentrations of cortisol in these fluids than healthy controls (Bjorntorp, 1996). The high concentrations associated with the above reviewed studies may result from failing to control for comorbid depression. Furthermore, free cortisol, found in saliva and urine, gives measurements of bioactive cortisol that is not bound to proteins. Measurements of cortisol taken from plasma or CSF may produce higher cortisol concentrations than is biologically active due to protein binding. Thus, is unclear whether increased cortisol concentrations in some of these studies indicate functional hypercortisolism (i.e.,

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increased free cortisol) or successful compensation via increased production of binding globulins (i.e., increased bound cortisol).

Hypocortisolism, a sign of HPA-axis negative-feedback hypersensitivity, is currently the leading theory regarding dysregulation associated with PTSD (Boscarino, 1996). Several studies support this theory. Simeon and colleagues (2007) examined urinary free cortisol and plasma cortisol in 46 individuals with dissociative disorders, 35 individuals with PTSD and 58 healthy controls. Urinary samples were collected over a 24-hour period, and blood samples were collected once per hour. Individuals with PTSD and dissociative symptoms had blunted basal plasma cortisol concentrations compared to healthy controls. Boscarino (1996) investigated morning serum cortisol in 1,972 Vietnam era veterans not diagnosed with PTSD and 2,490 Vietnam theater veterans diagnosed with PTSD. Cortisol concentrations were lower in Vietnam theater veterans diagnosed with PTSD than Vietnam era veterans. Lauc, Zvonar, Vuksic-Mihaljevic, and Flogel (2004) collected salivary cortisol from 12 veterans with PTSD with history of hospitalization, 14 veterans with PTSD and no history of hospitalization, and 16 veterans without a history of PTSD. The researchers collected six saliva samples: four post-awakening, one sample at 12:00pm and another at 6:00pm. They found blunted cortisol concentrations in veterans with current PTSD, regardless of hospitalization, compared to veterans without PTSD. Kanter and colleagues (2001) found lower basal cortisol concentrations and higher corticosteroid binding globulin in male Vietnam veterans with PTSD when compared to healthy controls. This effect was found by using Metyrapone to block cortisol synthesis and then reintroducing cortisol through injection, thus measuring negative feedback. MacMillan et al. (2009) examined resting and reactive concentrations

of salivary cortisol of 67 female youth ages 12-16 with a history of maltreatment and 25 age-matched controls. Saliva was collected as part of the Trier Social Stress Test (TSST). Female youth diagnosed with PTSD had blunted cortisol concentrations following the TSST.

Very few studies investigate PTSD in the female population and even fewer examine gender differences directly. Many studies that have examined both males and females do not compare HPA-axis function in terms of gender. One study, conducted by Freidenberg et al., (2010) investigated basal salivary cortisol in both males and females. Salivary cortisol was collected at three time points, in 6 females and 3 males with PTSD resulting from a motor vehicle accident. This study found that females' cortisol concentrations were lower than males' after awakening and decreased more slowly. However, given that this study did not compare individuals with PTSD to healthy controls, it is not clear if the gender differences found are specific to PTSD.

Little is known about the biological mechanisms involved in symptom expression in PTSD. However, clear criteria have been outlined as to what symptoms make up the diagnosis. This study will include a behavioral measure to quantify one aspect of PTSD symptom expression. Significant impairment in daily life is a diagnostic criterion that must be met to fulfill a PTSD diagnosis. The proposed study will include performance assessment on the Trier Social Stress Test (TSST). The TSST is a highly valid and reliable measure used to elicit HPA-axis activity in a laboratory setting (Kirshbaum & Hellhammer, 1993). This psychosocial stressor includes a five-minute speech and a five-minute mental arithmetic task. The speaking portion of the task is a mock job interview. Testing performance on the TSST will provide quantified support for the test's ability to

elicit a stress response. Our sample consisted of undergraduate college students who take part in similar tasks as part of their core curriculum. Thus, poor performance on the TSST may indicate impairment in daily functioning.

This study will examine two components involved in PTSD. First, we will assess HPA-function by measuring basal and stress related concentrations of salivary cortisol. Next, we will investigate behavioral performance on the Trier Social Stress Test (TSST). We will concentrate on tasks associated with an academic or workplace setting. To address gender differences in PTSD, we will collect data from both males and females. Comparing endocrine activity between genders will elucidate possible underlying factors involved in greater susceptibility to PTSD in women. We hypothesize males will have higher basal and stress reactive cortisol concentrations when compared to females, regardless of PTSD. Further, we hypothesize that individuals with PTSD, regardless of gender, will have blunted concentrations of cortisol. In terms of performance, we expect to find that individuals with PTSD will perform worse on the TSST compared to controls. Lastly, we expect higher levels of math anxiety to be associated with poor performance on the mental arithmetic task and higher levels of social and performance anxiety to be associated with poor performance on the speaking task.

Chapter 2: Methods

Participants

This study examined gender differences in genetic and endocrine aspects of PTSD. We recruited 38 females without PTSD, 14 females with PTSD, 32 males without PTSD and 5 males with PTSD. All participants were between the ages of 18 and 39. Participants were recruited from introductory psychology classes at the University of Nevada, Las Vegas. Participants were excluded if they had a diagnosis of major depressive disorder within the past year. Posttraumatic stress disorder status depended on scores obtained on the Posttraumatic Stress Diagnostic Scale (PDS).

Psychological Assessment

Participants completed questionnaires on the first and second days of the study. On the first day, participants completed a demographics form (appendices A and B) and three scales to measure anxiety and stress. We used the Leibowitz Social Anxiety Scale (LSAS) to measure performance and social anxiety (Heimberg & Holaway, 2007). The LSAS contains 13 questions that measure performance anxiety and 11 questions that measure social anxiety. The LSAS is a highly reliable measure of social and performance anxiety. The Cronbach alpha coefficient for the complete measure is .96. We used the Abbreviated Math Anxiety Scale (AMAS) to measure math anxiety (Hopko, Mahadevan, Bare & Hunt, 2003). The AMAS has a test-retest score of .85. Additionally, we created a Visual Analog Scale (VAS) to assess perceived stress before and following the Trier Social Stress Test (TSST). The VAS (appendix C) asks participants to rate how stressed out they feel on a scale from 1 (not stressed) to 10 (very stressed).

On the second day of the study, participants completed the Beck Depression Inventory II (BDI-II) and the PDS. We administered the BDI-II to account for depression levels in our participants. Depression may alter cortisol levels differently than PTSD (Bjorntorp, 1996). Specifically, depression is associated with higher than normal cortisol levels, whereas PTSD is associated with blunted cortisol levels. The BDI-II allowed us to account for this change and treat co-morbid depression as a co-variate. The BDI-II is a reliable measure used to assess current depressive symptoms, with a reliability coefficient alpha of .92 (Storch, Roberti, & Roth, 2004). We measured PTSD symptoms by administering the PDS. The PDS provides information about diagnostic criteria and symptom severity (Foa, 1995). Scores of 10 or below indicate low levels of PTSD symptoms. Scores above 10 indicate moderate to severe PTSD. We used a cut-off score of 10 to assign participants to PTSD+ and PTSD- groups for analysis. The PDS has a reliability Cronbach alpha of .92 and a test-retest kappa of .74.

Procedures

Participants took part in a two-day study. On the first day of testing, participants arrived to the testing location. The researcher greeted participants and administered the informed consent form for that day. The researcher gave participants a brief verbal description of the tasks they were to complete and then the participants began a 30-minute habituation phase. After the habituation phase, the researcher took the first saliva sample and explained the Trier Social Stress Test (TSST) in detail. Immediately before entering the TSST, the researcher asked participants to fill out the first VAS containing one question about their current level of stress. Then, the researchers administered the Trier Social Stress Test (Kirschbaum, Pirke & Hellhammer, 1993). The TSST began with

participants being instructed to spend ten minutes preparing a speech on why they are the

most qualified candidate for a management position. Then, the researcher escorted

participants to a room containing a committee. The researcher explained that the

committee members were trained to be experts on human behavior. The committee

consisted of two trained research assistants from the principle investigator's lab. The

committee members greeted the participants and prompted them to begin the prepared

speech. The committee followed a specific protocol and script (appendices D and E).

After completion of the public speaking task, participants began the mental arithmetic

section. A committee member verbally instructed the participant to start at 1,793 and

count backward (serially subtract) in increments of 13. The committee member advised

the participants to give answers aloud as quickly and accurately as possible. The

committee followed a script and protocol for handling participant errors. After an error

was made, a committee member instructed participants to begin again. After completing

the TSST, the researcher led participants to a private room to collect a post-stress saliva

sample and administer the second VAS. Before beginning the 60-minute recovery phase,

the researcher debriefed participants by informing them that the sole purpose of the

stressor was to elicit a stress response. After resting for 60 minutes, the last saliva sample

was collected. Participants were thanked for their participation and asked if they had any

questions. The researcher gave participants a packet containing instructions on evening

and morning saliva collection and Salivette collection devices. The participants were

verbally instructed on saliva collection procedures and instructed to return the packet at

their next appointment. The researcher gave participants written and verbal instructions

for setting up optional counseling through the Counseling and Psychological Services at the University of Nevada, Las Vegas.

On the second study day, participants gave written consent and completed the BDI-II and the PDS. Then, the researcher collected two buccal cell samples and one passive drool saliva sample on site for genetic analysis.

Protocol for Measuring Performance During the TSST

Aside from following the TSST protocol, the committee members were responsible for documenting participant performance. Both committee members had stopwatches to measure the length of time participants spoke before pausing. The committee members documented each time the participant ceased speaking for at least twenty seconds and recorded the total time spent silent. Committee members documented performance on the mental arithmetic task by noting how many times the participant was directed to begin again and length of pauses between answers if the participant was silent for more than 10 seconds. Additionally, committee members documented the most advanced step reached during this task.

Saliva Collection

All participants received two Salivettes and instructions for at-home collection. Participants collected samples of saliva before bedtime and within 30-minutes of awakening. The participants received instructions to refrain from eating, drinking, exercising, smoking and using chewing tobacco for 30 minutes prior to collection. Abstention from brushing or flossing teeth 30 minutes before sample collection was requested as to limit blood contamination. Also, participants received instructions to refrain from using medications containing steroids immediately before the collection. The

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researcher asked participants to provide a list of any medications they were taking on the demographics form. Collection of saliva occurred 3 times during the Trier Social Stress Test. Participants provided samples upon arrival, after the speaking and arithmetic tasks, and after a short waiting period upon completion of debriefing.

We stored saliva in salivette tubes at 4°C until samples were spun down in an Eppendorf centrifuge (5810R, Brinkmann Instruments Inc.) at 4000 rpm for 5 minutes. Afterwards, we transferred the saliva samples to microcentrifuge tubes and stored at -20°C. We performed cortisol assays using the Assay Designs cortisol enzyme imuunoassay (Enzo Life Sciences, Plymouth Meeting, PA) according to the manufacturer's instructions. A multi-mode microplate reader provided readings of the cortisol assays (SpectraMax M2, Molecular Devices). Each sample was assayed in duplicate, and cortisol concentrations were interpolated from a standard curve of serial cortisol dilutions provided with the kit. The intra-assay variability coefficients ranged from low, 7.3%, to high, 10.5% and the inter-assay variability coefficients ranged from low, 8.6%, to high, 13.4%.

Statistical Analyses

We analyzed all cortisol data by using a mixed-model analysis of variance (ANOVA). For measurement of diurnal cortisol levels, the between group factors were gender/PTSD status (female and males with PTSD, and healthy male and female controls). The within subject variables were time of saliva sample collection (nighttime and morning). We conducted a separate mixed-model ANOVA for the samples collected from the TSST. The between group factors were the same as outlined above for diurnal

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cortisol. The within subject variable was the time at which the three cortisol samples were collected during the TSST.

We conducted separate multiple correlations for performance on the TSST with performance measures and cortisol levels. Specifically, scores on the LSAS were correlated with performance on the speaking portion of the TSST. Similarly, scores on the AMAS were correlated with performance on the math portion of the TSST. Additionally, we conducted multiple correlations with the VAS, AMAS and LSAS to assess agreement between anxiety measures. Lastly, we conducted a two-way ANOVA to compare performance measures between groups.

Chapter 3: Results

Participants

All participants were recruited from introductory Psychology courses and were enrolled in classes at the University of Nevada, Las Vegas. At the time of data analysis, 90 individuals had participated in the study. Out of these individuals, 38 were women without PTSD, 14 were women with PTSD, 32 were men without PTSD and 5 were men with PTSD. We excluded data from 5 female participants without PTSD because we were unable to collect cortisol data. In addition, cortisol data from one female participant without PTSD were thrown out because the participant did not return for the second day of the study. We included data from 84 participants for analysis. Participant's ages ranged from 18-38; the mean age was 20.27 (SD=8.58). Female participants' ages ranged from 18-38 (M=20.38, SD=8.80). Male participants' ages ranged from 18-28 (M=20.13, SD=8.38). An independent t-test was conducted to compare the ages of male and female participants. There was no significant difference between male and female age [$t(88)=-.314$, $p=0.754$].

Table 1. Demographics

|	Male		Female	
	Mean	*St. Dev.*	*Mean*	*St. Dev.*
Age	20.13	8.38	20.36	8.8
Race/Ethnicity	*n*	*Percent*	*n*	*Percent*
American Indian	1	2.7	0	0
Black/African American	2	5.4	8	15.1
Asian/Pacific Islander	9	24.3	13	24.5
Spanish/Hispanic	7	18.9	11	20.8
Caucasian	12	32.4	18	34
Multiple	6	16.2	3	5.7
Education				
High School Graduate	17	45.9	30	56.6
Associates Degree	1	2.7	2	3.8
Technical Degree	2	5.4	0	0
Some Undergraduate	2	5.4	0	0

Table 1. *Education percentages do not sum to 100%. Many participants mislabeled their education status as having completed some high school courses, rather than some undergraduate courses.*

Cortisol

A mixed-model analysis of variance (ANOVA) was conducted to measure the difference of basal (awakening and bedtime) levels of cortisol between groups. A significant effect was found with regards to the time the sample was collected [$F(1,77)= 4.22, p = 0.039$]. Specifically, awakening cortisol levels were significantly higher than bedtime levels. No significant interactions were found between time and sex [$F(1,77)= .002, p=.952$], time and PTSD status [$F(1,77)= .532, p =.468$] or between PTSD status and sex [$F(1,75)=1.001, p=.320$]. Further, no significant interaction was found with regard to sex, time, and PTSD status [$F(1,75)=.004, p=.952$]. The main effects of sex [$F(1,75)=2.590, p=.112$] and PTSD [$F(1,75)=.815, p=.370$] status were not significant

Figure 1. Basal Cortisol Concentrations
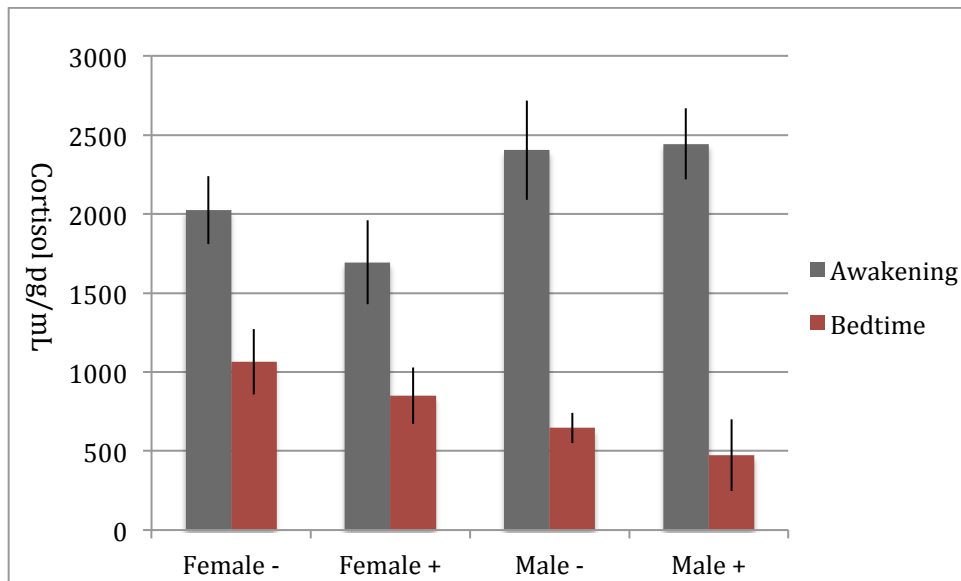
Figure 1. Group mean (SE) for basal salivary cortisol concentrations (pg/mL). The absence of PTSD is expressed with a – symbol and the presence of PTSD is expressed with a + symbol.

A separate mixed-model ANOVA was conducted to investigate differences in cortisol stress reactivity between groups. No significant effects were found for sex [$F(1,61)$=.743, p=.480] or PTSD status [$F(1,61)$=1.159, p= .321]. No significant interaction was found between sex, time, and PTSD status ($F(2, 12) = .176$, $p = 0.084$). We found a trend towards a significant main effect of time [$F(2,59)$=3.085, p=.053]. This trend is that cortisol increases between time point 1 and time point 2 and returns to baseline at time point 3. Males without PTSD had higher levels of cortisol than males with PTSD and females with and without PTSD, but this did not reach significance.

Figure 2. Stress Reactive Cortisol Concentrations
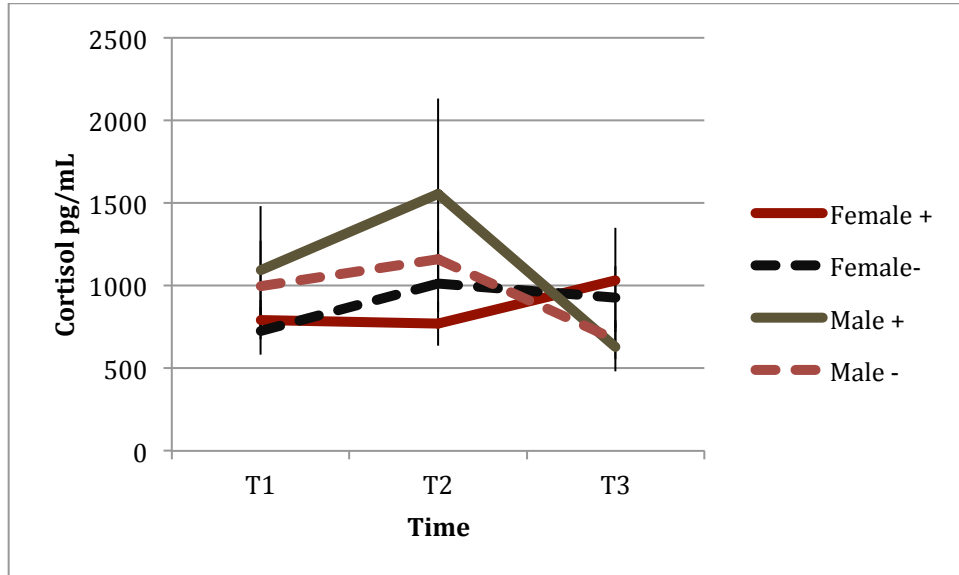
Figure 2. Group mean (SE) for stress reactive salivary cortisol levels (pg/mL). The presence or absence of PTSD is expressed with a + or – symbol respectively. Individuals with PTSD are expressed with a solid line, whereas individuals without PTSD are expressed with a dashed line.

Because sample sizes were uneven across groups, data were also analyzed using a non-parametric approach. The Aligned Rank Transform method (Wobbrock et al., 2011) allows for factorial analysis of nonparametric data, including interactions between factors, by first applying a transformation that aligns data for each effect, then ranking the data points. The aligned rank data are then subjected to a factorial ANOVA. The nonparametric analysis revealed no significant effects of sex, PTSD or sex by PTSD interactions for both basal and stress reactive cortisol levels at all time points.

Questionnaires

In order to investigate the effectiveness of the Trier Social Stress Test in eliciting a subjective stress response, we administered one VAS after the thirty-minute habituation phase and one VAS after the stressor. A mixed-model ANOVA was conducted to

investigate if the differences found between VAS time point 1 and VAS time point 2 were similar across groups. There was a significant effect of time [$F(1, 86)=109.166$, $p=.005$] but not for PTSD status [$F=(1,86)=1.202$, $p=.276$] or sex [$F(1,86)= 2.883$, $p=.093$] or PTSD by sex [$F(1,86)=2.559$, $p=.113$]. We also found that females rated the TSST (VAS 2) as being more stressful than males rated it [$F(1,88)=6.050$, $p=.016$].

Two-way ANOVAs were conducted to investigate sex and PTSD differences in AMAS and LSAS scores. We found a significant effect of sex on the AMAS [$F(1,86)= 4.972$, $p=.028$], such that females had higher scores than males. To further investigate the sex differences in anxiety ratings, we conducted a two-way ANOVA. We found that females endorsed higher levels of math anxiety compared to males [$F(1,88)=7.583$, $p=.007$]. Further, we found a significant effect of PTSD [$F(1,86)=4.123$, $p=.045$], such that individuals with PTSD had higher scores on the AMAS. We did not find an interaction between sex and PTSD on the AMAS [$F(1,86)=.397$, $p=.530$]. We did not find a significant effect of sex on the LSAS [$F(1,86)=.805$, $p= .372$] or a significant interaction between sex and PTSD [$F(1,86)=.160$, $p=.690$]. We found a significant effect of PTSD on scores on the LSAS [$F(1,86)=4.407$, $p=.039$], such that individuals with PTSD had higher scores.

Table 2. Questionnaire Descriptives

		AMAS		LSAS	
		Mean	St. Dev.	Mean	St. Dev.
PTSD-					
Female		21.331	5.12647	26.1795	12.02382
Male		18.6563	5.24625	21.625	12.04762
PTSD+					
Female		25.7857	8.21049	32.1429	14.8886
Male		21	7.17635	30.4	2.96648

Table 2. *Means and standard deviations for males and females with and without PTSD.*

Figure 3: Sex Differences in VAS
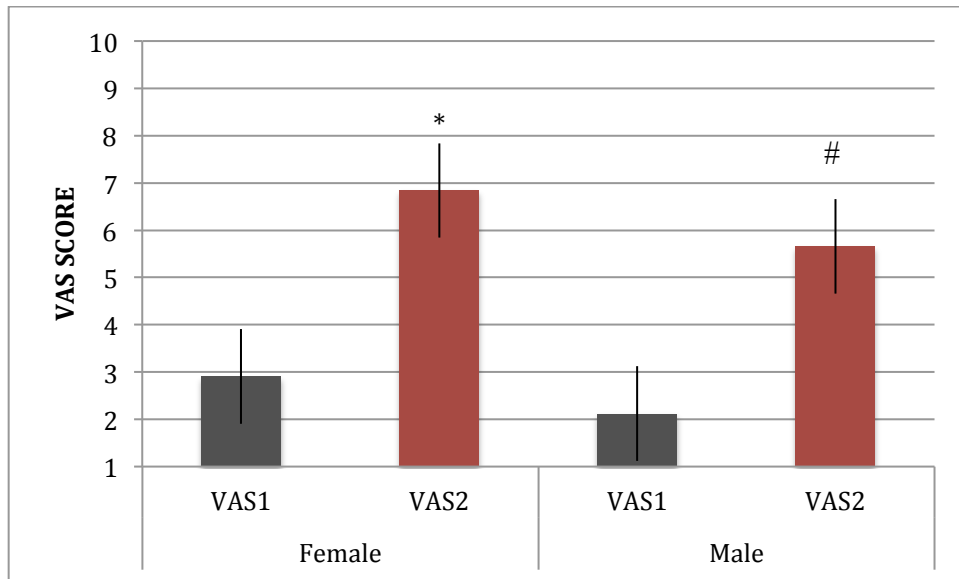
Figure 3. *Visual analogue scale mean ratings (SE) directly after habituation (1) and after the stressor (2). The asterisk denotes a significant change between VAS 1 and Vas 2. The pound sign denotes a significant difference between female VAS 2 and male VAS 2.*

We found several significant correlations when assessing the relationship between scores on the PDS, LSAS and AMAS and the VAS1 and VAS2. First, there was a significant positive correlation between PDS scores and VAS1 scores [r(88)=.297,

p=.005] and between PDS scores and VAS 2 scores [$r(88)$=.209, *p*=.048]. Individuals who scored higher on the PDS reported having higher stress levels after habituation and after the TSST than individuals with lower scores did. In addition, we found that individuals who scored higher on the PDS also scored higher on the AMAS [$r(1,88)$=.208, *p*=.049] and higher on the LSAS [$r(1,88)$=.211, *p*=.046].

Performance

We investigated participant performance during the speaking and arithmetic sections on the TSST. Two-way ANOVAs were conducted to investigate differences in length of continuous speech before being prompted. We predicted that individuals with PTSD would have less continuous speech than individuals without PTSD. We did not find a significant effect of sex [$F(1,86)$=1.462, *p*=.230], PTSD status [$F(1,86)$=.091, *p*=.763], or sex by PTSD status [$F(1,86)$=.454, *p*=.502].

We investigated how PTSD and sex affect performance on the speaking task by conducting two-way ANOVAs for total number of prompts. There were no significant effects of sex [$F(1,82)$=1.081, *p*=.302], PTSD status [$F(1,82)$=1.792, *p*=.311], or sex by PTSD status [$F(1,82$=.003, *p*=.956].

Next, we investigated how PTSD and sex affect performance on the mental arithmetic task. We conducted a two-way ANOVA to investigate these effects. There were no significant differences between males and females [$F(1,86)$=2.784, *p*=.099]. There was no significant effect of PTSD status [$F(1,86)$=1.395, *p*=.241], or sex by PTSD status [$F(1,86$=.149, *p*=.700].

We investigated how performance and math anxiety affected performance on the speaking and mental arithmetic tasks. First, we examined the relationship between math

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anxiety and performance during the math task. We found a significant negative correlation between AMAS scores and the number of steps reached [$r(88)$=-.333, p=.001]. Next, we examined the relationship between social and performance anxiety on speech performance. There was a significant positive correlation between LSAS scores and number of prompts needed for continuation [$r(88)$=.314, p=.002] and a significant negative correlation for amount of continuous speech before prompting and LSAS score [$r(88)$=-.284, p=.003].

Lastly, when investigating the effect of PTSD on performance measures, we found no relationship between measures of performance and PDS scores. Scores on the PDS were not correlated with steps reached [$r(88)$=-.080, p=.227] or error prompts [$r(88)$=-.127, p=.257] on the mental arithmetic task. PDS scores were not correlated with length of continuous speech [$r(88)$=.001, p=.996] or longest pause [$r(88)$=.079, p=.460]. Further, there was no correlation between PDS score and number of prompts [$r(88)$=-.113, p=.301] on the public speaking task.

Chapter 4: Discussion

Cortisol

We investigated differences in the circadian release of cortisol by giving participants a take-home saliva collection kit. Participants collected one saliva sample before going to bed and one saliva sample within 30-minutes of awakening on the next day. Research has shown that differences exist in the release of cortisol over the circadian cycle. Namely, awakening cortisol concentrations are significantly higher than evening concentrations in healthy populations (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). The higher concentrations in the morning act as part of a dynamic arousal system. At night, lower concentrations may serve as a way to recuperate energy expenses experienced during the day. Our results support previous research examining circadian cortisol release. We found significantly higher awakening cortisol concentrations compared to bedtime concentrations.

Research examining differences in circadian cortisol rhythms between trauma-exposed individuals and individuals with PTSD is discrepant. Many findings suggest that awakening cortisol concentrations are blunted in both males and females with PTSD (Meewise et al., 2007; Rohleder, Joksimovic, Wolf, and Kirschbaum, 2004; Wessa, Rohleder, Kirschbaum, and Flor, 2006; Violanti et al., 2007). Alternately, many studies do not find a differences in the circadian release of cortisol (Laudenslager et al., 2009; Klaassens et al., 2010; van Zuiden et al., 2011; Klaassens et al., 2012). This lack of cortisol response was found when comparing individuals with PTSD to non-trauma exposed controls, trauma exposed individuals to non-trauma exposed individuals and individuals with PTSD to controls whose trauma exposure was not controlled for.

Further, studies have found that differences between awakening and bedtime cortisol levels are more pronounced in females with PTSD when compared to female controls. Specifically, females with PTSD have blunted awakening cortisol response when compared to female controls (MacMillan et al., 2009). When investigating circadian cortisol concentrations in males, some studies have found males with PTSD to have a potentiated cortisol response to awakening when compared to males without PTSD (Lindley, Carlson and Benoit, 2004; Baker et al., 2006). Other studies have found that males with PTSD have a blunted cortisol response when compared to male controls (Simeon et al., 2007; Lauc, Zvonar, Vuksic-Mihaljevic, and Flogel 2004). Thus, because there have been more studies conducted examining this effect in males, there appears to be more variability in the direction of the circadian release of cortisol in males compared to females.

In line with some previous research, we did not find group differences in awakening versus bedtime cortisol concentrations. Our lack of significant findings may be a result of several factors. First, our group sample sizes were discrepant. Non-parametric analyses, which do not assume equal group sizes, also failed to reveal group differences in basal cortisol. Our total sample consisted of 17 individuals with PTSD, five of whom were male. This left us with inadequate power for examining PTSD effects in both sexes. Further, 77% of our sample was exposed to one or more qualifying traumatic events. Studies that did not control for trauma exposure often did not find differences in circadian cortisol release (Klassens et al., 2012; Eckart et al., 2009). In addition, our participants were not required to have a clinician-administered diagnosis of PTSD. All participants were placed into groups based on scores of symptom severity. While many

studies have followed the same design and found that individuals who experienced

trauma had blunted cortisol concentrations, (Klaassens et al., 2010; Heim, et al., 2000)

some studies found this HPA-axis dysregulation was only apparent in individuals with

diagnosed PTSD when compared to trauma-exposed controls and healthy controls (de

Kloet et al., 2008). It is possible that the HPA-axis dysregulation in these studies was

partly due to self-selection, in that individuals who enroll in a PTSD study that offers a

clinician-administered diagnosis may be treatment seeking and have more severe PTSD

symptoms than we observed in our sample from the undergraduate subject pool. Further,

we were unable to analyze trauma exposed versus non-trauma exposed individuals, as

most of our sample was trauma exposed.

　　　We measured stress reactive cortisol levels by collecting three saliva samples

during the TSST. The first sample was collected after a 30-minute habituation phase, the

second sample was collected after the stressor, and the third sample was collected after a

60-minute recovery phase. Acute cortisol release is also affected by exposure to physical

and environmental stressors (Kircshbaum & Hellhammer, 1993). When investigated

using laboratory stressors, cortisol concentrations follow a clear trajectory of increasing

in response to a stressor and decreasing during recovery from the stressor. Similar to

research examining basal cortisol concentrations, research findings of stress reactive

cortisol concentrations are discrepant. Many studies find the typical pattern of cortisol

release when investigating healthy, non-trauma exposed individuals. However, when

looking at sex differences, some studies show distinct differences in stress reactive

cortisol between males and females that are dependent on menstrual cycle phase (Zimmer

et al., 2003; Childs, Dlugos, & De Wit, 2010). Namely, females in the follicular phase of

the menstrual cycle have blunted stress reactive cortisol concentrations when compared to males and to females in the luteal phase. Females in the luteal phase have stress reactive cortisol concentrations comparable to males. Some studies, however, have found no sex differences when comparing stress reactive cortisol concentrations (Kidd, Carvalho, and Steptoe, 2014; Kelly et al., 2007). Studies have found discrepant results in stress reactivity between individuals with PTSD and controls. Many studies have found blunted cortisol reactivity in individuals with PTSD compared to controls (Boscarino, 1996; Lauc, Zvonar, Vuksic-Mihaljevic, and Flogel, 2004; Friedenberg et al., 2010) or no difference in cortisol reactivity (Bremner et al., 2003; Meewise et al., 2007, Klaassens et al., 2012).

We did not find a significant difference in stress reactive cortisol between groups. This lack of significant difference may be due to several factors. First, as mentioned previously, our group sizes were discrepant. Stress reactive cortisol concentrations from males both with and without PTSD and females without PTSD followed the typical stress reaction and recovery trajectory. However, stress reactive cortisol concentrations from females with PTSD were blunted, relative to other groups, during time point 1 and 2 and continued to rise during time point 3. Although we saw a pattern of differential cortisol reactivity emerge between groups, these differences were not significant. We used a 60-minute recovery phase for all participants, regardless of gender. Previous research indicates that males' and females' stress reactive cortisol recovers to baseline at different rates (Zimmer et al., 2003; Childs, Dlugos, & De Wit, 2010). Specifically, males recover within about 30-minutes, whereas females recover within about 60 to 75 minutes. Based on previous research, our recovery period was on the low end of the spectrum for

measuring sex differences in recovery. Further, many researchers take multiple samples during habituation, stress, and recovery. We collected one sample during each stage of the TSST. Taking additional samples could have provided useful information on differences in cortisol dynamics between groups.

In order to evaluate whether our cortisol findings are comparable to other studies, we investigated the magnitude of change between time points. We calculated percent change in cortisol concentrations for two time periods for each of three studies that used the TSST. The first time period was from baseline (T1) to immediately post-stressor (T2). The second time period was from the stressor (T2) to 60-minutes post recovery (T3). The changes in magnitude in female participants were most in line with a study conducted by MacMillan and colleagues (2009). This group examined stress reactive salivary cortisol concentration differences between females with PTSD and female controls. The magnitude of change in male participants with and without PTSD was more in line with the studies conducted by Zaba and colleagues (2015) and McRae and colleagues (2006).

The magnitude of change varied at each time point between studies. This may be due to several factors. First, two studies (McRae et al. 2006; Zaba et al. 2015) collected cortisol through blood plasma. Cortisol concentrations measured from blood plasma include both free and bound cortisol; thus these measurements tend to be higher than concentrations of free cortisol found in urine and saliva (Mendel, 1989). This may account for the larger magnitude of change between time 1 and time 2 when compared to our study and MacMillan et al. (2009). Second, because cortisol is released as part of the circadian rhythm, the time of day measures are collected may play a role in the variance observed (Childs, Dlugos, & De Wit, 2010). The times the TSST was conducted varied

between studies. We conducted the TSST between 10:00am and 6:00pm. The other

studies conducted the TSST in the evening between 4:00pm and 7:00pm (MacMillan et

al. 2009), at 2:00pm (McRae et al. 2006) and in the morning at 8:00am (Zaba et al. 2015).

Lastly, the comorbidity of other psychopathologies may account for variance between

studies. All of the studies compared used depression as a covariate but did not exclude

depressed individuals.

Table 3. Magnitude of Change

Magnitude of Change During the TSST		
Pierce (2015)	**T1 to T2**	**T2 to T3**
Female +	5.83%	35.16%
Female -	39.43%	-8.20%
Male +	42.17%	-59.58%
Male -	16.15%	-41.95%
MacMillan et al. (2009; Females)		
PTSD	9.09%	-20.83%
Control	39.90%	-7.14%
Zaba et al. (2015; Females)		
PTSD	42.85%	-40%
Control	60%	-31.25%
McRae et al. (2006; Males and females)		
PTSD	53.33%	-26.09%
Control	33.33%	-30.00%

Table 3. *Percentage in the magnitude of change in cortisol concentrations in response to stress (T1 to T2) and in recovery from the stressor (T2 to T3) in response to the TSST.*

Our results captured a stress response that is consistent with existing literature. The magnitude of change between T1 and T2 was smaller in females with PTSD compared to females without PTSD (MacMillan et al., 2009; Zaba et al. 2015). These results are consistent with the hypocortisolism hypothesis. Our male data are in line with the study conducted by McRae et al (2006). This group assessed stress reactive salivary cortisol in males and females both with and without PTSD using the TSST. Our male participants with PTSD had a larger magnitude of change between T1 and T2 than males without PTSD. Further, male participants had a larger magnitude of response compared to both females with and without PTSD. These differences support the great body of literature suggesting sex differences in the HPA-axis response to stress. Males have significantly higher cortisol concentrations in response to stress than females (Zimmer et al., 2003; Childs, Dlugos, & De Wit, 2010). In addition, many of the studies that support hypercortisolism in PTSD were conducted with male populations (Baker et al., 2004). Many of the studies that include females support hypocortisolism in PTSD. Thus, these results indicate that it is important to investigate sex differences in PTSD. Females are more likely to develop PTSD post trauma than males are. Differences in HPA-axis function may provide better insight into why these sex differences exist and may lead to better treatment options.

Questionnaires

We administered several questionnaires over the course of the study. First, we examined the subjective stress by administering a VAS after the 30-minute habituation period and another VAS directly following the TSST. As expected, we found that participants reported significantly higher stress levels following the stressor. However,

when looking at the relationship between VAS scores and PTSD symptom severity, we found that individuals with higher scores on the PDS reported being more stressed both after the habituation phase and after the stressor than participants with lower scores. This is consistent with previous findings; individuals with PTSD have higher levels of anticipatory anxiety than controls, especially when events are unpredictable (Grillon et al., 2009; Simmons et al., 2013). Before entering the habituation phase of our study, participants were given an informed consent that very briefly discussed the study for which they were to take part. This vague description may have sparked more anticipatory anxiety in individuals with moderate to severe PTSD symptoms because it was perceived as unpredictable. Further, individuals with PTSD ruminate on stressful events more than healthy populations (Hu et al., 2014; Egan, Hattaway, and Kane, 2014). Thus, it is probable that individuals with PTSD rated the TSST as being more stressful than controls because they were ruminating on the stressor itself and over their mistakes during the stressor.

We investigated two different types of anxiety, performance/social and math anxiety. We found a significant positive correlation between scores on the PDS and scores on the AMAS and on the LSAS. To date, no study has investigated of the relationship between math anxiety and PTSD. This finding provides more insight into how daily functioning is impaired in individuals with moderate to severe PTSD symptoms. Our findings suggest that the anxiety associated with PTSD may be generalized and not limited to areas of functioning associated with the traumatic event itself. It is important to note, however, that the AMAS and LSAS were administered 15 minutes after the stressor, during the 60-minute recovery period. It is possible that

individuals with PTSD were ruminating on the effects of the stressor or on their performance during the stressor, thus rating themselves as being more math and socially anxious. Future studies should control for the timing of scale administration.

Performance

Our data support previous studies that found differences in subjective appraisal of performance and stress in individuals endorsing anxiety symptoms (Lanzenberger, et al., 2010; Plag, Schumacher, Schmid and Strohle, 2013). We found that participants who scored higher on the LSAS, indicating higher levels of performance and social anxiety, performed worse on both speaking measures. Specifically, participants with higher levels of anxiety spoke for less time and needed to be prompted more during the speaking task. Similarly, individuals who scored high on the AMAS, indicating high levels of math anxiety, performed worse on the mental arithmetic task. Individuals with higher AMAS scores completed fewer steps, resulting in a higher number reached during the task, and longer lengths of pauses when trying to perform mental computations.

The significant correlations between performance scores and anxiety measures show that the performance measurements were sensitive to the specific types of anxiety provoked by the TSST. It is interesting in light of the fact that individuals with PTSD did not differ in performance from individuals without PTSD. Individuals with moderate to severe ratings of PTSD rated the stressor as being more stressful than those with no PTSD or mild symptom severity. In addition, individuals with higher scores on the PDS had higher AMAS and LSAS scores compared to participants with lower PDS scores. However, the individuals scoring higher on the PDS did not have worse performance on the math or speaking tasks, whereas individuals who scored higher on the AMAS and

LSAS did. These results may be due to the order the questionnaires were presented. As previously discussed, the correlation between PDS scores and math anxiety ratings was most likely due to rumination over errors made during the stressor itself. We posit that, due to excessive rumination, individuals with moderate to severe PTSD symptoms perceive their performance as worse than it actually is, and this perceived poor performance may trigger increased anxiety in the specific domains tested in the TSST (math and social performance). Individuals without PTSD symptoms may provide anxiety ratings that better predict their performance on the TSST tasks, either because their pre-existing levels of anxiety in these domains directly affect their performance or because they more accurately assess their performance and rate their anxiety in these domains accordingly. Because we did not ask participants about their performance, and because our results are correlational, we cannot test this hypothesis in this study, but future studies should examine the role of rumination and perceived performance on domain-specific anxiety ratings in individuals with PTSD.

To date, no study has systematically investigated performance on the TSST. Further, no study has examined either public speaking performance or mental arithmetic performance separately. Our study is the first to tackle both aspects of performance. First, we investigated how PTSD symptomatology affects participant performance on the TSST. The overarching hypothesis was that individuals with PTSD would perform worse on the TSST than individuals without PTSD. We did not find any significant differences in basic measures of performance for individuals with PTSD and without. This could be for several reasons. First, we assessed PTSD symptoms in individuals who had encountered a wide variety of traumatic events. Differences in hormonal response to

stress exist between individuals who have PTSD precipitated by different types of trauma (Lauc, Zvonar, Vuksic-Mihaljevic & Flogel, 2004; Baker, et al., 2005). Thus, differences in performance may be more reliant on the type of trauma experienced than the severity of symptoms of anxiety typically associated with PTSD.

To date, no study has investigated performance related changes in cortisol during the TSST therefore our explanations are speculative. Dysregulation of the HPA-axis may impact performance. For example, Lautenbach, Achtezehn, and Raab (2014) found that cortisol concentrations made a unique contribution to predicting performance in individuals performing a tennis serve task. This contribution was beyond the contribution of state anxiety. In addition, Leder, Housser, and Mojzisch (2013) found that participants who underwent the TSST-G performed more poorly on a strategy task then did non-stressed controls. The TSST-G is an adapted version of the TSST where groups of participants complete the TSST in front of two committee members. This effect was mediated by the stress-induced rise on cortisol. However, there is also evidence for complex interactions between anxiety, working memory and cortisol on cognitive performance (Mattarella-Micke et al., 2011). The extent and direction in which cortisol and anxiety affect math performance depends on whether participants have a high or low working memory capacity. Thus, changes in cortisol reactivity during the TSST may negatively impact performance, but other factors, such as working memory, which may also be affected by PTSD, could oppose the negative impact of cortisol.

PTSD was considered an anxiety disorder until 2013 (APA, 2000, 2013). Currently, PTSD is considered a stress and trauma related disorder. While performance differences in the TSST may not exist between individuals with PTSD and healthy

controls, we do see that individuals with PTSD score higher on measures of performance

and math anxiety. Therefore, anxiety associated with PTSD may generalize to different

areas of functioning but may not be severe enough to cause decreases in functioning.

Previous research has found similarities in performance ratings in individuals with math

anxiety, where these individuals perform worse during on-line math tasks than non-

anxious individuals (Ashcraft and Kirk, 2001).

Limitations

There are several limitations this study encountered worth mentioning. First, we

recruited participants from the undergraduate subject pool. Though our participants were

diverse in age and ethnicity, they did not have a clinician-administered diagnosis of

PTSD. We were, however, able to recruit participants with moderate and severe PTSD

symptoms, as measured by the PDS. Further, we were unable to recruit an adequate

number of males with PTSD. The current prevalence of PTSD for males in the United

States population is 3.6%, compared to 9.7% for females (APA, 2000). Our lack of male

participants with PTSD may mirror the larger PTSD population. This may also be the

result of gender differences in PTSD reporting. Males endorse different symptoms than

females (Carmassi et al., 2014). Specifically, males endorse fewer symptoms overall

when compared to females. Further, males endorse symptoms of hypervigilance and

reckless behavior, whereas females endorse all symptoms equally. The lack of symptom

endorsement by males may lead to the lack of diagnosis in the male population and may

have contributed to relatively low PDS scores in our male participants.

Cortisol concentrations are affected by several factors unrelated to stress. First,

timing can be a major confounding variable. The TSST sessions were scheduled during

late morning, early afternoon hours, or early evening hours. Most studies conduct the

TSST between the hours of 10:00am and 6:00pm. Although our timing was in line with

previous research (Kircshbaum & Hellhammer, 1993; Zimmer et al., 2003; Childs,

Dlugos, & De Wit, 2010), the cortisol fluctuations may have been skewed due to the

lunchtime schedule. Participants may have come to the study site directly from eating or

may have been anticipating a meal. Cortisol is synthesized from cholesterol and is

released during digestion (Slag et al., 1981; Anderson et al., 1987; Ennis, Kelly &

Lambert, 2001). Participants were instructed to refrain from eating, drinking or smoking

for one hour prior to their TSST appointment and before taking their at-home samples.

They were also instructed to collect samples within 30 minutes of awakening and retiring

to bed. However, participants may not have been compliant with instructions, thus

introducing additional variability in our cortisol concentrations. Future studies should

focus on scheduling the TSST appointments during midmorning and later afternoon

hours and collecting several at-home samples to account for possible non-adherence.

Further, Cortisol concentrations have also been found to increase after exercise

(Kirschbaum, Platte, Pirke, & Hellhammer, 1996). Our campus is large with limited

transportation. In order to gain access to our building, some participants were required to

walk a considerable distance before the start of their study session. We accounted for this

confound by lengthening our habituation phase. In addition, participants were instructed

to refrain from exercising for one hour prior to attending their session. Regardless of our

instruction, a few participants came from the gym or ran to the location to ensure an on-

time arrival. For these participants, an extra five minutes was added to the habituation

phase. Unfortunately, it was impossible to control for all participant compliance for physical activity, as participants were asked to self-report these data.

Anticipatory anxiety may play a role in discrepant progression of cortisol concentrations during the TSST (Ennis, Kelly, & Lambert, 2001). In particular, women in our study with PTSD symptoms did not show the typical immediate post-stress rise and delayed recovery to baseline. Instead, their cortisol concentrations were flat from time 1 to time 2 and continued to increase from time 2 to time 3. We found a significant correlation between PTSD symptom severity and subjective stress after habituation, such that individuals with more severe PTSD symptoms reported higher levels of stress before beginning the TSST. Thus, the baseline cortisol measure in this study may have been elevated relative to these participants' "true" baseline. Future studies should consider asking about current stressful events in conjunction with having participants rate their levels of stress in order to understand the intricate underpinnings of subjective stress.

Future Directions

As previously discussed, cortisol release is affected by many variables, most of which are difficult to control. While some PTSD researchers have been able to support their hypotheses of differences in cortisol, many others have not. Thus, this raises questions as to whether using cortisol as a marker of HPA-axis dysregulation in PTSD is still appropriate. Cortisol has proven to be a useful correlate of psychopathology; however our understanding of cortisol is mostly limited to peripheral activity. Currently, the most widely accepted hypothesis of HPA-dysregulation is the hypersensitivity of central glucocorticoid receptors. Use of peripheral biomarkers, like cortisol, can only provide an indirect measure of glucocorticoid receptor sensitivity. Thus, without utilizing

new methods, we cannot make accurate assumptions about the activity of cortisol at glucocorticoid receptors.

To understand the complexity of how cortisol is involved with psychopathology, future studies should examine the activity and function of glucocorticoid receptors. In the past, glucocorticoid receptor sensitivity has been assessed using the dexamethasone suppression test, however this method is problematic as it depends on measures of peripheral cortisol (Mehta et al., 2011). These studies have found PTSD to be associated with enhanced dexamethasone suppression of ACTH secretion, suggesting greater glucocorticoid receptor sensitivity. Expression of regulatory proteins plays an important role in glucocorticoid function. In order to understand how hypersensitivity is involved in PTSD, we must investigate genetic markers of glucocorticoid receptor sensitivity. The gene FK506 binding protein 51 (FKBP51), a co-chaperone for heat shock protein 90 (hsp90), is implicated in glucocorticoid receptor sensitivity (Velders et al., 2011). Chaperone proteins assist in reducing the levels of unfolded proteins in the nucleus (Krebs, Goldstein, & Kilpatrick, 2011). When cortisol enters the cell and activates the receptor, HSPs are released. Specifically, Hsp90 binds to the glucocorticoid receptors, displacing FKBP51, which is then replaced by another protein, FKBP4 (Binder et al., 2008). FKBP4 acts as a transcription factor for glucocorticoid receptor expression. FKBP4 binding reduces receptor binding affinity for cortisol. High and low expression of FKBP51 is associated with psychological disorders. Specifically, low expression of FKBP51 in a multitude of tissues, including the brain, is associated with PTSD (Sarapas et al., 2010). This research is consistent with lower expression of FKBP51 in individuals with PTSD. Lower expression of FKBP51 in individuals with PTSD can lead to an

increased translocation of GR to the nucleus, which in turn leads to enhanced HPA-axis negative feedback inhibition.

Studies also indicate that FKBP51 expression can be altered due to environmental factors. Specifically, FKBP51 expression may be altered by circulating stress hormones (Mehta et al., 2011). These studies have found that early life stress can result in underexpression of FKBP5 (Gillespie, Phiefer, Bradley, & Ressler, 2009). The risk for developing PTSD rises significantly when an individual encounters traumas earlier in life (Mehta et al., 2011). If an individual encounters early life stress or trauma, the activation of the stress response alters gene expression for regulators of glucocorticoid receptor sensitivity, leading to an increased susceptibility for developing PTSD.

Four single nucleotide polymorphisms in the FKPB51 gene have been found to be related to HPA-axis dysregulation in PTSD. Several studies have found the rs1360780, rs3800373, rs9296158 and rs4713916 SNP's to be associated with PTSD (Binder et al., 2008; Mehta et al., 2011; Sarapas et al., 2011). These SNP's have been associated with a higher risk of developing PTSD and with current PTSD. All four SNP's are likely to be involved in FKBP51 underexpression. For example, the SNP's rs1360780 and rs3800373 have been associated with impaired cortisol recovery after stress (Ising et al., 2008). Additionally, rs s9296158 is associated with greater levels of peritraumatic disassociation following a traumatic event (Mehta and Binder, 2012).

Thus, the combination of collecting peripherally active cortisol and genotyping for SNPs associated with FKBP51 or measuring FKBP51 expression will provide more useful information about the activity of cortisol in the central nervous system. FKBP51 can be genotyped using saliva and thus is a non-invasive method. Genotyping for

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FKBP51 SNPs will provide better understanding of the role that the central GRs play in the HPA-axis dysregulation observed in PTSD.

Two additional methods may also be useful when assessing HPA-axis function and glucocorticoid receptor sensitivity. First, the stress response is modulated by the synergistic relationship between the sympathoadrenomedullary axis (SAM-axis) and the HPA-axis (Glaser and Kiecolt-Glaser, 2005). The SAM-axis mediates the fight-or flight response. The HPA-axis responds to adrenergic input from the SAM-axis. Catecholamines and their metabolites can be measured in blood plasma and urine, thus providing a non-invasive measure of SAM activity. Studies have shown that individuals with PTSD have elevated levels of catecholamine activity relative to those without PTSD (Yehuda et al., 1992). Studies that have combined measures of cortisol and catecholamine concentrations have found higher concentrations of catecholamines in urine and blood plasma and lower concentrations of salivary and urinary cortisol in individuals with PTSD (Mason et al., 1986; Pitman and Orr, 1990; Lemieux and Coe, 1995; Young and Breslau, 2004). In healthy populations, catecholamine and cortisol concentrations rise and fall together. However, the discordant concentrations of cortisol and catecholamines suggest that PTSD may result from a dissociation of the two systems. Currently, research investigating the interplay of these systems is limited. Thus, future studies should investigate the relationship between the SAM- and HPA-axis in individuals with PTSD.

The second method that should be incorporated into studies that investigate cortisol concentrations and PTSD is investigating the secretogogues, ACTH and CRF. Because the measurement of CRF requires invasive measures (i.e. collection of

cerebrospinal fluid), more attention should be given to ACTH concentrations. ACTH can

be measured in blood plasma and CSF. Investigating concentrations of ACTH along with

cortisol will provide a CNS correlate of the hypersensitized negative feedback that has

been hypothesized in PTSD. Thus, if PTSD is associated with hypersensitization of

glucocorticoid receptors, ACTH concentrations should be lower in individuals with

PTSD compared to healthy controls in response to stress (Yehuda, Yang, Buchsbaum,

and Golier, 2006).

Appendix A: Female Demographics

Participant ID: _____   **Date:** _____
Age: _____
Race/Ethnicity (Please mark all that apply):
___ American Indian
___ Black or African American
___ Asian or Pacific Islander
___ Spanish/Hispanic/Latino
___ Caucasian

Highest level of completed education:
___ High School graduate or equivalency
___ Some Undergraduate
___ Technical school degree
___ Bachelor's degree
___ Graduate or professional degree

Have you ever participated in the United States Military? Yes ___ No ___

Have you been diagnosed with Major Depressive Disorder within the past 12 months?
Yes___ No ___

What was the first day of your last menstrual cycle? _____

Are you pregnant? Yes___   No____

Do you currently use oral contraceptives?
If so, please list: _____

Please list all medications used
daily: _____
_____

Do you smoke or use chewing tobacco? Yes___ No____

If yes, how many/often do you smoke/use tobacco
products? _____

Are you on a special diet? (i.e. low salt, low cholesterol, low carbohydrate, vegetarian, etc.). Please
describe _____
_____

Do you currently have a regular exercise routine? Yes ____ No _____

If yes, please describe (ie. typical days and times)_____
_____

Appendix B: Male Demographics

Participant ID: _____   **Date:** _____

Age: _____

Race/Ethnicity (Please mark all that apply):

___ American Indian
___ Black or African American
___ Asian or Pacific Islander
___ Spanish/Hispanic/Latino
___ Caucasian

Highest level of completed education:
___ High School graduate or equivalency
___ Some Undergraduate
___ Technical school degree
___ Bachelor's degree
___ Graduate or professional degree

Have you ever participated in the United States Military? Yes ___ No ___

Have you been diagnosed with Major Depressive Disorder within the past 12 months?
Yes ___ No ___

Please list all medications used daily: _____
_____

Do you smoke or use chewing tobacco? Yes ___ No ____

If yes, how many/often do you smoke/use tobacco products? _____

Are you on a special diet? (i.e. low salt, low cholesterol, low carbohydrate, vegetarian, etc.). Please describe _____
_____

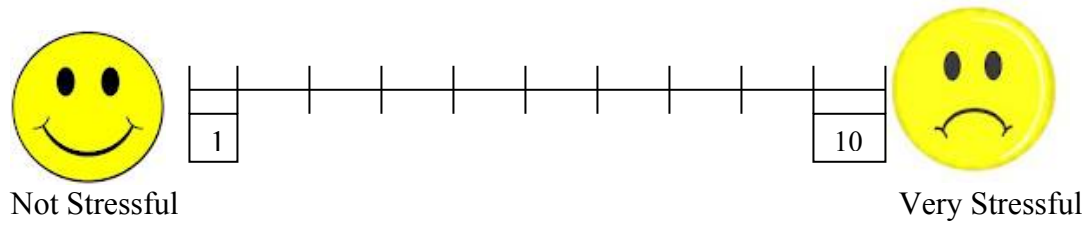**Do you currently have a regular exercise routine?   Yes ____ No _____**
If yes, please describe (ie. typical days and times) _____
_____

Appendix C: Visual Analogue Scale

Visual Analogue Scale

How stressed are you feeling right now?
Please place a mark on the scale to indicate your stress level.

Not Stressful   Very Stressful

Appendix D: Speech Script

** Reminder: Follow script word-for-word, do not deviate. **

"Hello, what is your participant ID?"

_____
(Please document in space provided)

"Please Begin."

Do not speak if the participant is speaking fluidly.
If the participant pauses for 20 seconds, respond with:

"You still have time left, please continue."

Document time of first <u>prompt</u> in the space provided below (ie. 3:22)

_____

Document time of second <u>prompt</u> in the space provided below

_____

If the participant ceases to speak after being prompted TWICE with the above statement,
proceed to the next step.

If the participant finishes early, remain quiet for 20 seconds before asking the questions
provided below. Please wait 20 seconds to speak during EACH pause.
Please document time of PROMPT in the space provided.

- **"Why do you think you are the best applicant for this position?"** _____
- **"What other experience have you had in this area?"** _____
- **"What about your studies/experiences identify a special aptitude and motivation for this position?"** _____
- **"Where else did you apply? Why?"** _____
- **"What would you do if your application here would not succeed?"** _____

If the participant is able to fill the <u>entire</u> allotted time with coherent speech, interrupt
with questions between the <u>third and fifth minute</u>.

When the speech is task is complete, please end and introduce the next task.

Use the space below to take notes when needed:

Appendix E: Arithmetic Script

** Please follow script word-for-word. Do not deviate.**

When the speech task is complete, please end and introduce the next task by saying:

"Thank you, that will be enough for now. We now want you to work on a second task. This task is about mental arithmetic. We will ask that you count backwards to zero in 13-numbered steps starting at 1793. If you miscalculate, we will notify you by asking you to restart at 1793. Do you have any questions?"

If the participant miscalculates, please respond with:

"Error, 1793"

At the five-minute mark, you will say:

"Thank you for your time, Meghan will escort you to room A."

Please document the following on the attached sheet:

- Place a check mark next to each number on which the participant makes an error.
- Circle the last number the participant correctly completes.
- Additionally, in the space provided, document the length of any pause over 10 seconds.

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University of Nevada, Las Vegas
Degree: Experimental Psychology, Ph.D.
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Graduation Date: December, 2014
Thesis: The hormonal, and performance correlates of posttraumatic stress disorder in males and females.

University of Nevada, Las Vegas
Degree: Counselor Education, M.S.
Emphasis: Clinical Mental Health Counseling/Addictions
Graduation Date: August, 2011
Thesis: The hormonal correlates of stress and posttraumatic stress disorder in female veterans.

University of Nevada, Las Vegas
Degree: Psychology, B.A.
Graduation Date: May, 2009

Professional Experience

08/2014-Present   **Doctoral Research Graduate Assistant**
- Supervisor: Stephen Benning, Ph.D.
- Duties: Conducting research on the hormonal and psychophysiological correlates of social support in psychopathy.

08/2014-Present   **Part-Time Instructor, Nevada State College**
- Duties: Preparing course content, assignments, and delivering lectures.
- Courses Taught: Physiological Psychology (PSY 403).

05/2013-Present   **Graduate and Professional Student Association**
- Supervisor: Peter Gray, Ph.D., Kate Korgan, Ph.D.
- Role: Treasurer (2014- Present), Secretary (2013-2014).

- Duties (Treasurer): Preparing and analyzing monthly budgets, chairing the government relations committee.
- Duties (Secretary): Preparing and posting meeting agenda and minutes, sitting as chair of the Publication Committee and holding a seats on Graduate College committees.

11/2012-Present **Research Intern, Cleveland Clinic, Lou Ruvo Center for Brain Health**
- Supervisor: Sarah Banks, Ph.D.
- Role: Visiting researcher
- Duties: Conducting research using MRI to examine structural and functional differences between wine experts and novices.

08/2013- 12/2014 **Part-Time Instructor, University of Nevada, Las Vegas**
- Duties: Preparing course content, assignments, and delivering lectures.
- Courses Taught: Psychopharmacology (PSY 422), Introductory Psychology (PSY 101).

06/2014- 08/2014 **Research Assistant, Nevada Institute for Children's Research and Policy**
- Supervisor: Amanda Haboush-Deloye, Ph.D.
- Duties: Writing reports, collecting and organizing data, and assisting in publishing original research.

06/2011-05/2013 **Graduate Research Assistant**
- Supervisor: Laurel Pritchard, Ph.D.
- Role: Research and Teacher Assistant
- Duties: Conducting research, teacher assistant duties, and other related projects.

05/2010-08/2011 **Harrah's Graduate Assistant in Problem Gambling Counseling**
- Supervisor**:** Larry Ashley Ed.S, LCADC, CPGC
- Role: Lead Counselor
- Duties: Conducting individual counseling, research and other related projects.

01/2010-08/2011 **Counseling Intern, U.S Veteran's Initiative**
- Supervisor: Shalimar T. Cabrera, MSW
- Role: Counseling Intern
- Duties: Conducting individual and group counseling.

6/2008-12/2008 **Research Assistant, Baby and Child Rebel Lab**
- Supervisor: Jennifer L. Rennels, Ph.D.
- Position; Undergraduate Research Assistant

- Duties: Recruiting infant participants, transforming video into usable data, and conducting studies. Project manager for scheduling, which entailed assigning weekly calls, ensuring protocol was followed, and answering other lab member's questions. I was also responsible for running reliability using Excel and SPSS.

Publications

Banks, S. J., Weintraub, D., Baldock, D., Pierce, M. E., Noback, M., Frasnelli, J., James, J., Beall, E., Sreenivasan, K. R., Zhuang, X., Cordes, D., Leger, G. C. (Submitted). This is your brain on fine wine: distinct structural and functional brain signatures in sommeliers.

Haboush-Deloye, A. L., **Pierce, M. E.,** Picker C. J. (Submitted) Exploring the relationship between exclusive infant feeding practices, weight status, and the consumption of sugar-sweetened beverages.

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Presentations

Pierce, M., Koenig, A., Zevallos, C., Egan, J., Orlewicz, M., Schumacher, T., Hensleigh, E., Pritchard, L. (2015). Hormonal Correlates of Posttraumatic Stress Disorder in Female Veterans. UNLV STEM Summit, Las Vegas, NV

Xiaowei, Z., Sreenivasan, K., Baldock, D., Weintraub, D., **Pierce, M.,** Frasnelli, J., Leger, G., Banks, S., Cordes, D. (2014). Insula Parcellation and Functional Connectivity Analysis in Sommeliers. International Society for Magnetic Resonance in Medicine, Toronto, Ontario, Canada.

Sreenivasan, K, R., Zhuang, X., Baldock, D., Weintraub, D., **Pierce, M**., Frasnelli, J., Beall, E., Leger, G. C., Banks, S. J. (2014). Activation and Functional Connectivity Patterns of Olfactory Perception in Sommeliers. International Society for Magnetic Resonance in Medicine, Toronto, Ontario, Canada.

Pierce, M., Koenig, A., Zevallos, C., Egan, J., Orlewicz, M., Schumacher, T., Hensleigh, E., Pritchard, L. (2013). The Hormonal Correlates of Posttraumatic Stress Disorder in Female Veterans. Society for Neuroscience Annual Convention, San Diego, CA.

Dold, K., Hensleigh, H., **Pierce, M.**, Lynch, S., Fowler, A., Abuali, K., Jager, A., Egan, J., Orlewicz, M., Pritchard, L. (2013). Early Life Stress, Drug Abuse, Exercise Effects on BDNF and Sex-Influenced Exercise Differences. Society for Neuroscience Annual Convention, San Diego, CA.

Pierce, M. E., Hensleigh, E., Egan, J., & Pritchard, L. (2013). The Hormonal Correlates of Stress and Posttraumatic Stress Disorder in Female Veterans. STEM Summit, University of Nevada, Las Vegas.

Pierce, M. E., Hensleigh, E., Egan, J., & Pritchard, L. (2012). The Hormonal Correlates of Stress and Posttraumatic Stress Disorder in Female Veterans. Association of Psychological Sciences Annual Convention, Chicago, IL.

Pierce, M., Egan, J., Jager, A., Schumacher, T, Pritchard, L. (2011). Correlates of Stress Response in Female Veterans with and without Posttraumatic Stress Disorder. Psi Chi Annual Poster Session, Las Vegas, NV.

Pierce, M. (2011). *Posttraumatic Stress and Mental Health Disorders*, Panel expert. Break-in session presented at Conference on Combat Trauma and Addiction, Las Vegas, NV.

Ashley, L. and Pierce, M. E. (2011). *The forgotten worker: the veteran*. Plenary Session presented at the EASNA Institute Annual Conference, Las Vegas, NV.

Pierce, M. E. and Ashley, L. (2011). *Working with female veterans.* Learning institute presented at the American Counseling Association Conference and Exposition, New Orleans, LA.

Hensleigh E, Lynch S, Semmel M, **Pierce M,** and Pritchard LM (2010). The effects of early postnatal separation and sex on methamphetamine reward. Poster presentation presented at *Society for Neuroscience*, 40th annual meeting.

Hensleigh, E., Lynch S., Semmel, M., and **Pierce, M. E.** (2010). *Sex and early maternal separation effects on methamphetamine reward and sensitivity*. Poster session presented at the Translational Research in Methamphetamine Addiction Conference, Bozeman, MT.

Pritchard, L. M., Hensleigh, E., Smith, L., Engel, S., Ridolfi, N., **Pierce, M**., and Dennehy, K. (2009). *Maternal separation produces sex-, dose-, and time-dependent alterations in amphetamine sensitivity*. Poster session presented at the Society for Neuroscience, Chicago, IL

Awards and Honors

Grants:
INBRE Small Grant, 2013: $4130.00
GPSA Research Grant, 2014: $1114.20
GPSA Travel Grant, 2011: $150.00

Awards:
Summer Session Scholarship, 2013
Patricia Sastaunik Scholarship, 2011, 2013, 2014, 2015
Chi Sigma Iota, Alpha Omega, Outstanding Research Contribution, 2010

Honors:
2007-2009   Dean's List

Nominations:
Nevada Regents Scholar, 2010

Chi Sigma Iota, Outstanding Masters Level Student, 2010
Service

2013-Present **Campus Representative**: Association for Psychological Sciences Student
Caucus
2014-Present **Chair:** GPSA Government Relations Committee
2014-Present **Chair:** GPSA Awards Committee
2014-Present Graduate College: New Program Evaluation Committee
2013-Present Graduate College: Program Review Committee
2013-2014   Graduate College: Curriculum Committee
2013-2014   **Chair**: GPSA Publications Committee
2013-2014   **Chair:** GPSA Social Media
2013- 2014   **President**: Experimental Students Committee
2012- 2014   **Peer Reviewer**: RISE award, Association for Psychological Sciences
2012- 2013: **Secretary**: Experimental Students Committee
2011-2012:   **First Year Cohort Representative**: Experimental Students Committee
2010-2013   **Secretary**: State of Nevada Association of Addiction Professionals
2010-2011   **President**: Student Organization of Addiction Professionals

Volunteer Experience

2010-2012   **U.S. Vets Stand down**
- Two-day event in which many organizations come together to provide services to Veterans.
- Services include: medical, housing, legal, dental, food, clothing, toiletries, mental health, etc.
- My main role in this event was doing intakes assessments.

Professional Affiliations

2008-Present Association for Psychological Sciences
2011-Present Society for Neuroscience
2014-Present Western Psychological Association
2010-2013   The Association for Addiction Professionals (NAADAC)
2010-2013   State of Nevada Association of Addiction Professionals (NAADAC
affiliate)
2009-2012   American Counseling Association

Student Affiliations

2010-Present Student Organization of Addiction Professionals
2011-Present Experimental Students Committee
2010-Present Chi Sigma Iota-Omega Alpha Chapter
2008-Present Psi Chi
2010-2011   Neuroscience Journal Club
2011-2013   Graduate Neuroscience Association

Certifications

2010-2012   Certified Problem Gambling Counselor, Intern

References

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