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Endocrine and psychophysiological correlates of jealousy and social anxiety in healthy adults: Elevated responses to inter-male competition.

An Honors Thesis

Presented to

the Department of Psychology
of the University of New Orleans

In Partial Fulfillment

of the Requirements for the Degree of
Bachelor of Science, with University Honors
and Honors in Psychology

by

Bethany H. McCurdy

May 2015

Acknowledgment

I would like to thank Dr. Elliott Beaton for his wisdom and guidance in this study. Thank you to Ashley Fortune Pruette Sanders for her invaluable assistance with E-Prime computer programming. I would also like to thank the University of New Orleans SCAN lab members for their assistance and support.

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Figure 1.

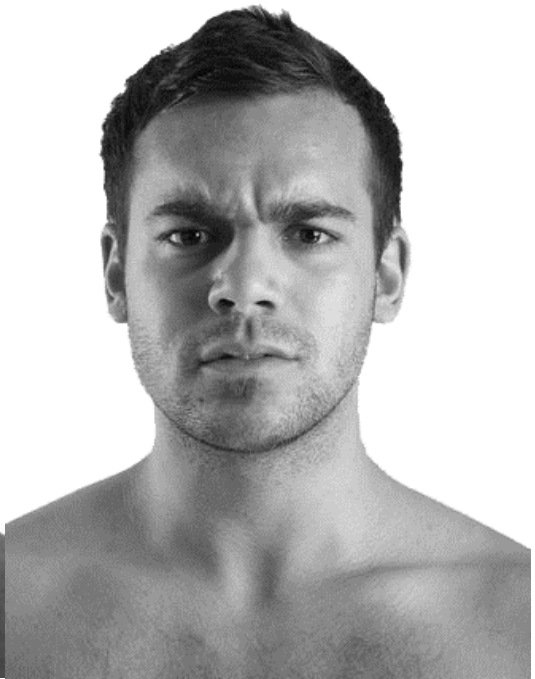


Figure 2.



Figure 3.

Figure 4.



Figure 5.



Figure 6.

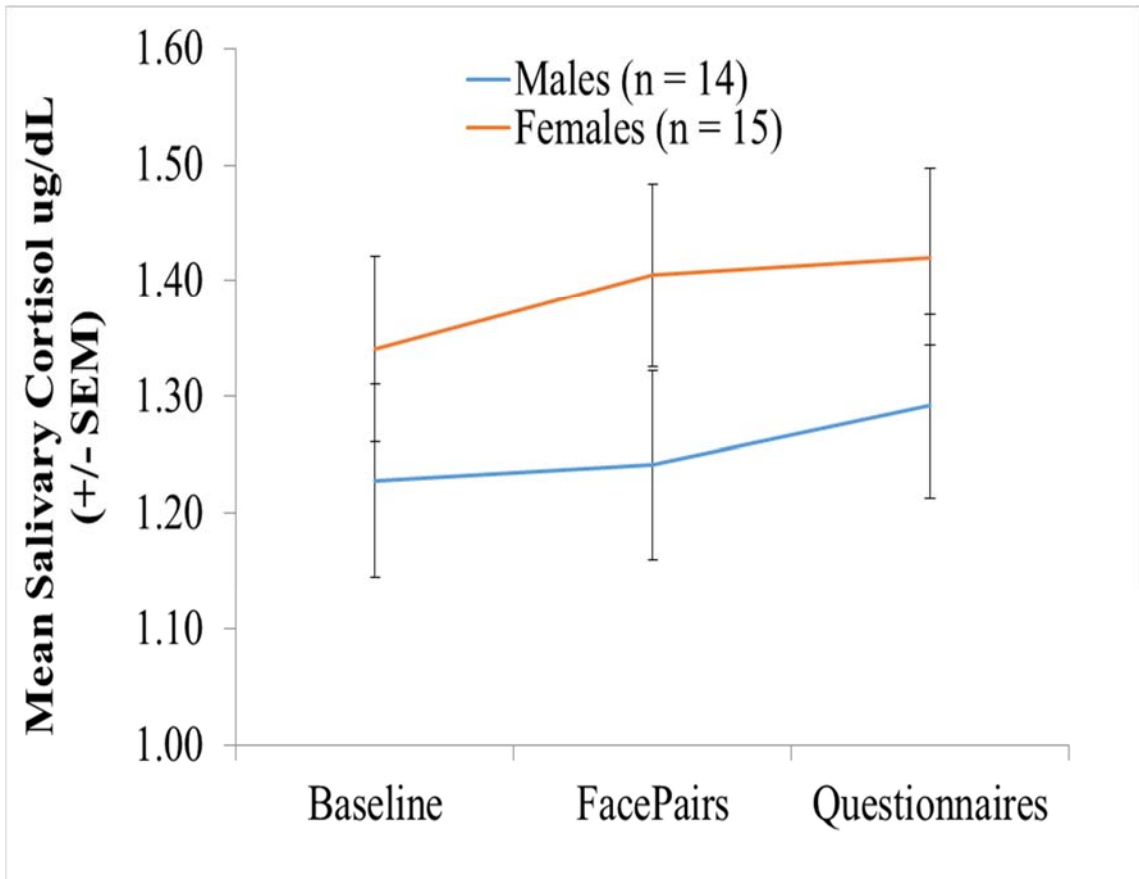


Figure 7.

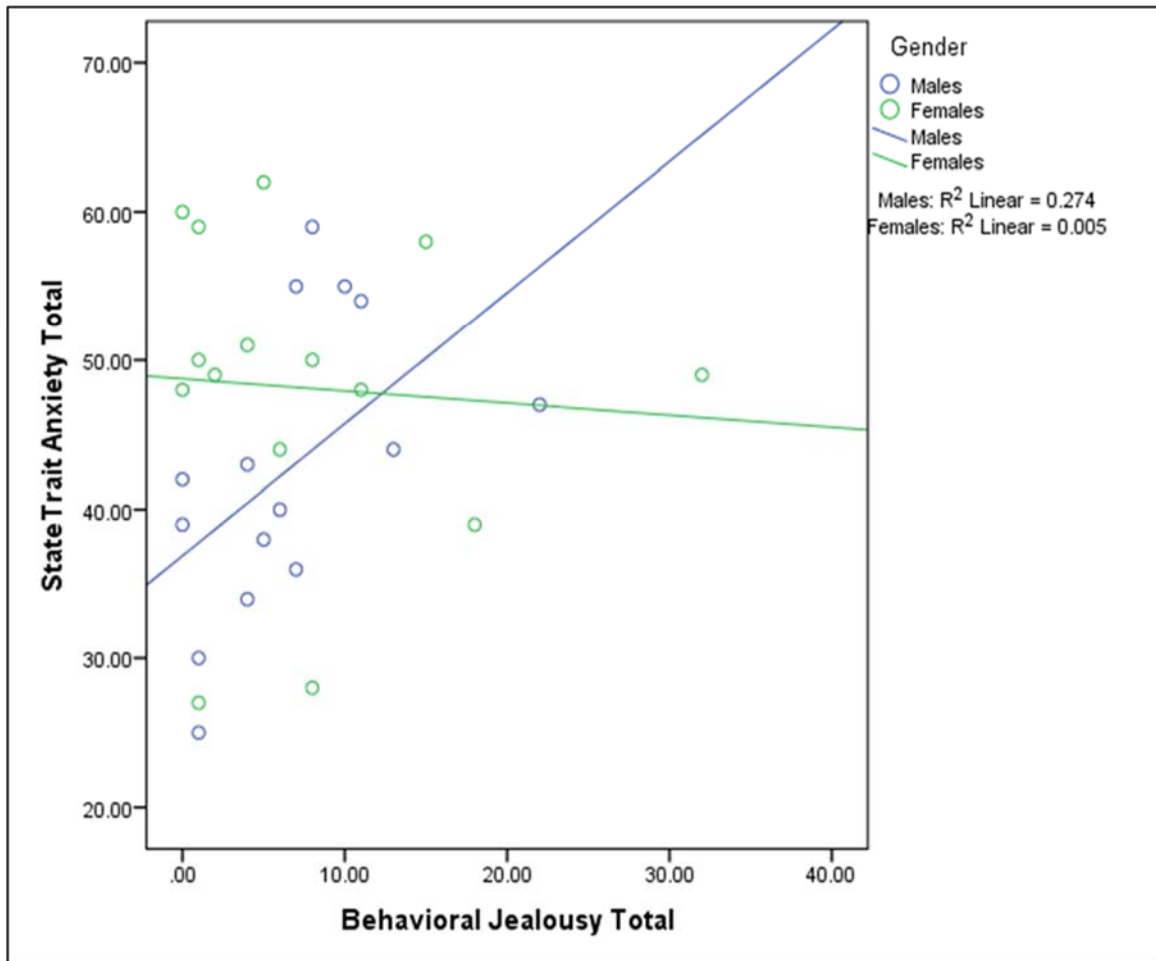


Figure 8.

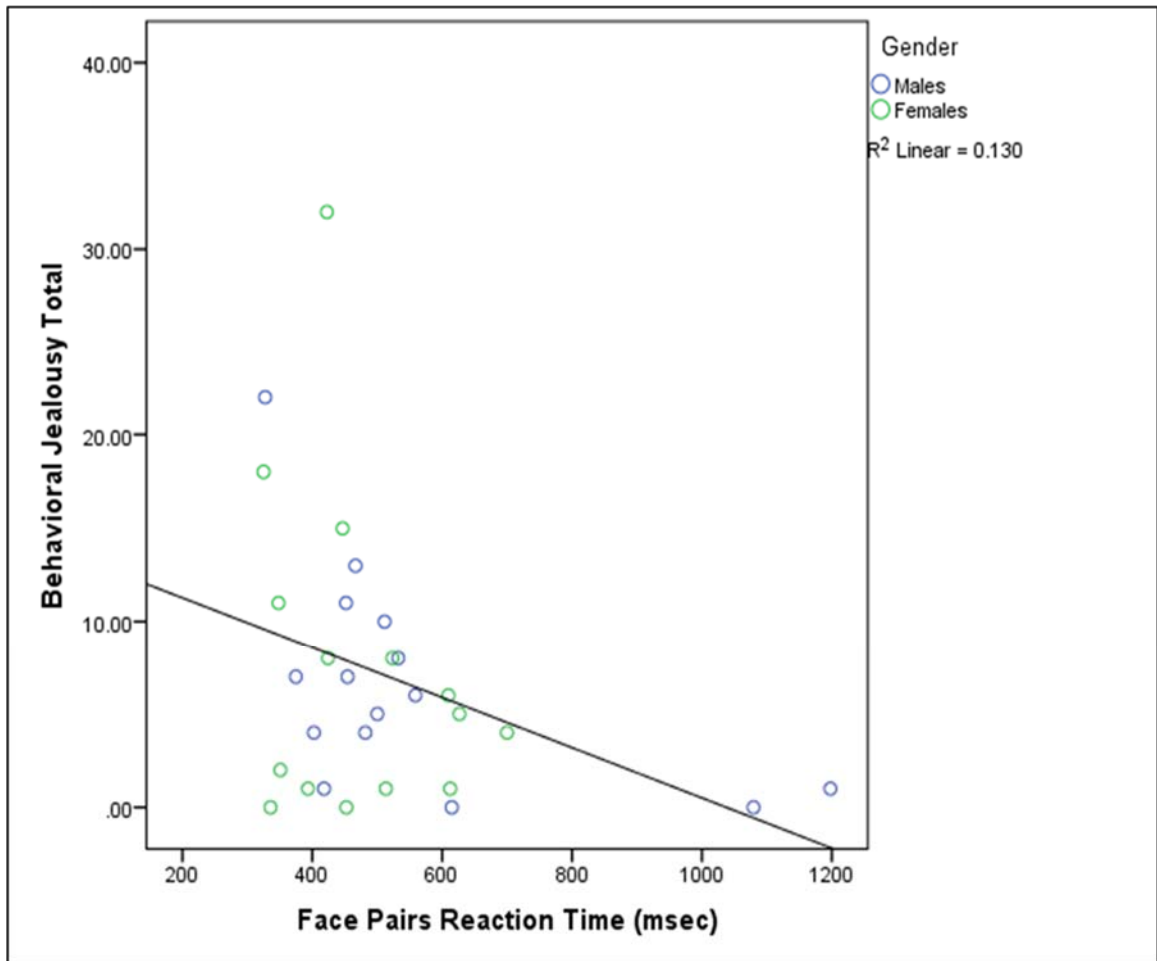


Figure 9.

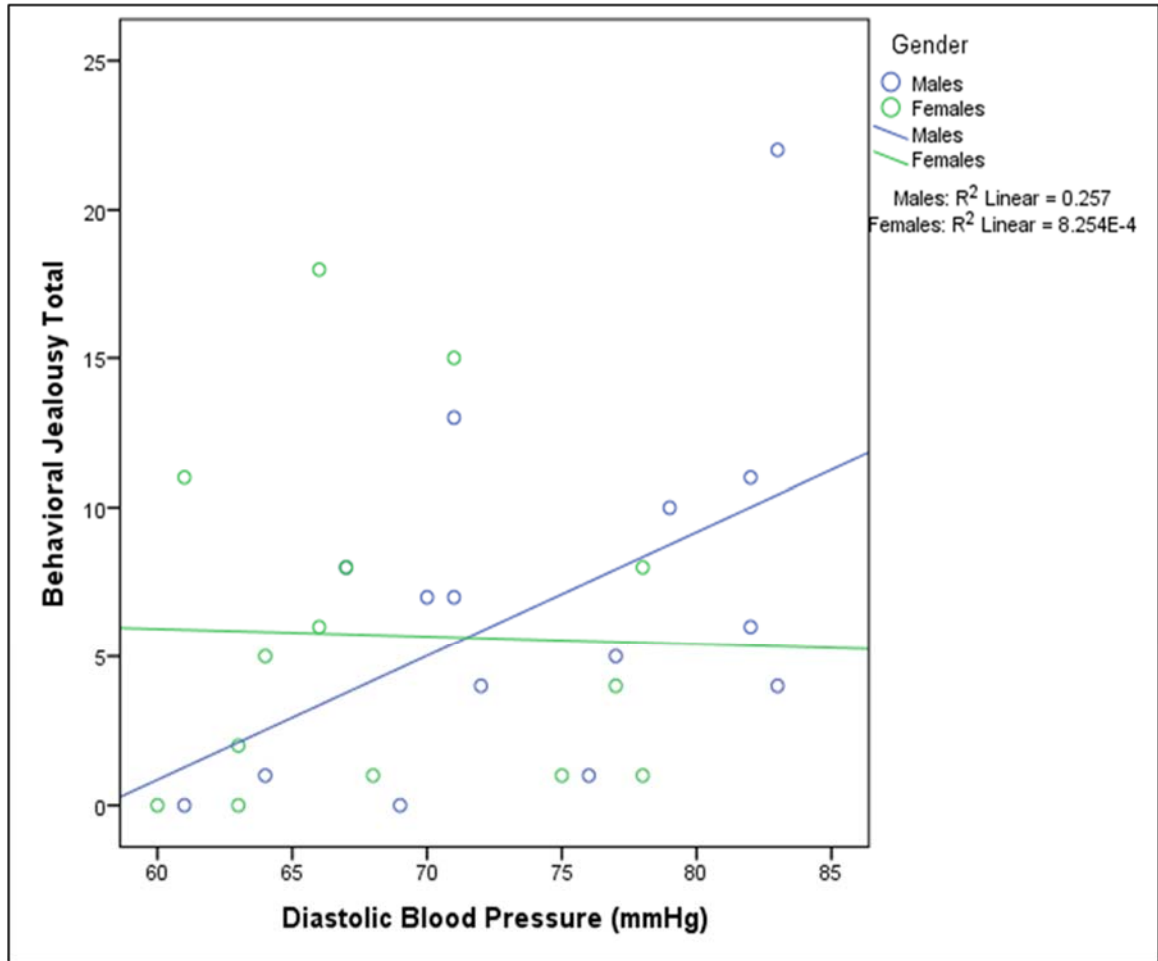


Figure 10.

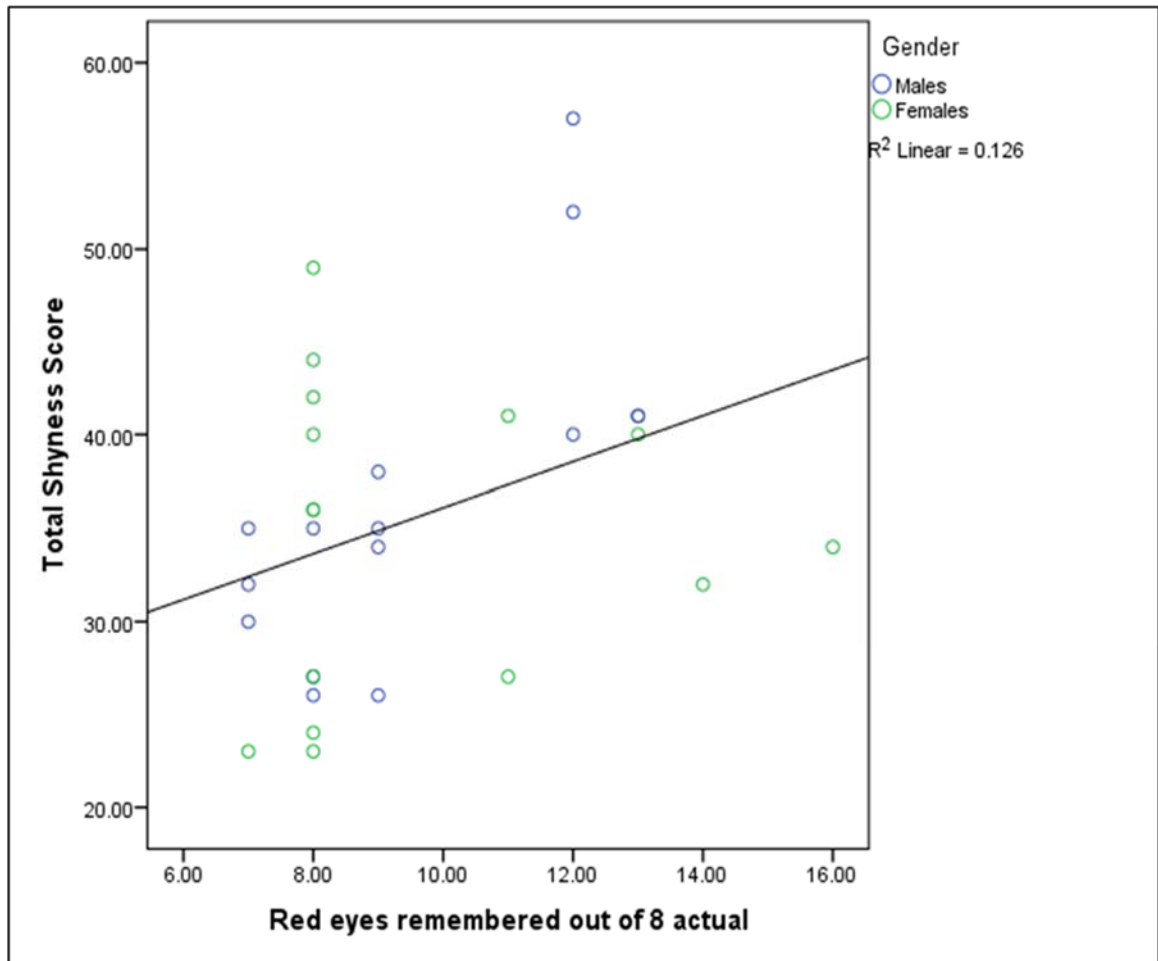


Figure 11a.

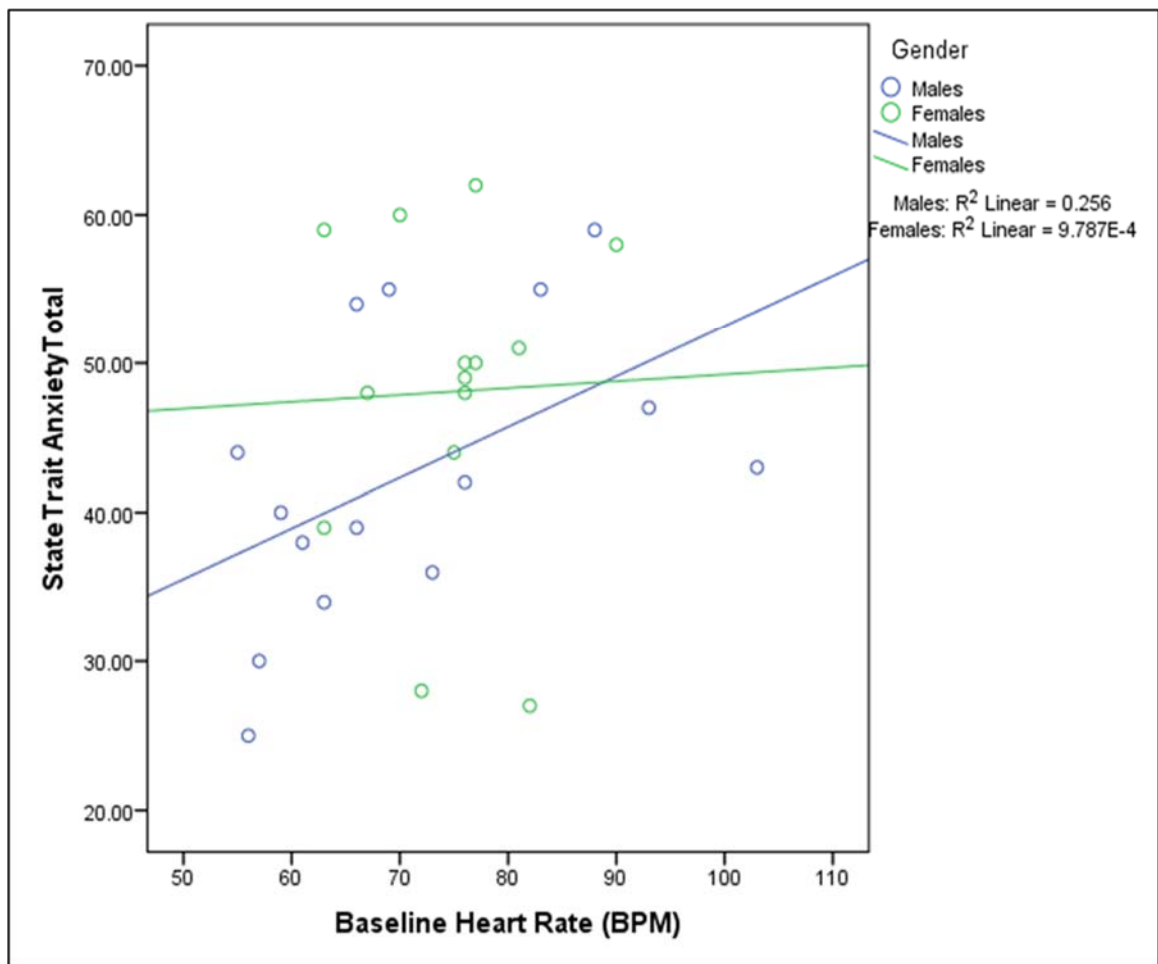


Figure 11b.

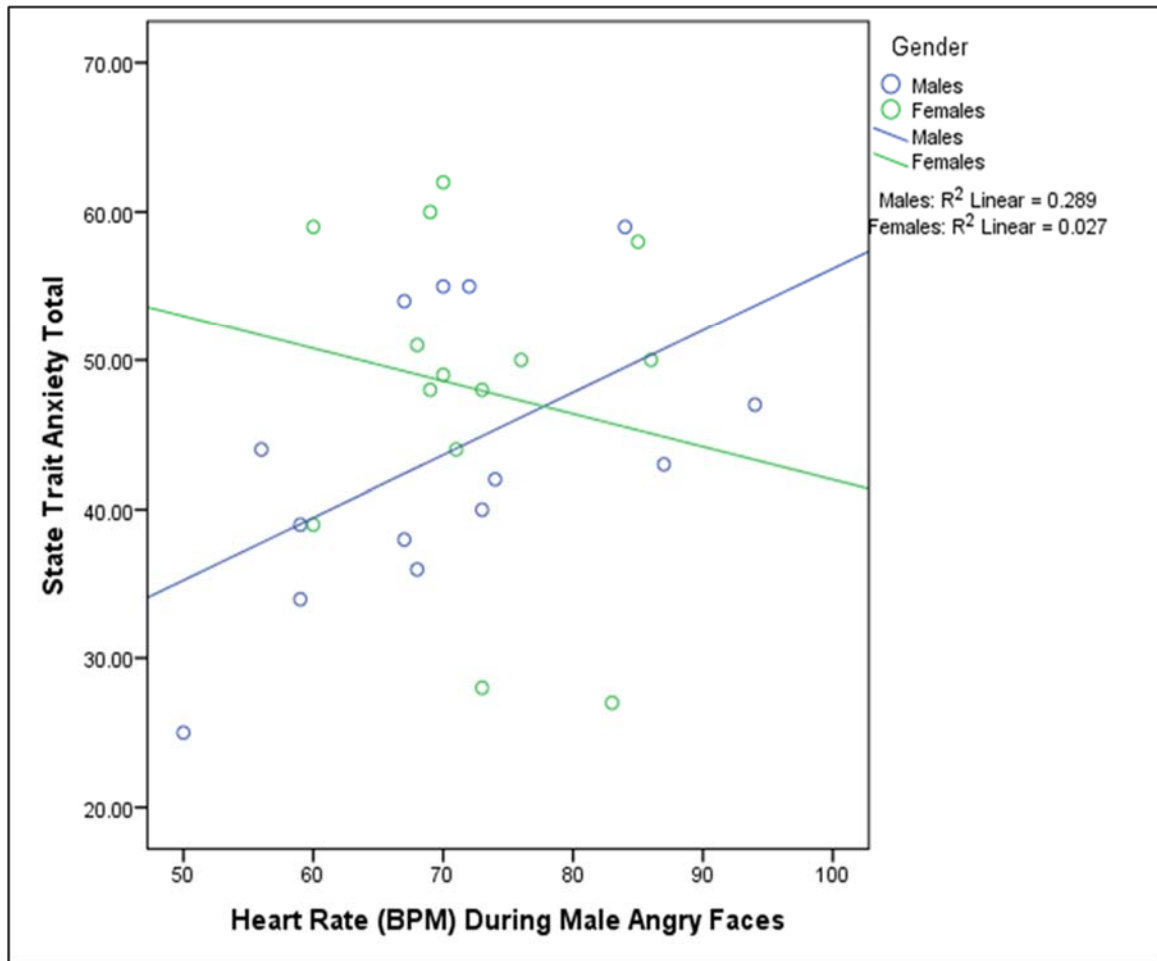


Figure 11c.

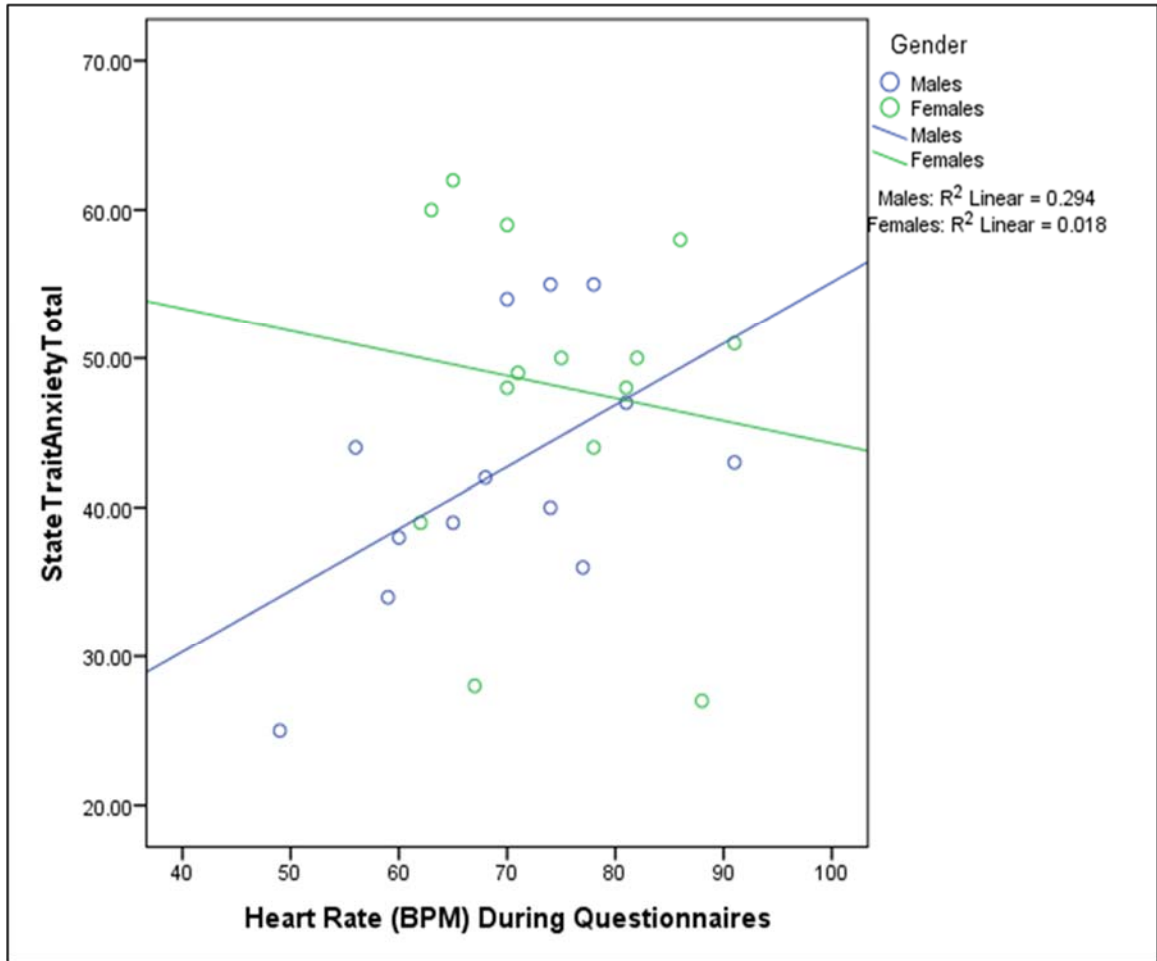


Figure 12.

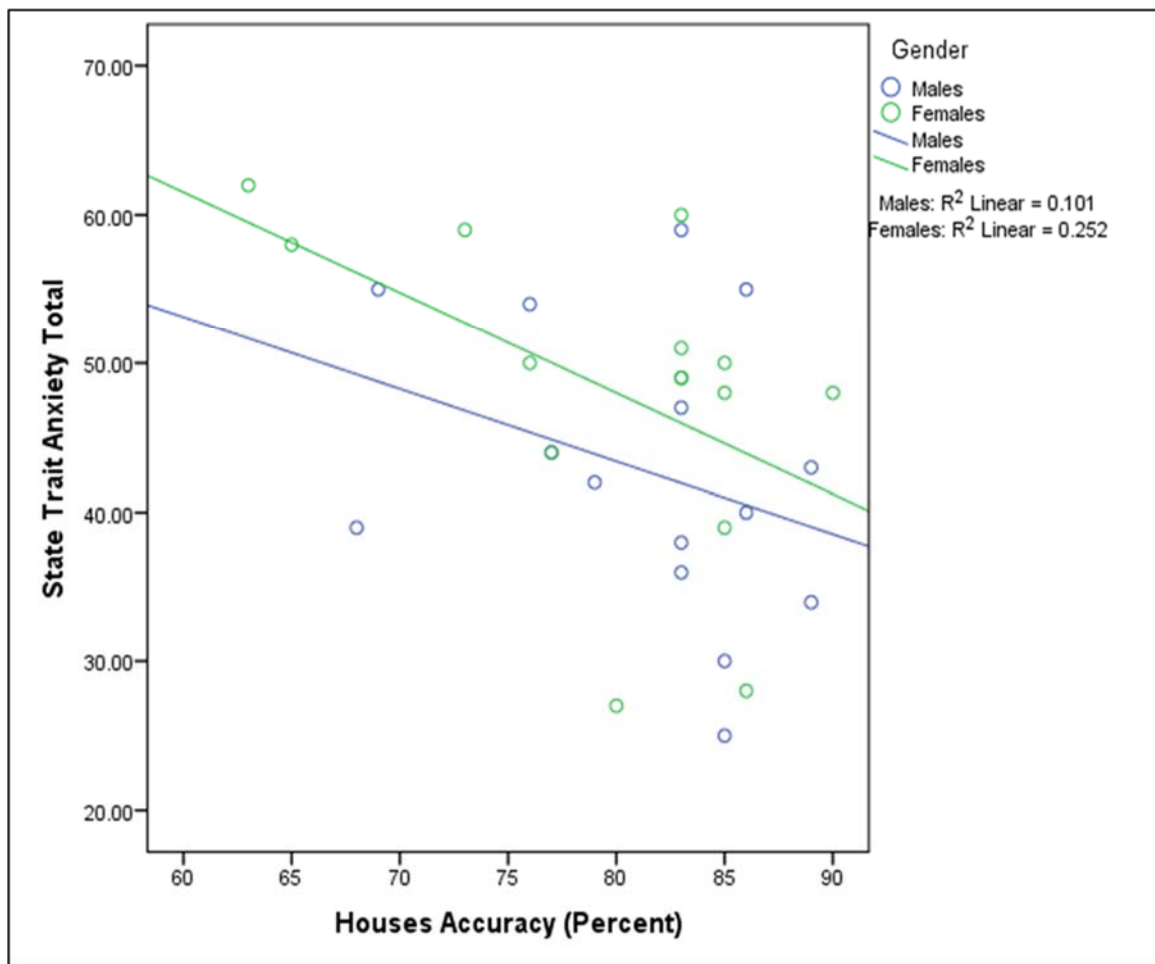


Figure Captions

- Figure 1.** – Example angry male face stimuli with red eyes modification. Eye color was either unchanged or modified to be red, yellow, or orange.
- Figure 2.** – Example angry male face stimuli without eye color modification.
- Figure 3.** – Example of angry male face accompanied by a supporting happy female face.
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- Figure 6.** – Male and female groups' mean salivary cortisol (ug/dL) plus or minus standard error of the mean over the three measures: baseline following the house task, following the paired face task, and immediately following the questionnaires.
- Figure 7.** – Scatterplot illustrating that elevated anxiety predicts behavioral jealousy in the male but not female sample.
- Figure 8.** – Scatterplot showing that in the combined group of males and females, higher behavioral jealousy predicted lower reaction times in indicating the placement of the angry male face in the paired faces task.
- Figure 9.** – Scatterplot illustrating that higher Behavioral Jealousy scores predicted elevated diastolic blood pressure in males but not females at the baseline measure.
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- Figure 12.** – Scatterplot illustrating that higher levels of anxiety predicted lower accuracy on the houses task in females but not males.

Abstract

Male mammals compete for reproductive access to females. Gaining and maintaining this access can be stressful and anxiety-provoking. In humans, anxiety and associated protective behaviors can manifest as jealousy. Physiological stress is likely to increase in relation to jealousy as it does with anxiety. Hypothetically, higher levels of anxiety and cortisol may indicate, and may even promote, strong territorial or jealous behavior. Chronically elevated cortisol has been shown to be deleterious to prefrontal and hippocampal neurons and result in emotional and stress-response dysregulation. In very anxious and jealous individuals, chronic stress activation could further promote these tendencies via emotional disinhibition. Cortisol production also related to vasopressin (AVP) levels and AVP has been shown to increase mate preference and territoriality. Furthermore, physiological measures may be more valid than self-report of less socially desirable behaviors such as jealousy and anxiety. As a preliminary study, we measured salivary cortisol, heart-rate, and blood pressure in relation to self-reported anxiety and jealousy in healthy men and women in response to threatening male faces paired with smiling female faces. Elevated anxiety positively predicted jealousy in men but not women. Anxiety and jealousy also predicted elevated heart rate and blood pressure. Cortisol levels in response to the threat task and in relation to jealousy approached statistical significance ($ps < 0.07$) and suggest the need for a larger sample size.

Keywords: Adults, Anxiety, Blood Pressure, Cortisol, Heart Rate, Face Processing, Jealousy

Introduction

A variety of hormones including both steroids and peptides respond to and influence social interactions in human and non-human animals (Brunnlieb, et al., 2013). For example, in female mammals, there is a release of the neurohypophysial hormone oxytocin during labor, birth, and lactation influencing maternal-offspring bonding. Along with causing smooth muscle contractions during birth and milk let-down as well as reducing memory for pain via action on the central nucleus of the amygdala, the interaction between hormone release and maternal behavior is essential for pair-bonding with offspring. Oxytocin is also released during states of anxiety, orgasm, and social recognition (Marieb, et al., 2013). Another pair-bonding nanopeptide found in both females and males – but in higher levels – is arginine vasopressin (AVP) also known as antidiuretic hormone (ADH; hereafter referred to as AVP). AVP has a slightly different structure from oxytocin by two amino acids. AVP is released primarily to retain water concentration in the body and to restrict blood vessels. By regulating the amount of water, salt, and glucose in the blood, vasopressin plays a crucial role in maintaining homeostasis including blood pressure (Marieb, et al., 2013). This hormone is dynamic in nature, and has not only physical effects, but influences psychophysiological aspects of behavior (Brunnlieb, et al., 2013).

An innate trait seen in mammals is the territorial and aggressive male protecting his mate, sisters, and mother(s) of his offspring as well as the offspring themselves (Guastella, et al., 2010). There is an emotional and behavioral attachment between the pair-bonds, and a mannerism of interest is the sexual jealousy provoked within the male. This jealousy is characterized in humans by mate-guarding techniques and

proprietaryness, which is regarded as an egocentric sense that the subject is the rightful and only owner of his belongings. When asking why this is evident in nature, keep in mind the differential cost of reproduction between males and females; sexual jealousy will potentially limit how much access is granted to multiple male partners. It is not unusual that there is a shortage of highly desirable females for the males to pursue or an abundance of attractive male rivals. In order to protect their generational offspring and access to future reproductive opportunities, males exhibit socially dominant behaviors that can increase their reproductive success (Daly and Wilson, 1992). What is described as jealousy in humans is seen across the spectrum of mammals including sheep (Bielsky, et al., 2004), mice, hamsters, (Ferris, 1997), and voles (Walum, et al., 2008). Ovulation is concealed in humans and in combination with the differential cost of reproduction between the sexes, mate seeking behavior by men and women is moderated by overlapping but differing goals. This has led to some interesting adaptations. For examples, during ovulation, women rate the scent of males with low fluctuating asymmetry [i.e. more symmetrical faces] and strong secondary sex characteristics as more attractive (Møller and Pomiankowski, 1993). So, via olfaction, females are able to detect subtle indicators of male mate quality (Bielsky et al., 2004).

It is important for females to be able to make rapid and accurate measures of genetic quality in potential male reproductive partners. Females maximize their genetic fitness by having fewer but high-quality offspring, while males potentially maximize their reproductive fitness by mating with as many females as possible. This can also lead to overlapping but different needs and potentially conflict – between males and females but also between males. This conflict arising over the differential cost of reproduction

with female selectivity results in male-male competition for what is essentially a limited reproductive resource. Thus, males seek to guard against potential female infidelity to avoid cuckoldry where the male would expend resources raising offspring that share none of his genes (Wilson and Daly, 1992). The neurobiological mechanisms that regulate these processes are not yet fully understood but are under investigation.

For example, AVP appears to regulate mate-guarding and monogamy in mammals. It is theorized that vasopressin acts the same way in human males as male voles (Walum, et al., 2008) or rats (Zbuzek & Wu, 1979). In animal models of monogamy and polygamy, AVP injections promote mate preference in male rats and voles towards females. There is evidence of seasonal variation in vasopressin in rats (Zbuzek & Wu, 1979) and genetic variation in humans (Walum, et al., 2008), indicating that levels of vasopressin may be higher in some individuals and not others. This may lead to individual differences in behavior regarding pair-bonding/emotional attachment and, more specifically, anxiety (Thompson, et al., 2006) and sexual jealousy.

Jealousy is not a pleasurable emotion and it arises from anxiety that one's mate or sexual partner is or could be engaging in extra-pair copulations or expending time and resources on another partner (Buss, et al., 1992). Individual differences in jealousy are also likely driven in part by differences in the expression of hormones such as AVP but in socially complex animals such as humans, jealousy is likely affected by the quality of the relationship, aggression, anxiety, social confidence, and social communication skills.

The tendency for jealousy likely contributes to social anxiety and arises from a lower threshold for social threat resulting in a greater physiological stress response (Buss,

et al., 1992). A tendency for jealousy is generally considered not to be a socially desirable trait although cultural variation exists (Buunk & Hupka, 1987). Therefore, people may not be completely honest on self-report measures as to how emotionally and behaviorally jealous they tend to be with sexual or romantic partners. Physiological markers of stress in response to an inter-male social threat in the presence of a female could be indicative of a tendency for jealousy. Elevated heart rate, blood pressure, and salivary cortisol are good indicators of a physiological stress response (Kirschbaum, et al., 2014). Cortisol is released naturally throughout the day, spiking in the early day of a normal sleep cycle and descending through the afternoon towards evening. Cortisol is released during specifically stressful situations, also to ensure homeostasis within the human body. Cortisol is relatively easy to measure in saliva. Besides being a measure of stress, elevated cortisol has also been reported in the context of elevated AVP which actually facilitates the release of cortisol from the adrenal glands via activation of V1 receptors (Perraudin, et. al., 1993).

Aims and Hypotheses

In this study, we were looking for signals of an increased stress response as measured by cortisol, heart rate (HR), and blood pressure (BP) to overt images of male threat and inter-male competition for females in relation to self-reported jealousy and social anxiety. Because cortisol is released in response to stress, we hypothesize that stressors triggering anxiety and sexual jealousy will lead to an increase in cortisol released. Those that are found in situations where anxiety and/or jealousy are higher may exhibit a higher psychophysiological stress response. Some individuals may have higher levels of vasopressin and thus exhibit more notable behaviors of aggression, pair-bonding, territory protection, and anxiety. While we are not directly measuring AVP, we hypothesize that higher tiers of sexual jealousy and social anxiety will predict higher levels of salivary cortisol, heart rate, and blood pressure in men and women when viewing images of threatening male and supportive female paired faces.

The task is designed to affect men more and elicit feelings of male-male competition for a female by displaying an attractive female smiling at a masculine attractive male who appears to be angry and looking at the participant. It was expected that men will be more affected than women by the male threatening face both alone and in the context of a smiling female face. Those that are affected and are more sensitive to the angry male faces were predicted to have an elevated stress response as measured by

cortisol, HR, and BP and that stress would remain high even during a relaxation phase compared to less jealous individuals.

Method

All procedures were approved by the Institutional Review Board at the University of New Orleans.

Participants

Thirty healthy men ($n = 15$) and women ($n = 15$) with a mean age of 22.16 (ranging from 17 to 47) from the University of New Orleans participated in the study.

Psychological Measures

Computer Tasks

Each of the 30 participants participated in a controlled environment by completing a computer-based E-Prime task that was broken into four parts: Phase I, II, III, and IV.

Phase I (also known as the baseline phase) consisted of 7 minutes and 30 seconds of differentiating between the neutral stimulus of one-story houses [Figure 5] and two-story houses by selecting a specific key on the keyboard.

In Phase II, participants were asked to observe a 6 minute long slideshow portraying faces of angry male faces, some of which had either yellow, orange, red [Figure 1], or unaltered [Figure 2] eyes. Participants were asked to remember how many

had red eyes and were notified they would be prompted for that number at the end of the study.

During Phase III, different pairs of attractive, angry male (facing forward) and attractive, happy female (profile/facing the angry male) faces [Figure 3] appeared on the screen for 7 minutes and 30 seconds. The participants were asked to select specific keys depending on whether the angry male was on the left or right of the happy female.

During phase IV, participants were given 5 minutes to relax by observing a slideshow of photographic nature [Figure 4]. At the end the E-Prime task, a questionnaire was given that prompted participants for information regarding demographics and health and asked questions based on how anxious, shy, and emotionally/physically jealous the participant was in everyday and current life.

Psychophysiological and Endocrine Measures

Between each phase and after the questionnaire, we measured blood pressure and heart rate using a portable hospital-grade digital sphygmomanometer (Welch-Allyn, Skaneateles Falls, NY).

After the baseline phase, Phase III, and the questionnaire, participants were asked to salivate at least one ml of saliva into three separate 2.5 ml cryotubes. Samples were stored at -80°C until assayed.

Cortisol Assays

Cortisol was measured in saliva using Salimetrics LLC (State College, PA) enzyme-based immunoassay kits. Saliva tubes were centrifuged to precipitate out mucus

and other contaminants. The wells of a 96-well microtitre plate were coated with monoclonal antibodies to cortisol and all samples and standards were run in duplicate. The plates were read under a 450 nm filter on an EON Absorbance microplate reader (BioTek, Winooski, VT) with BioTek Gen5 Data Analysis Software 2 (BioTek, Winooski, VT). The unknown amount of cortisol in the samples were interpolated to optical absorbance values on the standard curve of known amounts that make up the serially-diluted standard curve generating a value in $\mu\text{g}/\text{dl}$ saliva per well.

Questionnaires

Multiple questionnaires were merged to create a single questionnaire that assessed anxiety, social phobia, jealousy, shyness, and demographics. For self-reported jealousy, we modeled the Jealousy Instrument questionnaire created by Buss (1999). We also included the *Shyness and Sociability Scale* created by Cheek and Buss (1981). The *Social Phobia Inventory*, or SPIN, questionnaire was used to measure degree of social phobia. The self-reported anxiety was collected using Spielberger's *State-Trait Anxiety Scale* (1989). High and low levels of each trait were measured by scoring within the range of each questionnaire. The anxiety scale consisted of 20 questions, ranging on a scale of 1 for feelings least anxiety to 4 for feelings of strong anxiety. The jealousy scale asked 16 questions on emotional jealousy while the behavioral jealousy scale asked 8, ranging with scores on an ascending scale between 1 and 7. The shyness scale asked 13 questions ranging from scores 1 to 5 on an ascending scale.

Statistical Analyses

Salivary cortisol, HR, BP and anxiety were contrasted between the High- and Low-Jealousy groups using multivariate statistics controlling for age. Results of the questionnaire were recorded as a whole group and by gender, separating traits into the categories of shyness, state anxiety, social phobia, behavioral jealousy, and emotional jealousy. These factors were then tested against their abilities to accurately and quickly perform in Phase I and Phase III. Memory was also tested among these groups as it pertained to remembering the amount of red eyes. Relationships between these factors were examined using ANOVA linear regression, Pearson correlations, and repeated measures analyses of variance. The threshold for statistical significance was set at 0.05. Probability values less than 0.075 are reported as statistical ‘trends’.

Results

Anxiety

Collectively, the males and females did not show any relation between anxiety and jealousy. However, as Figure 7 illustrates, the males showed a positive correlation between state anxiety and behavioral jealousy, where $r[15] = 0.52, p = 0.045$. As a group, there were no significant effects on task performance concerning anxiety. For the females, seen in Figure 12, those who were more anxious scored lower on accurately depicting between one-story and two-story houses ($r[15] = -0.50, p = 0.057$). As a group, there was a trend towards a higher heart rate after Phase I ($r[30] = 0.347, p = 0.065$). Anxious males had higher heart rates after Phase I [Figure 11a] ($r[15] = 0.506, p = 0.055$) and Phase II [Figure 11b] ($r[15] = 0.538, p = 0.047$). A higher heart rate was also seen [Figure 11c] after the questionnaire for anxious males ($r[15] = 0.542, p = 0.056$). Contrasting men and women, they did not differ on any measure of anxiety or jealousy ($ps > 0.1$).

Shyness

For the group, as seen in Figure 10, there is a positive correlation between shyness and the number of red eyes remembered ($r[30] = 0.356, p = 0.054$). Shyness had no other immediate effect on performance of Phase I and III. Shy females showed slower heart rates after viewing angry male faces in Phase II ($r[15] = -0.549, p = 0.042$). Shyer

females also appeared to show lower heart rates after viewing pairs of angry male faces and happy females although this did not quite reach the commonly set level of statistical significance of 0.05 ($r[15] = -0.543, p = 0.055$).

Social Phobia

As a whole, socially phobic individuals were more accurate when mapping the placement of an angry male face accompanied by a happy female ($r[30] = 0.358, p = 0.052$). There was a trend towards an overall higher heart rate after Phase I ($r[30] = 0.346, p = 0.066$).

Behavioral Jealousy

For the group, higher self-reported Behavioral Jealousy predicted slower reaction times to forward facing threatening angry male faces paired with happy female faces facing the men with $r[30] = -0.361, p = 0.050$ [Figure 8]. Males who reported higher levels of Behavioral Jealousy showed a slower reaction time ($r[15] = -0.535, p = 0.40$) when indicating which side the target male was in relation to the female. Higher Behavioral Jealousy also predicted elevated diastolic blood pressure [Figure 9] at Baseline for the males only ($r[15] = 0.51, p = 0.054$).

Emotional Jealousy

As a group, self-reported Emotional Jealousy did not predict Shyness, Anxiety, Social Phobia or performance of any of the tasks (all $ps > 0.05$). However, emotionally jealous females showed a trend of reacting more quickly to the pairs of faces ($r[15] = 0.493, p = 0.062$). There was a trend for overall higher heart rates after Baseline ($r[30] =$

0.351, $p=0.062$). Emotionally jealous individuals also showed higher systolic blood pressure ($r[30]=0.384$, $p=0.040$) and diastolic blood pressure ($r[30]=0.369$, $p=0.49$) at Baseline (Phase I). Heart rate ($r[30]=0.374$, $p=0.042$) and diastolic blood pressure ($r[30]=0.382$, $p=0.041$) were elevated in relation to Emotional Jealousy following Phase IV when completing the questionnaire battery. Emotionally jealous females showed a higher diastolic blood pressure after Phase IV ($r[15]=0.536$, $p=0.048$).

Cortisol

A trend suggests that higher levels of emotional jealousy predict higher levels of cortisol immediately following the paired face task ($r[29]=0.34$, $p=0.074$). Shyness, state anxiety, social phobia, and behavioral jealousy do not predict cortisol at the group level. At the group level, cortisol measures did not predict accuracy or reaction time during Phase I or Phase III. Cortisol levels do not predict heart rate or blood pressure in any of the measures for the larger group.

Next, we conducted a repeated measures ANOVA examining salivary cortisol between men and women over time. There was no main effect of gender ($f[1, 27]=1.54$, $p=0.22$). While cortisol appeared to increase from measure one to measure three, a within-group main effect of time approached but did not reach statistical significance, as seen in Figure 6 ($f[1, 27]=3.63$, $p=0.067$). As a group, there was a statistical trend showing a possible relationship between elevated emotional jealousy and higher salivary cortisol levels in response to Phase III ($r[29]=0.34$, $p=0.074$).

Discussion

The results supported the hypothesis that males who were more socially anxious would also display traits of jealousy. As expected, these tasks had a greater effect on the male population in terms of psychophysiology. Images and scenarios in the tasks were designed to be more threatening and emotionally stimulating to male participants.

Limitations and Future Directions

The E-Prime tasks consisted of images that were targeted toward Caucasian heterosexual males. In order to achieve consistency, a demographic of Caucasian heterosexual males would need to be tested. Testing at a specific time of day would be ideal to even out varying levels of cortisol that are released naturally throughout the day. Many of the trends that were present in the results may become clearer with a larger sample of behaviorally and/or emotionally jealous participants. There were some consistencies in our results to our hypothesis with a small sample size; a more precise connection is predictive if there is a larger sample size.

Although these preliminary findings are intriguing, the study is not without weaknesses. Participant honesty in disclosing undesirable traits such as jealousy or health behaviors is a concern. Physiological measures can serve as corroborations of self-report measures but they too are imperfect. For example, the time of day that the

participant awoke can affect cortisol reactivity. Cortisol is also subject to significant individual variability and not always indicative of an elevated stress response (Kudielka, et al., 2009). Improvements include measuring dehydroepiandrosterone (DHEA), a putative anti-glucocorticoid that can be detected in saliva. Elevated cortisol is not always indicative of a problematic stress response if recovery is rapid. Elevated DHEA relative to cortisol may indicate psychological resilience (Oberbeck, et al., 1998). Therefore, a ratio of cortisol to DHEA may show more accurate metric hypothalamic pituitary adrenal axis activity in response to stress. Participants also chose to skip parts of the study that made them feel uncomfortable, such as salivation and the jealousy questionnaire. These missing data reduced statistical power. Faces for Phase II and Phase III were collected by members of the Stress, Cognition, and Affective Neuroscience (SCAN) Laboratory and tested for emotional valence. In a future study, potential improvement would be to use a well standardized, previously published face set. Furthermore, the addition of eye-tracking would provide valuable information about how long the participants looked at any given face and also where on the face they tended to look. It would be very interesting to know if jealous males spent more time looking at the threatening male face or at the smiling female face in the paired faces task.

Vasopressin is a dynamic hormone found in mammals: As an antidiuretic hormone, vasopressin controls the restriction of water in the body and constricts blood vessels. For example, male voles produce higher levels of vasopressin when territorially threatened in regards to his mate (Bielsky, et al, 2004). Positive correlates of vasopressin and sexual male jealousy may also be present in humans. Testing vasopressin directly can be problematic in certain populations. Currently, there are no ways to test for vasopressin

in the form of analyzing saliva, but methods do exist where AVP can be tested in plasma and serum. The experience of blood extraction may be more stressful to some than others, and this may itself cause fluctuations in stress hormones. One way to test for vasopressin non-invasively (ie. without blood extraction) would be to test for its precursor, copeptin in saliva. Copeptin has successfully been used as a proxy measure of AVP in humans. The gene for vasopressin encodes AVP and two other proteins: neurophysin II and copeptin. Copeptin is not biologically active but it is stable and produced at the same rate and the in the same amount as AVP. Also, it is found in higher concentrations in blood because it is unbound to blood platelets (Struck, et al., 2010). A valuable next step in the study would be to revisit the collected saliva samples to measure copeptin levels in the male participants with the working hypothesis that higher levels of behavioral and emotional jealousy would predict higher levels of copeptin. Before we determine any change in vasopressin in the more anxious and/or jealous males, it would be ideal to find differences in cortisol among the same group.

Conclusions

Why is the existence of jealousy important to understand in the natural world? Understanding how human psychology and physiology correlate can be crucial to interpreting underlying mechanisms for behavior. It appears that males have a higher psychophysiological response with a simple visual task, as opposed to a natural situation. These males may experience more intense physiological responses regarding jealousy in the natural world. Behaviorally, jealousy plays a role in mate-guarding and ensuring production of the best possible offspring. Higher levels of jealousy have a dangerous role in human culture and can be seen in cases of spousal homicide when the female displays

acts of infidelity, or if the male suspects it (Daly and Wilson, 1988). The question remains whether there are biological reasons for higher levels of jealousy in some and not others.

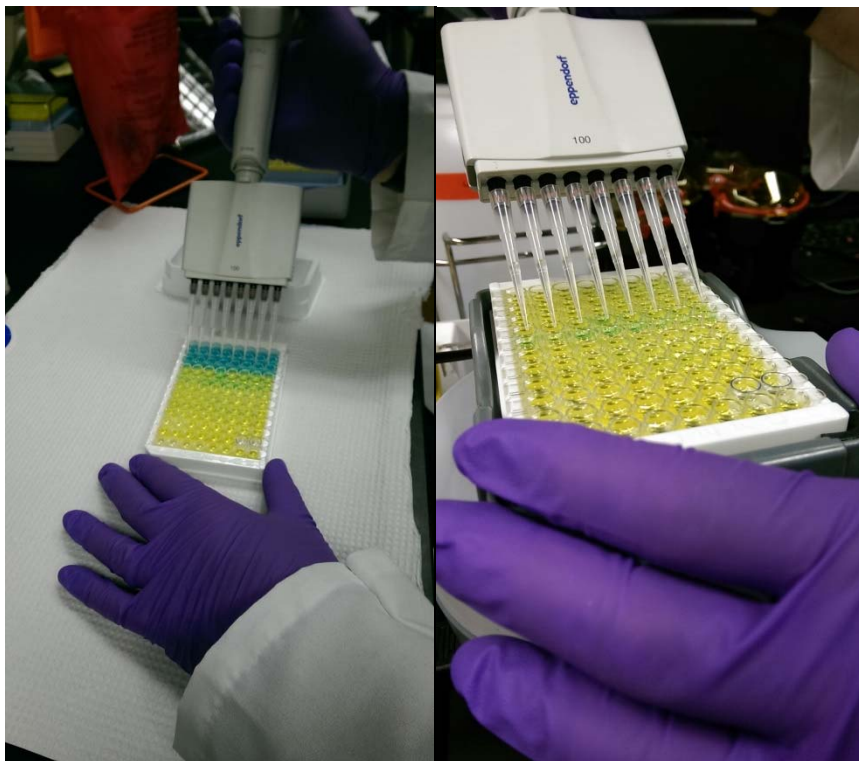
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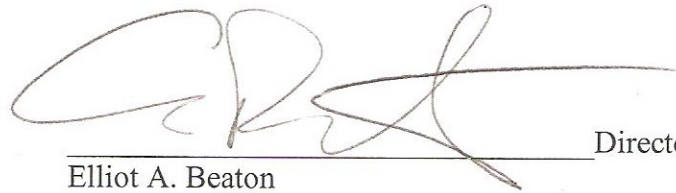
Appendix 1 – Images of the salivary cortisol analyses.



APPROVAL SHEET

This is to certify that Bethany McCurdy has successfully completed his Senior Honors Thesis, entitled:

Endocrine and psychophysiological correlates of jealousy and social anxiety in healthy adults: Elevated responses to inter-male competition.



Director of Thesis

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Abu Kabir Mostofa Sarwar Honors Program

April 30, 2015
Date