Effects of Social Research Methodology on Cortisol and Testosterone

by

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A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of Bachelor of Science

With Honors in Neuroscience from the

University of Michigan

2011

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Abstract

Previous research indicates that hormones respond to interactions with potential sexual partners and anticipation of being reunited with a long-distance romantic partner. Considering that many types of social stimuli can have endocrine effects, design in research studies may have confounding endocrine responses to the partner-based or sexual survey questions used to characterize the sample. The current study serves to analyze the effects of sexual vs. partner-based stimuli, in the form of survey questions, on endocrine responses. A sample of participants (n=136) filled out responses to one of three survey conditions randomly assigned: neutral, partner-based, and sexual. Participants also provided saliva samples immediately before and 15 minutes after their condition. The results showed a decline in cortisol levels for women that were assigned to the partner-based condition, and no effect of survey questions on testosterone in men or women. There were no significant effects of cortisol on men. Combined with previous research, the current study sheds light on the connection between cortisol levels and thinking about an ideal partner or romantic thoughts, which may have stress-relieving effects. These results can also serve to assist future researchers in the field in methodology, as they provide evidence that survey questions that are sexual in nature will not confound T or C measures in participants. Consideration must be given when asking partner-based questions, however, particularly in women, as the current study did show an effect on cortisol responses.

Effects of Social Research Methodology on Cortisol and Testosterone

In 1970, an anonymous man spent extended periods of time on a remote island in isolation (Anonymous, 1970). He began to notice that his beard grew less in isolated conditions, but grew more during his first few days back on the mainland and the days leading up to his return. After this discovery, he questioned if there was a correlation between his beard growth and sexual activity, which occurred when he returned to the mainland. By cutting his beard clippings each day, as a marker of testosterone secretion, he brought to light the relationship between hormones (particularly testosterone) and the anticipation of sexual activity. As larger experiments confirmed Anonymous's findings, many questions involving endocrine responses and sexuality arose (Loving, Crockett, & Paxson, 2009; Rose, Gordon, & Bernstein, 1972; van Anders, Hamilton, Schmidt, & Watson, 2007). From interventions to survey methodology, many research techniques are used to measure hormonal modulation and the effects of behavior on hormones. Particularly, one aspect of social neuroendocrinology includes the effect of thoughts on endocrine responses, especially in relation to testosterone and cortisol.

Testosterone (T) is an androgen in the sex steroid family. It is derived from cholesterol and is mainly synthesized in Leydig cells in men. T is regulated by the hypothalamic-pituitary-gonadal (HPG) axis, and is stimulated by the release of gonadotropin-releasing hormone (GnRH), which triggers follicle stimulating hormone (FSH) and luteinizing hormone (LH) release from the pituitary gland. FSH and LH then stimulate the production and release of T, and are regulated by a negative feedback loop with GnRH (van Anders & Gray, 2007). T can also be released from the adrenal glands, as part of the hypothalamic-pituitary-adrenal-gonadotropic axis.

Cortisol (C) is a glucocorticoid that is produced in the adrenal gland. Like T, C is derived from cholesterol, but is regulated by the hypothalamic-pituitary-adrenal (HPA) axis instead. Synthesis begins with the release of corticotropin-releasing hormone (CRH) in the hypothalamus, which then stimulates the production of adrenocorticotropic hormone, or ACTH, in the pituitary gland. ACTH stimulates the adrenal gland, which begins cortisol synthesis and release. Similar to testosterone, C production is regulated using a negative feedback loop. Cortisol is mostly known for its increased production and release when stress occurs, though it also has been found to have connections with pair bonding among humans and animals (Reburn & Wynne-Edwards, 1999; van Anders & Gray, 2007; Young, Murphy Young, & Hammock, 2005).

Cortisol and testosterone are both factors in competition and sexual activity, both of which are critical environmental variables that affect survival and reproduction (van Anders & Watson, 2006; Williams, 1975; Wingfield, Hegner, Dufty, & Ball, 1990). One researcher used multiple studies to evaluate the associations between testosterone and male-male interactions in birds, in regards to the male-male competition for female partners (Wingfield et al., 1990). Through this research with birds, Wingfield and colleagues (1990) developed the Challenge Hypothesis. According to the Challenge Hypothesis (Wingfield et al., 1990), androgens should be high when challenges for resources, such as potential partners or territory, are present. Theoretically, when sexual activity is desired, challenge/competition would occur, thereby increasing testosterone. While these predictions are theoretical, many experiments have investigated the association between endocrine responses and sexual arousal or anticipation based on non-physical sexual stimuli and found T increases in response to social stimuli in a variety of species (Amstislavskaya & Popova, 2004; Batty, 1978; Bronson & Desjardins, 1982;

Graham & Desjardins, 1980; Roney, Lukaszewski, & Simmons, 2007; Roney, Simmons, & Lukaszewski, 2010; Rose et al., 1972; Rupp & Wallen, 2007; van Anders et al., 2007).

Research conducted in mice and rhesus monkeys uncovered associations between testosterone and exposure to potential mates. When male rhesus monkeys were placed in an environment with a receptive female rhesus, their testosterone increased two to three fold (Rose et al., 1972). A few years later, various experiments analyzing the associations between sexual stimuli and testosterone found that testosterone might mediate sexual behavior and desire in the male house mouse (Batty, 1978). Graham and researchers then discovered that it was possible to classically condition mice in order to increase their testosterone levels in anticipation of sexual activity, showing that endocrine modulation could occur without the presence of a potential partner (Graham & Desjardins, 1980). Additionally, when male mice were exposed to a female mouse through a partition, blood testosterone increased (Amstislavskaya & Popova, 2004). This suggests the effect of female presence, not necessarily female interaction, on endocrine responses. It is possible, then, that endocrine responses occur due to arousal and anticipation, rather than direct sexual activity. A study on corticosterone, the main stress response regulator in rodents, shows increases when male mice see a receptive female (Bronson & Desjardins, 1982). This research indicates that non-physical sexual stimulation is associated with acute testosterone and corticosterone increases in rodents.

Beyond non-human research, however, many studies involving humans confirm the effect of sexual stimuli on testosterone and cortisol increase in humans. When men interacted with young women whom they perceived to be attractive, both testosterone and cortisol levels increased (Ronay & von Hippel, 2010; Roney, Mahler, & Maestripieri, 2003; Roney et al., 2007). Additional research found anticipatory testosterone responses before sexual activity,

indicating the potential for endocrine responses to sexual stimuli due to anticipation (van Anders et al., 2007). Another experiment conducted asked participants to think about a sexual encounter, resulting in increased T levels from merely thinking about a sexual situation (Goldey & van Anders, in press).

In addition to sexual activity, partnering, defined as human relationships based on long-term commitment and sexual activity, is also crucial for reproduction and survival. Partnering allows for the reduction of competition, and increases in bond maintenance, or behaviors that develop intimacy with a partner (van Anders & Watson, 2006). It has been shown that men in committed relationships have lower testosterone, which may be due to decreased daily competition for sex or romantic attention (van Anders & Watson, 2006; van Anders & Watson, 2007). Lower testosterone levels when singly partnered have also been shown in women (van Anders & Watson, 2006; van Anders & Watson, 2006; van Anders & Watson, 2007; van Anders & Goldey, 2010).While these studies follow the theoretical basis that partnering decreases testosterone, other studies have suggested testosterone increases during physical partner intimacy (i.e., cuddling) (van Anders et al., 2007).

There is some evidence of acute partner-based effects on hormonal modulation. Hamilton et al. showed that women's testosterone levels increased on the days leading up to a reunion with their long-distance partner (Hamilton & Meston, 2010). This effect would suggest an increase in testosterone based on partner-based stimuli, though there may be an effect due to the anticipation of sexual activity. In addition, studies where one thinks about a relationship partner or potential partner have also been shown to increase cortisol (Loving et al., 2009). This indicates that merely thinking of a relationship partner can increase testosterone and cortisol levels. However, people involved in relationships show decreased romantic stress, defined as typical stressors in regards

to finding a partner and maintaining a relationship, which might predict a decrease in cortisol when exposed to partner-based stimuli (Nieder & Seiffge-Krenke, 2001). In addition, Nieder and colleagues (2001) identified an increased amount of dyadic discussion among couples to cope with romantic stress, which led to more affection and intimacy, further explaining decreases in cortisol due to relationships and partner intimacy. This shows a slight contradiction, as Nieder et al. has shown findings aligned with the theoretical basis, yet Loving and colleagues (2009) has shown cortisol increases (Loving et al., 2009; Nieder & Seiffge-Krenke, 2001). It is important to note that these studies focused on people already in relationships, so it is unclear how partner-based thoughts might affect hormones in general, regardless of relationship status. Due to the effect of potential or current partner-based thinking on endocrine responses, it is suggested that stimuli that engage one in a partner-based mindset will change hormones, though some evidence predicts increases in testosterone and cortisol and other evidence predicts decreases.

There is also evidence of shared neural correlates for stimuli involving stress and love, which may be important for cortisol responses to partner-based stimuli. Recent findings have suggested that the ventral tegmental area (VTA) and the dorsal striatum are two neural areas that are associated with long-term romantic love (Acevedo, Aron, Fisher, & Brown, 2011; Bartels & Zeki, 2000; Fisher, 2004). Additionally, partner-based love has been associated with activity in the middle insula, the anterior cingulated cortex, and the posterior hippocampus (Bartels & Zeki, 2000). In regards to cortisol and its effect on stress responses, one study conducted by Buchanan (2009) found that lesioning the hippocampal area eliminated psychosocial stress responses. It is possible, then, that these neural areas may be involved with responses to stressful and partner-based stimuli. Based on responses, these neural areas may provide a mechanism for up- or down-regulation of the stress response upon exposure to partner-based stimuli.

Previous research has tended to confound partner-based and sexual motivations, leading to ambiguity as to which motivation an endocrine response is linked to. A majority of related studies look at hormone levels in relation to thinking about a partner, or being in the presence of an attractive potential mate (Goldey & van Anders, in press; Goldey & van Anders, under review; Loving et al., 2009; Roney et al., 2003; Roney et al., 2007). There is little acknowledgement of the separation between partner-based motivations and sexual desire. This is important because partnering is associated with relationship formation and bond maintenance, whereas sexual desire is related to sexual activity and not necessarily the partner the activity occurs with. For instance, two people could report desiring and engaging in sexual activity each night; however, one is single and involved with new partners each night, whereas the other is in a committed relationship and looks forward to returning to their same partner each night. Due to the theories of bond maintenance and competition, the endocrine responses of these two people in response to partner-based stimuli would be expected to be very different (van Anders & Watson, 2006). The first person, who engages in sexual activity each night with various people, would have high levels of competition, corresponding with increased levels in testosterone. On the other hand, the person in a committed relationship with sexual activity with their same partner each night has low levels of competition. This is due to less competition for sexual activity, and increased bond-maintenance (to develop bonding/intimacy with a partner) behaviors (van Anders & Watson, 2006). It would be difficult to differentiate how much of the endocrine response was due to sexual desire and how much was due to bond maintenance (or lack thereof), which may have opposing effects. Due to this, consideration must be given when selecting stimuli to ensure that there is a distinguishable divide between sexual and partner-based endocrine responses.

Although there have been many new discoveries, there is little to no research on social survey methodology potentially affecting endocrine responses of testosterone and cortisol through sexual activity or partner-based thoughts. One study (LaFerla, Anderson, & Schalch, 1978) found that interviews that included topics of a sexual nature increased levels of luteinizing hormones, which is a precursor for testosterone release in men. Since sexual and partner-based thoughts can affect hormones, as shown by Goldey & van Anders (2010), questionnaire methodology could also potentially be confounding. Beyond LaFerla and colleagues' finding, however, there are no studies related to methodology and its impact on endocrine responses. This current investigation aims to identify the effect of sexual and partner-based survey methodology on endocrine modulation in humans. Participants will provide saliva samples and complete a survey in one of three conditions: sexual, neutral, or partner-based. Partner-based stimuli in survey methodology encompass any types of questions associated with relationships and romantic partners. This can include topics such as partner trust and mate preferences (Buss & Bames, 1986; J. Rempel, Holmes, & Zanna, 1985). Sexual stimuli in survey methodology involve any questions related to sexuality, sexual activity, and desire. This can include topics such as sexual activity frequency, desire for activity, and sexual openness (Janda & O'Grady, 1980; J. K. Rempel & Serafini,).

Based on previous research, it is predicted that testosterone and cortisol levels will increase when exposed to sexual survey items. Theoretically, sexual contexts are competitive, and competition has shown associations with increased testosterone and cortisol responses (Ronay & von Hippel, 2010; Roney et al., 2003; Roney et al., 2007; Rupp & Wallen, 2007; van Anders et al., 2007). As sexual stimuli, the sexual survey items may also lead to arousal, which has been shown to increase testosterone (Alexander, 1991; Carani et al., 1990; Goldey & van

Anders, in press; van Anders et al., 2007). When exposed to partner-based survey items, it is hypothesized that testosterone and cortisol levels will decrease. Though previous research does not imply testosterone decreases, the theoretical basis of decreased competition due to partnering suggests a lowering of testosterone and cortisol, which is expected (van Anders & Watson, 2006). Due to the nature of overlap between sexual and partner-based motivation, this study aims to analyze endocrine responses based on these two motivations separately. As mentioned above, the two people engaging in equivalent levels of sexual activity would show differing endocrine responses due to competitive behaviors versus bond-maintenance behaviors. It is necessary to tease apart the difference between partner-based motivations and sexual motivations, as they often intertwine, but can have very different hormonal responses.

The results from this experiment are beneficial to researchers in that they identify potential endocrine responses that occur from the methodology, rather than the manipulated variable. In addition, the separation of sexual and partner-based stimuli can provide empirical evidence that may confirm or dispute the theoretical differences between sexual activity and partnering in relation to endocrine responses. Due to the fact that the theoretical basis of both sexual stimuli and partner-based stimuli set up contrasting endocrine response predictions, the current study is necessary to clarify features of this observed response.

Method

Participants

The participants (n=136) for this experiment were 83 women (mean age= 19.36±1.86 years) and 53 men (mean age= 21.13± 6.370 years), recruited from the undergraduate psychology pool and the larger community. Participants were compensated with partial fulfillment of a course requirement or $10. Most participants identified as heterosexual (n=131), with the exception of

one female and one male who identified as bisexual, one male who identified as questioning, and two males who reported a gay sexual orientation. Participants were diverse in religious background, and the majority (n=101) lived in the United States their whole lives, though approximately 20% (n=27) had lived outside of the country at some point in their life, and 8 did not respond. Participants also answered an open-ended question where they self-identified their race/ethnicity, which was classified into five categories: Asian (n=29), Black/African-American (n=4), Bi/multiracial (n=2), Hispanic/Latino(a) (n=3), while the majority identified as Caucasian/white (n=98). Participants also self-identified their relationship status, where 81 were single, 12 identified as dating but not in a committed relationship, and 41 participants were in a committed relationship.

Materials

Questionnaires.

Background Questionnaire. Participants filled out a background questionnaire that included questions regarding their demographics. This allowed for characterization of the sample, including measures of height and weight to compute BMI (body mass index, a measure of weight adjusted for height). The background questionnaire also addressed possible confounds for hormone measures such as age, time of day, and nicotine usage.

Partner-Based Condition. To induce participants to think about romantic partners or potential romantic partners, participants in the partner-based condition completed the Relationship Trust Scale, the Mate Preferences Questionnaire, Brennan Touch Scale, and the Investment Model Scale (Brennan, Wu, & Loev, 1998; Buss & Bames, 1986; Rempel et al., 1985; Rusbult, Martz, & Agnew, 1998). Some questionnaires in their original form ask participants to discuss their current partner. In order to allow participants of all relationship

statuses to participate equally, questions were altered to ask participants to imagine their responses to these questions if their partner was the one they were planning to marry or be with forever. In this way, participants would imagine their ideal partner, which would not skew the results if the participant was not particularly pleased with their current partner at the moment. In addition, asking participants to picture future partners allowed those that identified as single to potentially induce partner-based thoughts.

Sexual Condition. Participants in the sexual-based condition also completed questionnaires in order to induce thoughts based on sexual activity or sexuality. These questions were particular items drawn from the following questionnaires: the Sexual Arousal Assessment (SAA) (Rempel & Serafini,) and the Sexual Attitudes Scale (SAS) (Hendrick & Hendrick, 1987).

Each set of questions based on condition took approximately 15 minutes for the participants to complete.

Neutral Condition. In order to control for the romantic and sexual conditions, the neutral condition contained survey items that were not intended to evoke any type of emotional response. This included questions about the day, food preferences (a Likert scale from 1-dislike very much to 7-like very much with foods like hamburgers and tomatoes), transportation speed by method, odor preference, ranking smells like gasoline and cinnamon, and geographical placement of states.

Relationships and Sexuality Questionnaire. After participants completed the background questionnaire and their condition, they filled out a relationship and sexuality questionnaire about their own personal experiences. This included questions regarding frequency of masturbation, sexual encounters, and descriptions of sexual acts engaged in. Participants also

discussed interest and enjoyment of past and present sexual encounters and relationship experiences. In addition, items involving participants' future interest in sexual encounters, flirting, and relationships were asked. This was done so that the population of participants could be analyzed based on endocrine responses and the participants' sexual experience, current relationship status, and their desire for affiliation.

Procedure

Testing was completed between 12:00 and 19:00 PM, from September 2010 to November 2010. Women were tested in all phases of their menstrual cycles due to the analyses of change scores rather than individual sampling. This is supported by previous research that shows that unless menstrual cycle is being studied in research on T, menstrual phase does not need to be accounted for (Dabbs & de La Rue, 1991; van Anders & Watson, 2006; van Anders et al., 2007). Participants were asked to refrain from eating, drinking, brushing their teeth, or smoking one hour before testing. Upon arrival, participants first completed the informed consent. Next, they were asked to produce the baseline saliva sample while answering a health and demographics questionnaire online. Upon finishing their saliva sample and questionnaire, participants completed survey items relevant to their condition. Participants were randomly assigned to the partner-based (n=45), sexual (n=49), or neutral (n=42) condition. In order to allow for delayed endocrine responses to social stimuli in saliva, participants read an emotionally neutral picture book for 15 minutes after completing their condition (Roney et al., 2007; Schultheiss & Rohde, 2002). The two picture books contained landscape photos of mountains or trees with short textual captions. Once this time had passed, participants provided a second saliva sample while they completed a questionnaire regarding their past, present, and intended future experience with relationships and sexuality. These questions were different from the condition-related questions

in that they characterized the sample's experience with relationships, sexual encounters, and sexual activity. This questionnaire was completed last so that there would be no confounding endocrine responses to these questions. Due to the fifteen minute delay for endocrine changes to reflect in saliva samples, any endocrine responses that may occur based on the final questionnaire should not affect the results.

Saliva Samples

Participants provided unstimulated saliva samples by spitting into 17-ml polystyrene tubes after rinsing their mouths with water. Saliva collection is advantageous over blood samplings, due to the fact that it is less of a biohazard, non-invasive, and less likely to trigger a stress response due to the needle poke with blood sampling. Saliva sampling is often used in behavioral research and has been validated and well-established. Salivary testosterone correlates well with free blood serum testosterone (Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004; Khan-Dawood, Choe, & Dawood, 1984; Magrini, Chiodoni, Rey, & Felber, 1986; Swinkels, Meulenberg, Ross, & Benraad, 1988), and total serum testosterone (Granger et al., 2004; Shirtcliff, Granger, & Likos, 2002). Some evidence suggests that salivary testosterone measurements may not accurately estimate the strength of testosterone-behavior associations in women, therefore larger samples of women should be used in order to alleviate this issue (Granger et al., 2004; Shirtcliff, Granger, & Likos, 2002). Salivary cortisol also has a positive association with serum cortisol (Lippi et al., 2009; Lo, Ng, Azmy, & Khalid, 1992).

Upon completion of the study, participants' samples were frozen until they were assayed. Testosterone and cortisol were assayed by radioimmunoassay at the Core Assay Facility, University of Michigan, using a commercially available kit from Siemens. The intra-assay coefficient of variation (CV) was 12.83% for low T, and 3.16% for high T. Inter-assay

CVs for T were 18.42% for low T, 10.98% for medium T, and 8.19% for high T. The inter-assay for C was 13.03% for high C, and 14.48% for low C, while the intra-assay value for C was 2.86% for high C, and 7.84% for low C.

Results

Analyses

With the exception of hormonal contraceptive users, participants (n = 7) were eliminated from subsequent analyses if they had a medical condition that affects hormones or were using medications that affect hormones (e.g. corticosteroid-containing medications) or have sexual side effects (i.e. antidepressants). PASW Statistics 18 was used for data transformation and analysis. One male participant did not complete the study, so he was excluded from data analyses. Percent change in testosterone and cortisol (%T, %C) was calculated by subtracting participants' baseline testosterone (or cortisol) from post-activity testosterone (or cortisol) and dividing this change by baseline testosterone (or cortisol) and then multiplying by 100. The primary analysis was run using multivariate ANCOVA.

Women. There were outliers on the hormone measures (at least 3 standard deviations from the mean) who were excluded from the relevant analyses. There was one outlier for T1, two for %T, three for C1 and one for C2. None of the outliers were overlapping, so seven women were excluded in total for hormone outliers.

Age, time of day, nicotine use and BMI may interact with T and/or C levels (Axelsson, Ingre, Akerstedt, & Holmback, 2005; Burger, Dudley, Cui, Dennerstein, & Hopper, 2000; Van Cauter et al., 1991; Van Cauter, Leproult, & Kupfer, 1996; Wabitsch et al., 1995); however none of these were meaningful covariates for %C (Age, F[1,67]=3.24, p=.08; BMI, F[1,67]=.01, p=.91; Time, F[1,67] =1.89, p=.17; Nicotine, F[1,67]=.01, p=.91), and only nicotine was a

significant covariate for %T (Age, F[1,67]=.001, p=.98; BMI F[1,67]=.08, p=.77; Time, F[1,67]=.02, p=.89; Nicotine, F[1,67]=7.33, p=.009). Because of this, only nicotine usage was considered as a covariate. Hormonal contraceptive use was also analyzed as a confound, but including it as a covariate did not alter the pattern of results.

Responses to condition stimuli The effects of Condition on %T and %C were analyzed using multivariate analyses with %T and %C as the dependent variables, condition as the independent variable, and nicotine as the covariate. A trend was found for a multivariate effect (F[2,67]=2.49, p=.09), with a trend for an effect of condition on %C (F[1,67]=2.50, p=.09). There was no significant effect of condition on %T (F[2,67]=.72, p=.49), therefore the multivariate effect was specific to %C.

Post-hoc tests were conducted to examine the effect of condition on %C given the initial trend. An additional trend-level association was found between the partner-based and neutral conditions, where the partner-based condition decreased cortisol levels compared to the neutral condition (p=.08). Additionally, the partner-based condition significantly decreased cortisol levels in comparison to the sexual condition (p=.03). Overall, the partner-based condition was found to decrease cortisol levels in comparison to both the neutral and sexual conditions in women.

Men. Male outliers (over 3SD from the mean) were excluded from analyses; these included two for T1, one T2, one C1, and three C2. Once again, there was no overlap, so seven men were excluded from analyses for the relevant hormones.

As noted above, BMI, time, age, and nicotine usage are potential confounds and were analyzed as potential covariates. There were no significant covariates for either %C (Age, F[1,38]=0.32, p=.58; BMI, F[1,38]= 2.67, p=0.11; Time, F[1,38]=1.22, p=.28; Nicotine,

F[1,38]=.20, *p*=.66) or %T (Age, *F*[1,38]=1.44, *p*=.24; BMI, *F*[1,38]=.10, *p*=.76, Time, *F*[1,38]=.35, *p*=.56; Nicotine, *F*[1,38]=.002, *p*=.97). Accordingly, no covariates were entered into the following analyses.

Responses to condition stimuli. The effects of condition on %T and %C were analyzed using multivariate analyses with %T and %C as the dependent variables and condition as the independent variable. There was no significant multivariate effect (*F*[2,37]=.74, *p*=.48). There were no significant effects of condition on %C (*F*[2,38]=.66, *p*=.52) or %T (*F*[2,38]=.45, *p*=.64). Hormones changes in men were similar whether they completed the sexual, partner-based, or neutral surveys.

Discussion

In the present study, the effects of partner-based and sexual stimuli on T and C were examined. We found that partner-based stimuli decreased C levels in women in comparison to sexual and neutral stimuli, and this hormone response was seen within 15-20 minutes of being asked survey questions related to their ideal partner. No effect on T was found in women, regardless of neutral, sexual, or partner-based stimuli. Additionally, there was no effect on T or C in men, no matter what condition the participants were in.

Decreased C levels when exposed to partner-based stimuli in comparison to neutral and sexual stimuli can have a variety of implications. There is evidence that physically intimate couples show decreased cortisol secretion in comparison to couples that participate in less intimacy (Ditzen, Hoppmann, & Klumb, 2008), which is in line with the current study's finding. Additionally, there is prior evidence that warm touch and neck and shoulder massages from one's partner decrease cortisol (Ditzen et al., 2007; Holt-Lunstad, Birmingham, & Light, 2008). In our study, we asked participants to envision their ideal partner, so the decrease in cortisol

found in our current study is in line with previous research indicating that marital satisfaction plays a large role in cortisol levels in women (Saxbe, Repetti, & Nishina, 2008). Thoughts of an ideal relationship may also decrease C levels due to the idea that people in relationships have reduced stress due to the fact they do not need to be finding a partner, as concluded in previous research (Nieder & Seiffge-Krenke, 2001). Along these lines, if a participant thought about an ideal, or satisfactory, partnership, then there may be a relationship between cortisol levels in women and induced thoughts of partner satisfaction with their imagined ideal partner. Adam and Gunnar (2001) also found that the level of C decline increased as relationship satisfaction increased. This current study may indicate that the participants in the current findings did imagine their ideal partner and their (theoretically) happy relationship, leading to a decrease in cortisol after the partner-based stimuli. In this way, the current study may suggest that positive partner-based stimuli, whether through thoughts or interaction, decrease C. Based on previous studies, this may be due to stress-relieving effects when one thinks about their ideal partner, or merely due to romantic thoughts. Therefore, it is likely that thinking romantic thoughts, or thinking of an ideal partnership with a high level of satisfaction, can have a stress-reducing effect, reflected in lower C levels. On the other hand, Loving and colleagues (2009) found that women who engaged in relationship-focused thinking about a current partner showed an increase in cortisol levels. This variation may be accounted for by the fact that the current study's participants spanned a variety of relationship statuses and focused their partner-based responses to their ideal partner, rather than a current partner. Another possible explanation for the cortisol increase is that the participants in the study showed an increase in cortisol levels due to sexual thoughts rather than non-sexual partner-based thoughts.

Cortisol release is controlled by the hypothalamic-pituitary-adrenal (HPA) axis, and in the adrenal gland, in particular (van Anders & Gray, 2007). Increased cortisol production mediates alarm reactions to stress. Beyond the HPA axis, there is evidence that the hippocampus plays a role in cortisol responses to psychosocial stress, as lesioning the hippocampal area eliminated the stress response (Buchanan, Tranel, & Kirschbaum, 2009). Other studies have identified other neural areas that are associated with partner-based love, including the middle insula, the anterior cingulate cortex, and the posterior hippocampus (Bartels & Zeki, 2000). Additionally, recent findings implicate the ventral tegmental area (VTA) and the dorsal striatum as neural areas involved with long-term romantic love (Acevedo et al., 2011; Bartels & Zeki, 2000; Fisher, 2004). Based on the cortisol effect seen in women in the current study, it is possible that any of the above brain areas may have been involved with inducing the thoughts that led to our decreased C response from the HPA axis.

Contrary to previous studies (Lopez, Hay, & Conklin, 2009; van Anders et al., 2007), we did not find an effect of partner-based or sexual stimuli on T in women. Recent findings suggest that sexual thoughts can elicit a rapid (within 15-20 minutes) increase in T (Goldey & van Anders, in press), or have shown a T response to social stimuli exposure in women (Hamilton & Meston, 2010; Hellhammer, Hubert, & Schurmeyer, 1985; van Anders et al., 2007), so the current experiment's lack of effect was not in line with expected findings. In men, no effects were found on T or C, though there is some previous evidence suggesting that T responses can occur within 15-20 minutes if there is sexual or social stimuli exposure (Escasa, Casey, & Gray, 2010; Hellhammer et al., 1985; Roney et al., 2003; Roney et al., 2007). On the other hand, some research indicates that T responses do not occur quickly even if there is sexual or social stimuli exposure (Lee, Jaffe, & Midgley, 1974; Stearns, Winter, & Faiman, 1973). Other studies have

shown that T responses do not rapidly occur, but can be seen within 24 hours upon exposure to social stimuli (Hirschenhauser, 2002; Kraemer et al., 1976). Therefore, the lack of effect in the current study adds to the conflicting evidence previously found with men's T responses to social stimuli. Additionally, some findings imply that cortisol responses can be seen when men are exposed to social stimuli, or interaction with a young woman (Roney et al., 2007), while another study by van der Meij and colleagues (2010) showed that C levels in men increased from baseline if the woman they interacted with was perceived to be attractive. In this way, the HPA axis was indirectly implicated for human courtship, but this finding was not translated to the male participants in our study involving questions relating to human courtship, as the partner-based stimuli were.

It is possible that the reason that the current study did not replicate previous findings of T effects in women and both T and C effects in men is due to the need for personal investment in the social stimuli. For example, previous studies involved interactions with people (Roney et al., 2003; Roney et al., 2007), or a directed exercise to spend time imagining a sexual encounter, etc (Goldey & van Anders, in press; Goldey & van Anders, under review). Our study, however, used questions regarding partners or sexual-related items to indirectly induce partner-based or sexual thoughts. It is possible that participants were able to look at survey questions more objectively, whereas other studies produced situations in which the participants were fully immersed, inducing the endocrine changes that were found. Endocrine responses may also vary based on whether participants are envisioning an actual partner or an ideal partner, which might affect their personal investment in the questions being posed.

This present study has strengths that validate the use of partner-based or sexual survey questions for future endocrine studies. As researchers create studies using an independent

variable that is meant to produce hormonal responses, the current study provides reassurance that with the exception of C responses in women to partner-based stimuli, additional sexual questions can be asked to characterize the population without interfering with hormone responses. Additionally, this study adds to the minimal research that is conducted to tease apart endocrine response differences between sexual and partner-based stimuli, showing C increases in women who are provided partner-based stimuli.

No research study is without its limitations, however. Among both men and women, high variability among T responses may have hindered the ability to see significant effects. Larger sample populations can provide the ability to more thoroughly analyze T and C effects based on partner-based, sexual, and neutral stimuli. Additionally, to ask every partner-based or sexual survey question that is ever used is a bit unreasonable, so it is possible that other questions that are sexual or partner-based in nature may have induced thoughts, and therefore would show T or C effects. With only one post-activity sample, as well, it is possible that the T or C effects would be delayed, and that these stimuli did in fact create T or C changes, but not rapidly.

There are many directions that the current study could take to account for other potential factors. In humans, Gray, Chapman and colleagues (2004) found that single men with previous relationship experience had higher testosterone levels than single men who did not have previous experience. In rats, studies conducted showed that the presence of a female increased testosterone, but those with previous sexual experience showed a larger increase than those naïve rats lacking sexual experience (Bonilla-Jaime, Vazquez-Palacios, Arteaga-Silva, & Retana-Marquez, 2006; Wu & Gore, 2009). Further analysis into participants' past sexual experience may also show additional interactions between T and C responses to partner-based and sexual stimuli.

It is also possible that there may be associations between relationship status and endocrine responses. This is because the level of relationship commitment and interest in extrapair bonding is also correlated with levels of testosterone. One study conducted by McIntyre and colleagues (2006) found interactions between one's level of commitment to a relationship, their desire for romantic activity with others, and testosterone. This contrasts empirical evidence that men in committed relationships have lower levels of testosterone (van Anders & Watson, 2007). On the other hand, it follows theoretical evidence, in that lower mating effort (and in this case, lower extrapair sexual interest) is associated with lower testosterone levels (McIntyre et al., 2006; van Anders & Watson, 2006). Therefore, relationship status and level of commitment may have an impact on the endocrine responses after exposure to sexual and partner-based stimuli.

Additionally, the survey questions may not have provided enough of a real-life scenario for the induction of thought, and therefore endocrine responses, in participants. Specifically, the sexual condition asked questions regarding sexual behavior and attitudes that included sex toys, three-person sexual encounters, role-playing during intercourse, etc. Due to the young population of participants, as was present in our current study, questions relating to the sexual behavior and attitudes may have been uncomfortable for the participants to answer. Additionally, the behaviors and attitudes mentioned may not be experiences that the participants can relate to, which could be why T and C responses were not found. The partner-based condition, however, asked questions about an ideal and theoretical partner, which may have been too abstract for participants to imagine, and therefore have a T response to. On the other hand, there was a C response among women, so it is possible that there are other factors behind the lack of T response. Further analysis in endocrine response based on participants' relationship status may provide additional insight into this association.

There are still quite a few questions that remain regarding endocrine responses to partner-based and sexual stimuli. Why does a sexual thought exercise differ from sexual-based survey questions in regards to physiology and endocrine responses? Although C responses were found, can partner-based and sexual stimuli affect other hormones such as oxytocin, estradiol, etc? Can questions that are more closely relevant to encountering an attractive man or women produce the same hormone responses as previous findings? This study can shed light on the effect of partner-based and sexual survey questions on endocrine responses, but leaves room for further research on testosterone and cortisol responses in response to varying types of partner-based and sexual social stimuli.

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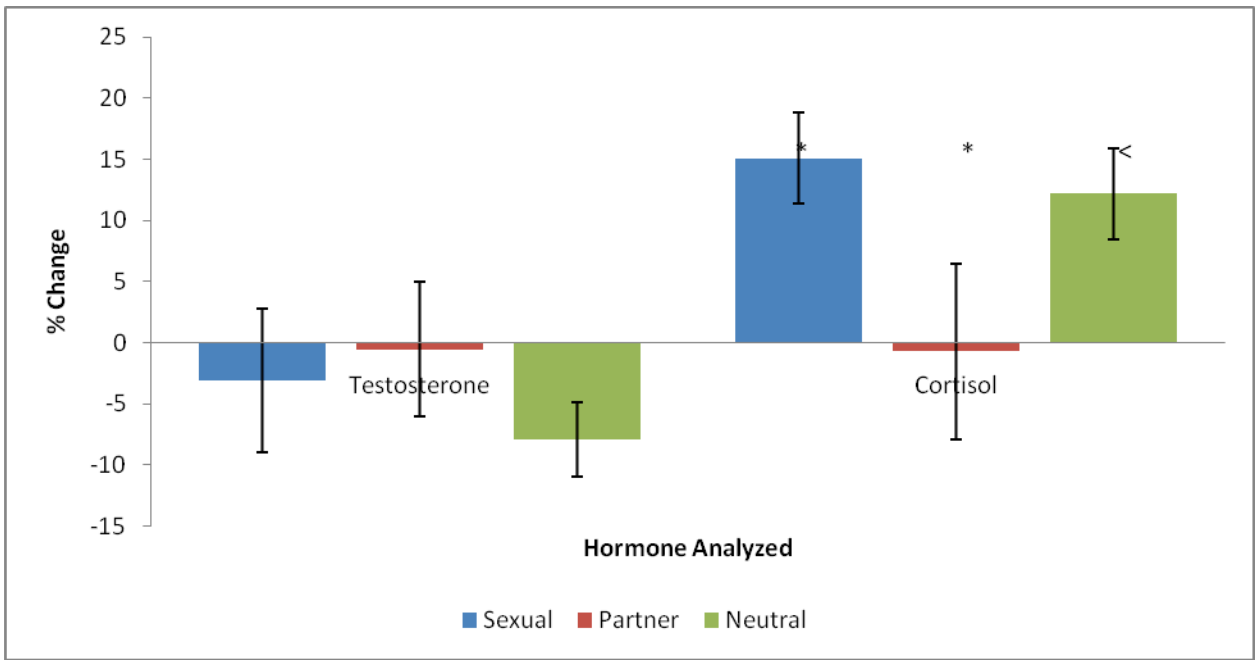

Figure 1. Mean Testosterone and Cortisol Change in Women. This figure illustrates the average change in testosterone and cortisol in women across the conditions. * indicate $p < .05$, and < indicates that $p < .10$

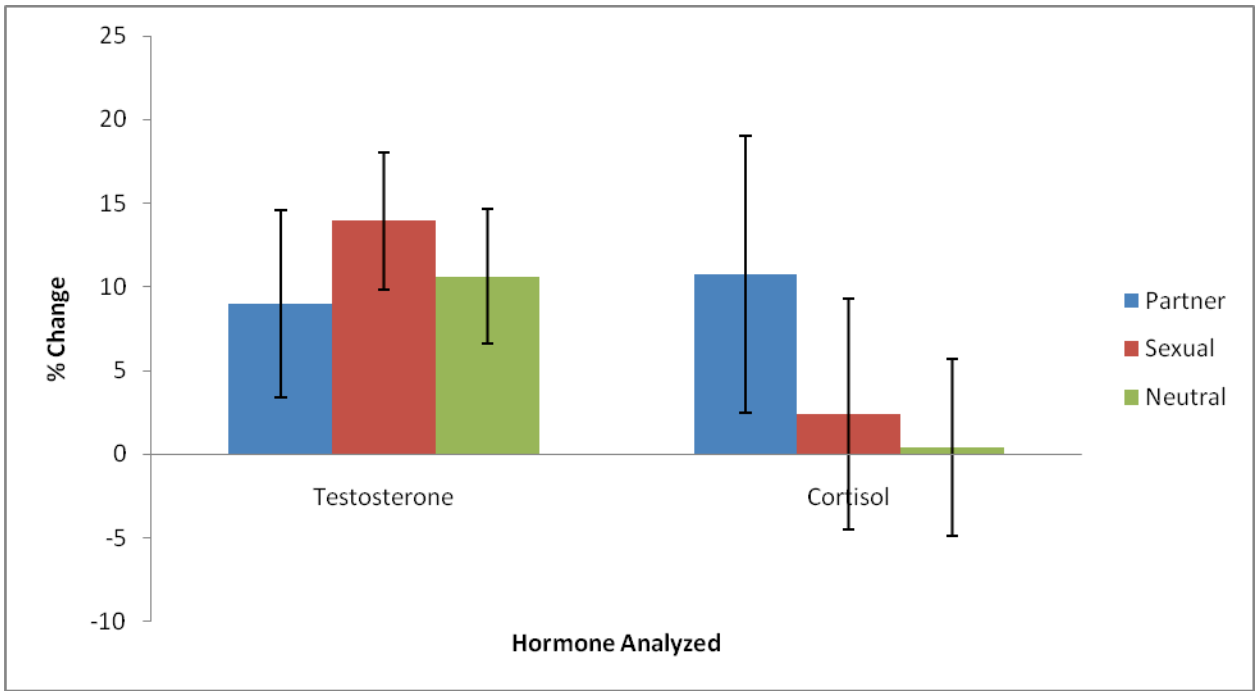

Figure 2. Mean Testosterone and Cortisol Change in Men. This figure illustrates the average change in testosterone and cortisol in men across the conditions.