CORTISOL, TESTOSTERONE, AND ALPHA-AMYLASE IN PSYCHOPATHY

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ABSTRACT

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A recently developed theory suggests that imbalances in hormone systems may contribute to psychopathy (van Honk & Schutter, 2006). Researchers have begun to emphasize the interconnectedness of hormone systems, and recommend examining multiple systems simultaneously in order to examine potential interactions. Very few studies have examined the role of hormones in psychopathy and results have been mixed, possibly due to the examination of only one hormone at a time. In a sample of 178 adults from the community demonstrating a wide range of psychopathy scores, I examine the relationship between psychopathy and two hormones and one enzyme that have been theoretically linked to psychopathy – cortisol, testosterone, and alpha-amylase. In Section 1, I focus on cortisol and testosterone – the end products of two hormonal axes that work together to maintain an appropriate balance between withdrawing in the presence of fearful or threatening stimuli, and approaching in the presence of reward – the hypothalamus-pituitary-adrenal (HPA) axis and the hypothalamus-pituitary-gonadal (HPG) axis. Psychopathy is associated with an apparent imbalance in these processes, as it is characterized by reduced fearfulness, insensitivity to punishment, reward-seeking, and aggression (Hare, 2003). Psychopathy was not associated with cortisol or testosterone measures individually, but was associated with the ratio between baseline testosterone levels and cortisol reactivity to a stressor. In Section 2, I focus on cortisol and alpha-amylase – indicators of the two primary components of the stress response system – the HPA axis and the sympathetic nervous system. Researchers have

hypothesized that deficits in this system contribute to the fearlessness and insensitivity to punishment observed in psychopathy, but the relative contribution of the two components, or how they may interact, has not been explored. Psychopathy was not associated with cortisol or alpha-amylase measures individually. However, an interaction was observed indicating that at high levels of alpha-amylase, cortisol was negatively associated with psychopathy. Overall, these results support the hypothesis that psychopathy is associated with an altered balance between highly interconnected hormone systems, and emphasize the importance of examining multiple systems simultaneously.

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INTRODUCTION

Psychopathy is a personality disorder that describes individuals who demonstrate pronounced emotional deficits, including a lack of guilt, remorse, and empathic concern for others. Psychopaths appear to lack emotional distress and are impervious to signals of distress in others. In addition, they are described as being superficially charming, manipulative, egocentric, and grandiose (Cleckley, 1941). They tend to be impulsive, risk-taking, and fail to plan for the future. They also demonstrate antisocial behavior and poor behavioral control. Individuals with psychopathy are unique in that they demonstrate an increased risk for both instrumental (i.e., predatory, goal-driven) and reactive aggression (i.e., in response to frustration or threat) (Cornell et al., 1996).

This study examines hormones in relation to psychopathy. There are several reasons why this is important. First, understanding the functioning of hormone systems in individuals with psychopathy helps us to gain a more complete picture of the biology of the disorder. This is important because it may provide clues regarding how psychopathy develops. For example, brain imaging studies have revealed structural and functional differences in regions such as the amygdala (Birbaumer et al., 2005; Glenn et al., 2009; Kiehl et al., 2001) and orbitofrontal cortex (Rilling et al., 2007; Yang et al., 2005) in psychopathic individuals. Impaired functioning in these brain regions is thought to underlie a wide range findings in psychopathy, including deficits in stress reactivity, sensitivity to punishment, autonomic functioning, fear conditioning, and decision-making (Blair, 2007). To date, the underlying causes of the impairments in brain structure and functioning in psychopathy remain unknown (Kiehl, 2006). Abnormalities in hormone systems may be a contributor to the disruption in brain structure and functioning. If so, we may be able to gain more information about how the deficits develop. For example, if psychopathy is found to be associated with abnormalities in hormones associated with the stress response system, then factors that are known to cause disruptions in this system, such as chronic

stress, could be potential contributors to psychopathy. Similarly, if levels of a hormone such as testosterone are found to be abnormal, this may indicate that prenatal factors such as maternal smoking, which affects prenatal testosterone (Rizwan et al., 2007), are involved. Insights into the environmental factors that may contribute to psychopathy can aid the development of prevention measures. In addition to environmental factors, knowing how hormones are involved in psychopathy can help to provide hypotheses for future molecular genetics studies (i.e., genetic polymorphisms associated with, for example, reduced cortisol reactivity, may be worth examining in relation to psychopathy).

Another reason why examining hormones is important is that it may aid in developing and refining treatment. Hormones influence the brain at many sites, so developing treatments that alter hormone levels is a potential way to improve brain functioning. One recent study found that a 22-week family-based psychosocial intervention increased levels of the stress hormone cortisol in preschoolers at high risk for antisocial behavior (Brotman et al., 2007), suggesting that even socially based forms of treatment can have an effect. A follow-up study found that the intervention effect on aggression was largely mediated by the intervention effect on the cortisol response (i.e., how much cortisol increased was related to how much aggression was reduced); this was only true among families that displayed lower warmth (O'Neal et al., 2010). This suggests that hormones have the potential play a large role in the development of future treatments, and that an understanding of how hormones change under different environmental conditions will also be important to understand.

Finally, because hormones are relatively easy to measure relative to brain imaging, hormones may be useful as an assessment tool for measuring an individual's biological functioning so that treatments could be designed that are tailored specifically to the individual. For example, individuals with high testosterone levels may respond best to reward- rather than punishment-based forms of learning. Such biological assessments could also be conducted widely

to identify individuals who may be at risk for psychopathy so that attempts at early intervention or enrichment could be made. Overall, understanding the functioning of hormone systems in psychopathy is important for several lines of future research.

Three systems in particular are theoretically relevant to psychopathy because they are involved in several functions that are impaired in psychopathy. The hypothalamus-pituitary-gonadal (HPG) axis has been hypothesized to be associated with psychopathy because its end product testosterone has been associated with approach-related behaviors including reward-seeking (Daitzman & Zuckerman, 1980), dominance (Archer, 2006), and aggression (Dabbs et al., 1991), all of which are features of psychopathy. The hypothalamus-pituitary-adrenal (HPA) axis, with its end product cortisol, and the sympathetic nervous system (SNS) are part of the stress response system, and are involved in potentiating the state of fear, generating sensitivity to punishment, and inducing withdrawal behavior (Schulkin et al., 1998); psychopathic individuals have deficits in these functions, suggesting that the HPA axis and/or the SNS may be hypoactive in these individuals.

Research has found that there is a large degree of interaction between the HPG and HPA axes, and also between the two components of the stress response system – the HPA axis and the SNS. Therefore, endocrinology experts have recommended examining multiple systems simultaneously in order to gain a clearer picture of how hormones may work together to predispose for a particular pattern of behavior (Bauer et al., 2002; Brown et al., 2008; Lovallo & Thomas, 2000), such as psychopathic traits; several studies have demonstrated the value of this approach (El-Sheikh et al., 2008; Gordis et al., 2006).

Only a few studies have examined hormones in psychopathy and results have been mixed. This may be, in part, due to the fact that previous studies have examined the relationship between a single hormone indicator and psychopathy, rather than exploring whether multiple hormones may interact. The study presented here examines the relationship between adult

psychopathy and the functioning of three separate biological systems and their interactions within the same context. The hormones cortisol, testosterone, and the salivary enzyme alpha-amylase, a biomarker for functioning of the SNS, were measured non-invasively via saliva samples.

Although this dissertation represents one large-scale study, I have divided it into two sections in order to provide a more focused presentation of the theoretical background and discussion of the results of different aspects of the study. The first section focuses on cortisol and testosterone – end products of the interconnected HPA and HPG axes, respectively. The second section focuses on cortisol and alpha-amylase – representing two interacting components of the stress response system. For theoretical reasons discussed in the respective sections, I hypothesized that (1) psychopathy would be associated with an increased ratio of testosterone to cortisol, and (2) individuals scoring highest in psychopathy would demonstrate reductions in both cortisol and alpha-amylase.

SECTION 1

Cortisol & Testosterone: End products of interacting systems

In this section of the study, I sought to test the main effects and interactions between cortisol and testosterone in relation to psychopathy, as well as in relation to the different aspects of psychopathy. Psychopathy has been divided into four facets roughly representing superficial charm, manipulativeness, and deceitfulness (Facet 1: Interpersonal), reduced guilt and emotional responsiveness (Facet 2: Affective), impulsivity and stimulation-seeking (Facet 3: Lifestyle), and antisocial behavior (Facet 4: Antisocial). These facets have two overarching factors (Factor 1: Interpersonal-Affective and Factor 2: Lifestyle-Antisocial). I will first review the role of the HPA axis and HPG axes and the previous research that has examined cortisol and testosterone in relation to psychopathy and related constructs.

The HPA axis is involved in potentiating the state of fear, generating sensitivity to punishment, and inducing withdrawal behavior (Schulkin et al., 1998), suggesting that this system may be hypoactive in psychopathic individuals. In antisocial groups in general, low cortisol levels have been observed in aggressive children (McBurnett et al., 2000), adolescents with conduct disorder (Pajer et al., 2001) and violent adults (Virkkunen, 1985). However, results from prior studies of psychopathy that measure cortisol are mixed. Holi et al. (2006) found reduced cortisol levels in young adult male psychopathic offenders with a history of violence. Similarly, Cima, Smeets, & Jelicic (2008) found lower average daily cortisol levels in a group of psychopathic offenders. However, others have failed to replicate this finding (van Honk et al., 2003). A study by O'Leary, Loney, & Eckel (2007) found reduced cortisol responses to a stressor in male undergraduates scoring higher in psychopathy, but no differences in pre-stressor levels of cortisol.

The HPG axis is hypothesized to be associated with psychopathy because its end product testosterone has been associated with approach-related behaviors including reward-seeking (Daitzman & Zuckerman, 1980), dominance (Archer, 2006), and aggression (Dabbs et al., 1991). Testosterone has been associated with a variety of antisocial behaviors including difficulties on the job, law breaking, marriage failures, drug use, alcohol abuse, and violent behavior (Mazur & Booth, 1998), which are commonly observed in psychopathy. However, only one study has tested the relationship between testosterone and psychopathy in adults. Stalenheim et al. (1998) found testosterone levels to be positively correlated with the impulsive and antisocial behavior aspects of psychopathy (Factor 2), but not with psychopathy as a whole. A study of youth with callous-unemotional traits, which are thought to be similar to psychopathic traits in adulthood, found no difference in testosterone levels in these youth compared to control participants (Loney et al., 2006).

A recently proposed theory suggests that the *ratio* between testosterone and cortisol may predispose to more severe forms of social aggression that include both instrumental and reactive

forms of aggression, as observed in psychopathy (Terburg et al., 2009). Terburg et al. (2009) base the ratio hypothesis on the Triple Balance Model of Emotion set forth by van Honk & Schutter (2006) which explains the role that cortisol and testosterone may play in the development of psychopathic traits. This model highlights that the HPA and HPG axes counteract each other, and that the relative activity of the two axes can significantly influence brain regions and pathways that have been implicated in psychopathy. The HPA and HPG axes are mutually inhibitory – testosterone inhibits functioning of the HPA axis at the level of the hypothalamus, whereas cortisol suppresses the activity of the HPG axis at all levels, diminishing the production of testosterone and inhibiting the action of testosterone at target tissues (Johnson et al., 1992; Tilbrook et al., 2000). Animal studies have shown that one of the primary brain regions where testosterone and cortisol have an effect is in the amygdala (Koolhass et al., 1990), a region that is consistently implicated in psychopathy (Blair, 2007). In the amygdala, cortisol is hypothesized to promote fearfulness and withdrawal behavior (Schulkin et al., 1998); testosterone has the opposite effect – it serves to promote reward-seeking and approach behavior (Daitzman & Zuckerman, 1980).

If the balance between these two hormones is changed so that there is more testosterone relative to cortisol acting on the amygdala, an individual may become less fearful and more reward seeking and aggressive (van Honk et al., 2010; van Honk & Schutter, 2006); these traits are associated with Facets 2 (Affective), 3 (Lifestyle), and 4 (Antisocial) of psychopathy, respectively. Furthermore, cortisol and testosterone affect the amount of communication between subcortical regions, such as the amygdala, and cortical regions, such as the orbitofrontal cortex. Cortisol strengthens the communication between these regions (as measured by the correlation in wave activity measured by EEG), whereas testosterone reduces it (Schutter & van Honk, 2005; van Peer et al., 2008; van Wingen et al., 2010). Increased testosterone relative to cortisol may reduce the communication between the amygdala and orbitofrontal cortex. This may mean that

there is less emotional input from the amygdala to guide decision making in the orbitofrontal cortex. This may result in the key components of Facet 2 of psychopathy – callousness, lack of empathy, and relatedly, increased instrumental aggression (Facet 4). Conversely, reduced communication between these regions may mean that the cortical regions that are important in emotion regulation and inhibition are less able to regulate input from sub-cortical regions, including impulsive, reward-seeking, and aggressive urges (i.e., related to Facets 3 and 4). In sum, a high testosterone/cortisol ratio may enhance sensitivity to reward relative to punishment, promote approach rather than avoidance reactions, and reduce the emotional input from the amygdala to the orbitofrontal cortex that is critical for empathy and recognizing cues that a decision may be risky or harmful. It may also impair the ability to regulate emotion and aggression. Researchers hypothesize that these mechanisms may predispose toward psychopathy (Terburg et al., 2009; van Honk & Schutter, 2006). Based on the mechanisms described, I hypothesized that the ratio of testosterone to cortisol would be associated with psychopathy, and that the strongest relationships would be with Facets 2, 3, and 4 of psychopathy.

Section 1: Methods

Participants

Participants were 178 adults (22 females) recruited from temporary employment agencies in the greater Los Angeles area. Because participation in an overarching study included magnetic resonance brain imaging, participants were excluded if they were under 18 or over 45 years of age; nonfluent in English; claustrophobic; or had a pacemaker, metal implants, or history of epilepsy. Participants were individually tested over two days. Prior to beginning data collection, the principal investigator obtained a certificate of confidentiality from the Secretary of Health pursuant to Section 303(a) of Public Health Act 42. Participants were informed that any

information they might provide about uninvestigated crimes could not be subpoenaed by any United States federal, state, or local court.

Psychopathy Assessment

Psychopathy was assessed using the PCL-R: 2nd Edition (Hare, 2003), which consists of a semi-structured interview and is supplemented by collateral data. The PCL-R: 2nd Edition consists of 20 items and reflects two overarching factors: interpersonal/affective characteristics (e.g., glibness/superficial charm, pathological lying, shallow affect) and antisocial behavior (e.g., impulsivity, need for stimulation/proneness to boredom, juvenile delinquency; Hare, 2003). Internal reliability (Cronbach's alpha) was .87, and the scale had good external validity in relation to Antisocial Personality Disorder symptom count ($r = .43$, $p < .001$).

The seven collateral data sources for assessing psychopathy were: (a) information gained from the Interpersonal Measure of Psychopathy (IM-P; Kosson et al., 1997), a measure designed to be completed by the interviewer which asks about the participant's psychopathic interpersonal behaviors that may have occurred during the session; the IM-P has demonstrated construct validity with the PCL-R in a prison sample, and has been validated for use with nonincarcerated samples (i.e., college students; Kosson et al., 1997); (b) self-reported theft, drug offenses, and violent crime as assessed by an adult extension (Raine et al., 2000) of the National Youth Survey self-report delinquency measure (Elliot et al., 1983); (c) official state Department of Justice criminal records; (d) professional nationwide criminal and court record database searches; (e) data derived from, and behavioral observations made during, the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I; First et al., 1996), and (f) the SCID Axis II Personality Disorders (SCID-II; First et al., 1997); and (g) independent IM-P ratings made by two different laboratory assistants during separate phases of testing provided a seventh source of collateral data.

Saliva Sample Collection

Cortisol and testosterone display diurnal rhythms with concentrations highest in the mornings and lowest in the evenings (Khan-Dawood et al., 1984). For cortisol, morning collection is optimal for detecting individual differences in resting levels, while afternoons are optimal for detecting differences in reactivity because of increased variability (Yehuda et al., 2003). The present study was designed to accommodate these factors by collecting resting hormone levels in the morning and reactivity measures in the afternoon.

Baseline. Due to the episodic secretion pattern of steroid hormones, the most reliable results come from multiple testing. As recommended (Salimetrics, 2006b), three resting saliva samples were collected in the morning of two consecutive days between 900 and 1000 h. The samples were obtained in 15-minute intervals. All six samples (three from each day) were assayed for cortisol and the values were averaged to obtain one "baseline" value; one sample from each morning was assayed for testosterone levels (as in Cohan et al., 2003) and the values were averaged to obtain a baseline value.

For each sample, participants were required to deposit 6 mL of saliva by passive drool through a short straw into separate collection vials (Granger et al., 2007). Participants were asked to abstain from exercise, smoking, eating, and consuming caffeinated beverages or alcohol for 1 hour prior to the collection of saliva in all instances. Samples were immediately frozen at -85°Celsius in a Revco upright Elite 13.4 ft^3 deep freezer.

Stressor Tasks. In the afternoon (1300 to 1800 h) on one of the testing days, five saliva samples were collected to track the hormone response to two consecutive stressor tasks. Timing of sample collection was determined based on the response curves of cortisol and alpha-amylase (alpha-amylase is discussed in Section 2). Cortisol peaks approximately 20 minutes post-stressor and requires approximately 20 minutes to return to baseline. Alpha-amylase peaks approximately 5 minutes post-task (Granger et al., 2007) and returns to baseline more quickly than cortisol. Two consecutive stressor tasks were used – one involving an uncontrollable stressor and one involving

a socially-relevant stressor. These tasks were chosen because a recent meta-analysis (Dickerson & Kemeny, 2004) found the two primary elements of psychological stressors that induce the greatest stress response are uncontrollability and social-evaluative elements (critical feedback, video recording). The study was designed to obtain an overall measure of reactivity, rather than separate analyses of the effects of each type of stressor. The following protocol for saliva collection during the stressor tasks was used and is summarized in Figure 1:

Sample 1: (baseline) obtained after an 8-minute rest period in which the participant was asked to sit still.

Stressor1: The participants performed a countdown task (uncontrollable stressor). In this task, participants were told that they would see numbers counting down from 12 to 0 on a computer monitor situated directly in front of them and that at the end of the countdown they would hear a loud, white noise through headphones. In addition to the anticipated loud noise following the countdown, there were two trials in which the noise was presented unannounced and could occur at any point during the countdown. The loud noise had a frequency of 5000 Hz, a rise and decay time of 0.5 ms, and was presented at 105 dB for a duration of 1s. There were 3 "expected countdown" and 2 "unnanounced" trials in total. The trials were presented in the following order: countdown (41 second ITI), unannounced (46 second ITI), countdown (45 second ITI), unannounced (49 second ITI), and countdown. Both the countdown and the unannounced trials lasted 12 seconds. This task lasted 6 minutes in total.

Sample 2: collected 5 minutes after the end of Stressor 1 to examine increases in alpha-amylase

Stressor 2: The second stressor was a modified version of the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), which was adapted for this sample by shortening its duration and increasing its relevance to the participants. During this task, the participant was asked to give a speech about the worst thing he/she has ever done. The participant was given two minutes to think about and prepare the speech. In the next two minutes, the participant presented the speech

about the worst thing he/she had ever done. The speech was videotaped and a research assistant remained in the room to enhance the stressfulness of the situation. If the participant had difficulty speaking continuously, the research assistant prompted him/her to elaborate and give specific examples to enhance social demands on the participant.

Sample 3: collected 5 minutes after the end of Stressor 2 to examine increase in alpha-amylase from Stressor 2, and increases in cortisol from Stressor 1 (approximately 20 minutes post-Stressor 1)

Sample 4: collected 20 minutes after Sample 3 to examine increases in cortisol from Stressor 2 and a return to baseline of alpha-amylase levels

Sample 5: collected 20 minutes after Sample 4 to examine a return to baseline of cortisol. During the time between Samples 4 and 5 participants were engaged in non-stressful tasks.

The timing between sample collection varied slightly between participants, and thus was recorded and included in analyses. Samples 1, 3, 4, and 5 were used for cortisol analyses.

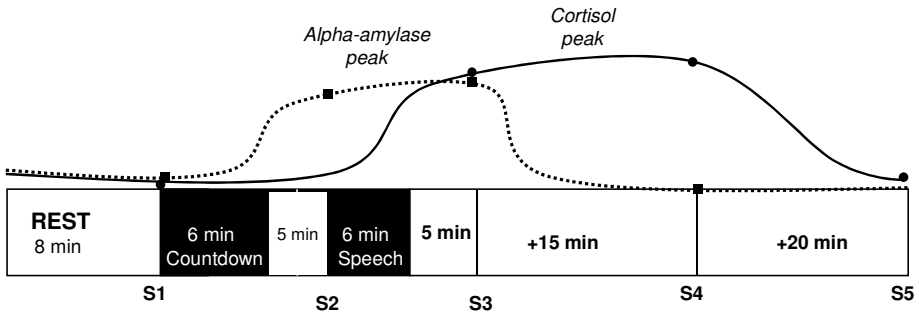

Figure 1. Time course of expected hormone responses and saliva sample collection.

Hormone Data Analysis

Saliva samples were analyzed using commercially available enzyme immunoassay kits without modification to the manufacturers recommended protocols (Salimetrics LLC; State

College, PA). Samples were assayed in duplicate and the averages of duplicate tests were used in analyses.

For cortisol, average recovery across saliva samples with known cortisol concentrations is 100.8%, and sensitivity of the cortisol kit is 0.003 μg/dL to 3.0 ug/dL. For this study, inter-assay and intra-assay precision (coefficient of variation) were less than 5.0%. For testosterone, average recovery across saliva samples of known concentrations of testosterone is 105.3%, and sensitivity of the kit is 1.0 to 600 pg/mL. Inter-assay and intra-assay precision (coefficient of variation) were, on average, less than 5.0%.

Statistical Analyses

Testosterone scores were not significantly skewed and did not require transformation. Cortisol scores were log-transformed to adjust for skewness. Subsequently, outliers defined as values more than three standard deviations from the mean group score were removed (Gordis et al., 2006). Deleted values were interpolated when sufficient remaining data were available. Five male participants were excluded from analyses involving cortisol reactivity because of insufficient remaining data after outliers were removed.

Cortisol reactivity to the stressors was measured by calculating the area under the curve (AUC) with respect to ground for the four samples (1, 3, 4, and 5) obtained during the stressor tasks (Gordis et al., 2006). The formula is given by:

$$\sum_{i=1}^{5} [t_i(m_i + m_{i+1}) / 2]$$

where t_i is the precise interval between sample i and sample i+1 (these times are specific for each subject and each interval) and m_i is the level of the hormone for sample i. This analysis resulted in one number representing a general index of cortisol reactivity for each subject. This was used in multiple regression analyses to test for associations with psychopathy scores, controlling for gender and age. The start time of the stressor session was also included as a covariate to account

for diurnal variation. Cortisol AUC has been used in the past to represent cortisol reactivity to a stressor. However, the reactivity to the stressor is overlaid on each participant's normal diurnal decline in cortisol. Thus, the area under the curve represents the combined effect of the response to the stressor, and declining cortisol levels due to the diurnal cycle. In some cases, a small response to the stressor may have been masked by the diurnal change (i.e., their slope may not have dropped as steeply as it might have had the stressor not been present, although the overall distribution appears to decline with time).

To examine the baseline testosterone/baseline cortisol ratio, distributions for baseline testosterone, baseline cortisol, and cortisol reactivity were standardized to t scores (mean 50; SD 10) and individual baseline testosterone/baseline cortisol and baseline testosterone/cortisol reactivity ratio scores were calculated, as in Hermans, Ramsey, & van Honk (2009).

Multiple regression analyses were used to examine main effects of hormone variables (baseline testosterone, baseline cortisol, cortisol reactivity, ratio scores) on total psychopathy scores, controlling for gender and start time of the stressor when necessary. In the event that a hormone variable was significantly associated with the total score, additional regression analyses were performed entering the two psychopathy factors simultaneously as predictors of the variable in order to determine if one factor of psychopathy uniquely contributed to the relationship. If one factor was a unique predictor, the two facets of that factor were then entered into the model, along with the other factor.

Multiple regression was also used to test for interactive effects between baseline cortisol and testosterone, and between baseline testosterone and cortisol reactivity. All variables were standardized prior to entry into the model.

Because the sample was primarily composed of males, and because hormone systems function differently in males and females, I also analyzed the data in the sample of males only. This analysis is presented in the Appendix. All analyses in the results sections include both males

and females. Age was significantly correlated with testosterone in the male sample, and therefore was included in regressions involving testosterone.

Section 1: Results

Descriptive statistics for the sample are provided in Table 1. Baseline levels of testosterone and cortisol are within range of previously reported studies (Brown et al., 2008). As expected, there were clear gender differences in baseline testosterone levels ($t(176) = 6.57$, $p < .001$); thus, gender was controlled for in analyses involving testosterone. There were no gender differences in baseline cortisol levels ($t(176) = 1.66$, $p = .25$). Age was not correlated with any of the hormone variables ($p > .06$) and therefore was not used as a covariate. In analyses of cortisol reactivity, the start time of the stressor session was also controlled for in order to account for variation in the diurnal cycle.

Table 1. Descriptive Information

	Mean (SD)
Age (years)	36.5 (8.8)
Psychopathy total scores	18.5 (9.0)
Baseline Cortisol (ug/dL)	.264 (.20)
Cortisol (Stressor session)	
Sample 1	.161 (.16)
Sample 3	.144 (.13)
Sample 4	.143 (.12)
Sample 5	.135 (.17)
Baseline Testosterone (pg/ml)	146.6 (65.5)

Testosterone. When controlling for gender, baseline testosterone was not significantly associated with psychopathy scores ($\beta(175) = .11$, $p = .22$).

Cortisol. In regression analyses, baseline cortisol levels were not significantly associated with total psychopathy scores ($\beta(176) = .06$, $p = .42$).

Cortisol reactivity was assessed by calculating the area under the curve for each participant (cortisol AUC). When controlling for start time, cortisol AUC was not significantly associated with psychopathy scores ($\beta(170) = -.08$, $p = .40$).

A repeated measures analysis of variance (ANOVA) indicated that there was a significant effect of time across the four samples measuring cortisol ($F(3, 169) = 12.9$, $p < .01$). Follow-up pairwise comparisons revealed that there was a significant decline in cortisol in the last three samples compared to the first (Sample 1> Samples 3, 4, and 5; all $p < .02$; no other pairwise comparisons were significant). This meant that there was not an overall increase in cortisol values in response to the stressors (i.e., at Sample 3 or Sample 4) (Figure 2).

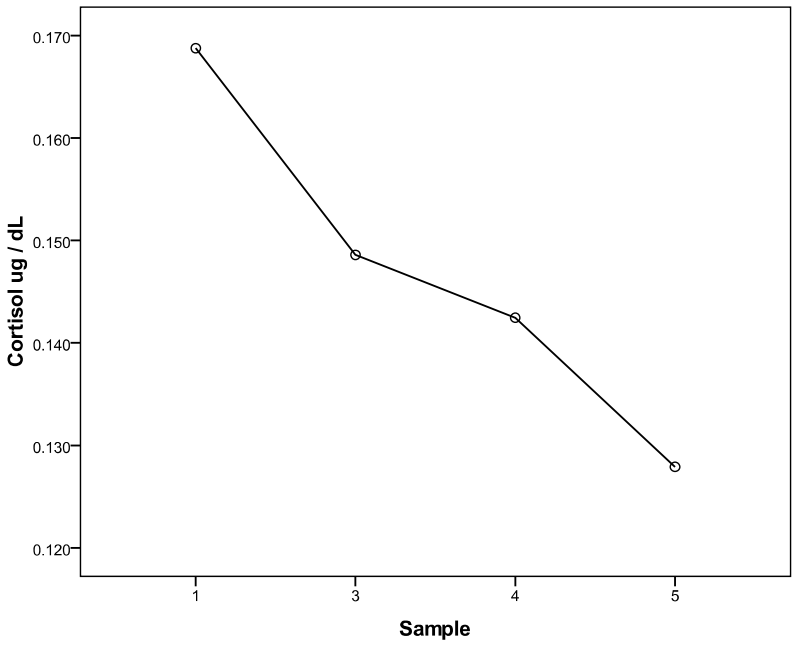

Figure 2. Average cortisol values at the four time points during the stressor.

Upon further examination, only 35% of participants showed an increase in cortisol from Sample 1 to Sample3. Of these subjects, the mean increase was 53.1%. Only 34% of participants showed an increase from Sample 1 to Sample 4. Of these subjects, the mean increase from Sample 1 to Sample 4 was 77.3%. Because not all participants demonstrated a cortisol response to the stressor task, I performed an additional analysis in which I divided the sample into groups of "Responders" and "Non-responders." Responders were defined as individuals with an increase in cortisol values of 10% or more at either Sample 3 or Sample 4, compared to Sample 1. Non-responders were defined as participants whose cortisol levels either decreased or stayed the same for Sample 3 and Sample 4. This resulted in 59 participants being classified as Responders and 77 being classified as Non-responders. Non-responders demonstrated marginally higher scores on total psychopathy ($t(129) = 1.7$, $p = .09$), as well as on Factor 2 (Lifestyle-Antisocial) ($t(129) = 1.8$, $p = .07$) and its Lifestyle Facet 3 ($t(134) = 2.0$, $p = .05$), although these did not reach significance. There was no significant difference on the remainder of the factors/facets (all $p > .11$). Furthermore, within the Responders there was no association between psychopathy scores and cortisol reactivity ($\beta(52) = \square.11$, $p = .42$).

Interactions. A multiple regression with psychopathy total scores as the dependent variable and with gender, baseline testosterone, baseline cortisol, and the interaction term (baseline testosterone × baseline cortisol) as independent variables revealed no significant interaction between baseline testosterone and cortisol levels ($\beta(174) = .23$, $p = .52$). A multiple regression with psychopathy total scores as the dependent variable and with gender, start time of the stressor, baseline testosterone, cortisol AUC, and the interaction term (baseline testosterone × cortisol AUC) as independent variables also revealed no significant interaction between baseline testosterone and cortisol AUC (β (169)= $\square.12$, $p = .23$).

Ratio. I tested the hypothesis set forth by Terburg et al. (2009) that psychopathy is associated with the *ratio* of testosterone to cortisol. After controlling for gender, psychopathy was

not significantly associated with the ratio of baseline testosterone to baseline cortisol ($\beta(175) = .06$, $p = .46$). However, psychopathy was associated with the ratio of baseline testosterone to cortisol reactivity (AUC) ($\beta(170) = .28$, $p < .01$), controlling for gender and the start time of the stressor. Controlling for the covariates, this ratio score accounted for 5% of the variance in psychopathy scores ($R^2 = .05$, $F(1, 169) = 7.1$, $p < .01$).

To determine whether one factor of psychopathy contributed uniquely to this relationship, Factors 1 and 2 were entered as predictors, along with gender and the start time of the stressor, and the ratio score (baseline testosterone/cortisol reactivity) was entered as the dependent variable. When entered simultaneously, Factor 2 (Lifestyle-Antisocial) significantly predicted ratio scores, but Factor 1 (Interpersonal-Affective) did not (Table 2). In an additional regression analysis replacing Factor 2 scores with Facets 3 (Lifestyle) and 4 (Antisocial), along with Factor 1, neither facet was a significant predictor, suggesting that the common variance between these facets is associated with the ratio score (baseline testosterone/cortisol reactivity).

Table 2. Regression Analyses Demonstrating the Association between Psychopathy Scores and the Ratio between Baseline Testosterone and Cortisol Reactivity

	Testosterone/Cortisol AUC	
	β	p
Total psychopathy scores[a]	.25*	<.01
Entered simultaneously[b]*:*		
Factor 1: Interpersonal-Affective	□.11	.41
Factor 2: Lifestyle-Antisocial	.32*	.01
Entered simultaneously[b]*:*		
Factor 1: Interpersonal-Affective	□.06	.64
Facet 3: Lifestyle	.19	.12
Facet 4: Antisocial	.11	.29

[a]Summary of estimates from multiple regression models predicting psychopathy from testosterone/cortisol AUC ratio score, gender, and start time of stressor session. Postive beta values represent higher ratio scores (higher testosterone, lower cortisol) in individuals with higher psychopathy scores.

[b]Summary of estimates from multiple regression models predicting Testosterone/Cortisol AUC from gender, start time of stressor, and psychopathy factors.

Additional analyses were conducted to determine whether the significant association between psychopathy and the ratio of baseline testosterone to cortisol reactivity was specific to psychopathy. Controlling for gender and the start time of the stressor, none of the following variables were associated with the ratio score: Antisocial Personality Disorder symptom count ($\beta(169) = .07$, p = .56), Interpersonal Measure of Psychopathy score ($\beta(169) = .07$, p = .51), or number of self-report violent offenses ($\beta(169) = .02$, p = .88).

Correlations. Zero-order correlations between psychopathy, hormone measures, and covariates appear in Table 3. Psychopathy was not significantly associated with morning-time baseline cortisol or testosterone, or the ratio between them. Nor was it associated with cortisol reactivity. However, psychopathy and its two factors were significantly associated with the baseline testosterone/cortisol reactivity ratio. In addition, Facets 2 (Affective) and 3 (Lifestyle) were correlated with the ratio.

Table 3. Intercorrelations among Psychopathy Scores, Hormone Measures, and Covariates for Section 1 (N =178)

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	Psychopathy	□												
2	F1:Interpersonal -Affective	.92**	□											
3	F2:Lifestyle-Antisocial	.94**	.75**	□										
4	Facet 1	.83**	.91**	.66**	□									
5	Facet2	.89**	.94**	.75**	.73**	□								
6	Facet 3	.85**	.68**	.90**	.59**	.69**	□							
7	Facet 4	.76**	.58**	.83**	.55**	.58**	.56**	□						
8	Baseline Testosterone	.10	.10	.08	.03	.10	.07	.00	□					
9	Baseline Cortisol	.05	.08	.03	.08	.04	-.01	-.01	.50**	□				
10	Cortisol AUC	□.07	□.03	□.10	□.04	□.05	□.11	□.11	.45**	.47**	□			
11	Ratio Testo/ Cortisol Baseline	.09	.06	.08	.01	.05	.06	.04	.51**	□.47**	□.02	□		
12	Ratio Testo/ Cortisol AUC	.21*	.19*	.20*	.10	.19*	.17*	.08	.53**	.02	□.51**	.52**	□	
13	Sex	□.09	□.18*	.01	□.09	□.10	.02	.18*	□.45**	□.09	□.05	.40	□.43**	□
14	Age	.01	.00	□.02	.01	□.01	.03	□.06	□.14	□.15	□.09	.05	□.06	□.11

Male Sample Analyses. The Appendix contains supplementary analyses restricted to the male participants only. Results largely paralleled those from the whole group analyses. Unlike the

total sample, the baseline testosterone/cortisol reactivity ratio score was not correlated with Factor 1 (Interpersonal-Affective) or either of its facets in the male sample. The ratio score was correlated with Facet 4 (Antisocial) in the male sample, as well as with Facet 3 (Lifestyle), which was also observed in the total sample.

Section 1: Discussion

In a large sample of adults, no significant relationships were observed between psychopathy and baseline testosterone or cortisol, or cortisol reactivity to a stressor. Furthermore, there were no significant interactions between these variables. Although there was no relationship between psychopathy and the ratio of baseline testosterone to cortisol, predicted by Terburg et al. (2009), there was a significant relationship between psychopathy and the ratio of baseline testosterone to cortisol reactivity. Individuals scoring higher in psychopathy had a higher ratio of baseline testosterone to cortisol reactivity; this accounted for 5% of the variance in psychopathic traits. These findings highlight the importance of a multi-system approach in hormone research.

The fact that I observed a significant relationship with the ratio score, but not with levels of the individual hormones or their interactions, may be indicative of the interconnected nature of the hormone systems. The ratio score indicates the level of testosterone relative to cortisol reactivity *within an individual*. This score could be viewed as a general index of the imbalance between the HPA and HPG axes within that individual. In contrast, the interaction term for cortisol × testosterone treats the hormones as two distinct variables, with the individual's score on each hormone being relative to the scores of the group. For example, an individual may have high testosterone (relative to the group) and low cortisol reactivity (relative to the group), but the important question seems to be 'how high is the individual's testosterone *relative to his own* cortisol reactivity?' This makes sense given the high degree of interconnectedness between the HPA and HPG axes. High HPG axis activity relative to HPA axis activity may affect the

sensitivity of brain regions such as the amygdala. Both testosterone and cortisol regulate and facilitate neuropeptide gene expression in the amygdala, and their influence on the probability of approach versus withdrawal is in opposing directions (Schulkin, 2003; Szot & Dorsa, 1994) – cortisol facilitates withdrawal and fearfulness, whereas testosterone facilitates approach and reward-seeking. Therefore, the relative contribution of each hormone is important in determining the reactivity of the amygdala to environmental stimuli. Similarly, the HPA and HPG axes act in opposite directions in their influence on the connectivity between subcortical and cortical regions. Increased levels of cortisol have been associated with enhanced functional connectivity between subcortical and cortical regions (Schutter & van Honk, 2005), whereas injections of testosterone reduce this communication (Schutter & van Honk, 2004; van Wingen et al., 2010). Therefore, the activity of these two systems relative to each other seems have a significant effect on brain systems that are relevant to psychopathy.

A high ratio of testosterone relative to cortisol reactivity may mean that amygdala functioning is driven more by testosterone than cortisol, so the individual becomes more likely to engage in approach-related or aggressive behavior, is more sensitive to reward, and is less fearful and less sensitive to cues of punishment or threat (Terburg et al., 2009; van Honk & Schutter, 2006). This may contribute to the fearlessness, reward-seeking, impulsiveness, and poor decision making observed in psychopathy. Furthermore, the decoupling between subcortical and cortical regions that results from increased testosterone relative to cortisol may have effects in two ways: 1) During decision making, emotion-related information from the amygdala that signals cues of threat, risk, or harm to others may not be able to reach cortical areas in order to inform the decision. This may result in the callousness, lack of empathy, risk-taking, and instrumental aggression observed in psychopathy. 2) Cortical regions may be less able to send inhibitory signals to subcortical regions, resulting in deficits in emotion regulation and inhibition (van Honk & Schutter, 2006), which contribute to reactive aggression and labile affect observed in

psychopathy. Thus, through these processes, a high ratio between testosterone and cortisol reactivity may contribute to a variety of psychopathic traits, including both instrumental and reactive forms of aggression.

The reason why the ratio involving cortisol *reactivity* was significant whereas the ratio involving baseline cortisol levels was not is unclear – I do not know whether this discrepancy is a result of measurement factors, or whether there is a neurobiological explanation. With regards to measurement, some researchers suggest that the degree of cortisol reactivity to a stressor is a more robust indicator than baseline cortisol of how an individual responds to cues of threat or punishment; baseline cortisol may be a less reliable and valid indicator of stress reactivity because it is influenced by a multitude of daily living factors that can affect cortisol levels (Loney et al., 2006). Stress induced changes in cortisol may provide a more precise measure of the functioning of the HPA axis and may be less susceptible to the influence of confounding factors (O'Leary et al., 2007). In the current study, the correlation between baseline cortisol and cortisol reactivity (AUC) was 0.47, suggesting that the two variables are clearly related, but that having high baseline cortisol levels does not directly translate into increased cortisol reactivity – other factors are involved in this process.

A possible neurobiological explanation is based on the idea that testosterone has more of an influence on cortisol reactivity than on baseline cortisol levels. In animals, castration and androgen replacement studies have found that androgens inhibit stress-stimulated cortisol release, but not baseline cortisol concentrations (Handa et al., 1994; Papadopoulos & Wardlaw, 2000). Similarly, in humans, testosterone decreases cortisol reactivity (as measured by area under the curve) to stress-stimulation, but not baseline cortisol levels (Rubinow et al., 2005). Therefore, the association between testosterone and cortisol reactivity may be the most relevant indicator of how the HPA and HPG axes interact. As we see in the present study, not all individuals with high testosterone levels had a high baseline testosterone to cortisol reactivity ratio, indicating that there

are individual differences in the degree to which testosterone suppresses the cortisol response. Individuals with high testosterone levels in which testosterone suppresses cortisol reactivity to a greater extent, may have the most pronounced alterations in amygdala functioning. In these individuals, the amygdala may be tuned to the testosterone-driven reward-seeking and approach-related behavior (Daitzman & Zuckerman, 1980), and much less responsive to cues of fear or threat that are facilitated by the HPA axis (Schulkin et al., 1998), which would predispose for psychopathic traits. Furthermore, the higher levels of testosterone may reduce the connectivity between the amygdala and orbitofrontal cortex, thus impairing decision making and inhibitory mechanisms (discussed above).

Analyses of the subfactors of psychopathy revealed that Factor 2 (Lifestyle-Antisocial) of psychopathy was a unique predictor of the baseline testosterone to cortisol reactivity ratio. When entered together neither of its subfactors (Lifestyle or Antisocial) were significant, suggesting that the common variance that is shared between the two factors is most associated with the ratio score. The altered imbalance between the HPA and HPG axis that may generally increase the probability of approach over withdrawal behavior and increase sensitivity to reward versus punishment (discussed above) may be associated with a latent factor that contributes to the specific features of Facets 3 and 4. Increased sensitivity to reward versus punishment, as well as an inclination toward approach behavior would likely result in the development of the more specific traits and behavior such as impulsivity, stimulation-seeking, irresponsibility (Facet 3), poor behavioral controls, and aggressive and deviant behavior (Facet 4).

Zero-order correlations also revealed a significant correlation between the baseline testosterone/cortisol reactivity ratio score and Facet 2 (Affective), suggesting that this factor may be related despite a lack of significance with the overall Interpersonal-Affective factor. This result may be potentially explained by reduced communication between the amygdala and cortical regions – if emotional input from the amygdala is unable to influence cognitive processes such as

decision making, the result may be that decision making becomes cold and calculated, and the individual is described as callous and unemotional.

Limitations of this section of the dissertation study include the fact that many participants did not respond to the stressor task. This may be due to the fact that this temporary employment agency is a high-risk sample that attracts disproportionately high numbers of antisocial individuals. Another possibility is that the resting period of 8 minutes prior to the baseline sample may not have been sufficient; prior to this rest period, electrodes were being applied to the participant for EEG assessment – a procedure that participants may have found stressful. Given that cortisol peaks approximately 20 minutes post-stressor, cortisol levels may have been elevated at baseline as well as at Sample 3 and Sample 4. A final limitation is that due to practical considerations, baseline saliva samples were acquired when participants came into the lab, rather than at waking, so one possibility is that effects may not have been detected. However, all correlations with baseline cortisol and testosterone were .1 or less, so any effects would have likely been small.

Although the present findings regarding cortisol and testosterone did not support the exact hypothesis of Terburg et al. (2009), the significant relationship between psychopathy and the testosterone/cortisol reactivity ratio provides support for the idea that the HPA and HPG axes may work in concert to predispose toward psychopathic traits, and highlights the importance of a multi-system approach. The interconnected nature of these systems may help to explain the mixed findings in previous studies examining individual hormones, as well as the lack of main effects of individual hormones in the present study. Future research will be necessary to elucidate the mechanisms of hormone action outlined by Terburg et al. (2009) and van Honk & Schutter (2006).

SECTION 2

Alpha-amylase and Cortisol: The Stress Response System

The stress response system is important to the biological understanding of psychopathy because it is involved in generating the body's responses to harmful or fearful situations, including punishment. When functioning properly, the stress response system increases the probability of *withdrawal* behavior by inducing fear and increasing sensitivity to punishment. Individuals with psychopathic traits have been described as fearless and insensitive to punishment, suggesting that the stress response system may be impaired. Without proper responses to cues of threat, individuals may be more likely to engage in risky and antisocial behavior with little fear of consequences.

The two main physiological systems of the stress response system are the HPA axis and the sympathetic nervous system (SNS). These two systems are thought to interact to maintain homeostasis and normal responding to stress (de Kloet et al., 2005). The SNS is a fast-acting system involved in regulating critical functions on a moment-to-moment basis; sympathetic responses include an increase in heart rate, skin conductance, and the release of neurotransmitters, primarily norepinephrine (NE). NE is released immediately in response to stress as part of the "fight-or-flight" response. The HPA axis, reviewed in Section 1, is involved in a second, slower-acting response that includes the release of cortisol.

Coordination of the two systems involved in the stress response occurs at several points in the brain where the SNS and HPA axis receive shared inputs and can be activated and inhibited simultaneously; one brain region where the two systems intersect is the amygdala. As discussed in Section 1, cortisol acts on the amygdala to potentiate the state of fear, generate sensitivity to punishment, and induce withdrawal behavior (Schulkin et al., 1998). Similarly, NE is an essential neurotransmitter for emotional processing in the amygdala (McGaugh, 2000); for example, when NE is blocked, the functioning of the amygdala (indicated by brain imaging) is inhibited when

individuals view highly emotional pictures, such as of mutilation or accidents (van Stegeren et al., 2005). Because both cortisol and NE have an effect on the amygdala, this is a region where the interaction of the HPA axis and SNS may affect behavior (Roozendaal et al., 2006). For example, van Stegeren et al. (2007) found that when NE is present, cortisol moderates amygdala responsivity to emotional stimuli (i.e., lower cortisol is associated with less amygdala activation). However, if NE is blocked using an adrenergic blocker, amygdala functioning is impaired regardless of cortisol levels. This suggests that NE in the amygdala is critical for cortisol to moderate amygdala functioning. Therefore, reduced functioning of SNS is also a potential contributor, in addition to reduced HPA axis functioning, to the reduced stress responsivity (and reduced amygdala activity) observed in psychopathy (Lykken, 1957; Patrick, 1994).

Previous studies have examined the functioning of the SNS in psychopathy using electrodermal and cardiovascular indicators; in general, psychopathic individuals tend to be electrodermally less responsive both when anticipating and reacting to aversive stimuli (Arnett, 1997; Hare, 1978; Lorber, 2004). However, psychopathic individuals do not show reliable differences in heart rate reactivity to aversive or stressful stimuli, or baseline level differences in heart rate or electrodermal arousal (Lorber, 2004). The reason for these discrepancies is unclear. One possibility is that other biological factors, such as the functioning of the HPA axis, may influence the relationship between SNS activity and psychopathy.

Two prior studies have examined the interaction of the SNS and HPA axis functioning in relation to aggression; both studies were conducted in children, and results were in opposite directions. El-Sheikh et al. (2008) found an interaction between baseline cortisol and alpha-amylase levels (an indicator of NE release / SNS functioning) when predicting externalizing behavior in children (aggression, impulsivity, disruptive behavior, delinquency, and noncompliance); higher baseline cortisol levels were positively associated with higher externalizing problems among children with higher SNS activity (alpha-amylase levels), as

compared to children with lower SNS activity. This supports an additive model in which high levels of HPA activity combined with high levels of SNS activity predict increased externalizing behavior. Gordis et al. (2006) also found an interaction between cortisol and alpha-amylase reactivity when predicting aggressive behavior in children; at high levels of alpha-amylase, cortisol was not related to aggression, yet at low alpha-amylase levels, low cortisol was associated with increased aggression. Thus, these results also support an additive model, but in the opposite direction. Low cortisol and low NE, when combined, may substantially increase the risk for aggression (i.e., a "double hit"). The source of the discrepancy between these two studies is likely due to heterogeneity in externalizing/aggressive samples, a topic which cannot be reviewed here. However, given the prior findings of reduced stress responsivity in psychopathy, a much more specific and homogenous category of aggressive individuals, I predicted that adult psychopathy would be associated with low levels of both SNS and HPA axis functioning.

As mentioned above, alpha-amylase is a salivary enzyme that reflects the release of NE into the blood during stress (Chrousous & Gold, 1992). Technological advances have allowed researchers to begin to implement alpha-amylase measures in biobehavioral studies, allowing for the simultaneous assessment of the HPA axis (cortisol) and SNS (alpha-amylase) functioning via non-invasive saliva samples. The novel advantage of measuring alpha-amylase is that it allows for a parallel investigation of two stress response systems (endocrine and neurotransmitter) through saliva samples, and is less invasive and stress-inducing than taking blood samples to measure NE, as salivary measures of NE do not reflect NE levels in the blood (Schwab et al., 1992). Salivary alpha-amylase levels are predictive of NE levels under a variety of stressors, including exercise, exposure to heat and cold, and psychological stressors such as written examinations (Chatterton et al., 1996).

The goal in the second section of the dissertation was to examine both systems involved in the stress response via cortisol and alpha-amylase. Because alpha-amylase is a relatively new biomarker, this study is the first to examine it in relation to psychopathy.

Section 2: Methods

The methods were the same as in Section 1 regarding the participants and psychopathy assessment. For the baseline alpha-amylase measure, all six samples (three from each day) were assayed for alpha-amylase and the values were averaged. During the stressor task, Samples 1 (baseline), 2 (5 minutes post Stressor1), 3(5 minutes post Stressor 2), and 4 (return to baseline) were used for alpha-amylase assessments.

Hormone Data Analysis

Saliva samples were assayed for alpha-amylase using commercially available kinetic reaction assay kits (Salmetrics, State College, PA), which employ a chromagnetic substrate, 2-chloro-*p*-nitrophenol, linked to maltotriose. The enzymatic action of alpha-amylase on this substrate yields 2-chloro-*p*-nitrophenol, which can be measured at 405 nm using a laboratory plate reader. The amount of alpha-amylase activity present in the sample is directly proportional to the increase, over a 2 min period, in absorbance at 405 nm.

Saliva samples (10μL) were diluted 1:200 in assay diluent. 8μL was pipetted into individual wells of a microtiter plate. 320μL of the chromagnetic amylase substrate solution, preheated to 37 C, were added to each well and rotated at 500 – 600 RPM at 37 C for 3 minutes. Optical density was read after exactly 1 and 3 minutes. Results were computed in U/mL of alpha-amylase using the formula: [Absorbance difference per minute × total assay volume (328 ml) × dilution factor (200)] / [millimolar absorptivity of 2-chloro-*p*-nitrophenol (12.9) × sample volume (.008 ml) × light path (.97)].

Average recovery across saliva samples with known alpha-amylase concentrations is 101.0%, and sensitivity of the alpha-amylase kit is 0.01 U/mL to 400 U/mL.

Statistical Analyses

Alpha-amylase scores were log-transformed to adjust for skewness. Subsequently, outliers defined as values more than three standard deviations from the mean group score were removed (Gordis et al., 2006). Deleted values were interpolated when sufficient remaining data were available.

Alpha-amylase reactivity to the stressors was measured using the same formula used for calculating cortisol reactivity (See Section 1 Methods). This resulted in one number representing a general index of alpha-amylase reactivity for each subject. This was used in multiple regression analyses to test for associations with psychopathy scores.

Section 2: Results

Means and standard deviations for the baseline and stressor task samples are as follows: baseline, 110.6 U/dL (72.9); Sample 1, 157.1 U/dL (92.9); Sample 2, 158.7 U/dL (101.6); Sample 3, 160.9 U/dL (98.4); Sample 4, 146.8 U/dL (96.6). There was no gender difference in baseline alpha-amylase levels ($t(176) = 0.138$, $p = .89$), but there was a significant difference in alpha-amylase reactivity, with males demonstrating increased reactivity to the stressor ($t(176) = 2.5$, $p = .01$). Therefore, gender was controlled for in analyses involving alpha-amylase reactivity. Age was not correlated with alpha-amylase baseline or reactivity measures.

In regression analyses, baseline alpha-amylase levels were not significantly associated with total psychopathy scores ($\beta(176) = .01$, $p = .93$). When controlling for gender, alpha-amylase reactivity (AUC) was also not associated with psychopathy scores ($\beta(175) = \square.43$, $p = .67$).

A repeated measures analysis of variance (ANOVA) indicated a significant effect of time across the four samples collected during the stressor task ($F(3,174) = 3.3$, $p = .02$) (Samples 1, 2,

3, and 4). A series of paired *t*-tests suggested that the significant effect of time was due to a decrease in the fourth sample relative to the second and third samples (Sample 2 > Sample 4, t = 2.1, p = .03; Sample 3 > Sample 4, t = 2.8, p = .01; all other pairwise comparisons were not significant). Thus, as with cortisol, there was not an overall increase in alpha-amylase values from Sample 1 in response to the stressors (i.e., Sample 2 or Sample 3) (see Figure 3). Only 31 participants showed an increase of at least 10% from Sample 1 to Sample 2 or 3. Those that showed a response did not have significantly higher psychopathy scores than those that did not ($t(176) = .18$, p = .86).

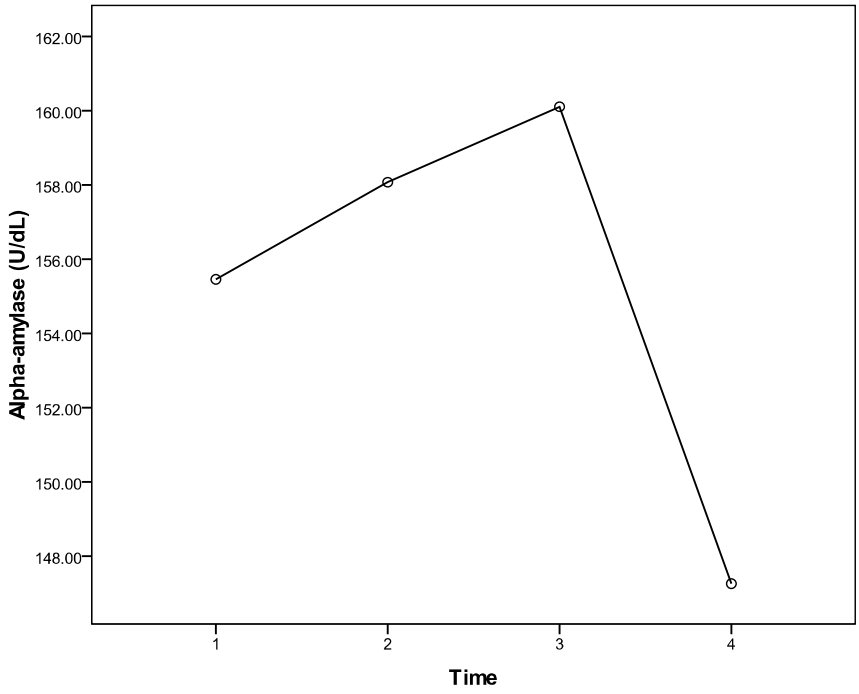

Figure 3. Average alpha-amylase values at the four time points during the stressor.

Interactions with cortisol. A multiple regression with psychopathy total scores as the dependent variable and with baseline alpha-amylase, baseline cortisol, and the interaction term (baseline alpha-amylase × baseline cortisol) as independent variables revealed no significant

interaction between baseline alpha-amylase and cortisol levels ($\beta(174) = .16$, $p = .87$). A multiple regression with psychopathy total scores as the dependent variable and with gender, baseline cortisol, alpha-amylase AUC, and the interaction term (baseline cortisol × alpha-amylase AUC) as independent variables also revealed no significant interaction ($\beta(173) = .28$, $p = .78$). Finally, a multiple regression with psychopathy total scores as the dependent variable, and with gender, start time of the stressor, cortisol AUC, alpha-amylase AUC and the interaction term (cortisol AUC × alpha-amylase AUC) as independent variables, a significant interaction was detected ($\beta(168) = \square.19$, $p = .03$). Controlling for the covariates, the interaction term accounted for 3% of the variance in psychopathy scores ($R^2 = .03$, $F(1, 169) = 4.0$, $p < .05$). The interaction remained significant when examining males only ($\beta(146) = \square.20$, $p = .04$).

To probe the significant interaction effect, the slope of the relation between cortisol AUC and psychopathy above and below the median on alpha-amylase AUC was plotted (1 SD above and below the mean resulted in insufficient sample sizes). A negative relationship was observed between cortisol AUC and psychopathy at high levels of alpha-amylase AUC ($\beta(84) = \square.28$, $p = .02$) but not at low levels of alpha-amylase ($\beta(85) = .18$, $p = .19$). As Figure 4 illustrates, psychopathy is negatively associated with cortisol reactivity when levels of alpha-amylase reactivity are high, but at low levels of alpha-amylase reactivity, the relationship with cortisol reactivity did not reach significance, although the beta value indicates a slightly positive relationship.

Correlations. Zero-order correlations between psychopathy, alpha-amylase measures, and cortisol measures appear in Table 4. Baseline cortisol levels were correlated with baseline alpha-amylase levels, and cortisol reactivity (AUC) was correlated with alpha-amylase reactivity (AUC). Psychopathy and the factors / facets were not significantly associated with baseline alpha-amylase measures or alpha-amylase reactivity.

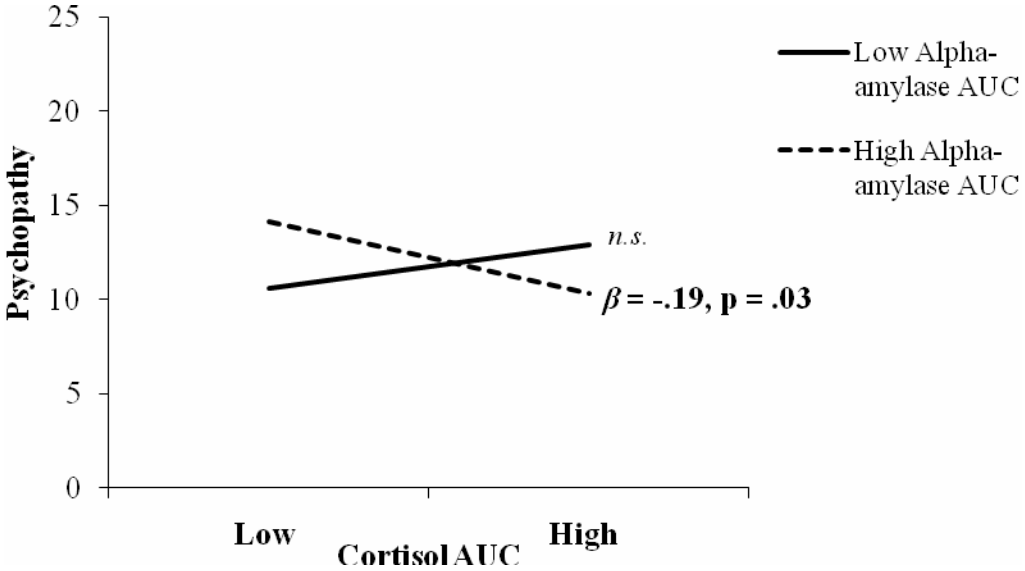

Figure 4. Relation between cortisol reactivity and psychopathy at high and low levels of alpha-amylase reactivity.

Table 4. Intercorrelations among Psychopathy Scores, Hormone Measures, and Covariates for Section 2 (N =178)

		1	2	3	4	5	6	7	8	9	10	11
1	Psychopathy	□										
2	F1:Interpersonal-Affective	.92**	□									
3	F2:Lifestyle-Antisocial	.94**	.75**	□								
4	Facet 1	.83**	.91**	.66**	□							
5	Facet2	.89**	.94**	.75**	.73**	□						
6	Facet 3	.85**	.68**	.90**	.59**	.69**	□					
7	Facet 4	.76**	.58**	.83**	.55**	.58**	.56**	□				
8	Baseline Cortisol	.05	.08	.03	.08	.04	□.01	□.01	□			
9	Baseline A-A	.01	.04	.02	.02	.03	.10	□.10	.22*	□		
10	Cortisol AUC	□.07	□.03	□.10	□.04	□.05	□.11	□.11	.47**	.08	□	
11	A-A AUC	.02	.05	□.00	.07	.01	.06	□.05	.19	.42**	.21*	□
12	Age	.01	.00	□.02	.01	.03	□.06	□.14	□.15	.00	□.09	.04

Section 2: Discussion

Although no direct relationships were observed between psychopathy and alpha-amylase baseline or reactivity levels, psychopathy was associated with an interaction between cortisol and alpha-amylase reactivity. Asymmetry between the two systems was associated with higher psychopathy scores. Specifically, at high values of alpha-amylase reactivity, the relationship between cortisol reactivity and psychopathy was significant and negative, but at low values of alpha-amylase reactivity, the relation was null.

This result was contrary to my prediction that higher psychopathy scores would be associated with low activity in both systems (i.e., a "double hit"); this is also in contrast to the findings by Gordis et al. (2006) involving aggression in adolescents. However, these results are in line with a theory proposed by Bauer et al. (2002), who suggest that asymmetry between the HPA axis and the SNS (i.e., inefficient or poor coordination) may contribute to behavioral problems (internalizing or externalizing). Bauer et al. (2002) suggest that although it is clear that the HPA axis and SNS work concurrently to generate the physiological changes associated with stress, there is evidence of differential activation and sometimes suppression between the two systems. For example, the SNS has been described as a "defense reaction" that responds more to controllable stressors and is more prominent in individuals with a personality tendency to exert high effort to obtain control. In contrast, activation of the HPA axis may be more of a "defeat reaction" – a passive response pattern characterized by emotional distress, behavioral withdrawal, and loss of control (Henry, 1992); activation of this system is especially likely to occur when situations are uncontrollable (Dickerson & Kemeny, 2004). Bauer et al. (2002) suggest that because the SNS and HPA systems can be activated in response to different situational demands and may be differentially activated depending on individuals' perception of events, there is potential for the responses of the two systems to become dissociated. As demonstrated in Figure 5, Bauer et al. (2002) proposes that optimal functioning is possible when activity of the SNS and

HPA axis are balanced; asymmetries in the two systems may increase risk for behavioral problems. This is in contrast to an "additive" model, which assumes that the HPA axis is redundant with the SNS such that symmetrical activity across these systems could result in hypoarousal (low SNS and HPA activity) or hyperarousal (high SNS and HPA activity), either of which could result in psychological problems.

One recent study has provided some support for this theory. Monteleone et al. (in press) also observed an asymmetry between the SNS and HPA axis (via cortisol and alpha-amylase measures) in patients with anorexia nervosa. In the present study, the significant interaction indicated that an asymmetry consisting of high SNS reactivity and low HPA axis activity is associated with higher levels of psychopathy.

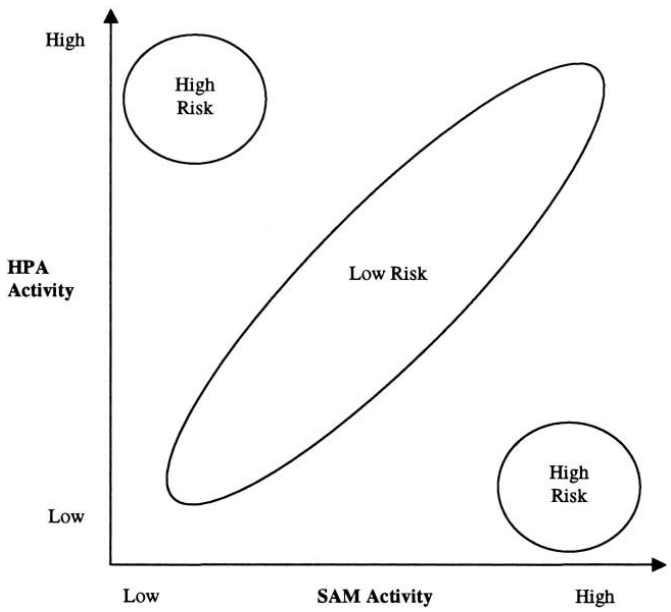

Figure 5. Model proposed by Bauer et al. (2002) representing the predicted risk for behavior problems based on asymmetry in the SNS and HPA systems. SAM (sympathetic-adrenal-medullary) is the equivalent of SNS. Reprinted from Bauer et al. (2002).

The stressor tasks that were selected in this study have elicited both alpha-amylase and cortisol responses in previous studies. Therefore, I do not think that the situational demands within the context of the study resulted in a dissociation of activation; this is supported by the fact that both baseline levels and reactivity indicators for cortisol and alpha-amylase were correlated, suggesting a coordinated stress response within individuals. Rather, the significant interaction may indicate a chronic dissociation between components in the stress response system in individuals scoring higher in psychopathic traits.

One potential source of such dissociation is chronic stress. Munck et al. (1984) found that products of the HPA axis can sometimes suppress the initial SNS activated stress response; reciprocal neural connections between the systems have been identified that allow each system to modulate the activity of the other (Stratakis & Chrousos, 1995). This suppression may occur when the individual is exposed to chronic or repeated stress, as a way of protecting the body from damage if the normal responses to stress went unchecked and could not return to a resting state (Munck et al., 1984). Such suppression may lead to a dissociation between the two systems of the stress response. Monteleone et al. (in press) suggested that the asymmetry may result from different habituation rates of the two systems to prolonged stress exposure. These interpretations should obviously be viewed as tentative, yet provide important avenues for future research examining multiple stress response systems.

The effect size of the interaction observed between cortisol and alpha-amylase reactivity was small, so additional studies are needed to verify whether an asymmetry between the SNS and HPA axis contributes to psychopathy. Limitations of this section of the dissertation study are similar to those discussed in Section 1. Although the plot of the alpha-amylase values over time suggests that there was a small increase from Time 1 to Time 2 and 3, this difference was not

significant, indicating that many participants did not respond to the stressor task. As with cortisol, this may be partly due to the carry-over effects of stress from the application of electrodes.

Another possibility, however, is that alpha-amylase is not a very reliable indicator of SNS functioning. In a recent publication, Bosch et al. (in press) argue that although initial evidence seemed compelling, there is currently no strong evidence for the use of salivary alpha-amylase as a reliable indicator of SNS functioning. There is evidence that parasympathetic activity also plays a role in alpha-amylase secretion, thereby invalidating it as an exclusive indicator of sympathetic activity. In addition to this problem, Bosch et al. (in press) argue that salivary flow rate, which is mediated by the parasympathetic system, affects alpha-amylase measurements, but this is not accounted for in most studies. Although salivary flow rate can be measured and controlled for, the problem still exists that the idea of alpha-amylase activity as a valid and reliable measure of SNS activity is too simplistic, as it can respond to a large number of contributing factors (Bosch et al., in press). Based on this argument, results of the current study should be interpreted with caution until future studies can examine these issues.

Future studies examining whether psychopathy is associated with asymmetry between the two stress response systems are necessary in order to confirm the present results. In addition to the amygdala, the HPA axis and SNS affect each other reciprocally at other brain sites, including the paraventricular nucleus and the locus coeruleus so there are several possible points of interaction. More information regarding what conditions might produce asymmetric effects in the two components of the stress response system and what the neurobiological consequences of such asymmetry are may help our understanding of how these systems may contribute to psychopathic traits.

GENERAL DISCUSSION

These findings support the contention that multi-system investigations can be more productive for understanding psychopathy compared to studies of singular physiological systems (Bauer et al., 2002; Granger et al., 2007; van Honk & Schutter, 2006). Indeed, interactions between the HPG and HPA axes, and between the SNS and HPA axis were associated with psychopathy, suggesting that the relation between each system and psychopathy depends on activity in interconnected systems. Zero order relations between psychopathy and each marker did not provide complete information about how these systems relate to psychopathy.

The finding in Section 1 of an association between psychopathy and the ratio of baseline testosterone to cortisol reactivity was more robust than the interaction observed in Section 2. The ratio score accounted for 5% of the variance in psychopathic traits. The interaction between SNS and HPA axis reactivity observed in Section 2 provides some initial support for the theory that asymmetry in the two systems may be a risk factor for behavioral problems (Bauer et al., 2002), but the effect was small. Recently raised concerns regarding the reliability of alpha-amylase as an indicator of SNS functioning raise concerns about these findings (Bosch et al., in press). Future studies should control for salivary flow rate and also examine other indicators of SNS functioning such as skin conductance reactivity.

Although the sample size in this study was large and psychopathy measures were extensive, strong conclusions cannot yet be drawn regarding the functioning of hormone systems in psychopathy. One of the most significant problems with the current study was the lack of responding to the stressor tasks in a large number of participants. Although this may partially be due to the fact that the sample contained a high proportion of antisocial individuals, in the future, pilot studies of a variety of stressors may help to identify ones that more reliably produce a response. Limitations of the study also include the fact that the sample was predominantly male, so findings cannot be generalized to psychopathic women, particularly considering the large

gender differences in hormones between males and females. Another limitation is that the study was cross-sectional, so information about how hormone systems may have changed over the course of development to result in psychopathic traits is not available. Abnormalities in hormone systems may be present from an early age and therefore affect development and socialization, or patterns of reactivity may co-develop as a function of environmental stress or other factors. Studies of hormones in children with psychopathic traits, as well as longitudinal studies examining changes across the lifespan in different environmental contexts may help to clarify these issues.

An advantage of studying hormones is that they can be assessed relatively easily via non-invasive saliva samples. If such research is further refined, hormone assessments could potentially be clinically useful in identifying response profiles of individuals in order to administer treatments that are specifically tailored to an individual's biological profile. Research on hormones may be a key element in aiding our understanding of the how brain abnormalities associated with psychopathy may arise, thus providing valuable clues regarding the origins of these deficits that may be useful targets of prevention and intervention attempts.

APPENDIX

Appendix Table 1. Descriptive Information (Males Only, n = 156)

	Mean (SD)
Age (years)	36.8 (8.6)
Psychopathy total scores	18.8 (9.0)
Baseline Cortisol (ug/dL)	.216 (.12)
Cortisol (Stressor session)	
Sample 1	.160 (.16)
Sample 3	.145 (.13)
Sample 4	.141 (.12)
Sample 5	.133 (.16)
Baseline Testosterone (pg/ml)	157.3 (62.0)

Appendix Table 2. Regression Values for Males Only (n = 156)

	Psychopathy Total Score	
	β	p
Baseline Testosterone	.10	.22[a]
Baseline Cortisol	.06	.45
Cortisol AUC	□.09	.34[b]
Ratio Testo/CortBaseline	□.04	.66[a]
Ratio Testo/Cort AUC	.30	<.01[a, b]

[a]Controlling for age

[b]Controlling for start time of stressor

Appendix Table 3. Regression Analyses Demonstrating the Association between Psychopathy Scores and the Ratio between Baseline Testosterone and Cortisol Reactivity for Males Only (n = 156)

	Testosterone/Cortisol AUC	
	β	p
Total psychopathy scores[a]	.30	<.01
Entered simultaneously[b]*:*		
Factor 1: Interpersonal-Affective	□.10	.47
Factor 2: Lifestyle-Antisocial	.41	<.01
Entered simultaneously[b]*:*		
Factor 1: Interpersonal-Affective	□.06	.66
Facet 3: Lifestyle	.25	.07
Facet 4: Antisocial	.15	.19

[a]Summary of estimates from multiple regression models predicting psychopathy scores from testosterone/cortisol AUC ratio score, age, and start time of stressor session. Postive beta values represent higher ratio scores (higher testosterone, lower cortisol) in individuals with higher psychopathy scores.

[b]Summary of estimates from multiple regression models predicting Testosterone/Cortisol AUC from age, start time of stressor, and psychopathy factors.

Appendix Table 4. Intercorrelations Among All Covariates, Predictors, and Outcome Variables (Males Only, n =156)

		1	2	3	4	5	6	7	8	9	10	11	12
1	Psychopathy	□											
2	F1:Interpersonal -Affective	.92**	□										
3	F2:Lifestyle-Antisocial	.94**	.76**	□									
4	Facet 1	.82**	.92**	.65**	□								
5	Facet2	.89**	.94**	.74**	.74**	□							
6	Facet 3	.85**	.68**	.90**	.57**	.69**	□						
7	Facet 4	.77**	.60**	.83**	.53**	.59**	.53**	□					
8	Baseline Testosterone	.07	.02	.10	□.02	.07	.09	.10	□				
9	Baseline Cortisol	.06	.08	.04	.07	.09	.06	.02	.53**	□			
10	Cortisol AUC	□.10	□.06	□.13	□.06	□.06	□.08	□.13	.52**	.49**	□		
11	Ratio Testo/ Cortisol Baseline	.03	□.04	.07	□.06	.00	.04	.11	.40**	□.54**	□.04	□	
12	Ratio Testo/ Cortisol AUC	.22*	.13	.25**	.08	.18	.23*	.20*	.41**	□.01	□.55**	.42**	□
13	Age	.00	□.03	□.01	□.02	□.02	.03	□.03	□.21*	□.16	□.13	.00	□.12

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