Comparing Stress Responses in Generalized Anxiety Disorder vs. Non-Clinical Populations: A Cortisol and Alpha-Amylase Study

A Thesis Presented for the

Master of Arts

Degree

University of Tennessee, Knoxville

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August 2013

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Dedication

This thesis is dedicated to my father for never putting a shred of doubt that I could accomplish anything I desired. He showed me how to work hard and taught me about reaping what you sew. Working with a new generation of children for the last 7 months has showed me the value of learning to work hard early in life. It sets a precedent and prepares one for challenges they face throughout their lifetime. But it's accomplishing the difficult tasks and the tasks you don't want to do that build character and work ethic. He taught me about the responsibility and pride in accomplishment. And I thank my father for instilling these qualities in me from a young age by working hard for me. This is my way of showing him what he has taught me.

Acknowledgements

I would like to show my appreciation for first to Dr. Rex Cannon and Dr. Debora Baldwin. I worked with both for several years. Although Dr. Cannon was never direct with his teaching, by forcing me to always research everything and look everything up I learned the research process. He was extremely influential in my growth as an individual and a professional. I would also like to thank Dr. Debora Baldwin, who really took me under her wing when I needed it. She has been extremely patient with me through writing a whole new thesis and working with me long distance while I have taken on this new job. I appreciate everything she has taught me and I cannot thank her enough for all the support she has given me through this abnormal and difficult situation.

I would also like to thank my thesis committee Dr. Debora Baldwin, Dr. Jake Levy and Dr. Matthew Cooper. Dr. Levy was my advisor as an undergraduate and guided me through the undergraduate process and has been providing me with great advice for the past several years. Dr. Cooper taught my two favorite classes in my college career thus far. I have a tremendous amount of respect for him and his knowledge of neuroscience. I am honored to have all three serve on my committee

And again, I would like to thank my family for everything they have done for me. They have always given me the resources I needed to succeed and have been supportive and motivational along the way. My mother has always helped me in any way she could and my sister always kept me motivated through sibling rivalry. I always wished I could have been a double major also.

Abstract

Debilitating anxiety affects 6.8 million Americans. Cortisol is an established measure of the stress response which reflects the hypothalamic-pituitary-adrenal (HPA) axis activity. However, salivary alpha-amylase (sAA) is a relatively new measure of the stress response, and it reflects the sympathetic-adrenal-medullary pathway (SAM pathway) activity. Our aim was to compare these two aspects of the stress response in a Generalized Anxiety Disorder (GAD) and a non-clinical population under a stressful stimulus (Knee replacement surgery video). To our knowledge this is the first time anyone has looked at both sAA and cortisol together with respect to GAD. We hypothesized that both cortisol and sAA levels would raise from pre-stimulus to post-stimulus, but not in concert. Forty-six college students were assessed for GADs and randomly assigned to watch a stressful or neutral video. Saliva samples were taken at the beginning of the study, immediately after the video, and 30 minutes after the video. Participants were also given the Taylor Manifest Anxiety Scale TMAS and Beck Anxiety Inventory BAI as measures of state and trait anxiety. There was a significant difference between GAD and non-clinical groups for the TMAS and a significant group by condition interaction for baseline cortisol. Our GAD, stress sub-group had a significantly raised baseline cortisol level. Although the GAD and Non-clinical groups did not differ significantly with regard to baseline cortisol levels, it was in the hypothesized direction. Moreover, baseline cortisol levels were inversely related to baseline sAA levels. The findings suggest that cortisol and sAA show contrary diurnal responses.

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Section 1: Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 1994), anxiety disorders include panic disorder, agoraphobia, post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), social anxiety disorder (social phobia), and generalized anxiety disorder (GAD). Estimates indicate that 6.8 million Americans exhibit debilitating anxiety (Kessler, Chiu, Demler, Walters, 2005). The common feature within anxiety disorders is excessive, irrational fear and avoidance of anxiety triggers. The impact of pathological anxiety extends to impaired workplace performance, hefty economic costs of up to 40 billion dollars each year (Greenberg, Sisitsky, Kessler, Finkelstein, Berndt, Davidson, 1999), greater risk of cardiovascular and cerebral vascular disease (Vogelzangs, Seldenrijk, Beekman, de Jonge, Penninx, 2010), and associations with mild cognitive impairment (Devier, Pelton, Tabert, Liu, Cuasay, Stern, Devanand, 2009). Thus, knowledge pertaining to this construct is impactful in a multitude of ways.

Anxiety has been conceptualized in many ways, consisting of several domains, making it a difficult topic to easily summarize or explain. According to Speilberger (1966), there are two forms of anxiety. The first form is *state anxiety*, which reflects a transitory emotional state or a condition that is characterized by subjective, consciously perceived feelings of tension and apprehension, and heightened autonomic nervous system activity. State anxiety is a variable state and may fluctuate. The second form is *trait anxiety* which refers to a general tendency to respond with anxiety to perceived threats in the environment, and it is a relatively stable characteristic of an individual. An individual with higher trait anxiety feels more threats in many situations than someone with low trait anxiety (Horikawa & Yagi, 2012).

Recent research has also categorized anxiety with fear as a physiological threat response. Fear is a phasic response to an immediate threat and anxiety is a prolonged worrisome state to some future threat (Walker, Toufexis, Davis, 2003). Barlow & Lehman, (1996) described anxiety as a future-oriented cognitive-affective-somatic state; the prominent feature being ‘‘a sense of uncontrollability focused on possible future threat, danger, or other upcoming, potentially negative events.’’ Paulus, Feinstein, Simmons, & Stein, (2004) propose anxiety as a manifestation of cognitions related to the self. What unites these theories is that in one way or another anxiety elicits a physical stress response.

Anxiety is the body’s natural reaction to real or perceived danger. The body then goes through a series of chemical and physical changes, called stress responses, to prepare for the danger. There are two stress responses in the body. When the body is presented with an immediate threat or danger, the sympathetic-adrenal-medullary pathway (SAM) is activated. This sympathetic nervous system pathway starts with biosynthesis in the adrenal medulla and releases several catacholomines including epinephrine, norepinephrine, and dopamine. This is commonly referred to as the fight or flight response. The second stress response pathway is the slower moving hypothalamic-pituitary-adrenal (HPA) axis. When a threat or danger is perceived, corticotrophin releasing factor (CRF) is produced in the hypothalamus and sent to the anterior pituitary gland in the brain. The pituitary gland then produces adrenocorticotropic hormone (ACTH) which is sent to the adrenal cortex where biosynthesis in the adrenal cortex produces the hormone cortisol. It allows the body to prolong the fight or flight response.

We aim to investigate possible differences between the two stress response systems in a GAD sample compared to a control sample. Throughout this thesis, we will review state vs. trait anxiety, several theories surrounding the concept of anxiety, and the endocrinology links to

stress. In the current study we will examine the neuroendocrinology of anxiety in a GAD populations compared to a normal population using salivary cortisol to test the response of the HPA axis and alpha amylase (sAA) to measure the (SAM) system under a stressful stimulus.

Trait v. State Anxiety

State and trait anxiety are commonly accepted as separate forms of anxiety. State and trait anxiety are distinctive in that state anxiety is classified as a temporary state of worry and trait anxiety is an enduring predisposition to anxiety that is consistent across situations (Spielberger, 1985). Trait and state anxiety have both been proposed to be uni-dimensional, bimodal, and multidimensional constructs (Davidson & Schwartz, 1976; Everitt, 1981; Spielberger, 1985). The uni-dimensional view is the separation of trait and state as single entities with state anxiety being the actual physical arousal to a stimulus and trait being the predisposition for arousal (Spielberger, 1985). The multidimensional view breaks state anxiety into possessing somatic and cognitive components (Davidson & Schwartz, 1976) and trait anxiety into social, ambiguous, danger, and daily routine components (Endler & Kocovski, 2001).

Trait anxiety comprises the tendency to respond anxiously to a wide variety of unspecific stressors (Spielberger, 1972). Interestingly, Wilkin, Smith, Tola, Mann, (2000) reported that low trait anxious subjects showed a greater stress response than high trait subjects. The authors explained this in several ways. First, the high trait anxious group could have higher baselines of physiological stress; thus, the presentation of the stimulus pushed them over the inverted “U” arousal curve. The authors further explained, low trait anxious individual showed a linear trend of arousal up one side of the inverted “U”. In addition, trait anxiety has been shown to be highly consistent with cognitive and somatic state anxiety ratings (Gould, Petlichkoff, Weinberg, 1984; Hanton, Mellalieu, Hall, 2002). Additionally, trait anxiety and subjective autonomic response

reports have shown a positive correlation with basal cortisol levels but not cortisol elevation to a social stress test in 20 healthy young men (Takahashi, Ikeda, Ishikawa, Kitamura, Tsukasaki, Nakama, Kameda, 2005).

Neurophysiologically, healthy subjects who scored high on trait anxiety have smaller prefrontal cortical volume (Spampinato, Wood, De Simone, Grafman, 2009) and less connectivity between the prefrontal cortex and the amygdala (Kim & Whalen, 2009). Paulus, Feinstein, Simmons, & Stein, (2004) however, found that activation of the anterior cingulated cortex and medial prefrontal cortex were significantly higher in high trait-anxiety subjects and was correlated with trait but not state anxiety. The prefrontal cortex has been shown to inhibit the HPA axis (Jahn, Fox, Abercrombie, Shelton, Oakes, Davidson, Kalin, 2010), and therefore may play a role in the dysfunction of the chemical stress response related to anxiety. Dysfunctions in the chemical stress response have been reported to correlate with generalized anxiety disorder. For example, Elevated cortisol levels have also been found in children ages 8 - 13 with anxiety disorders (McBurnett, Lahey, Frick, Risch, Loeber, Hart, Hanson, 1991) and older adults with GAD (Mantella, Butters, Amico, Mazumdar, Rollman, Begley, Reynolds, Lenze, 2008).

It is unclear in the literature how the HPA axis and SAM pathway work with respect to state or trait anxiety. However, this study will provide data in which to start differentiating between normal and pathological anxiety in relation to the HPA axis and SAM pathway.

Theories of Anxiety

Barlow's Anxious Apprehension Model

David Barlow proposed a bidirectional model of arousal and attention. He posits that negative affect causes a shift in attention to self-evaluative focus, which results in further increases of arousal and narrows attention to potential threats. This creates a constant state of

worry characterized by avoidance behaviors. He suggests that people tend to explain their arousal in terms of their negative view of the world. So as arousal rises, the more negative the world appears creating a negative feedback loop (Barlow, 1988).

Eysenck's Interversion/Extroversion

Eysenck proposed arousal on a continuum with introversion and extroversion at the polar ends. He argues that introverts are already highly aroused and aim to maintain or lower there arousal via avoidance of social situations. Extroverts are low in arousal and aim to increase their arousal level by seeking social stimulation. Eysenck proposes introversion and extraversion are located on a theoretical horizontal axis. In addition, a theoretical vertical axis contains neuroticism and stability at its polar ends. Neurotic individuals would be characterized by high autonomic function, while stable individuals would be characterized by low autonomic function (Eysenck, 1967). Grey (1982) then proposed an additional axis running from the introversion/neuroticism quartile through to the extroversion/stability quartile, which indicates types of symptomatic behavior. He believes, if an individual is too far in the introvert/neurotic quartile this leads to anxiety and too far in the extravert/stable quartile leads to impulsive behavior.

Aversive Conditioning

Recent research has focused on the aversive conditioning of an intertwined anxiety/fear network. In aversive conditioning, fear is described as a phasic response to imminent threat (Walker et al. 2003) typified by a surge of physiological arousal as in an alarm reaction (flight/fight) of the autonomic nervous system (Blanchard Sakai, McEwen, Weiss, Blanchard, 1993). Alternatively, anxiety is a sustained response to temporarily uncertain danger (Walker et al., 2003), which may be distal and distinguished via heightened apprehension and vigilance

(Blanchard et al., 1993). Behaviorally, anxiety is associated with avoidance and increased overall sensory sensitivity (Baas, Nugent, Lissek, Pine, Grillon, 2004; Cornwell, Baas, Johnson, Holroyd, Carver, Lissek, Grillion, 2007) commonly derived from aversive conditioning.

Aversive conditioning occurs when a discrete, conditioned stimulus (CS), such as a light, is paired with an aversive unconditioned stimulus (e.g., a shock). Fear will develop to the discrete cue and to the environmental context (LeDoux, 1996). For example, the repeated pairing of a rabbit with an electric shock may cause the rabbit to become an aversive stimulus for that individual.

Eventually, this notion led to an unfolding of several other theories. The Preparedness Theory (Seligman, 1971) results from Pavlovian conditioning, but postulates that some stimuli are naturally dispositioned for aversive conditioning. These neutral stimuli are graded along a continuum of biological predispositions for a fear condition. These predispositions extend from a pre-technological age that may include commonly feared stimuli such as snakes or spiders. Within this theory, these commonly feared stimuli should be conditioned more rapidly, be more resistant to extinction, and be more resistant to cognitive influences. The alternative to the Preparedness Theory is Equipotentiality Hypothesis, which assumes that all stimuli have equal potential to be fear eliciting. The equipotentiality theory was confirmed by McNally & Reiss (1982) when they used both snakes and flowers as unconditioned stimuli. They found that both predispositioned stimuli, such as a snake, and neutral stimuli, such as a flower, have potential for aversive conditioning.

Multi-Network Dysfunction

Sylvester, Corbetta, Raichle, Rodebaugh, Schlagger, Sheline, Zorumski, Lenze, (2012) reject the notion of a single circuit, such as the fear/anxiety circuit. Contrarily, they propose an

individual's fear or anxiety behavior may be the product of several dysregulated circuits. This theory is based on the idea that the study of functional networks will revolutionize our perspectives of psychological disorders. Findings suggest that psychological disorders may merely be disruptions in differential combinations of networks. Their research reveals for anxiety, dysregulation lies in the overactive cingulo-opercular and ventral attention network and underactive default mode in the frontal parietal network. The cingulo-operular network includes the dorsal AC, anterior thalamus, anterior PFC and the insula; it is important for cognitive control of error and conflict detection (Sylvester et al., 2012). Over activity in this network may establish rumination involving errors or conflict. The ventral attention system includes the ventrolateral PFC and temporal parietal junction and is involved in directing attention to newly appearing stimuli (Sylvester et al., 2012). Excessive activity of this network may cause an individual to focus on unnecessary stimuli.

Anxiety Sensitivity

The anxiety sensitivity construct has a similar concept to trait anxiety, with the distinguishing feature being "fear of fear" (Reiss & McNally, 1985). It is described as "an individual difference variable consisting of beliefs about the experience of anxiety/fear which causes illness, embarrassment or additional anxiety." For example, when the doctor tells an individual to avoid excitement in order to minimize the risk of a heart attack, the advice should increase the patients' motivation to avoid exciting stimuli. However, this advice increases the patients anxiety without experiencing a heart attack themselves. In fact, one criterion for panic disorder is fear of having another panic attack (DSM-IV, 1994). This possibly contributes further to anxiety disorders. It has been shown that anxiety sensitivity, as measured by the Anxiety

Sensitivity Index (ASI), is higher in patients with anxiety disorders and extremely higher in individuals with agoraphobia (Reiss and McNally, 1986).

Attentional Control Theory

A meta-analysis by Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, van IJzendoorn (2007) demonstrates that anxious individuals display an attentional bias towards threatening sources of information, and this effect is less consistent or typically not observed in non-anxious individuals. Eysenck and colleagues (Derakshan and Eysenck, 2009; Eysenck, Derakshan, Santos, Calvo, 2007) propose attentional control theory (ACT). This theory states that individual differences in trait anxiety are embedded in higher order cognitive function. High anxious individuals have an inclination to suppress their behavior in risk taking conditions. However, behavioral suppression is compensated by increased attention to external stimuli. Therefore anxiety is a bias toward the stimulus driven attention system over a goal directed attention system making task irrelevant stimuli become more intrusive than it does in low trait anxious individuals. Persons with high anxiety try to control their environment, as much as possible. Moreover, their attention to the environment and their own behavior is constantly maintained at a higher level. This attentional deficit requires them to recruit additional cognitive resources using more neuronal power than should be necessary; thus, leaving fewer resources for other cognitive processes. Neurophysiology of attentional control includes the lateral prefrontal cortex and the anterior cingulate cortex, particularly in the allocation of attentional resources and executive function (Miller & Cohen, 2001). These findings suggest that anxiety interferes with recruitment of prefrontal regions required for attentional control.

Negativity Bias

Likewise, in cognitive psychology, preferential attention to negatively valenced stimuli is interpreted as negativity bias (Ito, Larsen, Smith, Cacioppo, 1998). Excessive arousal due to trauma or stress may result in permanent brain changes that exacerbate a bias towards the expectation of threat. Beck (1976) highlighted how negativity biases are central to the development and maintenance of depression and anxiety. Biases towards fear stimuli in depression and anxiety have been observed in cognitive tasks that manipulate both nonconscious and conscious processing (Bradley Mogg, Millar, White, 1995). Anxious (GAD) and normal participants carried out a modified Stroop color-naming task with anxiety- and depression-related words. Compared with controls, the GAD subgroup showed slower color naming for negative than neutral words. In addition, within the anxious group, patients with GAD without concurrent depression showed more color-naming interference for anxiety words than neutral words than participants with comorbid GAD and depression. The neurocircuitry of negativity bias may be associated with anticipation of a fear cue (Carretié, Mercado, Hinojosa, Jose, Martin-Loeches, Sotillo, 2004). This unconscious processing of anxiety produces hyperactivation in the amygdala, mPFC and AC (Williams & Gordon, 2007).

Anxiety is an interesting construct that has derived many theories regarding its functionality and physiology. Regardless of the theoretical background used to explain anxiety, millions of people suffer from this disorder. Illumination on the physiological response to a given stressor may facilitate better understanding and lead to more effective treatments for individuals who suffer with GAD in particular.

Generalized Anxiety Disorder

Generalized anxiety disorder is characterized by excessive diffuse anxiety and worry that is difficult to control and debilitating to daily life (American Psychology Association, 2000). GAD's listed features in the DSM-IV are muscle tension, trembling, twitching, feeling shaky, and muscle aches/soreness. It also includes hypervigilance and hyper arousal comprising of an accelerated heart rate, shortness of breath and dizziness. Somatic symptoms such as sweating, nausea, diarrhea, and exaggerated startle response characterize it as well. GAD has a prevalence rate of 3% in one year and 5% in the lifetime. It frequently occurs with other mood disorders, anxiety disorders, and substance abuse disorders (APA, 2000). Different cultures seem to express GAD differently; somatic symptoms are more of a focus in some, while cognitive symptoms are weighted more heavily in others (APA, 2000).

A reduction of cerebral blood flow (CBF) has been found to be a customary characteristic of GAD (Mathews & Wilson, 1987; Nutt, 2001). In negative emotional processing, greater amygdala and insula activity was found in GAD compared to controls (Etkin & Wager 2007). However, some studies have not found exaggerated amygdala response in GAD (Blair, 2008). With regard to prefrontal activity, Monk, Nelson, McClure, Mogg, Bradley, Leibenluft, Blair, Chen, Charney, Ernst, Pine, (2006) found greater fMRI BOLD responses in the right ventrolateral prefrontal cortex to emotionally adverse stimuli in GAD compared to healthy controls. Many of these regions have been implicated in control of the HPA axis.

Stress Response Systems:

Stress responses are related to behavior, cognition, and psychopathology (Fortunato, Dribin, Granger, Bus, 2008). Both stress responses evoke a chain of neuroendocrine reactions. A variety of stressful events cause an increase in epinephrine in several brain regions including the

hypothalamus, amygdala, and locus coeruleus in a rat model (Tanaka, Yoshida, Emoto, Ishii, 2000). Another neurotransmitter, serotonin has been shown to be involved in the HPA axis (Hanley & Van de Karr, 2003). But the most predictable anxiolytic effects seem to be linked to the GABAergic system, specifically in the benzodiazepine receptors (Bailey & Nutt, 2008; Hoehn-Saric, 1982). The autonomic nervous system has been shown to play a role in stress related disorders such as depression and anxiety (Careny, Freedland, Weith, 2007; Guinjoan, Bernabo, Cardinali, 1995; Vaith, Lewis, Linares, Barnes, Raskind, Villacres, Murburg, Ashleigh, Castillo, Peskind, 1994; van Veen et al., 2008).

Two of the main physiological systems activated by stress are the SAM pathway (which is the short term "flight or flight" indicator of stress) and the HPA axis (which is more of a long term hormonal measure of stress). The SAM pathway starts with the hypothalamus stimulating the adrenal medulla. This begins the autonomic nervous system response by secreting the hormone adrenaline. Adrenaline stimulates the sympathetic nervous system (SNS) "fight or flight" response which includes decreased digestion, increased sweating blood pressure, and innervates the salivary glands. The SAM pathway and the HPA axis, together, play a role in homeostasis through catecholamine and glucocorticoid interaction (Engert, Efanov, Duchesne, Corbo, Pruessner, 2011).

SAM Pathway, Alpha-Amylase, and Stress

Under activation of the SNS and the SAM pathway, alpha-amylase (alpha-1, 4-alpha-D-glucan 4-glucanohydrolase) is one of the major protein components produced in saliva, accounting for 40-50% of gland produced protein (Zakowski & Bruns, 1985). It is produced via alpha and beta adrenergic mechanisms (Nater & Rohleder, 2009) in the epithelial acinar cells of the exocrine salivary glands (Baum, 1993). Its main function is the enzymatic digestion of

carbohydrates (Baum, 1993), but it is also important for mucosal immunity in the oral cavity, as it inhibits the adherence and growth of bacteria (Rohleder & Nater, 2009). Because sAA is produced with the saliva, it may be a better salivary marker of stress compared to cortisol. It is an active measurement, as opposed to cortisol which is passively transported by saliva (Baum, 1993).

Recently, the use of alpha-amylase (sAA) as an indicator of stress has become widely popular (Nater, Rohleder, Gaab, 2005). It is sensitive to physical and psycholological stress (DeCaro, 2008; Granger, Kivlighan, el-Sheikh, Gordis, Stroud, 2007; Nater & Rohleder, 2009; Strahler, Berndt, Kirschbaum, Rohleder, 2010). In addition, it has been shown to be an accurate indicator of autonomic function (Ehlert, Erni, Hebisch, Nater, 2006) and has been shown to correlate with norepinephrine in the blood under exercise and psychosocial stress (Chatterton, Vogelsong, Lu, Ellman, Hudgens, 1996). Moreover, it has been shown to be suppressed by beta-adrenorecepter blockade (van Stergen, Rohleder, Everaerd, Wolf, 2006). It tends to be elevated in pathological populations including generalized social anxiety disorder (van Veen et al., 2008), schizophrenia (Inagaki et al., 2010), and borderline personality disorder (Nater Chrousos, Kino, 2010).

In a study of the genetics of sAA, correlations were larger for monozygotic than dizygotic twins, although both groups had large correlations. Out et al. (2011) concluded that there is evidence for one common genetic factor that accounted for 51% of the variance of sAA levels at baseline and between 56%and 62% during a stressful baby cry paradigm. It has been shown that age has no effect of sAA levels (Aguirre, Levine, Cohen, Tabak, 1987; Salvolini, Mazzanti, Martarelli, Di Gorgio, Fratto, Curatola, 1999), but more recent evidence has suggested that basal sAA levels increase with age (Strahler, Berndt, Kirschbaum, Rohleder, 2010).

Almost every physiological system has some sort of circadian rhythm. Disruptions in this biological rhythm have been associated with several psychological and physical disorders including renal disease (Kock et al., 2009), depression (Tan et al., 2007), and post traumatic stress disorder (Wessa et al., 2006). Both Alpha amylase (sAA) and cortisol follow diurnal rhythms. The diurnal pattern of sAA activity decreases sixty-minutes after waking and increases at throughout the day (Natar, Rohleder, Schlotz, Ehlert, Kirschbaum, 2007) while cortisol is highest in the morning then decreases throughout the day. sAA responds faster to acute challenges and has a lower activation threshold than cortisol (Gordis et al., 2006). Few studies have compared the differences between sAA and cortisol levels as a function of pathological anxiety. We examined both (diagnostic classification and pathways) in this study.

HPA Axis and Stress

Secretion of cortisol, through the HPA axis, is a necessary physiological response to emotional and physical stress. It promotes survival in life threatening situations. Salivary cortisol levels are highly positively correlated with serum levels of cortisol ($r>.9$) (Umeda, Hiramatsu, Iwaoka, Shimada, Miura, & Sato, 1981). Salivary cortisol is actually advantageous to serum cortisol due to the fact that only free hormone fraction will be determined, removing the buffer of other influences such as binding proteins (Foley & Kirschbaum, 2010; Follenius & Brandenberger, 1986). Moreover, it is a non-invasive measure, which reduces potential "carry-over" effects that may be associated with more invasive methods (plasma cortisol).

Stimulation of the paraventricular nucleus of the hypothalamus results in activation of the HPA axis producing ACTH from the pituitary gland. ACTH stimulates the adrenal cortex and produces cortisol, which enables the body to maintain steady supplies of blood sugar. Adequate and steady blood sugar levels help a person cope with prolonged exposure to a stressor

by ensuring it has enough energy to meet the demand and help the body to return to homeostasis. It takes about 30 minutes to peak, although it shows vast variation in latency to peak within people (Foley & Kirschbaum, 2010). The PFC, hippocampus, and parahippocampal gyrus have been implicated in the modulation of the HPA axis function by acting as a site for glucocorticoids to exert negative feedback (Diorio, Viau, & Meaney, 1993; Hurley-Guis & Neafsey, 1986; Jahn et al., 2010; Pruessner, Dedovic, Pruessner, Lord, Buss, Collins, Dagher, Lupien, 2010; Sapolsky, Romero, Munck,2000).

Chronic psychological distress can lead to hyperactivity of the HPA axis (Baldwin, Cannon, Fischer, Kivisto, 2008; Mantella et al., 2008; O'Brien, Lloyd, McKeith, Gholkar, Ferrier, 2006) and increase cortisol activity at these sites. This can cause damage to the hippocampal neurons (Sapolsky et al., 2000), which results in deleterious effects on memory and executive functions (Bremner, Vythilingam, Vermetten, Anderson, Newcomer, Charney, 2004; Li, Cherrier, Tsuang, Petrie, Colasurdo, Craft, Schellenburg, Peskind, Raskind, Wilkinson, 2006; Lupien, Lecours, Lussier, Schwartz, Nair, Meaney, 1994; Mantella et al., 2008). Additionally, aging alters the basal cortisol rhythmicity and possibly diminishes the ability to regulate the HPA axis. (Van Cauter, Leproult, Kupfer, 1996; Lupien et al., 1994).

A relationship has also been found between anxiety and cortisol levels. For example, Vreeburg, Zitman, van Pelt, Derijk, Verhagen, van Dyke, Hoogendijk, Smith, & Penninx (2010) conducted a cortisol investigation in populations with and without anxiety disorders. They found a significant positive association with all anxiety disorders and morning cortisol. This included panic disorder, agoraphobia, major depressive disorder, and generalized anxiety disorder. However the largest contributors to the correlation were panic disorder with agoraphobia and anxiety disorders comorbid with depression. However, Vedhara, Miles, Bennett, Plummer,

Tallon, Brooks, Gale, Munnoch, Schreiber-Kounine, Fowler, Lightman, Sammon, Rayter, & Farndon (2003) did not find a significant correlation between stress, anxiety and absolute cortisol levels in 54 women attending a diagnostic breast clinic. However they did find a non-linear relation between time of day and cortisol levels. More specifically, cortisol levels decreased as the day progressed. Finally, studies show that cortisol levels often do not correlate with alpha amylase during stress (Chatterton et al., 1996; Nater & Rohleder, 2009; Schenkels, Veerman, Nieuw Amerongen, 2005, Strahler et al., 2010) reaffirming their activity due to two different stress systems.

Objectives:

van Veen et al. (2008) examined 43 general social anxiety disorder participants regarding HPA axis and ANS functioning. They found elevated levels of basal sAA and diurnal sAA, but not cortisol in participants with generalized social anxiety. Similarly, Takai et al. (2004) examined the effects of a psychosocial stressor and soother on salivary cortisol and sAA levels in young adults and compared them to the trait version of the State-Trait Anxiety Inventory (STAI). The participants provided samples every three minutes throughout a stressing and/or soothing video. sAA levels rose at the start of the stressor and fell to baseline at the end. Cortisol levels rose to a lesser extent and remained elevated for a longer period of time than the sAA. Also, sAA levels, but not cortisol levels, correlated with the trait version of the STAI. The question remains whether there are differences in resting and response conditions in the SAM pathway and HPA axis activity in generalized anxiety compared to non-clinical populations.

To our knowledge, we are the first to examine sAA and cortisol response in a GAD population in the same study. The objective of the current study is to examine the neuroendocrine measures of stress in non clinical populations compared to a generalized anxiety

disorder population using cortisol as a measure of the HPA axis and sAA as a measure of the SNS. We will evoke stress in the participants by using a six-minute knee replacement surgery video clip. The endocrine measures will then be compared with the Beck Anxiety Inventory (BAI), a diagnostic inventory measuring state anxiety, and the Taylor Manifest Anxiety Scale (TMAS), a scale measuring trait anxiety.

Hypothesis

- We hypothesize that the GAD group will have greater basal cortisol than the non-clinical group, but there will be no differences between groups for basal sAA levels.
- We hypothesized that baseline cortisol and sAA will be positively related irrespective of group.
- We hypothesized that there will be a greater increase in cortisol and sAA levels from pretest to posttest stimulus in the clinical group compared to the normal population.
- We hypothesized that high trait anxiety as measured by the TMAS will be associated with higher basal cortisol levels, but not sAA levels.
- We hypothesized that higher two week state anxiety scores as measured by the BAI will be associated with higher basal cortisol levels, but not sAA levels.

Section 2: Methods

Participants

The study included 46 university students between the ages of 18 and 25 divided (*M*=20.609, *SD* =1.9262) into two groups of a GAD (n=22; *M*=20.542, *SD* =2.1865) and a normal population (n=24; *M*=20.682, *SD* =1.6442). The sample was 76% female (n=36) and 83% Caucasian (n=38), 13% African American (n=6), 3% Hispanic (n=2). The GAD participants had a diagnosis of GAD as confirmed by the Miniature International Neuropsychiatric Interview (M.I.N.I.) or a BAI score of >15. All subjects were recruited from the University of Tennessee Knoxville's Human Participation in Research pool. The students were offered extra credit for their participation. All participants completed an Informed Consent document (see appendix) and held the right to drop out of the study at any time.

Materials:

Miniature International Neuropsychiatric Interview -

All participants underwent a minimally intrusive standardized clinical interview at the start of this study. The clinical interview took approximately 10 minutes to complete and was completed after the first cortisol sample and before the video. This study used the M.I.N.I., which follows DSM-IV-TR (2004) AXIS I diagnostic criteria for relevant clinical interviewing process inquiring about current psychopathological diagnosis, present symptoms (i.e., within past two-weeks) and life-time history of psychopathological episodes (i.e., initial onset and reoccurrence). Confirmation of the diagnostic outcome was supervised by a licensed counseling psychologist.

Knee Replacement Surgery Video –

The video is a YouTube video showing a knee replacement surgery. It is a six minute video clip and was shown on a computer screen in the Biopsychology Laboratory at the University of Tennessee. Viewing videos of surgery is a widely used stimulus that activates hormonal stress response (Bosch et al., 2003; Takai et al., 2004; Takai, Yamaguchi, Aragaki, Eto, Uchihashi, Nishikawa, 2007).

Beck Anxiety Inventory (BAI) –

The BAI is a state dependent measure of anxiety (Beck, 1993). Engagement in the BAI places the participant in the anxiety-reminiscent context, therefore eliciting a state-dependent anxiety response. It is a diagnostic tool; therefore, scores on the BAI are directly related to participant symptoms. It is also used to assess clinical treatment. The BAI consists of twenty-one questions expressed as common symptoms of anxiety (e.g. numbness and tingling, sweating not due to heat, and fear of the worst happening). It is designed for an age range of 17–80 years old. Each question has the same set of four possible answer choices, which are arranged in columns and are answered by marking the appropriate one with a cross. These are: Not at all (0 points), Mildly: It did not bother me much. (1 point), Moderately: It was very unpleasant, but I could stand it. (2 points), Severely: I could barely stand it. (3 points). The BAI has a maximum score of 63. It is rated such that: 0-7: minimal level of anxiety, 8-15: mild anxiety, 16-25: moderate anxiety, 26-63: severe anxiety. The BAI is psychometrically sound as indicated by internal consistency ranges from .92 to .94 for adults and test-retest (one week interval) reliability is .75 (Beck, 1993).

Taylor Manifest Anxiety Scale-

The TMAS is a 50 item scale used as a general indicator of trait anxiety (Taylor, 1953). True-false responses are used for each item, and the responses indicating anxiety are summed. The scores range from 0 to 50 with higher scores indicating greater trait anxiety. It is up to the discretion of the investigator to decide where they fit in the "manifest anxiety" interpretation. Items judged by clinicians as being indicative of manifest anxiety were selected from the Minnesota Multiphasic Personality Inventory. Correlations of 0.72 and 0.75 were reported between the TMAS and Evsenck's measure of neuroticism in two samples. High correlations of 0.81 and 0.92 were reported between the TMAS and the psycho-asthenia scale of the MMPI and a low of 0.64 with the Beck Depression Inventory. For the original 50-item version, retest correlations of 0.89, 0.82, and 0.81 over intervals of three weeks, five months and nine to 17 months (Taylor, 1953).

Saliva Sampling-

Participants were seated and instructed to expectorate into a sanitized 50 mL collection tube once per minute over a three minute period (Navazesh, 1993). Once collected, saliva samples were centrifuged for ten minutes, alloquated in microtubes (two per sample), and stored at -70 degrees Celsius for subsequent analysis. We also obtained medications, time of day, and data relative to menstrual cycle for female participants among other variables as indicated by (Kudielka, Hellhammer, & Wust, 2009). We asked that participants not smoke, eat, or exercise within an hour of sampling (Salimetrics Inc.). We collected one saliva sample prior to the stressful video, and this sample was the baseline for both the cortisol and sAA measures. We collected a second sample right after the video which corresponds to the expected peak of the sAA response and a

third sample 20 minutes after the second sample which corresponds to the expected peak of 30 minutes for the cortisol response.

Analyzing sAA-

Supernatants were analyzed for total sAA concentration using the Salivary Alpha-Amylase Kit (Salimetrics Inc, PA). This method utilizes a chromagnic substrate, 2-cloro-p-nitrophenol, linked with maltotriose (Wallenfells et al., 1978) the enzyme action of the sAA on this substrate yields 2-chloro-p-ntrophrenal, which can be spectrophotometrically measured at 405nm. The amount of sAA activity present in the sample is directly proportional to the increase in absorbance at 405nm. Assay concentrations are given in opitical density and were converted to U/mL and range from 3.1 – 423.1 U/mL. The Assay has been shown to be test-retest reliable for high, medium, and low with a coefficient of variation of 2.5%, 6.7%, and 7.2% respectively (n=10) (Salimetrics Inc., PA). Samples were divided randomly and equally between two plates.

Analyzing Cortisol-

Supernatants were analyzed for total cortisol concentration using the High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics Inc., PA). The bound cortisol peroxidase is measured by the reaction of peroxidase enzyme on the substrate tetramathylbenzidine (TMB). Assay concentrations are given in optical density that must be converted to μg/dL, and the assay can detect cortisol levels from 0.003 to 3.0 μg/dL. Optical density is read on a standard plate reader at 450nm. Samples were divided randomly among 4 plates.

Procedure-

The subjects signed up for a designated time on the University of Tennessee Human Participation in Research pool (HPR) and signed an Informed Consent Statement. We collected the first saliva sample from each participant and then administered the (M.I.N.I.). Subjects were then seated in front of a computer monitor where they were shown a 6-minute video clip of a knee replacement surgery or travel documentary on the Blue Mountains of Australia. The latter video acted as a non-stressed control condition. . This provided an appropriate mechanism for examining activation or non activation of the HPA axis and SAM pathway. Immediately, after the video we collected the second saliva sample and collected the third saliva sample 20 minutes after the second. Next, the participants filled out the BAI and TMAS to assess their state and trait anxiety levels respectively. It is important to do these after the post cortisol sample to avoid any confounds in the stressor. Participants held the right to drop from the study at any time.

Data Analysis

The study design is a 2 x 2 x 2 x 3(Group [GAD vs Control] x Condition [stress vs no stress] x Stress Measure [sAA vs Cortisol] x Time [T1, T2, T3]). All data analyses were performed in SPSS 19 32-bit statistical mining program. We used an alpha level of .05 for all statistical tests. First a data transformation was performed creating difference time series data for cortisol and sAA measures which entered the data as cort2-1, cort3-1, sAA 2-1, sAA3-1. In order to determine differences between groups and stress conditions, several multivariate generalized linear analyses were performed on the dependent measures (physiological and self-report variables). The fixed factors were the group (GAD vs Control) and the condition (experimental vs control). A bivariate correlation was performed between BAI, TMAS, Cort T1, and sAA T1. Means and standard deviation for the study variables are presented in Table 1.

Section 3: Results

Cortisol

Figure 1 presents the cortisol results as a function of group, condition, and time. It was hypothesized that basal salivary cortisol levels would be elevated in the GAD group compared to the non-clinical group. The analysis revealed a marginally significant difference between groups for baseline cortisol levels [F (1,46) = 2.875, p=.098]. There was a significant group by condition interaction [F (1,46) = 4.351, p=.044]. This means that the non-clinical (M=.385, SD= .181) and GAD groups (M=.531, SD=.298) had no difference in baseline cortisol, but the GAD, stress condition (M=.645, SD=.328) had significantly elevated baseline cortisol level before seeing the video (see Figure 3). We hypothesized there would be an increased cortisol response in the GAD at T2 (M=.468, SD=.244) and T3 (M=.396, SD=.209) compared to the non-clinical group at T2 (M=.605, SD.463) and T3 (M=.540, SD=.540). There were no significant differences between groups in the cortisol response for T2 [F (1,46) = .286, p=.596] and T3 [F (1,46) = .149, p=.702] (see Table 5). Moreover, there were no significant interactions ($p > .05$). However, there was a significant difference is cortisol response from T1 to T2 between the stress (M=.146, SD=.585) and non- stress conditions (M=-.179, SD=.454), [F (1,46) = 4.519, p=.04]. This suggests that the surgical video elicited a greater neuroendocrine stress response compared to the control video.

Correlational data are presented in Table 4. We hypothesized that the BAI would be positively associated with baseline cortisol levels. The analysis yielded no significant correlation [r (44) = .07, p=.46] between baseline cortisol and the BAI. We hypothesized that trait anxiety, as measured by the TMAS, would be positively correlated with baseline salivary cortisol. The analysis yielded no significant correlation between the TMAS and baseline cortisol levels [r

(1,46) = .185, *p* = .109]. Finally, we hypothesized a positive correlation between cortisol levels and sAA. Contrary to our hypothesis, a significant inverse correlation was found between baseline cortisol levels and baseline sAA levels [*r* (42) = -.26, *p*=.041]. As baseline cortisol levels increased, baseline sAA levels decreased.

Alpha-Amylase

Figure 2 presents the sAA results as a function of group, condition, and time. We hypothesized that the GAD group would not have an elevated baseline sAA compared to the non-clinical group. The analysis indicated no significant main effect between the non-clinical (*M*=24.480, *SD*=28.936) and GAD groups (*M*=17.428, *SD*=19.964) for baseline sAA levels [*F* (1,44) = .999, *p*=.324] (see Table 5). We also hypothesized there would be a greater sAA response in GAD compared to non-clinical groups. There were no significant differences in the increases of sAA levels between the non-clinical group for T2 (*M*=-.0156, *SD*=32.816) or T3 (M=-1.843, SD=35.417) and GAD groups at T2 [(*M*=4.547, *SD*=25.585), *F* (1,44) = .263 *p*=.611] or T3 [(*M*=4.323, *SD*=28.770), *F* (1,44) = .508, *p*=.480]. Also, there was no difference for sAA responses for condition at T2 [*F* (1,44) = .533, p=.470] or T3 [*F* (1,44) = 1.650, p=.207]. However, there was a marginally significant group by condition interaction for T2 sAA response [*F* (1,44) = 3.696, *p* = .062] but not T3 [*F* (1,44) = .000, *p*=1] (See Table 5). This suggests that breaking the groups into stress, no-stress subgroups had some effect on the sAA response. The non-clinical, non-stress subgroup had a significantly different stress response from the other three subgroups. Although it was not significant, the GAD, stress condition (*M*=12.135, *SD*=33.320) and the GAD, control condition (*M*=10.233, *SD*=27.060) both had elevated sAA levels T1 to T2 to T3 (see Table 3).

We hypothesized that there would be no significant correlation between baseline sAA and BAI or TMAS measures. As predicted there was no significant relationship between baseline sAA and BAI [r (1,44) = -.111, p = .236] or baseline sAA and TMAS measures [r (1,44) = .-135, p = .191]. Neither the BAI nor TMAS showed a relationship with baseline sAA measures.

Anxiety Scales

There was a significant main effect for the TMAS [F (3,46) = 12.445, p=.000] according to group. The GAD group (M=23.864, SD=7.213) showed a significantly higher TMAS score than the non-clinical group (M=11.667, SD=5.189). We did not include the Beck because we used it as part of our grouping criteria. However, also as predicted, the BAI and TMAS had a significant positive correlation [r (44) = .792, p=.000] (see Table 4). The scores of one questionnaire predicted scores for the other; or as the BAI scores increased, the TMAS scores increased.

Section 4: Discussion

The aim of our study was to examine the two stress responses in GAD compared to non-clinical populations using cortisol and sAA measures. Our hypotheses were partially supported. Although the groups did not differ significantly with regard to baseline cortisol levels, it was in the hypothesized direction. Moreover, baseline cortisol levels were inversely related to baseline sAA levels.

Cortisol

We did not find a significant difference between GAD and non clinical groups for baseline cortisol, however it was in the hypothesized direction. This lack of a relationship has been found previously by Takahashi et al. (2005) but there are other studies that have also found a positive relationship between increased baseline cortisol and pathology (Careny, et al., 2007; Guinjoan, et al., 1995; Vaith, et al., 1994). One reason for our finding may be our disproportional amount of females in our GAD group (77%). Highly anxious females have been found to have significantly lower cortisol levels than highly anxious males (Takai, Yamaguchi, Aragaki, Eto, Uchihashi, Nishikawa, 2007). However, we performed a multivariate general linear ANOVA with sex as a covariate that determined that sex did not influence our results. Alternatively, our non-significant finding may be due to diagnostic technique. In our study, the anxiety group was determined using the M.I.N.I. We did not follow-up with other techniques for determining GAD. In addition, it is possible that our GAD group was confounded with other disorders because we only controlled for comorbid anxiety disorders. For example, depression has been found to be significantly associated with GAD (Kessler et al., 2005). It is also possible that our GAD group was not GAD. The BAI may not add the same level of anxious individuals as the M.I.N.I.

We found a significant group by stress condition interaction, which indicates that our GAD, stress subgroup had significantly elevated baseline cortisol compared to the other groups. This was determined with a tukey's post hoc test of the means. This suggests that the groups were different at the start of the study and could have altered several of the cortisol comparison results including the stress-induced cortisol response.

With regard to the stress manipulation, we used a knee replacement surgery clip. Previous studies (Bosch et al., 2003; Takai et al., 2004, Takai et al., 2007) have employed the viewing of surgical clips as a method for inducing stress and anxiety. We did find a significant increase in salivary cortisol levels for the stress condition. Regardless of group, individuals viewing the knee replacement video displayed greater elevation in salivary cortisol than the participants in the no-stress condition. This suggests that our stressor caused a cortisol stress response providing evidence that our stressful video was successful eliciting a stress response. Behavioral observations supported this conclusion of the knee replacement video as stressful. For example, several participants showed eye aversion and fidgeting while viewing the video.

Alpha Amylase

With regard to the protein sAA, we found no significant differences between groups on baseline measures. The null hypothesis was supported in our study and is in line with previous research regarding basal sAA and GAD (Fisher, Granger, Newman, 2010). We expected this result because sAA is a response to arousal as it is correlated with the release of epinephrine. Epinephrine is released in response to stress in the ANS. Although we found that the GAD group reported greater trait anxiety compared with the non-clinical control participants, there was no significant difference on sAA levels between the groups contrary to Takai et al. (2004).

Moreover, sAA did not correlate significantly with any of the self-report measures, yet we found a significant inverse relationship between baseline cortisol levels and baseline sAA levels. We are unaware of any previous research that has found this result. The inverse relationship may be due to our data collection being limited to morning hours. sAA drops to its lowest in the morning and cortisol peaks at its highest in the morning hours. The diurnal patterns continue to oppose each other throughout the day. Alternatively, Het, Schoofs, Rohleder, and Wolf (2012) found a negative correlation between cortisol and negative affect. They also found a positive correlation between sAA levels and negative affect. They explained that the negative affect of the stressor is associated with sAA levels then cortisol acts as a "mood buffer" later on. While this is not a direct inverse relationship it may provide us with insight to our findings. Neither group experienced an increase in sAA. Therefore it is possible the GAD group entered the study with negative affect due to everyday stressors of university life causing an up regulation of baseline cortisol to try to buffer the circular negativity bias associated with anxiety.

There have been a number of studies that have reported no relationship between sAA and cortisol (Chatterton et al., 1996; Fisher, Granger, Newman, 2010; Nater & Rohleder, 2009; Schenkels, Veerman, Nieuw Amerongen, 2005, Strahler et al., 2010, van Veen et al., 2008). van Veen et al. (2008) did not find significant differences for cortisol levels, but did find significant differences between baseline sAA levels and diurnal sAA levels between SAD and control groups. They suggested an imbalance in the two stress systems. It may be that GAD does not evoke autonomic arousal at baseline. Fisher, Granger, and Newman, 2010 reported that the DSM-IV removed the autonomic activity (e.g. sweating, increased heart rate, etc.) from its criteria for generalized anxiety (DSM-IV, 1994) because there was trouble replicating autonomic function in a clinical setting. In concordance to this, they found no significant difference between

GAD and controls for baseline sAA levels. They sampled 107 participants and divided them using the MINI into Control (n=62), GAD (n=21), and GAD comorbid (n=24). They measured sAA levels at baseline and post stressful video. They found that the GAD and GAD comorbid group act very differently in terms of their sAA response. Of their GAD cormorbid group, 15 were depressive disorders. They provided evidence that GAD shows diminished physiological flexibility and that comorbidity must be taken into account when examining sympathetic arousal of anxiety disorders. It is possible that our lack of account for depressive disorders skewed our results. In the same way, Fisher & Newman (2013) reported diminished HR responses and concluded suppression of adrenergic sympathetic response in GAD. Therefore, it is possible that GAD individuals exhibit a diminished SAM pathway response and an over active HPA axis. Granted that both the SAM pathway and HPA axis both start in the hypothalamus, it may be that in GAD individuals the hypothalamus responds to minor stressors as "chronic" therefore activating HPA axis to acute stress as opposed to the SAM pathway in GAD. Or there may be a blocked system that causes a one-way activation path to the HPA axis for all stressors. This would consequently deprive the SAM pathway of resources and activation reducing the amount of alpha-amylase produced, but increase cortisol. Because our groups did not show a sAA response, our data neither supports nor refutes this hypothesis. Future studies should investigate this further. It is also possible that general academics or the process of coming in for an unfamiliar experiment causes activation of one stress response but not the other. Further studies are warranted to understand the relationship between the two stress response systems in GAD.

We also hypothesized that the stress manipulation would generate greater levels of sAA in the GAD group compared to the non-clinical participants. This hypothesis was not supported. However, Takai et al (2004) reported the use of a stressful video to consequently increase sAA

levels, but these levels returned to baseline by the end of the video watching. They took saliva samples throughout the video manipulation. In our study, saliva samples were collected immediately after the 6 minute video viewing and 30 minutes later.

BAI vs TMAS

We found a significant positive correlation between the state and trait measures of anxiety. This was an expected finding and it is consistent with other researchers (Gould et al., 1984; Hanton et al., 2002). The significant correlation between the BAI and TMAS is interesting because they are supposed to measure state and trait anxiety respectively. Our results suggest they may share more similar components than previous thought.

Limitations

Some limitations to the study include methodology, choice of stressor, sample size, and sample acquisition. First, our sample was a convenience sample. The HPR pool allows for students to sign up for studies in exchange for class credit. There may have been a bias of students that were drawn to the title of the study due to personal issues. Likewise, the time period for participations was limited to the morning hours. This may have influenced the type of participants for this study. Also sample size was a limiting factor. It was difficult to find 24 people with GAD. We therefore included people who qualified as medium anxiety (score >15) according to the BAI, which has been used for diagnostic purposes. It was also difficult to find

24 people that did not have any underlying anxiety problem. Cleaner group delineation may have yielded richer data and a better understanding regarding this subject matter.

Part of our study that may have been limiting for us was our choice of stressor. Choosing the surgical video was designed to have the participant view something that would make them uncomfortable enough to produce a physiological response without having to frighten them or produce a specific type of anxiety such as a social situation. In those cases for example, we would no longer be measuring GAD; we would be measuring Phobias or Social Anxiety Disorder (SAD). This gives us a narrow range of stressor to choose from, but according to our data the stressor was effective. It is also possible that the video made some people anxious, because they are scared of surgeries. There is also the possibility that the participants entered the study nervous and therefore their arousal levels were already elevated.

Finally, it must be noted that the sAA analysis was amended due to equipment issues. The protocol requires that the sAA substrate solution be warmed for 20 minutes to 37º C in plate incubator. This is a kinetic assay and the substrate must be warmed in order to facilitate binding. We did not have a plate incubator, but warmed the solution on a hot plate. We used a thermometer to gage the temperature of the solution during this 20 minutes time period. It is plausible that this amended protocol may have influenced our sAA findings.

Section 5: Future Directions and Conclusion

In the future, this experiment should be replicated with a larger sample, cleaner diagnostic groupings, and different types of stressors. This would provide insight into the different ways each anxiety disorder differs with respect to physical and psychological stressors and the neuroendocrine correlates associated with such an environmental stimulus. Similar to the Takahasi et al. (2005), repeated sampling of saliva during the stressor would improve upon the sensitivity of the current study. Finally, incorporating a 37º C plate incubator for analyzing sAA is also warranted.

In conclusion, we found differences in cortisol response between conditions supporting our premise that the surgical video was stressful. We found marginal differences in baseline cortisol between groups, a marginally significant interaction between groups and condition that suggest that the GAD, treatment subgroup had a greater T2 response than the other subgroups. We found no significant difference between groups or subgroups that would suggest deviant autonomic activation. We also found a negative correlation between baseline sAA and cortisol levels. This provides evidence of opposing diurnal patterns between the two stress response systems. And the marginally significant effect of baseline cortisol between groups suggests that cortisol may be a better determinate of stress in GAD. Finally, there was also a significant difference between groups on the TMAS. This may provide evidence for the TMAS to be used clinically as a diagnostic tool.

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Appendix

Table1.

Total Means

	N	Mean	Std. Deviation
Age	46	20.609	1.926
Beck	46	12.174	9.379
TMAS	46	17.500	8.717
Cort 1	46	.455	.252
Cort2	46	.533	.368
Cort 3	46	.465	.350
sAA 1	44	20.954	24.825
sAA 2	44	18.666	26.210
sAA 3	44	23.466	24.556
DIFF(Cort2,1)	45	-.005	.547
DIFF(Cort3,1)	45	-.003	.514
DIFF(sAA2,1)	43	2.318	29.076
DIFF(sAA3,1)	43	1.312	31.952
Valid N (listwise)	43		

Table 2.

Non-clinical Group Means				
	Condition	Mean	N	Std. Deviation
Age	Stress	20.583	12	2.644
	Non-Stress	20.500	12	1.732
	Total	20.542	24	2.186
Beck	Stress	6.000	12	4.285
	Non-Stess	6.000	12	3.437
	Total	6.000	24	3.799
TMAS	Stress	11.667	12	6.242
	Non-Stress	11.667	12	4.163
	Total	11.667	24	5.189
Cort 1	Stress	.350383	12	.210
	Non-Stress	.420858	12	.147
	Total	.385621	24	.181
Cort2	Stress	.454908	12	.260
	Non-Stress	.481992	12	.237
	Total	.468450	24	.244
Cort 3	Stress	.369783	12	.202
	Non-Stress	.422433	12	.220
	Total	.396108	24	.209
sAA 1	Stress	15.654	11	13.379
	Non-Stress	33.306	11	37.522
	Total	24.480	22	28.936
sAA 2	Stress	13.835	11	23.758
	Stress	11.569	11	22.48
	Total	12.702	22	22.602
sAA 3	Stress	13.597	11	22.947

Table 2. Continued

	Non-clinical Group Means			
	Condition	**Mean**	**N**	**Std. Deviation**
	Non-Stress	20.723	11	20.415
	Total	17.160	22	21.506
DIFF(Cort2,1)	Stress	.138	12	.339
	Non-Stress	-.112	11	.341
	Total	.018	23	.356
DIFF(Cort3,1)	Stress	.090	12	.295
	Non-Stress	-.079	11	.343
	Total	.009	23	.323
DIFF(sAA2,1)	Stress	12.136	10	33.320
	Non-Stress	-11.062	11	29.541
	Total	-.015	21	32.816
DIFF(sAA3,1)	Stress	-8.495	10	42.651
	Non-Stress	4.204	11	28.056
	Total	-1.843	21	35.417

Table 3.

GAD Group Means				
	Condition	**Mean**	**N**	**Std. Deviation**
Age	Stress	20.333	12	1.497
	Non-Stress	21.100	10	1.792
	Total	20.682	22	1.644
Beck	Stress	17.667	12	6.125
	Non-Stress	20.400	10	11.834
	Total	18.909	22	9.033
TMAS	Stress	23.417	12	7.573
	Non-Stress	24.400	10	7.121
	Total	23.864	22	7.213
Cort 1	Stress	.644	12	.328
	Non-Stress	.395	10	.197
	Total	.531	22	.298
Cort2	Stress	.778	12	.542
	Non-Stress	.397	10	.230
	Total	.605	22	.463
Cort 3	Stress	.715	12	.5384
	Non-Stress	.330	10	.179
	Total	.540	22	.451
sAA 1	Stress	14.978	12	15.139
	Non-Stress	20.368	10	25.144
	Total	17.428	22	19.964
sAA 2	Stress	19.789	12	17.102
	Non-Stress	30.438	10	38.603
	Total	24.629	22	28.658
sAA 3	Stress	28.645	12	24.489
	Non-Stress	31.127	10	29.506

Table 3. Continued

	GAD Group Means			
	Condition	**Mean**	**N**	**Std. Deviation**
	Total	29.773	22	26.246
DIFF(Cort2,1)	Stress	.154	12	.775
	Non-Stress	-.252	10	.564
	Total	-.030	22	.703
DIFF(Cort3,1)	Stress	.173	12	.705
	Non-Stress	-.244	10	.567
	Total	-.016	22	.666
DIFF(sAA2,1)	Stress	-.191	12	24.428
	Non-Stress	10.233	10	27.060
	Total	4.547	22	25.585
DIFF(sAA3,1)	Stress	-1.448	12	26.433
	Non-Stress	11.250	10	31.298
	Total	4.323	22	28.770

Table 4.
Table of Correlations

		TMAS	Cort 1	sAA T1
Beck	Pearson Correlation	.729	.007	-.111
	Sig. (1-tailed)	.000*	.480	.236
	N	46	46	44
TMAS	Pearson Correlation		.185	-.135
	Sig. (1-tailed)		.109	.191
	N		46	44
Cort T1	Pearson Correlation			-.265
	Sig. (1-tailed)			.041*
	N			44

Table 5.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	TMAS	1616.420[a]	3	538.807	12.628	.000*
	Cort 1	.549[b]	3	.183	3.196	.034*
	sAA 1	2245.176[c]	3	748.392	1.225	.314
	CortT2-T1	1.438[d]	3	.479	1.595	.206
	CortT3-T1	1.200[e]	3	.400	1.498	.230
	sAAT2-T1	3635.488[f]	3	1211.829	1.483	.234
	sAAT3-T1	2133.008[g]	3	711.003	.681	.569
Intercept	TMAS	13486.556	1	13486.556	316.091	.000*
	Cort 1	8.976	1	8.976	156.846	.000*
	sAA 1	19674.803	1	19674.803	32.195	.000*
	CortT2-T1	.001	1	.001	.003	.959
	CortT2-T1	.001	1	.001	.003	.954
	sAAT2-T1	330.158	1	330.158	.404	.529
	sAAT3-T1	81.151	1	81.151	.078	.782
Group	TMAS	1615.633	1	1615.633	37.866	.000*
	Cort 1	.165	1	.165	2.875	.098
	sAA 1	610.375	1	610.375	.999	.324
	CortT2-T1	.086	1	.086	.286	.596
	CortT3-T1	.040	1	.040	.149	.702
	sAAT2-T1	214.940	1	214.940	.263	.611
	sAAT3-T1	530.673	1	530.673	.508	.480
Condition	TMAS	3.916	1	3.916	.092	.764
	Cort 1	.100	1	.100	1.747	.194
	sAA 1	1240.066	1	1240.066	2.029	.162
	CortT2-T1	1.358	1	1.358	4.519	.040*
	CortT3-T1	1.056	1	1.056	3.955	.054
	sAAT2-T1	435.988	1	435.988	.533	.470
	sAAT3-T1	1723.723	1	1723.723	1.650	.207
Group * Condition	TMAS	1.527	1	1.527	.036	.851
	Cort 1	.249	1	.249	4.351	.044*
	sAA 1	309.502	1	309.502	.506	.481
	CortT2-T1	.028	1	.028	.092	.763
	CortT3-T1	.115	1	.115	.430	.516
	sAAT2-T1	3020.872	1	3020.872	3.696	.062
	sAAT3-T1	6.599E-7	1	6.599E-7	.000	1.000
Error	TMAS	1663.998	39	42.667		

Table 5. Continued

	Tests of Between-Subjects Effects					
	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
	Cort 1	2.232	39	.057		
	sAA 1	23833.556	39	611.117		
	CortT2-T1	11.720	39	.301		
	CortT3-T1	10.416	39	.267		
	sAAT2-T1	31873.556	39	817.271		
	sAAT3-T1	40746.316	39	1044.777		
Total	TMAS	17033.000	43			
	Cort 1	12.121	43			
	sAA 1	45820.348	43			
	CortT2-T1	13.158	43			
	CortT3-T1	11.616	43			
	sAAT2-T1	35740.265	43			
	sAAT3-T1	42953.342	43			
Corrected Total	TMAS	3280.419	42			
	Cort 1	2.780	42			
	sAA 1	26078.732	42			
	CortT2-T1	13.158	42			
	CortT3-T1	11.616	42			
	sAAT2-T1	35509.045	42			
	sAAT3-T1	42879.325	42			

a. R Squared = .493 (Adjusted R Squared = .454)

b. R Squared = .197 (Adjusted R Squared = .136)

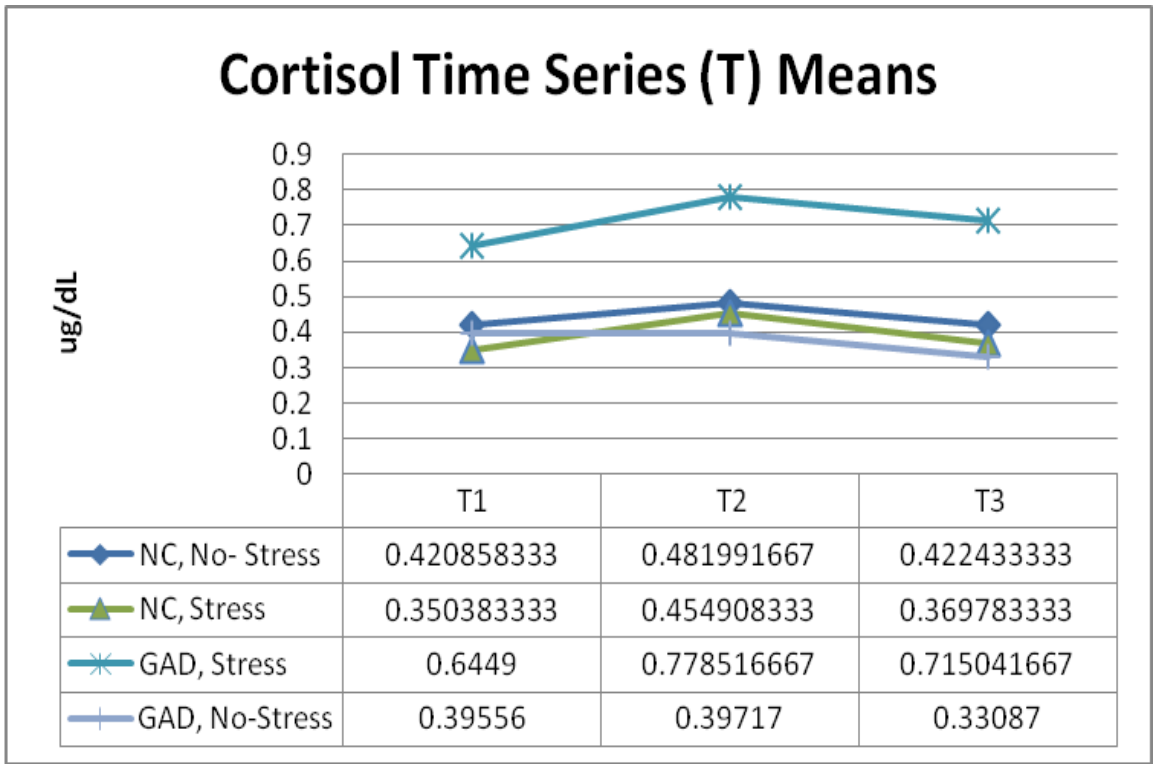
c. R Squared = .086 (Adjusted R Squared = .016)

d. R Squared = .109 (Adjusted R Squared = .041)

e. R Squared = .103 (Adjusted R Squared = .034)

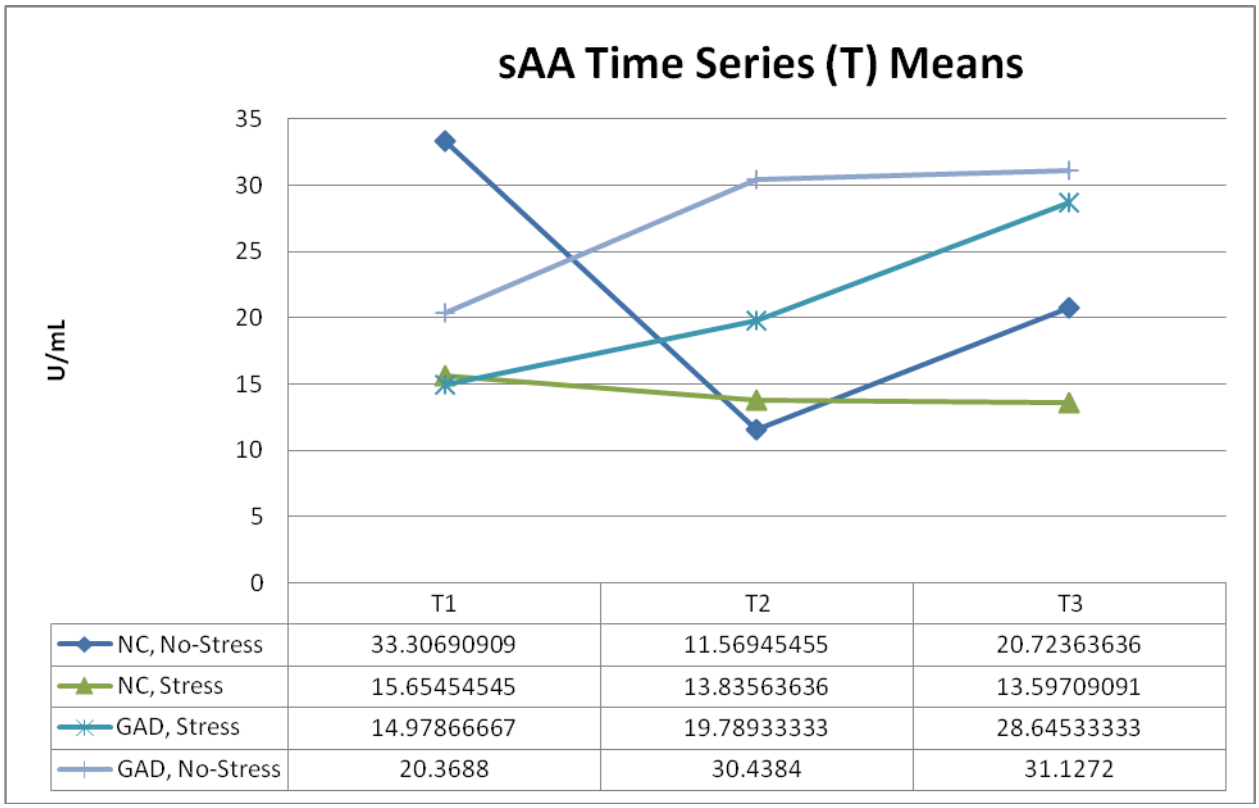
f. R Squared = .102 (Adjusted R Squared = .033)

g. R Squared = .050 (Adjusted R Squared = -.023)



	T1	T2	T3
NC, No- Stress	0.420858333	0.481991667	0.422433333
NC, Stress	0.350383333	0.454908333	0.369783333
GAD, Stress	0.6449	0.778516667	0.715041667
GAD, No-Stress	0.39556	0.39717	0.33087

Figure 1. Cortisol Time Series (T) Means



	T1	T2	T3
NC, No-Stress	33.30690909	11.56945455	20.72363636
NC, Stress	15.65454545	13.83563636	13.59709091
GAD, Stress	14.97866667	19.78933333	28.64533333
GAD, No-Stress	20.3688	30.4384	31.1272

Figure 2. sAA Time Series (T) Means

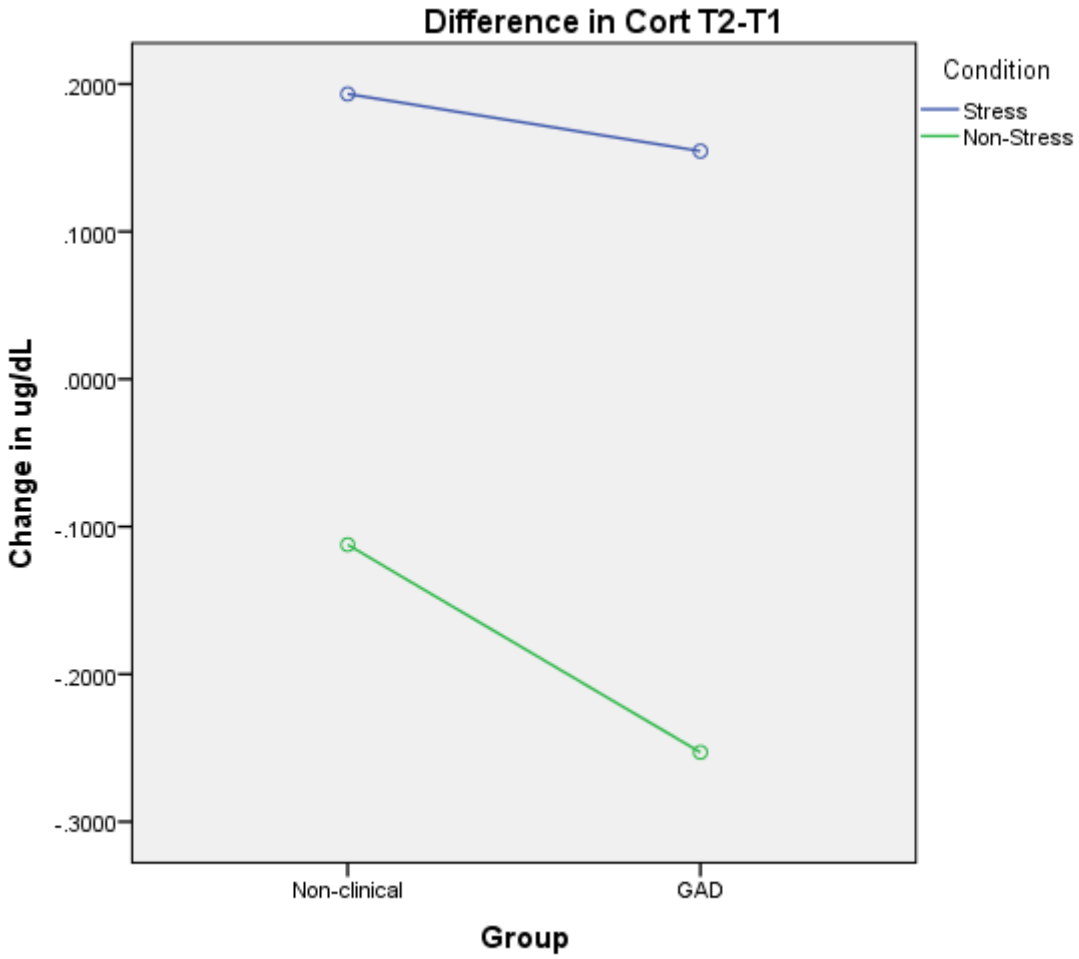


Figure 3. Difference in Cortisol T2-T1 (Group*Condition)

Vita

Dominic Joseph Di Loreto was born in Indianapolis, Indiana to the parents of Daniel and Julie Di Loreto. He was raised in Franklin, TN and attended the University of Tennessee, Knoxville where he began working in the Biopsychology and Neuropsychology lab under Drs. Baldwin and Cannon. Under their guidance he began working with EEG and Cortisol as mechanisms of measuring behavior. He was published as an undergraduate on a paper entitled "A 9-year old male with multifocal Encephalomalacia: EEG LORETA andLifespan database, Magnetic Resonance Imaging and Neuropsychological agreement." In the *Journal of Neurotherapy: Investigations in Neuromodulation, Neurofeedback and Applied Neuroscience*. He graduated Cum Laude with a Bachlors of Arts degree in psychology. Then he accepted a Graduate Teaching Assistant position at the University of Tennessee, Knoxville in their Experimental Psychology department where he has contributed to over 10 poster presentations, a few publications, and several papers that are being prepared for publication. He won a student travel award for his oral presentations entitled "LORETA Neurofeedback and the Morphology of Working Memory and Processing Speed" from the International Society for Neurofeedback and Research and was featured in the student issue of *Neuroconnections* magazine to highlight up and coming student researchers (Fall 2012). Over his four years working in the Biopsychology Laboratory, Dominic has accumulated over 2000 lab hours collecting data, working with participants, working on papers and presentations, running statistics, etc. Dominic graduated in August 2013 with a Masters of Arts and has accepted a position as a Clinical Supervisor at the function health clinic Unique Mind Care in Houston, TX, where he plans to work part time and pursue a PhD.