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NECESSARY VERSUS SUFFICIENT CAUSES OF IMPAIRED PHYSIOLOGICAL
FUNCTIONING IN GENERALIZED ANXIETY DISORDER

A Dissertation in
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Aaron J. Fisher

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The dissertation of Aaron J. Fisher was reviewed and approved* by the following:

Michelle G. Newman
Professor of Psychology
Dissertation Adviser
Chair of Committee

William J. Ray
Professor of Psychology

Kristin Buss
Associate Professor of Psychology

Douglas A. Granger
Professor of Biobehavioral Health

Melvin M. Mark
Professor of Psychology
Head of the Department of Psychology

*Signatures are on file in the Graduate School

ABSTRACT

An extensive body of literature has established the role of impaired autonomic nervous system (ANS) functioning in individuals with clinically-diagnosed generalized anxiety disorder (GAD; c.f. Hoehn-Saric, McLeod, Funderburk, & Kowalski, 2004). Worry is the cardinal feature and central facet of GAD specifically, as well as a major component of anxiety and mood disorders more broadly (Barlow, 2004). Worry has been shown to disrupt the adaptive responsiveness of physiological systems in anxious individuals as well as controls. Worrisome thinking prior to exposure to a phobic image has been shown to preclude cardiovascular response to the image in anxious individuals (Borkovec & Hu, 1990; Borkovec, Lyonfields, Wiser, & Deihl, 1993) and the induction of worry in non-anxious controls has been shown to cause significant reductions in heart rate variability (HRV) – a proxy for parasympathetic control of heart rate (HR; Hofmann, et al., 2005; Lyonfields, Borkovec, & Thayer, 1995; Thayer, Friedman, & Borkovec, 1996).

If worry is both necessary and sufficient for physiological impairment, it would be expected that a non-clinical, high-worry control group would exhibit impairments in physiological functioning equivalent to clinically-diagnosed GAD participants at resting baseline, and that phasic increases in state worry would impair physiological reactivity across healthy controls, high-worry controls, and GAD participants in response to a stressful task. However, although prior research seems to indicate that worry is necessary, it may be that clinical distress, significant impairment, or other aspects of the clinically-diagnosed GAD syndrome – such as the inability to control worry – are required for the development of significant physiological dysfunction. It is precisely this question that the present study aimed to address.

The present study compared clinically-diagnosed GAD patients to both healthy controls, and high-worry controls. The lattermost group demonstrated significant elevations in worry that placed them within a clinical range, but did not meet clinical criteria for GAD or any other Axis I disorder as determined by structured interview. Moreover, phasic variability in state worry was tracked across baseline, during the experimental induction of worry versus relaxation, during two laboratory stressors, and finally following a 20-minute recovery period. Measures of HR, HRV, salivary alpha-amylase – an index of adrenergic sympathetic tone, and cortisol were taken across the study. Thus, we examined the relative effects of state, trait, and clinical worry (GAD) on sympathetic, parasympathetic, and hypothalamic-pituitary-adrenal (HPA) axis systems at resting baseline, in response to a laboratory stressor, and following recovery.

Results indicated that clinically-diagnosed GAD accounted for impairments in parasympathetic, sympathetic, cardiovascular, and HPA systems above and beyond state and trait worry. Individuals with GAD exhibited reduced vagal cardiac control at baseline and recovery, poor coordination of sympathoexcitatory-HPA axis stress reactivity, and rigidity in cardiovascular reactivity resulting from experimentally induced worry. In addition, it was demonstrated that impairments in vagal cardiac control following recovery were a function of expressed anger and the degree to which individuals with GAD were able to express sadness and/or contentment counteracted the negative effect of anger.

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CHAPTER 1

Autonomic and hypothalamic-pituitary-adrenal responses to stress after experimental induction of worry versus relaxation in healthy, high-worry, and generalized anxiety disorder individuals.

Generalized anxiety disorder (GAD) is the most commonly occurring anxiety disorder and one of the most highly co-occurring disorders in the diagnostic and statistical manual of mental disorders-fourth edition (DSM-IV; Kessler, Berglund, Demler, Jin, & Walters, 2005; Maier, et al., 2000). A chronic and disabling disorder, GAD is associated with substantial personal, societal, and economic costs (Ballenger, et al., 2001; Wittchen, 2002). Individuals with GAD have been shown to frequently use primary and specialty health care resources (Ormel, et al., 1994; Schonfeld, et al., 1997); making GAD the second most prevalent mental health problem found in primary care settings (Maier, et al., 2000). Moreover, GAD has been associated with cardiovascular disease such as cardiac ischemia, sudden cardiac death, and myocardial infarction (Frasure-Smith & Lesperance, 2008; Martens, et al., 2010).

Several mechanisms have been proposed for the connection between GAD and negative health outcomes. Perhaps most prominently, GAD has been shown to be related to diminished physiological flexibility in response to naturalistic and laboratory stressors (c.f. Hoehn-Saric, McLeod, Funderburk, & Kowalski, 2004). Diminished physiological flexibility (Hoehn-Saric & McLeod, 2000; Hoehn-Saric, et al., 2004) refers to an absence of expected variability in arousal as measured by physiological indices such as heart rate (HR) and heart rate variability (HRV) – a proxy for parasympathetic control of HR via vagal efferents. A lack of flexibility in autonomic regulation is potentially problematic, as rigidity in physiological systems has been related to mortality and physical morbidity (Lipsitz & Goldberger, 1992; Peng, et al., 1994). Worry has

similarly been shown to be related to diminished physiological flexibility (c.f. Borkovec & Hu, 1990) and Brosschot, Gerin, and Thayer (2006) have implicated perseverative cognition – which subsumes worry and the related construct, rumination – as a potential causal mechanism in negative health outcomes.

Worry is the cardinal feature of the current diagnostic criteria for GAD and both excessiveness and uncontrollability of worry are required for the diagnosis of GAD. Worry has been shown to preclude autonomic nervous system (ANS) response in anxious individuals as well as controls. Worrisome thinking prior to exposure to a phobic image has been shown to preclude cardiovascular response to the image in anxious individuals, whereas relaxed thinking prior to phobic image presentation increased HR response (Borkovec & Hu, 1990; Borkovec, Lyonfields, Wiser, & Deihl, 1993; Llera & Newman, 2010). Additional studies showed that laboratory inductions of worry also led to reduced HRV in nonanxious controls (Lyonfields, Borkovec, & Thayer, 1995; Thayer, Friedman, & Borkovec, 1996). Worry generated higher HR and lower HRV when compared to a resting baseline in 43 healthy males (Hofmann, et al., 2005), as well as when compared to relaxation in 53 healthy adults of mixed gender (Verkuil, Brosschot, Borkovec, & Thayer, 2009). Finally, Brosschot, Van Dijk, and Thayer (2007) recently showed in a 24-hour ambulatory study of 52 healthy individuals that worry frequency predicted higher HR and lower HRV during waking, whereas worry duration predicted lesser HRV during both waking and sleeping; and Pieper, Brosschot, van der Leeden, and Thayer (2007) similarly found in a 4-day ambulatory study that worry predicted elevated HR and decreased HRV in 73 Dutch secondary school teachers. Taken together, these studies suggest a possible causal association between worrisome thinking and ANS impairment, most notably reduced parasympathetically-mediated HRV and increased HR.

Consistent with findings for laboratory-induced and naturalistic worry, the broader clinical syndrome of GAD has been related to diminished ANS responses following stressors. In one of the first studies to examine the role of the ANS in GAD, 20 females with GAD exhibited narrower ranges of HR than did controls in response to two laboratory stressors (Hoehn-Saric, McLeod, & Zimmerli, 1989). Thayer and colleagues hypothesized that these effects were mediated by poor parasympathetic regulatory tone and in two separate studies demonstrated that individuals with GAD exhibited both lower levels of HRV at baseline as well as invariant parasympathetic responses to laboratory stressors (Lyonfields, et al., 1995; Thayer, et al., 1996). Llera and Newman (2010) found that not only did worry elicit lesser HRV in GAD analogues, the induction of worry prior to the presentation of fearful material precluded robust parasympathetic withdrawal in response to the feared stimulus. Finally, in an ecological assessment of these effects, persons with GAD exhibited lesser HRV compared to controls over the course of a 4-day ambulatory study (Hoehn-Saric, et al., 2004). Somewhat inconsistent with these findings, Fisher, Granger, and Newman (2010) found that diminished physiological flexibility in adrenergic sympathetic arousal was limited to GAD participants with elevated SNS arousal at baseline. This finding points to a possible dissociation of sympathetic and parasympathetic systems, and calls for further investigation of the role of sympathoexcitatory systems in GAD. However, taken together these studies demonstrate that, across measurements of the ANS and within both laboratory and ecological settings, persons with GAD exhibit diminished physiological responses following stressors, especially reduced parasympathetic inhibitory control of HR.

Autonomic nervous system control of HR – predominantly regulated by the parasympathetic branch of the ANS and often referred to as vagal tone – is vital to

cardiovascular health. Heart rate is chronically inhibited by afferent vagal signals at the sinoatrial node. The inhibitory signals of the PNS are necessary for cardiac stability and flexibility. Dysregulation of these effects has been shown to limit appropriate cardiac responsiveness (Levy, 1990; Verrier, 1987). Diminished vagally mediated HRV has been shown to predict congestive heart failure and hypertension (Curtis & O'Keefe, 2002) and has been used as a proxy for cardiovascular disease altogether (Stys & Stys, 1998). Low tonic HRV likewise has been shown to be a significant risk factor for all-cause mortality (Tsuji, et al., 1994), whereas survival after myocardial infarction is positively related to greater levels of HRV (Stein, Bosner, Kleiger, & Conger, 1994). Therefore, it is critical to distinguish the degree to which impaired HRV and PNS functioning are implicated in the process of worry and clinically-diagnosed GAD.

Unfortunately, the delineation of the specific causal factors underlying diminished flexibility in GAD has been confounded by the use of persons with GAD, non-clinical samples stratified by level of trait anxiety or trait worry, and general populations in worried states. This conflation of disparate populations likely belies differences in the amount, duration, severity, and function of worry within each group. Thus, the utilization of methodologies such as high-worry control groups, experimental inductions of worry, and momentary assessments of phasic worry may help to identify whether the construct of worry or the greater clinical syndrome of GAD is responsible for ANS rigidity. Given the often synonymous treatment of worry and GAD in the literature (Brown, Chorpita, & Barlow, 1998) it is important to investigate whether the phenomenon of chronic worry is responsible for the diminished autonomic flexibility demonstrated in GAD, or if the greater clinical syndrome is required to observe significant effects. This is an important distinction to make given that worry has been implicated in nearly all mood and anxiety disorders (Barlow, 2004; Brosschot, et al., 2006).

Several authors have argued that worry is a mediator, rather than a moderator of the effects of psychological and psychosocial stressors on long-term health (Brosschot, et al., 2006; Brosschot, et al., 2007; Thayer & Lane, 2002). Within this model worry perpetuates the effects of passing stressors by maintaining the cognitive representation and thereby the physiological presence of the stressor, prolonging the impact of that stressor on the body. Such an argument would seem to support the hypothesis that worry and not the larger clinical syndrome of GAD is the causal mechanism in autonomic inflexibility. Consistent with this hypothesis, Kubzansky et al. (1997) demonstrated that worry proneness predicted a second myocardial infarction. However, no work to date has compared clinically-diagnosed GAD and non-clinical, high-trait-worry populations directly. This is a potentially vital distinction to be drawn given that Martens et al. (2010) found that the presence of GAD predicted a 62% higher rate of negative cardiovascular outcomes, and this effect was not affected by the presence of comorbid psychological disorders. Thus, despite the prevalence of worry across the general population and within multiple DSM disorders, the broader clinical syndrome of GAD may make an independent contribution to poor cardiovascular health.

Although the work of researchers such as Borkovec (1990; 1993) Thayer (1995, 1996) and Hoehn-Saric (1989, 2004) has been instrumental in beginning to delineate the role of physiological flexibility and rigidity in GAD, these data have skewed heavily toward the assessment of HR and HRV. Thus, inferences about health broadly or cardiovascular functioning specifically may be questionable due to the relative independence of parasympathetic-inhibitory and sympathoexcitatory systems. Within the ANS, separate measurement modalities may capture phenomena mediated by differential central nervous system efferents (Berntson, Cacioppo, & Quigley, 1991), complicating the generalization of a

single index to the ANS globally, or even one branch specifically. Moreover, the balance of sympathetic versus parasympathetic control of specific organs can be variably reciprocal, nonreciprocal, or uncoupled given specific environmental demands (Berntson, et al., 1991; Koizumi & Kollai, 1981). Although SNS predominance can be facilitated at times by the active withdrawal of inhibitory PNS effects (Beauchaine, 2001; Porges, 1995), fight or flight symptoms are largely the result of the release of epinephrine by the adrenal medulla, a process mediated centrally by norepinephrine in presynaptic neurons (Noback, Strominger, Demarest, & Ruggiero, 2005, p. 354) and facilitated, amplified, and conversely suppressed by glucocorticoids (Sapolsky, Romero, & Munck, 2000). Often overlooked in research on GAD (Mantella, et al., 2008; Steudte, et al., 2011), but equally important to an adaptive stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis can play important permissive and suppressive roles in determining the length, breadth, and impact of physiological responses to stress (Sapolsky, et al., 2000). Thus, studies of diminished physiological flexibility in worry and GAD should endeavor to include measures of HPA functioning as well.

Within an adaptive stress response, the occurrence of a physical and/or psychological stressor is met with an almost immediate activation of the SNS. In a matter of seconds, epinephrine is released into the bloodstream by the adrenal medulla and soon after reaches cells throughout the body. Results of this activation are well-documented and include alertness, vigilance, psychological and emotional distress, increased rate and contractility of the heart, increased metabolic rate, and bronchodilation (Noback, et al., 2005). In addition, the mobilization of the stress response stimulates increased immune functioning, which, in turn promotes the release of pro-inflammatory cytokines. Also occurring within seconds are the release of corticotropin releasing hormone and then adrenocorticotrophic hormone (ACTH),

which promote increased production of glucocorticoids, most notably cortisol. Glucocorticoids have both permissive and suppressive functions within the stress response (Sapolsky, et al., 2000). For instance, they permit – and enhance – the cardiovascular effects of catecholamines by increasing the binding capacity and affinity of β -adrenergic receptors (Collins, Caron, & Lefkowitz, 1988). Conversely, glucocorticoids play a crucial role in the suppression of the stress response, inhibiting the inflammatory action of cytokines and creating a negative feedback loop in which the enhanced presence of available cortisol down regulates the production of ACTH and thus the further production of cortisol. In concert, these responses are engaged to meet the metabolic needs of the organism and, following the removal of the stressor, return it to homeostasis.

Thus, in addition to assessments of cardiovascular functionality through the investigation of vagal tone and HR, research on physiological flexibility and rigidity within GAD should also incorporate measures of sympathetic and HPA axis activity. Steudte et al. (2011) recently found that, despite no differences in momentary assessments of salivary cortisol between GAD patients and controls, the GAD group exhibited significantly lower concentrations of hair cortisol. Whereas the former method is valuable for measuring acute levels of available cortisol, the latter is a more stable reflection of typical within-person HPA arousal. Given that the availability of glucocorticoids such as cortisol facilitate the engagement and initial responsiveness of the SNS, chronic reductions in available cortisol could potentially impair this response. Thus, due to the intimate interrelations between the functional behavior of the SNS and HPA axis, modeling of concurrent and interactive effects of these systems on adaptive versus maladaptive – i.e. flexible versus rigid – responses to stress would likely prove maximally informative.

The aim of the current study was to use strong inference (Platt, 1964) to determine the independent versus additive contributions of worry and the broader clinical GAD syndrome to diminished physiological flexibility. Strong inference is an empirical approach wherein one devises alternative hypotheses and then creates an experimental study which is designed to rule out one or more of the initial hypotheses. Within the present context alternative hypotheses exists that phasic worry periods, conditions of high trait worry, or a clinically-severe syndrome (GAD) are primary agents in the generation of negative health outcomes. The present study utilized a healthy control group, a clinically-diagnosed GAD group, and a high-worry control group, with significant elevations in trait worry and no Axis I diagnoses. In order to control for and test against the influence of phasic worry, an experimental manipulation was additionally employed whereby all study participants were randomly selected to receive a worry or relaxation induction and levels of phasic worry were measured at five points throughout the study.

Physiological flexibility and rigidity were measured via two target processes. Consistent with much of the research on diminished physiological flexibility in GAD, the first process examined within the present study was the relationship between parasympathetic inhibitory control and HR. Thus, both HR and HRV were measured across an initial baseline, experimental induction of worry versus relaxation, two iterations of a mental arithmetic task (of increasing difficulty), and a 20-minute recovery period. In addition, both salivary alpha-amylase, a marker of adrenergic sympathetic activity, and cortisol were measured at baseline, following the stressor, and following the recovery period so that we could model the concurrent and interactive effects of the SNS and HPA axis. This study marks the first time that clinically-diagnosed GAD participants have been compared to both healthy and high-worry controls in an examination of

physiological flexibility and rigidity and the first time that the concurrent and interactive effects of SNS and HPA axis stress responses have been measured in a GAD population.

Method

Participants

Participants ($n=151$) were mostly female (65.6%) and Caucasian (76.8%), with a mean age of 19.1 ($SD = 1.40$) years. The GADQ-IV (Newman, et al., 2002) and Penn State Worry Questionnaire (PSWQ; Meyer, Miller, Metzger, & Borkovec, 1990) were administered to 1,665 Penn State University students in order to screen for the presence of elevated worry and GAD. In all, 198 individuals were recruited to the study. GAD status required a GADQ-IV score of 9 or higher and diagnosis of GAD using the Mini International Neuropsychiatric Interview (MINI; Sheehan, et al., 1998). Agreement between GADQ-IV and MINI diagnoses was high using these criteria ($kappa = .75, p < .001$). High-worry control status required that individuals not meet clinical criteria for any Axis I diagnosis on the MINI and that they either score within the diagnostic range on the GADQ-IV (≥ 5.7 , see below) or fall within the upper tertile of scores on the PSWQ. Individuals falling within the diagnostic range on the GADQ-IV who failed to meet GAD criteria via structured interview endorsed significantly elevated and intrusive worry but judged that worry to be controllable and/or not severe enough to cause significant impairment. Healthy control status required that individuals not meet clinical criteria for any Axis I disorder and that they fall below the median score on both the GAQ-IV and the PSWQ.

Healthy Controls accounted for 27.8% ($n = 42$) of the sample, high-worry controls accounted for 21.9% of the sample ($n = 33$), and individuals with GAD accounted for 50.3% ($n = 76$) of the sample. Thirty-two GAD participants met criteria for at least one comorbid disorder

(major depressive disorder, n=9; dysthymia, n=2; panic disorder, n=4; social phobia, n=9, post-traumatic stress disorder n=2; obsessive-compulsive disorder, n=4, anorexia nervosa, n=3).

Exclusion criteria for all groups included the current use of tricyclic and SSRI antidepressants, beta blockers, or benzodiazepines. Beta blockers chronically inhibit beta-adrenergically mediated SNS tone and therefore significantly impact a key dependent variable in the present study. Benzodiazepines and SSRIs have been shown to alter vagal outflow both positively (Tucker, et al., 1997; Tulen, et al., 1994) and negatively (Adinoff, Mefford, Waxman, & Linnoila, 1992; Rissanen, Naukkarinen, Virkkunen, Rawlings, & Linnoila, 1998), whereas tricyclics have been shown to unidirectionally decrease vagal activity (Jakobsen, Hauksson, & Vestergaard, 1984; Mezzacappa, Steingard, Kindlon, Saul, & Earls, 1998; Yeragani, et al., 1992). Twenty-two individuals were excluded from the present study due to current prescribed use of one of these medications. An additional 20 individuals were excluded because they did not meet the criteria set forth for inclusion within the GAD, healthy control, or high-worry control groups. One individual initiated, but did not complete the study protocol and therefore was excluded from the present study.

Procedure

After providing written consent, all participants were administered the Mini International Neuropsychiatric Interview (MINI; Sheehan, et al., 1998), a brief structured interview for the diagnosis of mood, anxiety, substance abuse, and eating disorders, as defined by the DSM-IV (see below). Immediately following the administration of the MINI, participants were asked to provide a saliva sample. Self-adhesive electrodes were then placed at the wrist of the non-dominant hand, as well as on the inner portion of the lower legs, three inches above the ankles.

Participants were then seated in a 4 by 6 foot room equipped with a computer, desk, and chair. While the experimenter sat in an adjacent room, participants sat quietly for a 10-minute adjustment period, during which they were instructed to remain still. A two-minute baseline measurement was then taken. Immediately following the conclusion of the baseline measurement, participants were prompted to indicate on the computer the degree of worry they were experiencing *right now*. Answers were given on a nine-point, Likert-like scale from “none” to “as much as possible.” Having been randomly assigned to the worry or relaxation conditions, participants then received one of the following scripts:

Worry: Pick your most worrisome topic and worry about it as intensely as you can in your usual way for the next few minutes. If at any point your mind wanders off track, simply refocus your thoughts back onto your worry topic.

Relaxation: Shift to breathing from your stomach rather than from your chest. Allow your stomach to rise and fall without expanding your chest. Also, slow your breathing down to a rate slower than usual but not so slow that it is unpleasant or uncomfortable. You might do this by counting from one to three as you breathe in evenly and then again as you evenly exhale.

Participants engaged in the respective induction procedures for three minutes. Immediately following the conclusion of the induction, they were prompted to indicate the degree to which they felt worried and relaxed. As before, responses were given on a nine-point Likert-like scale. After a brief instructional session, in which the experimenter introduced the Paced Auditory Serial Addition Task (PASAT; see below) to the participants and allowed them to complete 10 practice items, the first iteration of the PASAT was completed with an interstimulus interval of three seconds. This was followed by the second iteration of the PASAT, with an interstimulus interval of two seconds. After each presentation of the PASAT,

participants were prompted to indicate the degree of worry they were experiencing. Five minutes after the completion of the second PASAT iteration, a second saliva sample was taken. Finally, participants completed a 20 minute recovery phase, during which time they completed a set of auxiliary measures and sat quietly. At the conclusion of the recovery phase a final saliva measurement was taken and participants were asked to indicate how worried they felt *right now*, using the 9-point Likert-like scale described above.

This study was approved by the Institutional Review Board of Penn State University.

Diagnostic Instruments

Generalized Anxiety Disorder Questionnaire-IV (GADQ-IV; Newman, et al., 2002). The GADQ-IV is a 9-item self-report measure designed to diagnose GAD based on DSM-IV criteria. Participants with GADQ-IV scores > 9 were recruited to the study as potential GAD participants, whereas individuals exhibiting scores < 5 were recruited to the study as potential control participants. Newman et al. (2002) demonstrated that while the optimal balance between sensitivity and specificity is found at a cutoff of 5.7, the GADQ-IV demonstrates 97% specificity, with a false positive rate of only 4% at scores of 9 or above. Using Receiver Operating Characteristic analyses, the GADQ-IV showed 89% specificity and 83% sensitivity when compared to structured interview diagnoses of individuals with GAD, social phobia, panic disorder, and non-anxious controls. The GADQ-IV also demonstrated retest reliability, convergent and discriminant validity, and kappa agreement of .67 with a structured interview (Newman, et al., 2002). College students diagnosed with GAD by the GADQ-IV were not significantly different from a GAD community sample on the PSWQ, and both groups had significantly higher PSWQ scores than students identified as not meeting criteria for GAD, demonstrating the clinical validity of the GADQ-IV.

MINI (Sheehan, et al., 1998). The MINI is based on diagnostic criteria from the DSM-IV and ICD-10 and has been validated against the Structured Clinical Interview for DSM Disorders (SCID; First, Spitzer, Gibbon, & Williams, 1997) and Composite International Diagnostic Interview (CIDI; Robins, et al., 1988). It is designed to be utilized as a short but accurate structured clinical interview for clinical trials and epidemiology studies. Kappa agreement with SCID diagnoses ranged from .50 (simple phobia) to .90 (anorexia), with a .70 for GAD. Sheehan et al., (1998) found sensitivity on the MINI to be 70% or greater for all diagnoses but dysthymia and obsessive-compulsive disorder (67% and 62% respectively), while specificities were 85% or greater for all diagnoses (Sheehan, et al., 1998). The MINI was administered by undergraduate research assistants, trained by an advanced doctoral student in clinical psychology and supervised by a licensed clinical psychologist.

Video recordings were made of each diagnostic interview so that a second rater could independently assess whether participants met criteria for Axis I disorders. Reliability was excellent across all disorders on the MINI ($kappa = .89, p < .001$) and for the GAD diagnosis specifically ($kappa = .95, p < .001$).

Measures and Assays

HR and HRV. The electrocardiogram (ECG) signal was continuously recorded at 500 hertz throughout the duration of the study. The ECG waveform was subjected to a band pass filter with a low cutoff of .5 Hz and a high cutoff of 35 Hz. A raw tachogram was then generated in order to inspect the ECG waveform for anomalies. Such anomalies were cleaned by simply raising or lowering a given R spike so that the *AcqKnowledge* 4.1 software algorithm was able to appropriately detect the beat-to-beat intervals. When the ECG waveform was free of anomalies, a separate HR channel was generated, from which the mean HR for each period was calculated.

Respiratory sinus arrhythmia (RSA), the fluctuation of heart rate related to the frequency of respiration is a widely used and non-invasive method of estimating vagal tone. It has been demonstrated that in the process of respiration, vagal cardiomotor neurons are inhibited during inspiration and activated during expiration (Richter & Spyer, 1990). This inhibition and excitation of vagal nerve traffic results in heart period variations that fluctuate within a frequency band between .15 and .40 Hz (Task Force, 1996). Fourier transformation of the interbeat intervals in the electrocardiogram signal can yield a periodogram from which the spectral power of the respiratory band can be estimated and modeled as RSA. Thus, a fast-Fourier transformation was applied to the RR intervals of the ECG signal for the baseline, induction, PASAT 3", PASAT 2", and recovery periods, yielding periodograms for each. The average spectral power in the high-frequency band (.15 Hz to .40 Hz) for each period was then calculated and used as a proxy for parasympathetic activity.

Cortisol. Saliva was collected on three occasions: 1. at the conclusion of the diagnostic interview, 2. five minutes after the conclusion of the second PASAT presentation, and 3. twenty minutes after the conclusion of the second PASAT presentation.

All saliva samples were collected between 11:00 am and 4:00 pm. Each sample consisted of approximately 200- 500 ul of whole unstimulated passive drool. Following Granger et al. (2007), participants were asked to imagine that they were chewing their favorite food and move their jaws as if they were chewing, allowing saliva to pool under their tongue. Once a pool had developed they were instructed to gently force the sample through a short plastic straw into a 2 mL cryogenic vial. Samples were kept cold (on ice) and then frozen at -20 c. Saliva samples were assayed for cortisol using a highly sensitive enzyme immunoassay US FDA (510k) cleared for use as an in vitro diagnostic measure of adrenal function (Salimetrics, State College, PA).

The test used 25 μl of saliva (for singlet determinations). Further, the test had a lower limit of sensitivity of .007 $\mu\text{g}/\text{dl}$, and a range of sensitivity from .007 to 1.8 $\mu\text{g}/\text{dl}$, and average intra- and inter-assay coefficients of variation of less than 5 and 10%.

Salivary alpha-amylase. Sympathetic nervous system activity was measured via salivary alpha-amylase (sAA) level. Although electrodermal activity indices such as skin conductance level (SCL) are some of the most widely used measures of SNS activity, several researchers have raised concerns about the generalizability of SCL findings to the SNS as a whole (Duncko, Makatsori, Fickova, Selko, & Jezova, 2006; Hoehn-Saric, et al., 1989; Thayer, et al., 1996), arguing that EDA is a potentially anomalous sympathetic system, given its cholinergic, as opposed to adrenergic innervations. Skin conductance is sensitive to a wide range of stimulus parameters, such as novelty, intensity, and significance (Dawson, Schell, & Filion, 2007) and responds equally to both positively and negatively valenced stimuli (Lang, Greenwald, Bradley, & Hamm, 1993). Although such characteristics make SCL a useful and flexible index for the measurement of SNS responses to various stimuli and laboratory paradigms, the lack of specificity underlying the activation of the electrodermal system may be problematic.

Salivary alpha-amylase (sAA) is a salivary enzyme involved in the digestion of starch in the oral cavity that has been shown to reflect stress-related changes in arousal (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Granger, Kivlighan, el-Sheikh, Gordis, & Stroud, 2007). Secretion of sAA is mediated by adrenergic mechanisms in the SNS (Anderson, et al., 1984; Batzri & Selinger, 1973; Ehlert, Erni, Hebisch, & Nater, 2006; Skov Olsen, et al., 1988; van Stegeren, Rohleder, Everaerd, & Wolf, 2006). Salivary alpha-amylase reflects changes in SNS activity in response to mental arithmetic (Noto, Sato, Kudo, Kurata, & Hirota, 2005), the Trier Social Stress Test (Nater, et al., 2006; Nater, et al., 2005; Rohleder, Nater, Wolf, Ehlert, &

Kirschbaum, 2004), and passive viewing of aversive video and still images (Takai, et al., 2004; van Stegeren, et al., 2006). In addition to being highly sensitive to laboratory stressors, sAA levels reflect trait-like features such as chronic stress (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007), and depression (Rohleder, Chen, Wolf, & Miller, 2008). Further evidence for the association between sAA and SNS activity comes from pharmacological studies. Beta-adrenergic receptor blockade reduces stress-induced sAA increases (van Stegeren, et al., 2006), whereas administration of the alpha-2-adrenergic receptor antagonist yohimbine hydrochloride significantly increases sAA levels relative to a placebo condition (Ehlert, et al., 2006).

Saliva samples were assayed for sAA by kinetic reaction assay (Granger, Blair, et al., 2007).

PASAT

The PASAT is an effective psychological stressor, uniquely suited to the study of ANS functioning in GAD. Although the perseverative and pathological functions of worry prescribe that it is a non-productive process, worry in the non-pathological realm has been conceptualized as an attempt at constructive mental problem solving (Brosschot, et al., 2006). In one study, nearly 50% of daily worries across both high and low worriers were attempts at problem solving (Szabo & Lovibond, 2002). The PASAT is a serial-addition task used to assess working memory, divided attention, and information processing speed. In this task, a random series of single-digit numbers 1-9 is presented, and participants are required to add consecutively presented numbers such that each number is added to the number that immediately preceded it. At the end of each trial, the interstimulus interval – the time between each digit presentation – is decreased. The PASAT has been shown to reliably induce psychological stress during laboratory inductions (Diehr, et al., 2003; Lejuez, Kahler, & Brown, 2003).

Results

Approach to linear mixed-effect models.

The present study examined the trajectories of HR, HRV, cortisol, and sAA during and in response to the induction of worry versus relaxation. Linear mixed effect models – often referred to as hierarchical linear models or random effects models – were constructed in order to examine these relative trajectories. For each dependent variable a separate model was constructed for each group, in order to examine the function of induced worry versus relaxation on healthy controls, high-worry controls, and individuals with GAD before, during, and following the application of a laboratory stressor. For both HR and HRV, mixed-effect models were constructed using a piecewise analysis of time, as opposed to a single time coefficient. Piecewise analyses allow for the representation of discrete multiple time periods by modeling separate variables (and therefore separate coefficients and slopes) for these periods. Piece 1 represented the period from baseline to the conclusion of the induction, Piece 2 represented the period from the conclusion of the induction until the conclusion of the PASAT 2”, and Piece 3 represented the period from the conclusion of the PASAT 2” until the conclusion of the recovery period.

The number of salivary observations precluded the representation of these measures in piecewise fashion. Thus, for the model representing the interaction between cortisol and sAA described below, a linear and quadratic term were employed in order to represent the onset during stress and offset during recovery of the sympathoexcitatory-HPA axis stress response.

Within each group, and for each dependent variable, a null model was first constructed with fixed effects for time (Piece 1, Piece 2, and Piece 3 for HR and HRV, linear and quadratic for sAA) and a random effect for between-subjects variation at the intercept. Random effects for

time were iteratively added to each model and tested for significance via chi-square difference tests. For each model, only those random effects found to be significant were retained.

Preliminary results: Baseline differences between groups.

Multivariate analyses of variance (MANOVAs) were run to test for baseline differences between groups. Group differences were examined in HR, HRV, cortisol, sAA, and scores for the GADQ-IV and PSWQ. No significant group differences were found in HR ($F(2,146) = 2.18$, $p = .12$, $\eta^2 = .03$), HRV ($F(2,146) = 2.06$, $p = .13$, $\eta^2 = .03$), cortisol ($F(2,148) = .69$, $p = .51$, $\eta^2 = .01$) or sAA ($F(2,148) = 1.99$, $p = .14$, $\eta^2 = .03$). Significant group effects were found for the GADQ-IV ($F(2,150) = 162.01$, $p < .001$, $\eta^2 = .69$), PSWQ ($F(2,150) = 126.94$, $p < .001$, $\eta^2 = .64$), and state worry at baseline ($F(2,150) = 17.96$, $p < .001$, $\eta^2 = .18$). Post hoc analyses revealed significant differences in the GADQ-IV between all groups (all $ps < .001$), wherein the GAD group exhibited the highest scores, followed by the high-worry controls and then the healthy controls. Significant differences were likewise found between all groups on the PSWQ. Here the mean differences between the GAD and healthy control group and between the high-worry and healthy control groups were both significant at $p < .001$ ($MDs = 29.18$ and 23.41 respectively). The mean difference between the GAD and high-worry control groups was substantially smaller ($MD = 5.77$) and significant at the .01 level.

Manipulation check – degree of worry.

In order to assess the effect of the experimental inductions on levels of worry, piecewise linear mixed effect models were constructed wherein condition by time interactions were modeled for the induction, stress, and recovery periods (i.e. Piece 1, Piece 2, and Piece 3).

An intercept only linear mixed effect models was conducted for the total sample, as no significant random variation was found within the slopes for Pieces 1-3. Main effects for Piece 1

($\beta = -.09$, $SE = .05$, $t = -1.64$, $p = .10$, $d = .19$) and condition ($\beta = -.18$, $SE = .36$, $t = -.49$, $p = .62$, $d = .06$) were non-significant, whereas the main effects for Piece 2 ($\beta = .15$, $SE = .03$, $t = 5.18$, $p < .001$, $d = .60$) and Piece 3 ($\beta = -.06$, $SE = .01$, $t = -5.83$, $p < .001$, $d = .67$) were significant. Interactions between condition and Piece 1 ($\beta = .67$, $SE = .08$, $t = 8.77$, $p < .001$, $d = 1.01$) and condition and Piece 2 ($\beta = -.36$, $SE = .04$, $t = -9.08$, $p < .001$, $d = 1.05$) were likewise significant, while the interaction between condition and Piece 3 was found to be non-significant ($\beta = .02$, $SE = .02$, $t = 1.58$, $p = .12$, $d = .18$). All tests of group differences in worry trajectories, either by Piece ($ts = .02 - 1.00$, $ps = .32 - .99$), or Piece x condition ($ts = .01 - 1.56$, $ps = .13 - .99$) were non-significant. These results indicate that the experimental induction of worry generated significant increases in worry during the induction period and significant decreases in worry during the stress period, with no significant effect on level of worry during the recovery period. In contrast, participants in the relaxation condition exhibited no significant changes in degree of worry during the induction period, significant increases in worry during the stress period and significant decreases in worry during the recovery period. Figure 1 displays the relative trajectories of worry within the relaxation and worry conditions.

Heart Rate

We first examined the degree of physiological flexibility in HR responses within healthy control, GAD, and high-worry control groups. Trajectories for each group were examined by condition, in order to test the effect of experimentally induced worry versus relaxation.

Healthy control group. An intercept only linear mixed effect models was conducted for the healthy control group, as no significant random variation was found within the slopes for Pieces 1-3. Main effects for Piece 1 ($\beta = .07$, $SE = .36$, $t = .20$, $p = .84$, $d = .04$) and condition ($\beta = -.61$, $SE = 4.10$, $t = -.15$, $p = .88$, $d = .03$) were non-significant. Slopes for Piece 2 ($\beta = .66$, SE

= .19, $t = 3.53$, $p = .001$, $d = .77$) and Piece 3 ($\beta = -.32$, $SE = .07$, $t = -4.50$, $p < .001$, $d = .98$) were significant. Interactions between condition and Piece 1 ($\beta = -.04$, $SE = .51$, $t = -.09$, $p = .93$, $d = .02$), 2 ($\beta = .05$, $SE = .27$, $t = .17$, $p = .87$, $d = .04$), and 3 ($\beta = .04$, $SE = .10$, $t = .39$, $p = .70$, $d = .09$) were all non-significant. Thus, while there was no main effect for change in HR during the induction period, there was a significant increase in HR during the stress period, and a significant decrease in HR during the recovery period, with no significant difference between individuals in the worry and relaxation conditions at baseline. Moreover, condition did not significantly moderate the slopes for Piece 1, 2, or 3.

GAD group. Random effects at intercept and in the slopes for change in HR over Piece 2 and Piece 3 were found to be significant and retained within the model. Consistent with the healthy control group, main effects for Piece 1 ($\beta = .001$, $SE = .18$, $t = .01$, $p = .99$, $d = .002$) and condition ($\beta = 4.16$, $SE = 2.70$, $t = 1.54$, $p = .13$, $d = .25$) were non-significant, while slopes for Piece 2 ($\beta = .99$, $SE = .16$, $t = 6.32$, $p < .001$, $d = 1.02$) and Piece 3 ($\beta = -.36$, $SE = .06$, $t = -5.64$, $p < .001$, $d = .91$) were significant. However, while slopes for the interactions between condition and Pieces 1 ($\beta = .22$, $SE = .26$, $t = .83$, $p = .41$, $d = .13$) and 3 ($\beta = .16$, $SE = .09$, $t = 1.70$, $p = .09$, $d = .28$) were non-significant, contrary to the healthy control group, the interaction between condition and Piece 2 ($\beta = -.52$, $SE = .23$, $t = -2.26$, $p = .03$, $d = .37$) was significant. Thus, within the GAD group there was no significant change in HR during the induction period, regardless of condition, yet the induction of worry precluded robust elevations in HR during the stress period when compared to the relaxation condition. There were no significant differences in HR at baseline between the two conditions and no effect of condition on the slope for change in HR during recovery. Figure 1 illustrates the effect of induced worry versus relaxation on the trajectory for HR.

High-worry control group. Random effects at intercept and in the slope for change in HR over Piece 3 were found to be significant and retained within the model. Once again, main effects for Piece 1 ($\beta = .07$, $SE = .18$, $t = .38$, $p = .71$, $d = .09$) and condition ($\beta = 3.20$, $SE = 2.10$, $t = 1.52$, $p = .14$, $d = .37$) were non-significant, while those for Piece 2 ($\beta = .84$, $SE = .09$, $t = 9.03$, $p < .001$, $d = 2.22$) and Piece 3 ($\beta = -.35$, $SE = .04$, $t = -8.43$, $p < .001$, $d = 2.08$) were significant. Regarding the interaction effects, only the interaction between condition and Piece 1 ($\beta = .11$, $SE = .27$, $t = .42$, $p = .68$, $d = .10$) was non-significant. The interactions between condition and both Piece 2 ($\beta = -.35$, $SE = .13$, $t = -2.59$, $p = .01$, $d = .64$) and Piece 3 ($\beta = .14$, $SE = .06$, $t = 2.28$, $p = .03$, $d = .56$) were significant. Therefore, within the high-worry control group, the induction of worry precluded robust elevations in HR during the stress period and inhibited HR recovery during the recovery period. There were no significant differences in HR between conditions at baseline. Figure 1 illustrates the effect of induced worry versus relaxation on the trajectory for HR.

Heart rate variability (RSA).

We next examined the degree of physiological flexibility in RSA each of the three groups. Trajectories for each group were again examined by condition, in order to test the effect of experimentally induced worry versus relaxation. An intercept only linear mixed effect models was conducted for all groups, as no significant random variation was found within the slopes for Pieces 1-3.

Healthy control group. Main effects for Piece 1 ($\beta = .01$, $SE = .007$, $t = 1.67$, $p = .10$, $d = .36$) and condition ($\beta = -.006$, $SE = .06$, $t = -.12$, $p = .90$, $d = .003$) were non-significant. Slopes for both Piece 2 ($\beta = -.02$, $SE = .004$, $t = -4.98$, $p < .001$, $d = 1.09$) and Piece 3 ($\beta = .006$, $SE = .001$, $t = 4.29$, $p < .001$, $d = .94$) were significant. Interactions between condition and Piece

1 ($\beta = -.01$, $SE = .009$, $t = -1.18$, $p = .24$, $d = .26$), 2 ($\beta = .005$, $SE = .005$, $t = .93$, $p = .36$, $d = .20$), and 3 ($\beta = .0006$, $SE = .002$, $t = .29$, $p = .77$, $d = .06$) were all non-significant. Thus, while there was no main effect for change in PNS activity during the induction period, there was a significant reduction in PNS level during the stress period, and a significant increase in PNS level during the recovery period, with no significant difference between individuals in the worry and relaxation conditions at baseline. Moreover, induction condition did not significantly moderate the slopes for Piece 1, 2, or 3.

GAD group. As in the healthy control group, main effects for Piece 1 ($\beta = .02$, $SE = .009$, $t = 1.83$, $p = .07$, $d = .30$) and condition ($\beta = -.14$, $SE = .08$, $t = -1.67$, $p = .10$, $d = .27$) were non-significant. Also consistent with the healthy control group, slopes for both Piece 2 ($\beta = -.04$, $SE = .005$, $t = -8.28$, $p < .001$, $d = 1.34$) and Piece 3 ($\beta = .01$, $SE = .002$, $t = 5.26$, $p < .001$, $d = .85$) were significant. Interactions between condition and Piece 1 ($\beta = -.02$, $SE = .01$, $t = -1.18$, $p = .24$, $d = .19$) and Piece 3 ($\beta = -.002$, $SE = .003$, $t = -.81$, $p = .42$, $d = .13$) were non-significant. However, the interaction between condition and Piece 2 ($\beta = -.02$, $SE = .007$, $t = -2.20$, $p = .03$, $d = .37$) was significant. Thus, while the induction of worry did not affect PNS activity during the induction period, it did preclude a robust vagal withdrawal during the stress period when compared to the relaxation condition. Figure 1 depicts the interaction between condition and time on the trajectory for HFPSD within the GAD group.

High-worry control group. Consistent with both other groups, main effects for Piece 1 ($\beta = .01$, $SE = .02$, $t = .90$, $p = .37$, $d = .20$) and condition ($\beta = .005$, $SE = .13$, $t = .04$, $p = .97$, $d = .01$) were non-significant, while slopes for both Piece 2 ($\beta = -.03$, $SE = .008$, $t = -3.74$, $p < .001$, $d = .92$) and Piece 3 ($\beta = .01$, $SE = .003$, $t = 3.03$, $p = .005$, $d = .75$) were significant.

Interactions between condition and Piece 1 ($\beta = -.01$, $SE = .02$, $t = -.59$, $p = .56$, $d = .15$), 2 ($\beta =$

.009, $SE = .01$, $t = .79$, $p = .44$, $d = .19$), and 3 ($\beta = -.005$, $SE = .004$, $t = -1.11$, $p = .28$, $d = .27$) were all non-significant. As with the healthy control group, there was a significant reduction in PNS level during the stress period, and a significant increase in PNS level during the recovery period, with no significant difference between individuals in the worry and relaxation conditions at baseline, and the induction condition did not significantly moderate the slopes for Piece 1, 2, or 3.

Moderating effect of cortisol availability on sympathetic stress response, by condition.

In order to examine the permissive and suppressive effects of available cortisol on sympathetic stress response a linear mixed effect model was constructed for each condition within each group with linear and quadratic effects for time and interactions between time, time-varying cortisol, and condition. Both cortisol and sAA were log-transformed to correct for non-normal distributions.

Healthy control group. Random effects at intercept and in the slope for change in sAA over time were found to be significant and retained within models for relaxation and worry conditions. Results for the moderating effect of available cortisol on sympathetic stress response in healthy controls are provided in table 2. Interactions between cortisol and both linear and quadratic effects for time were significant within the relaxation condition. While neither main nor moderated effects of linear or quadratic time were significant within the worry condition, effect sizes for the interactions between cortisol and linear time and cortisol and quadratic time were $d = .50$ and $d = .44$ respectively. Given the small sample size per condition in the healthy control group ($n=21$ per condition), these results may therefore be interpreted, albeit with some caution. Altogether, within the worry condition, greater levels of cortisol promoted a significant increase in sympathetic arousal during the stress period and a significant decrease in sympathetic

arousal during recovery with no effects seen in the presence of low available cortisol.

Conversely, in the relaxation condition, the opposite was true. Here, lower levels of cortisol led to increases in sympathetic arousal during the stress period, and a significant decrease in sympathetic arousal during the recovery period, with no significant changes in SNS arousal observed in the presence of elevated cortisol. Moderation effects in the relaxation condition were significant at the $p < .005$ level, with large effect sizes ($ds = .95$ and $.94$). Figure 4 depicts the moderating effect of available cortisol on the sympathetic stress response within the worry and relaxation conditions for healthy controls.

GAD group. Random effects at intercept and in the slope for change in sAA over time were found to be significant and retained within the model. Results for the moderating effect of available cortisol on sympathetic stress response in GAD participants are provided in table 2. Consistent with the healthy control group, for GAD participants in the worry condition, greater available cortisol predicted a significant increase in sympathetic arousal during the stress period and a significant decrease in sympathetic arousal during the recovery period. However, contrary to the healthy controls, for GAD participants in the worry condition lesser available cortisol predicted a significant decrease in sympathetic arousal across the duration of the study. In the relaxation condition, both main and moderated effects for quadratic time were found to be non-significant ($\beta = .0001$, $SE = .0002$, $t = .52$, $p = .61$, $d = .11$, and ($\beta = -.00007$, $SE = .0003$, $t = .25$, $p = .80$, $d = .06$, respectively). The model was thus rerun with only linear effects for time. Results indicated that within the relaxation condition, greater levels of cortisol promoted a significant decrease in sympathetic arousal across the duration of the study and lesser levels of cortisol predicted a significant increase in sympathetic arousal across the duration of the study.

Figure 4 depicts the moderating effect of available cortisol on the sympathetic stress response within the worry and relaxation conditions for GAD participants.

High-worry control group. Random effects at intercept and in the slope for change in sAA over time were found to be significant and retained within the model. All main and moderating effects were found to be non-significant ($ps = .95 - .30$). Thus, within the high-worry control group, no significant increases or decreases in sympathetic arousal were observed for the stress or recovery periods, nor were levels of sympathetic arousal significantly moderated by the level of available cortisol.

Discussion

The present study examined the trajectories of HR, HRV, cortisol, and sAA preceding, during, and following a psychological stressor. In order to examine the relative contributions of state worry, trait worry, and clinically-diagnosed GAD to deficits in autonomic and HPA systems, a three by two design was employed wherein healthy controls, high-worry controls, and individuals with GAD were randomly assigned to receive experimental inductions of either worry or relaxation following baseline and preceding the psychological stressor. State levels of worry were recorded at baseline, following the induction of worry versus relaxation, after the 3rd interstimulus iteration of the PASAT, after the 2nd interstimulus interval iteration of the PASAT, and after a 20-minute recovery period. Thus, we employed a strong inference approach (Platt, 1964) to assessing the independent and additive effects of state worry, trait worry, and clinically-diagnosed GAD to ANS and HPA reactivity to laboratory stress.

A piecewise linear mixed effect model revealed that, for those who received the worry induction, worry significantly increased during the induction period, significantly decreased during the stress period, and showed no significant change during recovery. For those

individuals who received the relaxation induction, there was no change in worry observed during the induction period, a significant increase in worry over the stress period and a significant decrease in worry during the recovery period. These data reveal that the experimental induction of worry succeeded in generating increased levels of worry, and that this induction precluded significant increases in worrying in response to the stressor. These findings were consistent across all three groups.

Piecewise analysis of HR trajectories revealed no effect of worry or relaxation on HR during the induction period in any of the three groups. Additionally, the induction of worry versus relaxation had no impact on changes in HR during the stress period or recovery in the healthy control group. For healthy controls there was a significant increase in HR during the stress period and a significant decrease in HR during recovery across both conditions. For both the GAD and high-worry groups, the experimental induction of worry precluded robust increases in HR during the stress period. Condition had no additional effects on individuals with GAD during the recovery, as these individuals exhibited significant decreases in HR across both conditions. However, in the high-worry controls, the effects of induced worry appear to have carried over into the recovery period, significantly inhibiting HR recovery during this time.

RSA trajectories for healthy controls were consistent with, and thus the inverse of, those found for HR. Across both conditions, healthy controls showed no change in RSA during the induction period, a significant decrease in RSA during the stress period, and a significant increase in RSA during recovery. Results for GAD participants were also consistent with those found for HR. Individuals with GAD did not exhibit significant changes in RSA during the induction period however, the induction of worry precluded robust parasympathetic withdrawal during the stress period when compared to the relaxation condition. There was no moderating

effect of condition on RSA recovery during the recovery period; GAD participants exhibited a significant increase in RSA during recovery across both conditions. RSA trajectories for high-worry control participants were inconsistent with those found for HR. High-worry controls exhibited no change in RSA during the induction period, a significant decrease in RSA during the stress period, and a significant increase in RSA during recovery. There were no moderating effects of condition on any period. Thus, while the experimental induction of worry reduced both HR response and recovery, there were no corresponding effects of worry on parasympathetic inhibitory signals in the high-worry control group.

Results for both the GAD and high-worry groups are consistent with prior findings that worry precludes HR response in anxious individuals (Borkovec & Hu, 1990; Borkovec, et al., 1993) and inhibits robust parasympathetic withdrawal in response to a laboratory stressor (Llera & Newman, 2010; Lyonfields, et al., 1995; Thayer, et al., 1996). However, our findings were inconsistent with Lyonfields et al. (1995) and Thayer et al. (Thayer, et al., 1996), as we did not observe any significant effects of worry on cardiac or PNS response in our healthy control group. Importantly, we observed coordination of cardiac and parasympathetic systems for healthy controls and persons with GAD. That is, for both of these groups increases in HR were mirrored by decreases in RSA for each period and across both conditions. Of note, the significant effect of worry on diminished physiological flexibility in HR response in the high-worry controls was not mirrored by a significant effect of worry on vagal withdrawal during the stressor or vagal recovery.

Finally, we examined the permissive and suppressive effects of available cortisol on the sympathetic stress response by condition. To this end we constructed mixed effect models with linear and quadratic effects of time and interactions between time and time-varying cortisol for

each condition, within each group. For healthy control participants who received the worry induction, higher levels of cortisol predicted a significant increase in SNS arousal in response to the stressor and a significant decrease in SNS arousal following recovery. However, for healthy control participants in the relaxation condition, *lesser* levels of cortisol predicted a significant increase in SNS arousal in response to the stressor and a significant decrease in SNS arousal following recovery, whereas greater levels of cortisol predicted no change in SNS arousal across the duration of the study. These disparate results are likely due to an interaction between the aversive versus pleasant nature of the worry and relaxation inductions and the glucocorticoid response latency. While catecholamine secretion by the SNS and corresponding releases of corticotrophin releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) by the HPA axis occur within seconds of stress initiation, the stimulation of glucocorticoid secretion typically takes more than 10 minutes to reach its peak (Sapolsky, et al., 2000).

Thus, for healthy controls in the worry condition, cortisol secretion likely began in earnest during the worry induction and was available to enhance the SNS stress response. Conversely, for healthy controls in the relaxation condition, the PASAT represented the first encounter with an aversive stimulus within the study. Thus, the amplitude of the SNS stress response to the PASAT would likely have been mediated more directly by sympathetic systems. The decay of the stress responses in the two conditions support such an interpretation: both the onset and decay of the SNS response have pronounced slopes (simple slopes = .61 and -.56 respectively) in the relaxation condition, whereas the SNS arousal decay in the worry condition was approximately two times smaller in magnitude of effect ($d = .44$ versus $.94$) and slope (simple slopes = $-.31$ versus $-.56$).

Consistent with healthy controls, greater available cortisol predicted a significant increase in SNS arousal in response to the stressor and a significant decrease in SNS arousal following recovery, for GAD participants in the worry condition. However, contrary to healthy controls, lower levels of cortisol predicted a significant decline in SNS arousal across the duration of the study. Thus, the lack of available cortisol precluded the initiation of a SNS stress response. Additionally, for GAD participants in the relaxation condition, greater available cortisol predicted a significant decrease in SNS arousal across the duration of the study, while lesser levels of cortisol predicted a significant increase in SNS arousal across the duration of the study. Thus, for GAD participants in this condition, we observed a breakdown in the coordination of the SNS and HPA axis stress responses. Glucocorticoids such as cortisol facilitate and enhance the initial SNS response to stress (Sapolsky, et al., 2000) however; through their inhibition of inflammatory immune systems and negative feedback system, these hormones also serve a vital role in limiting the overall stress response and preventing it from overshooting. The failure of cortisol to facilitate a downregulation in the stress response of GAD participants in the relaxation condition potentially leaves these individuals vulnerable to increased immunological inflammation and chronic elevations in sympathetic arousal.

Interestingly, we did not observe any significant main or moderating effects for changes in sympathetic arousal in the high-worry control group. Thus, once again results for the high-worry control group were inconsistent with both the GAD and healthy control groups. It is notable that, overall, the physiological trajectories of high-worry controls did not align with either GAD or healthy control groups. For instance, only high-worry controls exhibited deficits in cardiac recovery extending from the experimental induction of worry. These individuals also exhibited a dissociation of cardiac and RSA responses, with diminished physiological flexibility

in HR, but not in RSA. Finally, whereas both healthy controls and GAD participants in the worry condition exhibited significant SNS responses to the stressor, high-worry controls showed no significant changes in sympathetic arousal, regardless of condition or study period. Thus, within both target processes – parasympathetically-mediated cardiac regulation and sympathetic-HPA stress response – high-worry controls exhibited idiosyncratic patterns of responding that neither mirrored GAD nor healthy control trajectories.

A possible explanation for the idiosyncratic response profile of the high-worry control group is that this group may have been composed of a clinically heterogeneous population with latent, as yet unexpressed clinical disorders other than GAD. As noted above, worry has been implicated in nearly all mood and anxiety disorders (Barlow, 2004; Brosschot, et al., 2006). Thus, it is plausible that the high-worry control group was comprised of individuals in prodromal stages of heterogeneous Axis I disorders. In this event, underlying cognitive and physiological phenomena related to panic disorder, social phobia, or major depressive disorder would potentially contribute to the observed idiosyncratic response pattern observed for this group.

Studies of physiological dysfunction in GAD have focused primarily on rigidity resulting from diminished physiological flexibility and have skewed heavily towards assessments of cardiac and parasympathetic systems. In addition, the phenomenon of worry has been hypothesized as a casual factor in diminished physiological flexibility and has been shown to be related to impaired HR and HRV in healthy controls in laboratory and ambulatory studies. Moreover, Brosschot and colleagues (2006) have hypothesized that perseverative cognition plays an important role in translating transient stressors into chronic morbidity and even mortality. The present study contained two important innovations in the study of the relationships between GAD, worry, and negative health outcomes. First, we utilized a high-worry control group and an

experimental induction of worry in order to separately control for state and trait worry. Thus, we could better identify whether the construct of worry or the greater clinical syndrome of GAD was responsible for ANS rigidity and diminished physiological flexibility. Secondly, the present study marks the first time that the interactive effects of the SNS and HPA axis were modeled within a GAD population. Here we propose that deficits in the coordination of the sympathoexcitatory-HPA stress response may represent an additional contributing factor to negative health outcomes in GAD.

Regarding the relative roles of state and trait worry, and clinical GAD we found that phasic worry did not significantly impact cardiac or parasympathetic stress responses or recovery in healthy controls. The experimental induction of worry precluded robust increases in HR in both GAD and high-worry control groups, but precluded robust parasympathetic withdrawal in the GAD group only. Thus, coordinated diminished physiological flexibility in parasympathetic cardiac inhibitory control was observed in GAD but not in high-trait-worry. Regarding the coordination of the SNS-HPA stress response, the present study found that greater levels of cortisol promoted the adaptive onset and decay of the SNS stress response in GAD participants in the worry condition, but that cortisol failed to facilitate a downregulation of sympathetic arousal in the relaxation condition. Additionally, for GAD participants in the worry condition with lower levels of available cortisol, there was a failure to initiate a sympathetic stress response altogether. Thus, within both target processes – parasympathetically-mediated cardiac regulation and sympathetic-HPA stress response – we found evidence that the clinical GAD syndrome predicted greater physiological impairment.

Altogether, the present study represents a potential bridge between findings that worry in healthy populations is related to diminished physiological flexibility and prolonged impact of

transient stressors (Brosschot, et al., 2007; Pieper, et al., 2007) and the recent finding that GAD diagnosis predicted a 60% increase in negative cardiac events, above and beyond the presence of comorbid disorders and important behavioral and biological parameters such as age, weight, level of exercise, and smoking behavior (Martens, et al., 2010). Although phasic worry did not affect PNS-mediated cardiac responses in healthy controls, it did promote the onset of a SNS-mediated stress response in these individuals. Moreover, high-worry controls with significant elevations in trait worry, but nevertheless free of Axis I pathology, exhibited impaired cardiac responses to stress after the experimental induction of worry. In addition, the induction of worry in high-worry controls precluded the engagement of a sympathoexcitatory response to the laboratory stressor. Thus, the present study provides support for the hypothesis that phasic worry can have deleterious effects in non-clinical populations. However, in addition to exhibiting impaired parasympathetic and cardiovascular responses to stress following worry, individuals with GAD exhibited impaired coordination of SNS-HPA axis activation in two ways: lower levels of cortisol following worry predicted a failure to initiate a sympathetic stress response and in the relaxation condition, available cortisol failed to promote the downregulation of sympathetic arousal.

The inability of glucocorticoid concentrations to successfully promote the offset of the sympathetic stress response could negatively impact health in at least two ways. First, it could lead to chronically elevated sympathetic arousal, which in turn could promote physiological rigidity. Secondly, the SNS-mediated stress response heightens immunological functioning, including the activation and distribution in the body of inflammatory cytokines. Ineffective inhibition of immunological inflammation could result in chronic inflammatory states which, in turn, could contribute to various morbidities such as hypertension and atherosclerosis. Whereas

Martens et al. (2010) and Frasure-Smith and Lesperance (2008) were able to report the cardiovascular risk represented by the GAD diagnosis, these authors noted that they could not suggest a potential mechanism by which this risk was incurred. The findings of the present study potentially point to a possible mechanism that could account for the cardiac morbidity reported by these authors.

One possible weakness of the current study is the method of recruitment and its impact on the generalizability of the present findings. GAD participants were screened and recruited from a large undergraduate subject pool. Although GAD status required agreement between a self-report diagnostic inventory and a structured clinical interview, these individuals were not treatment-seeking and represented a limited age range. Furthermore, although diagnosis required that they endorse their worry as uncontrollable and their distress as significantly impairing, it is unknown whether these individuals held such beliefs about their experiences when unprompted. However, Suls and Bunde (2005) note that anxiety may play a more important role in cardiopathogenesis than in the progression of already established cardiovascular disease. Thus, it could be argued that the population utilized for the present study is a strength as research on younger, premorbid participants may facilitate the delineation of potential mechanisms by which to identify possible at risk populations. Nevertheless, further research should seek to replicate these findings with treatment-seeking clinical populations from a community sample. Additionally, the present sample was predominantly female and Caucasian. Replication with more diverse populations would further strengthen the generalizability of the present findings.

CHAPTER 2

State, trait, and clinical worry: Necessary versus sufficient causes of impaired vagal cardiac control in generalized anxiety disorder and implications for increased adrenergic tone.

Generalized anxiety disorder (GAD) is the most commonly occurring anxiety disorder and one of the most highly co-occurring disorders in the diagnostic and statistical manual of mental disorders-fourth edition (DSM-IV; Kessler, et al., 2005; Maier, et al., 2000). A chronic and disabling disorder, GAD is associated with substantial personal, societal, and economic costs (Ballenger, et al., 2001; Wittchen, 2002). Those with GAD are among the heaviest users of primary care, specialty clinic, and emergency room services, contributing considerably to the medical cost associated with anxiety disorders in the US (Fogarty, Sharma, Chetty, & Culpepper, 2008; Jones, Ames, Jeffries, Scarinci, & Brantley, 2001; Mehl-Madrona, 2008; Ormel, et al., 1994; Wittchen, 2002). Moreover, GAD has been shown to be a risk factor for cardiovascular disease such as cardiac ischemia, sudden cardiac death, and myocardial infarction (Frasure-Smith & Lesperance, 2008; Martens, et al., 2010).

Importantly, individuals with GAD have been shown to exhibit reduced parasympathetic nervous system (PNS) tone, often referred to as vagal tone (Lyonfields, et al., 1995; Thayer, et al., 1996). Vagal tone represents the outflow of nervous traffic along the 10th cranial (Vagus) nerve, which innervates the viscera. Heart rate (HR) is chronically inhibited by afferent vagal signals at the sinoatrial node. These inhibitory signals are necessary for cardiac stability and flexibility. Dysregulation of these effects has been shown to limit appropriate cardiac responsiveness (Levy, 1990; Verrier, 1987). Diminished vagally mediated heart rate variability (HRV) has been shown to predict congestive heart failure and hypertension (Curtis & O'Keefe, 2002), myocardial infarction (Bigger, Fleiss, Rolnitzky, & Steinman, 1993) and sudden cardiac

death (Martin, et al., 1987; Mølgaard, Sørensen, & Bjerregaard, 1991), and has been used as a proxy for cardiovascular disease altogether (Stys & Stys, 1998). Low tonic HRV has likewise been shown to be a significant risk factor for all-cause mortality (Tsuji, et al., 1994), whereas survival after myocardial infarction (Stein et al., 1994) was positively related to greater levels of HRV.

Worry, the cardinal feature of GAD, has likewise been associated with reduced vagal tone and HRV. Work by Thayer and colleagues demonstrated that laboratory inductions of worry lead to reduced vagal tone in nonanxious controls as well as individuals with GAD (Lyonfields, et al., 1995; Thayer, et al., 1996). Additionally, worry generated higher HR and lower HRV when compared to a resting baseline in 43 healthy males (Hofmann, et al., 2005), as well as when compared to relaxation in 53 healthy adults of mixed gender (Verkuil, et al., 2009). Finally, Brosschot, Van Dijk, and Thayer (2007) recently showed in a 24-hour ambulatory study of 52 healthy individuals that worry frequency predicted higher HR and lower HRV during waking, whereas worry duration predicted lesser HRV during both waking and sleeping; and Pieper, Brosschot, Van Der Leeden, and Thayer (2007) similarly found in a 4-day ambulatory study that worry predicted elevated HR and decreased HRV in 73 Dutch secondary school teachers. Taken together, these studies suggest a possible causal association between worrisome thinking and reduced vagal tone and increased HR. Although worry has been largely ignored as a potential predictor of outcome in studies of stress, coping, and disease (Brosschot, et al., 2006), Kubzansky et al. (1997) found that worry proneness predicted incidence of myocardial infarction and fatal coronary heart disease (CHD) during a 20-year follow up period in the Normative Aging Study.

Brosschot and colleagues have recently argued that worry is a mediator, rather than a moderator of the effects of psychological and psychosocial stressors on long-term health (Brosschot, et al., 2006; Brosschot, et al., 2007; Thayer & Lane, 2002). These authors argue that worry maintains the presence of passing stressors by maintaining the cognitive representation and thereby the physiological presence of the stressor, prolonging the impact of that stressor on the body. This hypothesis would suggest a role for worry as a causal mechanism in the promotion of cardiovascular disease processes. Given that both GAD (Frasure-Smith & Lesperance, 2008; Martens, et al., 2010) and worry (Kubzansky, et al., 1997) have been associated with hard endpoints of cardiac morbidity and mortality, a mechanistic understanding of the relative contributions of clinical and non-clinical worry to cardiovascular health could help to identify the necessary and sufficient factors contributing to cardiovascular disease.

Although historically research on autonomic correlates of anxiety has been dominated by SNS phenomena (Friedman, 2007; Lader, 1975), research on GAD and worry has skewed heavily toward vagal tone and HRV. In fact, recent conceptualizations of GAD have contended that the disorder is marked by an absence or even a suppression of sympathetic arousal and accompanying symptomatology (Brown, et al., 1998; Watson, 2005). Symptoms of autonomic hyperarousal – as they were characterized in the DSM, third edition, revised (DSM-III-R; American Psychiatric Association, 1987) – were removed from the diagnostic criteria for GAD in the DSM-IV (American Psychiatric Association, 1994). These symptoms, including palpitations or accelerated HR, dry mouth, dizziness, and lightheadedness were removed because they were found to be the least reliable and most infrequently endorsed of the GAD criteria from the DSM-III-R (Marten, et al., 1993). However, Fisher, Granger, and Newman (2010) recently demonstrated that these symptoms are significantly elevated in at least a subset of the GAD

population and that the severity of such symptoms was directly related to concurrent elevations in adrenergic sympathetic tone. Indeed, Curtis and O'Keefe (2002) assert that elevated HR and reduced HRV are the result of chronic and excessive sympathetic tone. Whether elevations in sympathetic tone are prevalent in GAD is an important question. Over time, chronic or sustained SNS arousal represents an excessive energy demand that can tax the organism (Thayer & Lane, 2007), leading to deleterious increases in inflammation and suppression of immunological functioning (Sapolsky, et al., 2000).

Debates about the relative importance of sympathetic or parasympathetic systems in anxiety broadly or GAD specifically ultimately rely on false dichotomizations of autonomic functioning. Berntson, Cacioppo, and Quigley (1991) have demonstrated that the traditional doctrine of autonomic reciprocity, in which autonomic functioning lies along a single continuum from sympathetic to parasympathetic control, is insufficient for representing the ANS and that – at minimum – a two-dimensional space is required. Thus, investigations of autonomic effects on target organs should endeavor to include parallel measurements of both the SNS and PNS. Sympathetic and parasympathetic effects can be coupled or uncoupled and, in the case of the former, reciprocal or nonreciprocal (Berntson, et al., 1991). Early research demonstrated that coactivation of the PNS and SNS contributed to sexual functioning (Root & Bard, 1947) and response to emotional stress (Gellhorn, Cortell, & Feldman, 1941). More recently it has been shown that mild hypoxia and hypercapnia – both concomitants of anxious states – can provoke sustained coactivation of PNS and SNS cardiac efferents (Fukuda, Sato, Suzuki, & Trzebski, 1989; Koizumi, Terui, & Kollai, 1983; Kollai & Koizumi, 1979), and that chronotropic control of HR can be subject to coactivation of excitatory SNS and inhibitory PNS effects in response to stressful conditioned stimuli (Obrist, Wood, & Perez-Reyes, 1965) and attentional demands

(Quigley & Berntson, 1990). Thus, these findings point to the importance of concurrent measurement of sympathetic and parasympathetic systems in evaluating dually innervated organs such as the heart.

Nevertheless, the independent and concurrent measurement of the two branches of the ANS is complicated by methodological challenges in assessing parasympathetic effects. Vagal tone is not directly measurable in humans. Although tonic and phasic levels of vagal tone can be inferred from pharmacologic blockade (Berntson, et al., 1991; Taylor, Myers, Halliwill, Seidel, & Eckberg, 2001), less invasive and more readily available techniques for estimation of vagal efferents have been sought. Respiratory sinus arrhythmia (RSA), the fluctuation of heart rate related to respiratory oscillations is a widely used and non-invasive method of estimating vagal tone. In the process of respiration, vagal cardiomotor neurons are inhibited during inspiration and activated during expiration (Richter & Spyer, 1990). This inhibition and excitation of vagal nerve traffic results in heart period variations that fluctuate within a frequency band between .15 and .40 Hz (Task Force, 1996). Thus, Fourier transformation of the interbeat intervals in the electrocardiogram signal can yield a periodogram from which the spectral power of the respiratory band can be estimated and modeled as RSA.

The purity of RSA an index of vagal tone has been argued to result from the relative response latencies of sympathetic and parasympathetic signals. Whereas, vagal signals operate on an order of milliseconds, sympathetic signals exhibit a latency of 1 – 2 seconds (Karemaker, 1985). Thus, it has been assumed that sympathetic chronotropic effects on HR are too slow to affect the high-frequency modulations associated with respiration (Berntson, Cacioppo, & Quigley, 1993) and it has commonly been reported that SNS influences on RSA roll off at ~ .15 Hz (Task Force, 1996; Saul, 1990). However, Taylor et al. (2001) have demonstrated that the

influence of sympathetic outflow on .14-.50 Hz spectral power is ~ 56% and can be as high as 90%. Importantly, these authors stress a considerable degree of individual variation in this effect, observing a range of sympathetic restraint of vagally-mediated RSA from 0 to 90%. Additionally, the neuromodulator neuropeptide Y can inhibit vagal innervations of the heart (Hall & Potter, 1990). Neuropeptide Y is released from cardiac sympathetic nerve terminals, and its inhibitory effect on vagal tone is typically found in instances of moderate to high sympathetic activity (Hall & Potter, 1990; Warner & Levy, 1989). Finally, due to sympathetic-parasympathetic interactions, moderate to large changes in sympathetic outflow can have significant impact on vagal tone and the measurement of RSA (Berntson, Cacioppo, & Grossman, 2007). Thus, because of its multiply determined nature, Berntson et al. (2007) note that RSA should not be considered a one-to-one representation of vagal tone.

Echoing the call of Berntson et al. (2007) we argue that the multiply determined nature of RSA precludes the assumption that this index can be held as a direct representation of vagal tone. We therefore adopt the terminology of “vagal cardiac control” offered by these authors and propose a novel method for modeling this effect. As noted above, parasympathetic efferents conducted via the vagus nerve chronically inhibit heart rate. Higher levels of parasympathetic influence are reflected in lower heart rate and lower levels of parasympathetic influence reflected in higher heart rate. Note that we stress the *influence* of the parasympathetic nervous system via vagal efferents and not the absolute level of parasympathetic activity. As mentioned above, the ANS is a two dimensional system, wherein sympathetic activity can be complementary or competitive to parasympathetic activity. This is an important distinction in dually innervated organs such as the heart, where the influence of the two branches of the ANS can be coupled or uncoupled, reciprocal or nonreciprocal, and where coactivation of both branches has been

empirically observed (Berntson, et al., 1991). Assessing only absolute levels of one branch of the ANS does not account for possible coactivation of a target organ. Thus, effects of the measured branch could be over or underestimated. Moreover, RSA, the gold standard for noninvasive measurement of parasympathetic effects, has been shown to be subject to sympathetic influence (Taylor, et al., 2001).

To operationalize vagal cardiac control we argue that one must represent not only the mere level of RSA but the relationship between RSA and heart rate. In the present study the construct “vagal cardiac control” is operationalized as the linear relationship between RSA and HR. This relationship is represented as $HR \sim \beta_1(RSA) + e$, wherein the coefficient β_1 represents vagal cardiac control. The degree of negative correlation between HR and RSA reflects adaptive vagal cardiac control, whereas the degree to which this relationship is less negative – flatter – demonstrates poorer vagal cardiac control. The influences of worry or group status (see below) on vagal cardiac control are represented as moderation effects, moderating the linear relationship between HR and RSA.

Although moderators are characterized statistically by interaction terms, they can be understood conceptually as the *direct effect* of the moderator on the *relationship* between two constructs. Baron and Kenny note that a moderator is a “variable that affects the direction and/or strength of the relation between an independent or predictor variable and a dependent or criterion variable” (pg. 1174; 1986). As we have indicated here, vagal cardiac control is operationalized as the relationship between RSA and HR, represented as the coefficient β_1 .

The aim of the present study was to use strong inference (Platt, 1964) to assess the necessary versus sufficient contributions of state, trait, or clinical worry (GAD) to impaired vagal cardiac control. Strong inference is a method of inductive reasoning and scientific inquiry

by which multiple alternative hypotheses are devised and an experiment is conceived and carried out with multiple possible outcomes, each of which will potentially rule out one or more of the hypotheses. Prior evidence points to three potential hypotheses: 1. phasic increases in worry are sufficient to promote impaired vagal cardiac control, 2. conditions of high trait worry are necessary to promote impaired vagal cardiac control, or 3. the clinical syndrome of GAD is necessary to promote impaired vagal cardiac control. Thus, the present study measured phasic worry at baseline, in response to a mental arithmetic stressor, and following a 20-minute recovery period in healthy controls, persons with diagnosed GAD, and high-worry controls. The lattermost group demonstrated significant elevations in worry that placed them within a clinical range, but did not meet clinical criteria for GAD or any other Axis I disorder as determined by structured interview.

Given the unique contribution of GAD to cardiovascular disease recently reported by Martens et al. (2010), we hypothesized that those with GAD would exhibit impaired vagal cardiac control above and beyond the effects of state or trait worry. Moreover, consistent with Curtis and O'Keefe's (2002) assertion that reduced parasympathetically-mediated HRV is the result of elevated sympathetic tone, we hypothesized that impairments in vagal cardiac control would be the result of elevations in adrenergic sympathetic tone in those with GAD.

Method

Participants

Participants ($n=151$) were mostly female (65.6%) and Caucasian (76.8%), with a mean age of 19.1 ($SD = 1.40$) years. The GADQ-IV (Newman, et al., 2002) and Penn State Worry Questionnaire (PSWQ; Meyer, et al., 1990) were administered to 1,665 Penn State University students in order to screen for the presence of elevated worry and GAD. In all, 198 individuals

were recruited to the study. GAD status required a GADQ-IV score of 9 or higher and diagnosis of GAD using the Mini International Neuropsychiatric Interview (MINI; Sheehan, et al., 1998). Agreement between GADQ-IV and MINI diagnoses was high using these criteria ($kappa = .75, p < .001$). High-worry control status required that individuals not meet clinical criteria for any Axis I diagnosis on the MINI and that they either score within the diagnostic range on the GADQ-IV (≥ 5.7 , see below) or fall within the upper tertile of scores on the PSWQ. Individuals falling within the diagnostic range on the GADQ-IV who failed to meet GAD criteria via structured interview endorsed significantly elevated and intrusive worry but judged that worry to be controllable and/or not severe enough to cause significant impairment. Healthy control status required that individuals not meet clinical criteria for any Axis I disorder and that they fall below the median score on both the GADQ-IV and the PSWQ.

Healthy Controls accounted for 27.8% ($n = 42$) of the sample, high-worry controls accounted for 21.9% of the sample ($n = 33$), and individuals with GAD accounted for 50.3% ($n = 76$) of the sample. Thirty-two GAD participants met criteria for at least one comorbid disorder (major depressive disorder, $n=9$; dysthymia, $n=2$; panic disorder, $n=4$; social phobia, $n=9$, post-traumatic stress disorder $n=2$; obsessive-compulsive disorder, $n=4$, anorexia nervosa, $n=3$).

Exclusion criteria for all groups included the current use of tricyclic and SSRI antidepressants, beta blockers, or benzodiazepines. Beta blockers chronically inhibit beta-adrenergically mediated SNS tone and therefore significantly impact a key dependent variable in the present study. Benzodiazepines and SSRIs have been shown to alter vagal outflow both positively (Tucker, et al., 1997; Tulen, et al., 1994) and negatively (Adinoff, et al., 1992; Rissanen, et al., 1998), whereas tricyclics have been shown to unidirectionally decrease vagal activity (Jakobsen, et al., 1984; Mezzacappa, et al., 1998; Yeragani, et al., 1992). Twenty-two

individuals were excluded from the present study due to current prescribed use of one of these medications. An additional 20 individuals were excluded because they did not meet the criteria set forth for inclusion within the GAD, healthy control, or high-worry control groups. One individual initiated, but did not complete the study protocol and therefore was excluded from the present study.

Procedure

After providing written consent, all participants were administered the Mini International Neuropsychiatric Interview (MINI; Sheehan, et al., 1998), a brief structured interview for the diagnosis of mood, anxiety, substance abuse, and eating disorders, as defined by the DSM-IV (see below). Immediately following the administration of the MINI, participants were asked to provide a saliva sample. Self-adhesive electrodes were then placed at the wrist of the non-dominant hand, as well as on the inner portion of the lower leg, three inches above the ankle. A respiration belt was centered just below the solar plexus and fitted firmly around the torso.

Participants were then seated in a 4 by 6 foot room equipped with a computer, desk, and chair. While the experimenter sat in an adjacent room, participants sat quietly for a 10-minute adjustment period, during which they were instructed to remain still. A two-minute baseline measurement was then taken. Immediately following the conclusion of the baseline measurement, participants were prompted to indicate on the computer the degree of worry they were experiencing *right now*. Answers were given on a nine-point, Liker-like scale from “none” to “as much as possible.” A worry versus relaxation induction period then followed. A more detailed description of this procedure, and analyses thereof, can be found in Chapter 1. Notably, results of Chapter 1 demonstrated that the phasic variability captured in continuously measured

state worry accounted for the variance in physiological response patterns attributable to the categorical induction of worry versus relaxation.

Following the induction period, participants were given a brief instructional session, in which the experimenter introduced the Paced Auditory Serial Addition Task (PASAT) and provided 10 practice items. The first iteration of the PASAT was completed with an interstimulus interval of three seconds. This was followed by the second iteration of the PASAT, with an interstimulus interval of two seconds. After each presentation of the PASAT, participants were prompted to indicate the degree of worry they were experiencing. Five minutes after the completion of the second PASAT iteration, a second saliva sample was taken. Finally, participants completed a 20 minute recovery phase, during which time they completed a set of auxiliary measures and sat quietly. At the conclusion of the recovery phase a final saliva measurement was taken and participants were asked to indicate how worried they felt a final time.

This study was approved by the Institutional Review Board of Penn State University.

Diagnostic Instruments

Generalized Anxiety Disorder Questionnaire-IV (GADQ-IV; Newman, et al., 2002). The GADQ-IV is a 9-item self-report measure designed to diagnose GAD based on DSM-IV criteria. Participants with GADQ-IV scores > 9 were recruited to the study as potential GAD participants, whereas individuals exhibiting scores < 5 were recruited to the study as potential control participants. Newman et al. (2002) demonstrated that while the optimal balance between sensitivity and specificity is found at a cutoff of 5.7, the GADQ-IV demonstrates 97% specificity, with a false positive rate of only 4% at scores of 9 or above. Using Receiver Operating Characteristic analyses, the GADQ-IV showed 89% specificity and 83% sensitivity

when compared to structured interview diagnoses of individuals with GAD, social phobia, panic disorder, and non-anxious controls. The GADQ-IV also demonstrated retest reliability, convergent and discriminant validity, and kappa agreement of .67 with a structured interview (Newman, et al., 2002). College students diagnosed with GAD by the GADQ-IV were not significantly different from a GAD community sample on the PSWQ, and both groups had significantly higher PSWQ scores than students identified as not meeting criteria for GAD, demonstrating the clinical validity of the GADQ-IV.

MINI (Sheehan, et al., 1998). The MINI is based on diagnostic criteria from the DSM-IV and ICD-10 and has been validated against the Structured Clinical Interview for DSM Disorders (SCID; First, et al., 1997) and Composite International Diagnostic Interview (CIDI; Robins, et al., 1988). It is designed to be utilized as a short but accurate structured clinical interview for clinical trials and epidemiology studies. Kappa agreement with SCID diagnoses ranged from .50 (simple phobia) to .90 (anorexia), with a .70 for GAD. Sheehan et al., (1998) found sensitivity on the MINI to be 70% or greater for all diagnoses but dysthymia and obsessive-compulsive disorder (67% and 62% respectively), while specificities were 85% or greater for all diagnoses (Sheehan, et al., 1998). The MINI was administered by undergraduate research assistants, trained by an advanced doctoral student in clinical psychology and supervised by a licensed clinical psychologist.

Video recordings were made of each diagnostic interview so that a second rater could independently assess whether participants met criteria for Axis I disorders. Reliability was excellent across all disorders on the MINI ($kappa = .89, p < .001$) and for the GAD diagnosis specifically ($kappa = .95, p < .001$).

Measures and Assays

Heart Rate. ECG was continuously recorded at 500 hertz throughout the duration of the study. The ECG waveform was subjected to a band pass filter with a low cutoff of .5 Hz and a high cutoff of 35 Hz. A raw tachogram was then generated in order to inspect the ECG waveform for anomalies. Such anomalies were cleaned by simply raising or lowering a given R spike so that the *AcqKnowledge* 4.1 software algorithm was able to appropriately detect the beat-to-beat intervals. When the ECG waveform was free of anomalies, a separate HR channel was generated, from which the mean HR for each period was calculated.

High-frequency power spectral density (RSA). A fast-Fourier transformation was applied to the RR intervals of the ECG waveform for the baseline, PASAT 2”, and recovery periods, yielding periodograms for each. The average spectral power in the high-frequency band (.15 Hz to .40 Hz) for each period was then calculated and used as a proxy for RSA.

Salivary alpha-amylase. Salivary alpha-amylase (sAA) is a salivary enzyme involved in the digestion of starch in the oral cavity that has been shown to reflect stress-related changes in arousal (Chatterton, et al., 1996; Granger, Kivlighan, el-Sheikh, et al., 2007). Secretion of sAA is mediated by adrenergic mechanisms in the SNS (Anderson, et al., 1984; Batzri & Selinger, 1973; Ehlert, et al., 2006; Skov Olsen, et al., 1988; van Stegeren, et al., 2006). Salivary alpha-amylase reflects changes in SNS activity in response to mental arithmetic (Noto, et al., 2005), the Trier Social Stress Test (Nater, et al., 2006; Nater, et al., 2005; Rohleder, et al., 2004), and passive viewing of aversive video and still images (Takai, et al., 2004; van Stegeren, et al., 2006). In addition to being highly sensitive to laboratory stressors, sAA levels reflect trait-like features such as chronic stress (Nater, et al., 2007), and depression (Rohleder, et al., 2008). Further evidence for the association between sAA and SNS activity comes from pharmacological

studies. Beta-adrenergic receptor blockade reduces stress-induced sAA increases (van Stegeren, et al., 2006), whereas administration of the alpha-2-adrenergic receptor antagonist yohimbine hydrochloride significantly increases sAA levels relative to a placebo condition (Ehlert, et al., 2006).

Saliva was collected on three occasions: 1. at the conclusion of the diagnostic interview, 2. five minutes after the conclusion of the second PASAT presentation, and 3. 20 minutes after the conclusion of the second PASAT presentation. All saliva samples were collected between 11:00 am and 4:00 pm. Each sample consisted of approximately 200- 500 ul of whole unstimulated passive drool. Following Granger et al. (2007), participants were asked to imagine that they were chewing their favorite food and move their jaws as if they were chewing, allowing saliva to pool under their tongue. Once a pool had developed they were instructed to gently force the sample through a short plastic straw into a 2 mL cryogenic vial. Samples were kept cold (on ice) and then frozen at -20 c. Saliva samples were assayed for sAA by kinetic reaction assay (Granger, Blair, et al., 2007).

PASAT

The PASAT is a serial-addition task used to assess working memory, divided attention, and information processing speed. In this task, a random series of single-digit numbers 1-9 is presented, and participants are required to add consecutively presented numbers such that each number is added to the number that immediately preceded it. At the end of each trial, the interstimulus interval – the time between each digit presentation – is decreased. The PASAT has been shown to reliably induce psychological stress during laboratory inductions (Diehr, et al., 2003; Lejuez, et al., 2003).

Results

Preliminary analyses: Baseline variables.

A three-way multivariate analysis of variance (MANOVA) was run to test for group differences in baseline variables. No significant effects were found for group differences in HR ($F(2,145) = 2.18, p = .12, \eta^2 = .03$), RSA ($F(2,145) = 2.06, p = .13, \eta^2 = .03$), or BMI ($F(2,145) = 1.20, p = .30, \eta^2 = .02$). Significant group effects were found for the GADQ-IV ($F(2,145) = 162.01, p < .001, \eta^2 = .69$), PSWQ ($F(2,145) = 126.94, p < .001, \eta^2 = .64$), and state worry at baseline ($F(2,145) = 17.96, p < .001, \eta^2 = .18$). Post hoc analyses revealed significant differences in the GADQ-IV between all groups (all $ps < .001$), wherein the GAD group exhibited the highest scores, followed by the high-worry controls and then the healthy controls. Significant differences were likewise found between all groups on the PSWQ. Here the mean differences between the GAD and healthy control group and between the high-worry and healthy control groups were both significant at $p < .001$ ($MDs = 29.18$ and 23.41 respectively). The mean difference between the GAD and high-worry control groups was substantially smaller ($MD = 5.77$) and significant at the .01 level.

Effect of trait and state moderators on vagal cardiac control.

In order to examine study hypotheses related to trait and state contributions to impaired vagal cardiac control we chose to pursue a two-step regression approach. In the first step main effects for RSA, GAD status, and high-worry control status, and the interactions between RSA and GAD and RSA and high-worry were modeled via regression analysis. In the second step, the main effect for state worry and the interaction between state worry and RSA was added to the model. This allowed us to determine the effects of GAD status and non-clinical, high-trait worry

on vagal cardiac control independent of variability in state worry, and then to assess the additive impact on the model represented by phasic worry.

Baseline. Table 1 provides the regression results for the effect of trait and state moderators on vagal cardiac control at baseline. In the initial step, greater RSA significantly predicted lower HR. In the GAD group, but not the high-worry group, there was a significant reduction in this relationship demonstrating impaired vagal cardiac control. In the second step, state worry did not significantly moderate the relationship between RSA and HR, however, controlling for state worry significantly reduced the variance accounted for by the moderating effect of GAD, reducing the effect size from $d = .57$ to $d = .42$. Moreover, when controlling for state worry, the effect sizes for GAD and high-worry status were comparable ($ds = .42$ and $.45$ respectively).

Following stress task. In the initial step greater levels of RSA significantly predicted lower HR ($\beta = -10.61$, $SE = 1.76$, $t = -6.19$, $p < .001$, $d = .71$). Main effects for GAD status ($\beta = -.43$, $SE = 2.00$, $t = -.22$, $p = .83$, $d = .04$) and high-worry control status ($\beta = 1.24$, $SE = 2.46$, $t = .50$, $p = .62$, $d = .12$) were non-significant. In addition interactions between GAD and RSA ($\beta = 3.19$, $SE = 2.03$, $t = 1.58$, $p = .12$, $d = .26$) and high-worry and RSA ($\beta = -1.03$, $SE = 2.92$, $t = -.35$, $p = .73$, $d = .09$) were both non-significant. The addition of the main effect for state worry ($\beta = -.39$, $SE = .37$, $t = -1.04$, $p = .30$, $d = .12$) and interaction between state worry and RSA ($\beta = .02$, $SE = .34$, $t = .06$, $p = .95$, $d = .007$) were both non-significant. Controlling for state worry did not have any meaningful impact on the main effects for RSA ($\beta = -10.88$, $SE = 1.76$, $t = -6.18$, $p < .001$, $d = .71$), GAD status ($\beta = .49$, $SE = 2.17$, $t = .23$, $p = .82$, $d = .04$), or high-worry status ($\beta = 1.62$, $SE = 2.54$, $t = .64$, $p = .52$, $d = .16$), nor did it impact interactions between GAD

and RSA ($\beta = 3.47, SE = 2.26, t = 1.54, p = .13, d = .25$) or high worry and RSA ($\beta = -.41, SE = 3.04, t = -.14, p = .89, d = .03$).

Recovery. Table 2 provides the regression results for the effect of trait and state moderators on vagal cardiac control following the 20 minute recovery period. In the initial step, greater RSA significantly predicted lower HR. As with measurements at baseline, there was a significant reduction in this relationship in the GAD group, but not the high-worry group, demonstrating impaired vagal cardiac control in the former. Whereas at baseline the inclusion of state worry in the second step reduced the effect of GAD on impaired vagal cardiac control – redistributing a portion of this variance to phasic worry – following the recovery period a suppression effect was observed. Here the inclusion of state worry was significant and predicted *greater* vagal cardiac control. In addition, controlling for state worry strengthened the impairing effect of GAD on vagal cardiac control, increases the size of this effect from $d = .37$ to $d = .56$.

Post-hoc analysis of moderating effect of state worry on vagal cardiac control in GAD. Given the presence of a suppression effect in the final model, whereby controlling for phasic levels of worry increased the moderating effect of GAD status on vagal cardiac control, we conducted a post-hoc analysis of the moderating effect of state worry on vagal cardiac control in the GAD group. The main effect for RSA was significant ($\beta = -4.90, SE = 1.65, t = -2.98, p = .004, d = .54$) and the main effect for state worry non-significant ($\beta = -1.20, SE = 1.70, t = -.71, p = .48, d = .13$). However, the interaction between RSA and state worry was found to be significant ($\beta = -3.55, SE = 1.34, t = -2.65, p = .01, d = .48$), indicating that, following the recovery period, greater levels of phasic worry predicted *greater* vagal cardiac control within the GAD group.

Moderating effect of SNS activity on vagal cardiac control in GAD group.

To test the hypothesis that impaired vagal cardiac control in GAD was related to elevated levels of SNS activity, we examined the moderating effect of sAA on vagal cardiac control in the GAD group at baseline, following the stress task, and following the recovery period.

Baseline. The main effect for RSA was significant ($\beta = -10.62$, $SE = 1.05$, $t = -10.12$, $p < .001$, $d = 1.65$) and the main effect for sAA was non-significant ($\beta = 1.72$, $SE = .90$, $t = 1.91$, $p = .06$, $d = .31$). The interaction between the two was significant ($\beta = 3.45$, $SE = 1.17$, $t = 2.96$, $p = .004$, $d = .48$), indicating that, at baseline, higher levels of SNS activity were related to impaired vagal cardiac control.

Following stress task. The main effect for RSA was significant ($\beta = -8.61$, $SE = 1.19$, $t = -7.24$, $p < .001$, $d = 1.18$) and the main effect for sAA was non-significant ($\beta = 1.46$, $SE = 1.19$, $t = 1.23$, $p = .22$, $d = .20$). Consistent with baseline, the interaction between the two was significant ($\beta = 3.16$, $SE = 1.33$, $t = 2.37$, $p = .02$, $d = .39$), indicating that, following the stress task, higher levels of SNS activity continued to be related to impaired vagal cardiac control.

Recovery. Consistent with baseline and stress periods, the main effect for RSA was significant ($\beta = -8.06$, $SE = 1.50$, $t = -5.38$, $p < .001$, $d = .88$), the main effect for sAA was non-significant ($\beta = 1.60$, $SE = 1.41$, $t = 1.14$, $p = .26$, $d = .19$), and the interaction between the two was significant ($\beta = 6.00$, $SE = 1.68$, $t = 3.56$, $p < .001$, $d = .58$). Notably, the effect of SNS level on vagal cardiac control was most pronounced at recovery. Figure 2 illustrates the moderating effect of SNS activity on vagal cardiac control in the GAD group following recovery.

Discussion

The present study provided innovations or extensions of prior findings in three domains. First, we demonstrated that vagal cardiac control can be effectively represented as the linear

relationship between HR and RSA and that such a representation accommodates potential confounds or interference in the quantification of vagal tone via RSA by modeling the degree of inhibitory control of RSA on HR instead of treating RSA as directly analogous to vagal tone. Secondly we found that the clinical GAD syndrome contributed to impaired vagal cardiac control above and beyond phasic variability in state worry and significant elevations in trait worry; and finally, we showed that decrements in vagal cardiac control in GAD were directly related to increased adrenergic sympathetic tone.

Despite the consistent use of RSA as a direct proxy for vagal tone, research has shown that this index is influenced by parameters other than vagal outflow, including adrenergic sympathetic tone (Berntson, et al., 2007; Taylor, et al., 2001). Thus, the degree of vagal cardiac control cannot be directly assessed by RSA. We presented a method of estimating vagal cardiac control whereby this effect was modeled as the *relationship* between RSA and HR in the linear regression $HR \sim \beta_1(RSA) + e$. Thus the coefficient β_1 represented vagal cardiac control and all direct effects of independent variables on vagal cardiac control were modeled as moderators of the relationship between RSA and HR. This approach to modeling vagal cardiac control accommodates the potential influence of factors that might interfere with the purity of the RSA/vagal tone relationship. That is, the degree to which the correlation between HR and RSA is negative represents effective inhibitory vagal cardiac control, and the degree to which this effect is less negative – flatter – represents impaired vagal cardiac control. Moreover, we were able to demonstrate that reductions in vagal cardiac control were directly related to increased adrenergic sympathetic tone.

The present study used strong inference (Platt, 1964) to assess the necessary versus sufficient contributions of state, trait, and clinical worry (GAD) to impaired vagal cardiac control

at baseline, following a stressor, and following a 20-minute recovery period. Study participants – including individuals meeting clinical criteria for GAD via structured interview, healthy controls, and high-worry controls with significant elevations in trait worry – rated their level of worry throughout the study. In addition, measures of HR and RSA were taken at each occasion. Preliminary analyses revealed no group differences in BMI, HR, or RSA at baseline. Significant groups differences were found in GAD and worry severity via the GADQ-IV and PSWQ respectively. Both high-worry controls and GAD participants exhibited significantly higher GADQ-IV and PSWQ scores than healthy controls.

Those with GAD scored substantially higher on the GADQ-IV, but only modestly higher on the PSWQ than high-worry controls. Thus, the clinical GAD group exhibited greater GAD symptomatology including irritability, difficulty controlling worry, muscle tension, distress, functional impairment and feelings of restlessness, being keyed up or on edge. However, these groups did not differ greatly in amount or severity of worry. In fact, when controlling for the single GADQ-IV item “Do you find it difficult to control your worry (or stop worrying) once it starts?,” the difference between participants with GAD and high-worry controls became non-significant on the PSWQ ($t = 1.61, p = .11$) and the effect size for the difference between the two groups was reduced from $d = .44$ to $d = .26$. Thus, it can be inferred that the substantive difference in worry between the two groups was a function of the *controllability* of worry.

A two-step regression approach was taken whereby in the first step group status moderated the effect of RSA on HR. The interaction between state worry and RSA was added in the second step. At baseline, GAD status predicted a significant reduction in vagal cardiac control, but high-worry status did not. When state worry was added to the model, it was found to be non-significant as either a main or moderating effect. However, the inclusion of state worry

seemed to partially mitigate the effect of GAD status, reducing the effect size from $d = .57$ to $d = .42$. Although this effect remained significant, after the inclusion of state worry the effect for the GAD group was roughly equivalent to the high-worry group ($ds = .42$ and $.45$ respectively). Thus, the non-significant effect of high-worry status on vagal cardiac control at baseline should be taken with some caution. Given that the size of the high-worry group was less than half the size of the GAD group ($n = 33$ and 76 respectively), a lack of power might have precluded finding significant effects in this group. Thus, individuals with significant elevations in trait worry may exhibit impaired vagal cardiac control at baseline. Future research should examine this possibility, ideally within groups larger than the current high-worry sample ($n = 33$).

The PASAT, a mental arithmetic task commonly used for stress inductions, was administered in two iterations, with interstimulus intervals of 3 seconds and then 2 seconds. Regression analysis of vagal cardiac control during the administration of the 2nd PASAT revealed no effect of group status on vagal cardiac control. State worry was also found to have no significant effect on vagal cardiac control. Finally, controlling for the effect of state worry had no effect on the relationships between GAD or high-worry status on vagal cardiac control. Thus, group differences observed at baseline were not maintained during the stress procedure.

Finally, we assessed the effects of state, trait, and clinical worry (GAD) on vagal cardiac control following a 20-minute recovery period. After recovery, only the GAD group exhibited significantly reduced vagal cardiac control. Whereas the high-worry control group had exhibited a non-significant but moderate size effect at baseline ($p = .07$, $d = .45$), following recovery high-worry status had no effect on vagal cardiac control ($p = .79$, $d = .06$). Thus, the high-worry control group exhibited an adaptive recovery from the stressor equivalent to the healthy control group. State worry was found to have a significant impact on vagal cardiac control following

recovery. Of note, greater levels of state worry predicted *increased* vagal cardiac control at recovery. Moreover, the inclusion of state worry in the model created a suppression effect whereby the effect of GAD status on vagal cardiac control was amplified from $d = .37$ to $d = .56$. Given this suppression effect, we conducted a post hoc analysis of the moderating effect of state worry on vagal cardiac control within the GAD group. Here we found that greater levels of state worry during recovery predicted *greater* vagal cardiac control in the GAD group.

Finally, we hypothesized that the impaired vagal cardiac control observed within the GAD group at baseline and following recovery would be accompanied by increased levels of adrenergic sympathetic tone. Thus, we examined the moderating effect of sAA on vagal cardiac control in the GAD group at baseline, during the stress task, and following the recovery period. At all three measurement occasions, greater levels of adrenergic sympathetic tone predicted lesser vagal cardiac control. Notably, the effect of SNS level on vagal cardiac control was most pronounced at recovery.

Both reduced vagal cardiac control and increased adrenergic sympathetic tone are predictors of cardiovascular morbidity and mortality. As noted above, diminished vagally mediated HRV has been shown to predict congestive heart failure and hypertension (Curtis & O'Keefe, 2002), myocardial infarction (Bigger, et al., 1993) and sudden cardiac death (Martin, et al., 1987; Mølgaard, et al., 1991). In addition, chronic sympathetic arousal increases cardiovascular workload and is a risk factor for endothelial dysfunction, left ventricular hypertrophy, and arrhythmias (Curtis & O'Keefe, 2002; Metra, et al., 2000). Conversely, β -blockers reduce the risk of myocardial infarction, sudden cardiac death, and stroke (Peters, 1990). The relative time courses of the SNS and PNS dictate differential levels of flexibility in cardiac responsiveness. Whereas sympathetic signals are relayed on an order of seconds,

parasympathetic signals function on an order of milliseconds. Thus, a parasympathetically-mediated heart is more flexible and responsive a sympathetically-mediated heart more rigid and less responsive. A lack of flexibility in cardiac regulation is potentially problematic, as rigidity in physiological systems has been related to mortality and physical morbidity (Lipsitz & Goldberger, 1992; Peng, et al., 1994).

Little work to date has incorporated adrenergic sympathetic markers in GAD populations (Fisher, et al., 2010). When SNS activity has been measured, it has typically been assessed via electrodermal activity (c.f. Hoehn-Saric, et al., 2004). Although electrodermal activity indices such as skin conductance level (SCL) are some of the most widely used measures of SNS activity, several researchers have raised concerns about the generalizability of SCL findings to the SNS as a whole (Duncko, et al., 2006; Hoehn-Saric, et al., 1989; Thayer, et al., 1996), arguing that EDA is a potentially anomalous sympathetic system, given its cholinergic, as opposed to adrenergic innervations. Skin conductance is sensitive to a wide range of stimulus parameters, such as novelty, intensity, and significance (Dawson, et al., 2007) and responds equally to both positively and negatively valenced stimuli (Lang, et al., 1993). Although such characteristics make SCL a useful and flexible index for the measurement of SNS responses to various stimuli and laboratory paradigms, the lack of specificity underlying the activation of the electrodermal system may be problematic. Moreover, sympathetic signaling at the sinoatrial node and across the myocardium is mediated by alpha- and beta-adrenergic transmission, making interpretations of peripheral cholinergic efferents problematic for investigations of cardiovascular functioning and health. Thus, future studies should seek to incorporate measures of adrenergic sympathetic tone and examine potential effects of SNS arousal on cardiac control.

The present finding, that increases in adrenergic sympathetic tone promote reductions in vagal cardiac control are likely to be interpreted as inconsistent with much of the empirical work on GAD to date. As noted previously, SNS-related symptoms of physiological arousal were removed from the criteria for GAD with the publication of DSM-IV (American Psychiatric Association, 1994). In addition, few studies have examined adrenergic sympathetic indices directly (Fisher, et al., 2010), and baseline differences in HR – a dually innervated process – have consistently been attributed to deficits in RSA (c.f. Hoehn-Saric & McLeod, 2000). However, we have outlined in detail how RSA should not be considered a direct proxy for vagal tone and how both decrements in RSA and increases in HR can result from increases in adrenergic sympathetic tone. Measurements of absolute differences in either sympathetic or parasympathetic markers do not take into account the potential interactive effects of these two systems, including possible coactivation. Additionally, absolute levels of SNS or PNS activity may be secondary to the relative balance between these systems. Thus, if chronic elevations in adrenergic sympathetic tone can be seen to exist *relative* to vagal influences, then the present findings are consonant with the empirical evidence on autonomic functioning in GAD. We offer here a brief outline of how sympathetic arousal may function within GAD.

Individuals with GAD exhibit cognitive biases for threat, in which they interpret ambiguous environmental cues as negative or threatening (Bradley, Mogg, White, Groom, & Bono, 1999; Mathews & MacLeod, 2005; Mogg & Bradley, 2005; Wilson, MacLeod, Mathews, & Rutherford, 2006). Additionally, individuals with GAD exhibit both an increased attention to and superior perception of symptoms of physiological arousal (Andor, Gerlach, & Rist, 2008; Fisher, et al., 2010), demonstrating a cognitive attentional bias for internal stimuli as well. Finally, those with GAD are fearful of the negative consequences of emotions (Mennin,

Heimberg, Turk, & Fresco, 2005; Turk, Heimberg, Luterek, Mennin, & Fresco, 2005) and report greater distress associated with a range of emotions compared to nonanxious controls (Llera & Newman, 2010). Thus, individuals with GAD exhibit a heightened sensitivity to a range of subjectively aversive stimuli resulting from cognitive attentional biases for both internal and external threat cues.

Borkovec and colleagues have proposed that worry is an attempt at problem solving aimed at obviating or removing potential threats (Borkovec, Alcaine, & Behar, 2004). Consistent with this, worry in the non-pathological realm has been conceptualized as an attempt at constructive mental problem solving (Brosschot, et al., 2006). In one study, nearly 50% of daily worries across both high and low trait worriers were attempts at problem solving (Szabo & Lovibond, 2002). In addition, worry precludes emotional processing during engagement with fearful stimuli, consequently suppressing autonomic reactivity (Borkovec, et al., 2004). Myriad evidence has supported the inhibitory effect of worry on emotional and somatic responses to stressors (Borkovec & Hu, 1990; Borkovec, et al., 1993; Llera & Newman, 2010; Lyonfields, et al., 1995; Thayer, et al., 1996; Chapter 1). Worry is negatively reinforced by both “preventing” negative future outcomes and mitigating aversive somatic experiences. Within the GAD syndrome, clinical worry is uncontrollable, perceived to be excessive, and present across domains. Individuals with GAD “often worry about everyday, routine life circumstances” and such worries occur more days than not (American Psychiatric Association, 1994). Thus, within GAD, worry is a ubiquitous and ineffective coping process, utilized for the avoidance of negative emotional and somatic experiences and the mitigation of potential future threats.

The magnitude of physiological responses to stress have been tied to the degree of active engagement with the stressor (Obrist, 1976, 1981). Obrist et al. (1978) demonstrated that task

difficulty was non-linearly related to increases in HR and blood pressure (BP) – increases were largest when the task was difficult as opposed to easy or impossible. Importantly, these increases in cardiovascular activity were reduced under beta-adrenergic pharmacologic blockade, demonstrating that increased adrenergic sympathetic tone was driving cardiovascular arousal. Because individuals with GAD perceive near constant stressors with which to cope, they are likely recruiting active engagement coping systems related to increased adrenergic sympathetic tone throughout the day on a day-to-day basis. Increased adrenergic sympathetic tone was observed in the GAD group at baseline, during the stressor, and following recovery. However, reduced vagal cardiac control compared to healthy controls was not observed during the laboratory stressor. Importantly, the stressor employed in the current study – the PASAT – is a mental arithmetic task that is cognitively challenging and subjectively distressing. Thus, the lack of group differences during the stressor is likely due to increased engagement of active coping systems and concomitant increases in SNS cardiac control across all three groups. That this phenomenon was idiosyncratically related to GAD at resting baseline and recovery indicates that impaired vagal cardiac control in GAD is likely due to chronic engagement of active coping via adrenergic sympathetic arousal.

However, it has been proposed that perceptions of arousal in GAD are met with bouts of worry (Andor, et al., 2008). Because worry inhibits the emotional processing of and somatic reactivity to stressors (Borkovec, et al., 2004; Llera & Newman, 2010) these bouts of worry may serve as a regulatory mechanism, preventing more acute, severe, or distressing physiological experiences. Worry has been shown to interfere with both the strengthening and extinction of anxious associations during repeated exposure and unconditioned stimulus rehearsal (Davey & Matchett, 1994) and has been posited to inhibit the adequate representation of negative stimuli

required for the engagement of an adaptive stress response (Borkovec, et al., 2004; Foa & Kozak, 1986). Moreover, Borkovec and colleagues have shown that attentional avoidance reduces the cardiovascular impact of exposure to fearful stimuli (Borkovec, 1974; Grayson & Borkovec, 1978). Thus, it is plausible that during periods of acute duress worry serves a subjectively protective function for individuals with GAD, precluding full engagement with emotional stimuli and thereby inhibiting reductions in vagal cardiac control. However, it should be emphasized that despite this apparently cardioprotective role of worry *within GAD participants* following the stress procedure, GAD status nevertheless predicted impaired vagal cardiac control compared to both healthy and high-worry controls following recovery. In addition, phasic variability in state worry did not serve a protective function at baseline or during stress. Thus, these transient “protective” effects likely come at the cost of more chronic disruptions in vagal cardiac control and elevations in adrenergic sympathetic tone.

One possible weakness of the current study is the method of recruitment and its impact on the generalizability of the present findings. GAD participants were screened and recruited from a large undergraduate subject pool. Although GAD status required agreement between a self-report diagnostic inventory and a structured clinical interview, these individuals were not treatment-seeking and represented a limited age range. However, it should be noted that the present study was able to identify decreased vagal cardiac control and increased adrenergic sympathetic tone – both strong prospective predictors of cardiovascular morbidity and mortality – in relatively healthy, college-aged participants. Suls and Bunde (Suls & Bunde, 2005)(2005) note that anxiety may play a more important role in cardiopathogenesis than in the progression of already established cardiovascular disease. Thus, it could be argued that the population is a strength of the present study as research on younger, premorbid participants may facilitate the

delineation of potential mechanisms by which to identify possible at risk populations.

Nevertheless, further research should seek to replicate these findings with treatment-seeking clinical populations from a community sample. Additionally, the present sample was predominantly female and Caucasian. Replication with more diverse populations would further strengthen the generalizability of the present findings.

CHAPTER 3

Positive and negative emotional responses to laboratory-induced stress: relation to worry and cardiovascular functioning in generalized anxiety disorder.

Generalized anxiety disorder (GAD) is the most commonly occurring anxiety disorder and one of the most highly co-occurring disorders in the diagnostic and statistical manual of mental disorders-fourth edition (DSM-IV; Kessler, et al., 2005; Maier, et al., 2000). A chronic and disabling disorder, GAD is associated with substantial personal, societal, and economic costs (Ballenger, et al., 2001; Wittchen, 2002). Those with GAD are among the heaviest users of primary care, specialty clinic, and emergency room services, contributing considerably to the medical cost associated with anxiety disorders in the US (Fogarty, et al., 2008; Jones, et al., 2001; Mehl-Madrona, 2008; Ormel, et al., 1994; Wittchen, 2002). A common misconception about GAD is that it is a relatively innocuous disorder (Persons, Mennin, & Tucker, 2001) however; GAD has recently been shown to be a risk factor for cardiovascular disease (CVD) such as cardiac ischemia, sudden cardiac death, and myocardial infarction (Frasure-Smith & Lesperance, 2008; Martens, et al., 2010). Additionally, GAD has been shown to be related to decreased parasympathetic cardiac control (Lyonfields, et al., 1995; Thayer, et al., 1996; Chapter 2), increased adrenergic sympathetic tone (Fisher, et al., 2010; Chapter 2), and elevated heart rate (HR; Hoehn-Saric, et al., 2004; Thayer, et al., 1996), all independent risk factors for cardiac morbidity and mortality (Gillum, Makuc, & Feldman, 1991; Singh, et al., 1998).

Parasympathetic inhibitory control of HR is necessary for cardiac stability and flexibility, and dysregulation of these effects has been shown to limit appropriate cardiac responsiveness (Levy, 1990; Verrier, 1987). Diminished parasympathetically-mediated heart rate variability (HRV) has been shown to predict congestive heart failure and hypertension (Curtis & O'Keefe,

2002), myocardial infarction (Bigger, et al., 1993) and sudden cardiac death (Martin, et al., 1987; Mølgaard, et al., 1991), and has been used as a proxy for cardiovascular disease altogether (Stys & Stys, 1998). Low tonic HRV has likewise been shown to be a significant risk factor for all-cause mortality (Tsuji, et al., 1994), whereas survival after myocardial infarction (Stein, et al., 1994) was positively related to greater levels of HRV. Worry, the cardinal symptom of GAD, has also been linked to impaired HRV, and has been shown to reduce parasympathetic tone – often referred to as vagal tone (Lyonfields, et al., 1995; Thayer, et al., 1996; Chapter 1), promoting increased HR and reduced HRV (Pieper, et al., 2007).

Autonomic effects of worry may be exerted via the generation of negative emotionality, as both theoretical and empirical evidence point to an association between worry and the provocation of negative emotional experiences for individuals with GAD. Mennin and colleagues have demonstrated that GAD is characterized by intense emotional reactions to negative stimuli (McLaughlin, Mennin, & Farach, 2007; Mennin, et al., 2005). Because individuals with GAD find such emotional experiences threatening, worry is employed to suppress negative emotionality (Mennin, et al., 2005), provide distraction from emotional topics (Borkovec & Roemer, 1995), and preclude the emotional processing of fearful stimuli (Borkovec, et al., 2004). Nevertheless, worry itself promotes increased incidence and intensity of emotional reactions (Borkovec, Robinson, Pruzinsky, & DePree, 1983; McLaughlin, et al., 2007), promoting a cycle of elevated worry and negative emotionality. Moreover, Newman and Llera (2011) have recently demonstrated that individuals with GAD are motivated to both worry and generate negative emotional content in order to avoid the experience of a negative emotional contrast. This contrast theory of worry and avoidance (Newman & Llera, 2011) predicts that even mild perturbations will generate elevated worry in individuals with GAD and that the

generation of worry will, in turn, predict increases in negative emotionality and decreases in positive emotionality. These relative changes in valenced emotion help to lessen the likelihood of a negative emotional contrast and likewise propagate the cycle of concurrently elevated worry and negative emotions.

Negative emotions have been shown to increase the incidence of cardiovascular disease (Kubzansky, 2007). Prospective epidemiological data has demonstrated that chronic anxiety carries a relative risk (RR) of coronary heart disease (CHD) from 1.5 (Thurston, Kubzansky, Kawachi, & Berkman, 2006) to 7 (Eaker, Pinsky, & Castelli, 1992). Rugulies (2002) found that, while the RR for clinically-severe depression was 2.69, the RR for individuals with non-clinical depressed mood was 1.49. Additional studies have likewise found significant risk associated with non-clinical depressed mood (Empana, et al., 2005; Kamphuis, et al., 2006; Marzari, et al., 2005; Rowan, Haas, Campbell, Maclean, & Davidson, 2005; Wulsin, et al., 2005). Counter to these findings, the acute emotional experience of sadness has been shown to promote increased parasympathetic activity (Gross, Fredrickson, & Levenson, 1994; Llera & Newman, 2010) and decreased sympathetic activity (Gross & Levenson, 1997; Kreibig, Wilhelm, Roth, & Gross, 2007). Thus, there may be important differences between chronic depressive symptomatology and transient depressed affect. Finally, higher levels of anger symptoms have been associated with prospective RRs for CHD ranging from 1.5 to 3 (Kubzansky, 2007) and anger has been shown to increase sympathetic and decrease parasympathetic control of HR (McCraty, Atkinson, Tiller, Rein, & Watkins, 1995).

There is some evidence to suggest that GAD may be related to the disposition to experience and express anger. For instance, GAD has been shown to correlate with endorsements of hypersensitivity, suspiciousness, and the tendency to be easily slighted

(Gasperini, Battaglia, Diaferia, & Bellodi, 1990; Mavissakalian, Hamann, Haidar, & de Groot, 1995) and those with GAD have reported greater unresolved anger toward childhood caregivers compared to controls (Cassidy, 1995). Moreover, GAD is associated with aggressive behavior independently and when controlling for other mood and anxiety disorders and Axis II pathology (Posternak & Zimmerman, 2002). Finally, Hawkins and Cogle (2011) recently demonstrated that 12-month GAD diagnosis within the National Comorbidity Study – Replication was significantly predictive of anger experience over the last 30 days and 12-month intermittent explosive disorder-related impairment. Importantly, these associations were maintained when controlling for other anxiety disorders, major depressive disorder, substance use disorder, and borderline personality disorder.

Despite strong and wide-ranging evidence for the deleterious effects of negative emotionality, the potentially protective role of positive emotionality in the development of CVD has only just begun to be established (Kubzansky & Thurston, 2007). Kubzansky and Thurston (2007) recently demonstrated that a sense of positive energy and positive well-being – termed emotional vitality – prospectively predicted a reduced risk of developing CHD over a 15-year follow up period. A possible mechanism for this effect may be parasympathetic tone. Positive emotionality has been related to greater parasympathetic tone and cardiac control (Kok & Fredrickson, 2010; McCraty, et al., 1995) and Kok and Fredrickson (2010) have found that greater positive emotionality promoted greater increases in parasympathetic tone over time. Given that the absence of negative emotions is not directly analogous to the presence of positive emotions (Richman, et al., 2005), investigations of the effects of emotionality on psychological and physiological functioning should seek to assess the relative and concurrent impact of multiple positive and negative emotions.

The present study was interested in examining the role that worry plays in promoting the experience and expression of negative emotionality and suppressing the experience and expression of positive emotionality. We chose two negatively valenced and two positively valenced emotions – anger, sadness, contentment, and happiness. In addition, we sought to assess the impact that positive and negative emotions had on vagal cardiac control. Chapter 2 recently demonstrated that individuals with GAD exhibited impaired vagal cardiac control compared to controls and that, despite these impairments, there was a significant effect *within the GAD group* such that higher worry predicted greater vagal cardiac control following the presentation of a laboratory stressor. The present study represents a secondary analysis of these data, in which we sought to examine temporal relationships between worry, positive and negative emotionality, and vagal cardiac control.

We hypothesized that levels of worry elicited in response to an initial mild stressor would increase expressions of negative emotionality and decrease expressions of positive emotionality in response to a subsequent, more evocative stressor, and that both impairments in vagal cardiac control and the paradoxically cardioprotective effect of worry within individuals with GAD would be a function of negative emotionality. Specifically, given the unequivocal role of anger in the development of cardiovascular disease, we hypothesized that the expression of anger would account for the between groups effect of impaired vagal cardiac control. In contrast to this, based on evidence that transient expressions of sadness promote increased parasympathetic tone, we hypothesized that the protective effect of worry *within GAD* found in Chapter 2 would be a function of expressed sadness. That is, we predicted that worry would significantly predict increases in the experience and expression of both sadness and anger and that the former would promote cardioprotective effects, while the latter would promote cardiopathogenic effects.

Method

Participants

Participants ($n=118$) were mostly female (61%) and Caucasian (72.9%), with a mean age of 19.2 ($SD = 1.49$) years. The GADQ-IV (Newman, et al., 2002) and Penn State Worry Questionnaire (Meyer, et al., 1990) were administered to 1,665 Penn State University students in order to screen for the presence of elevated worry and GAD. In all, 198 individuals were recruited to the study. GAD status required a GADQ-IV score of 9 or higher and diagnosis of GAD using the Mini International Neuropsychiatric Interview (MINI; Sheehan, et al., 1998). Agreement between GADQ-IV and MINI diagnoses was high using these criteria ($kappa = .75, p < .001$). Healthy control status required that individuals not meet clinical criteria for any Axis I disorder and that they fall below the median score on both the GAQ-IV and the PSWQ.

Healthy Controls accounted for 35.6% ($n = 42$) of the sample and individuals with GAD accounted for 64.4% ($n = 76$) of the sample. Thirty-two GAD participants met criteria for at least one comorbid disorder (major depressive disorder, $n=9$; dysthymia, $n=2$; panic disorder, $n=4$; social phobia, $n=9$, post-traumatic stress disorder $n=2$; obsessive-compulsive disorder, $n=4$, anorexia nervosa, $n=3$).

Exclusion criteria for all groups included the current use of tricyclic and SSRI antidepressants, or benzodiazepines. Benzodiazepines and SSRIs have been shown to alter vagal outflow both positively (Tucker, et al., 1997; Tulen, et al., 1994) and negatively (Adinoff, et al., 1992; Rissanen, et al., 1998), whereas tricyclics have been shown to unidirectionally decrease vagal activity (Jakobsen, et al., 1984; Mezzacappa, et al., 1998; Yeragani, et al., 1992). Twenty-two individuals were excluded from the present study due to current prescribed use of one of these medications. An additional 53 individuals were excluded because they did not meet the

criteria set forth for inclusion within the present study. One individual initiated, but did not complete the study protocol and therefore was excluded from the present study.

Procedure

After providing written consent, all participants were administered the Mini International Neuropsychiatric Interview (MINI; Sheehan, et al., 1998), a brief structured interview for the diagnosis of mood, anxiety, substance abuse, and eating disorders, as defined by the DSM-IV (see below). Immediately following the administration of the MINI, participants were asked to provide a saliva sample. Self-adhesive electrodes were then placed at the wrist of the non-dominant hand, as well as on the inner portion of the lower leg, three inches above the ankle. A respiration belt was centered just below the solar plexus and fitted firmly around the torso.

Participants were then seated in a 4 by 6 foot room equipped with a computer, desk, and chair. While the experimenter sat in an adjacent room, participants sat quietly for a 10-minute adjustment period, during which they were instructed to remain still. A two-minute baseline measurement was then taken. Immediately following the conclusion of the baseline measurement, participants were prompted to indicate on the computer the degree of worry they were experiencing *right now*. Answers were given on a nine-point, Likert-like scale from “none” to “as much as possible.” A worry versus relaxation induction period then followed. A more detailed description of this procedure, and analyses thereof, can be found in Chapter 1. Notably, results of Chapter 1 demonstrated that the phasic variability captured in continuously measured state worry accounted for the variance in physiological response patterns attributable to the categorical induction of worry versus relaxation.

Following the induction period, participants were given a brief instructional session, in which the experimenter introduced the Paced Auditory Serial Addition Task (PASAT) and

provided 10 practice items. The first iteration of the PASAT was completed with an interstimulus interval of three seconds. This was followed by the second iteration of the PASAT, with an interstimulus interval of two seconds. After each presentation of the PASAT, participants were prompted to indicate the degree of worry they were experiencing. Five minutes after the completion of the second PASAT iteration, a second saliva sample was taken. Finally, participants completed a 20 minute recovery phase, during which time they completed a set of auxiliary measures and sat quietly. At the conclusion of the recovery phase a final saliva measurement was taken and participants were asked to indicate how worried they felt a final time.

This study was approved by the Institutional Review Board of Penn State University.

Diagnostic Instruments

Generalized Anxiety Disorder Questionnaire-IV (GADQ-IV; Newman, et al., 2002).

The GADQ-IV is a 9-item self-report measure designed to diagnose GAD based on DSM-IV criteria. Participants with GADQ-IV scores > 9 were recruited to the study as potential GAD participants, whereas individuals exhibiting scores < 5 were recruited to the study as potential control participants. Newman et al. (2002) demonstrated that while the optimal balance between sensitivity and specificity is found at a cutoff of 5.7, the GADQ-IV demonstrates 97% specificity, with a false positive rate of only 4% at scores of 9 or above. Using Receiver Operating Characteristic analyses, the GADQ-IV showed 89% specificity and 83% sensitivity when compared to structured interview diagnoses of individuals with GAD, social phobia, panic disorder, and non-anxious controls. The GADQ-IV also demonstrated retest reliability, convergent and discriminant validity, and kappa agreement of .67 with a structured interview (Newman, et al., 2002). College students diagnosed with GAD by the GADQ-IV were not

significantly different from a GAD community sample on the Penn State Worry Questionnaire (PSWQ; Meyer, et al., 1990), and both groups had significantly higher PSWQ scores than students identified as not meeting criteria for GAD, demonstrating the clinical validity of the GADQ-IV.

MINI (Sheehan, et al., 1998). The MINI is based on diagnostic criteria from the DSM-IV and ICD-10 and has been validated against the Structured Clinical Interview for DSM Disorders (SCID; First, et al., 1997) and Composite International Diagnostic Interview (CIDI; Robins, et al., 1988). It is designed to be utilized as a short but accurate structured clinical interview for clinical trials and epidemiology studies. Kappa agreement with SCID diagnoses ranged from .50 (simple phobia) to .90 (anorexia), with a .70 for GAD. Sheehan et al., (1998) found sensitivity on the MINI to be 70% or greater for all diagnoses but dysthymia and obsessive-compulsive disorder (67% and 62% respectively), while specificities were 85% or greater for all diagnoses (Sheehan, et al., 1998). The MINI was administered by undergraduate research assistants, trained by an advanced doctoral student in clinical psychology and supervised by a licensed clinical psychologist.

Video recordings were made of each diagnostic interview so that a second rater could independently assess whether participants met criteria for Axis I disorders. Reliability was excellent across all disorders on the MINI ($kappa = .89, p < .001$) and for the GAD diagnosis specifically ($kappa = .95, p < .001$).

Study Measures

Emotion responses. Changes in self-reported emotional states were measured using a 16-item emotion checklist (Gross & Levenson, 1993, 1997). This checklist is a self-report inventory consisting of 16 emotion terms (amusement, anger, arousal, confusion, contempt,

contentment, disgust, embarrassment, fear, happiness, interest, pain, relief, sadness, surprise, tension), adapted from Gross and Levenson (1993, 1997). Participants rated the degree to which they were experiencing each emotion using a 9-point Likert-type scale (0 = *none*, 8 = *as much as possible*). The emotion checklist was administered four times: at baseline, following each of the iterations of the PASAT, and at the conclusion of the recovery period. Emotion data for one GAD participant was missing throughout the study, leaving 75 GAD participants for analysis.

Worry. Worry was similarly assessed throughout the study along with emotion responses. Self-reports of phasic worry were rated using a 9-point Likert-type scale from 0 (none) to 8 (as much as possible).

Heart rate. ECG was continuously recorded at 500 hertz throughout the duration of the study. The ECG waveform was subjected to a band pass filter with a low cutoff of .5 Hz and a high cutoff of 35 Hz. A raw tachogram was then generated in order to inspect the ECG waveform for anomalies. Such anomalies were cleaned by simply raising or lowering a given R spike so that the *AcqKnowledge* 4.1 software algorithm was able to appropriately detect the beat-to-beat intervals. When the ECG waveform was free of anomalies, a separate HR channel was generated, from which the mean HR for each period was calculated.

RSA. A fast-Fourier transformation was applied to the RR intervals of the ECG signal for the baseline, induction, PASAT 3", PASAT 2", and recovery periods, yielding periodograms for each. The average spectral power in the high-frequency band (.15 Hz to .40 Hz) for each period was then calculated and used as a proxy for parasympathetic activity.

PASAT

The PASAT is a serial-addition task used to assess working memory, divided attention, and information processing speed. In this task, a random series of single-digit numbers 1-9 is

presented, and participants are required to add consecutively presented numbers such that each number is added to the number that immediately preceded it. At the end of each trial, the interstimulus interval – the time between each digit presentation – is decreased. The PASAT has been shown to reliably induce psychological stress during laboratory inductions (Diehr, et al., 2003; Lejuez, et al., 2003).

Dichotomization of emotion responses.

Distributions of responses for anger (*skewness* = .59, *kurtosis* = 2.13), sadness (*skewness* = 1.70, *kurtosis* = 5.58), happiness (*skewness* = 1.02, *kurtosis* = 3.07), and contentment (*skewness* = 1.13, *kurtosis* = 3.23) were found to be highly non-normal, with substantial zero-inflation on all counts (*percentage of zeros* = 25%, 51%, 48%, and 49% respectively). In order to accommodate the non-normality of the data, and to facilitate more practical interpretations, responses for each emotion were dichotomized such that responses of zero retained their zero value and all responses greater than zero were coded as one. Thus, the newly coded emotion variables related to the presence versus absence of each emotion and not to degree. In contrast, self-reports of phasic worry captured following the stressor were significantly less skewed (*skewness* = .14) and kurtotic (*kurtosis* = 1.68). These responses were kept intact and are to be interpreted as reflecting the degree of worry present at baseline and following the stressor.

Operationalization of vagal cardiac control.

Parasympathetic efferents conducted via the vagus nerve chronically inhibit heart rate. Higher levels of parasympathetic influence are reflected in lower heart rate and lower levels of parasympathetic influence reflected in higher heart rate. Note that we stress the *influence* of the parasympathetic nervous system via vagal efferents and not the absolute level of parasympathetic activity. The ANS is a two dimensional system, wherein sympathetic activity

can be complementary or competitive to parasympathetic activity (Berntson, et al., 1991). This is an important distinction in dually innervated organs such as the heart, where the influence of the two branches of the ANS can be coupled or uncoupled, reciprocal or nonreciprocal, and where coactivation of both branches has been empirically observed (Berntson, et al., 1991). Assessing only absolute levels of one branch of the ANS does not account for possible coactivation of a target organ. Thus, effects of the measured branch could be over or underestimated. Moreover, RSA, the gold standard for noninvasive measurement of parasympathetic effects, has been shown to be subject to sympathetic influence (Taylor, et al., 2001).

To operationalize vagal cardiac control we argue that one must represent not only the mere level of RSA, but the relationship between RSA and heart rate. In the present study the construct vagal cardiac control is operationalized as the linear relationship between RSA and HR. This relationship is represented as $HR \sim \beta_1(RSA) + e$, wherein the coefficient β_1 represents vagal cardiac control. The degree of negative correlation between HR and RSA reflects adaptive vagal cardiac control, whereas the degree to which this relationship is less negative – flatter – demonstrates poorer vagal cardiac control. The influences of emotion or group status (see below) on vagal cardiac control are represented as moderation effects, moderating the linear relationship between HR and RSA.

Although moderators are characterized statistically by interaction terms, they can be understood conceptually as the *direct effect* of the moderator on the *relationship* between two constructs. Baron and Kenny note that a moderator is a “variable that affects the direction and/or strength of the relation between an independent or predictor variable and a dependent or criterion

variable” (p. 1174; 1986). As we have indicated here, vagal cardiac control is operationalized as the relationship between RSA and HR, represented as the coefficient β_1 .

Results

Path model.

A longitudinal path model was constructed in Mplus (version 5.2.1; Muthen & Muthen, 2009) and estimated with maximum likelihood estimation robust to non-normality (Satorra & Bentler, 2001). This model was constructed to in order to test the proposed study hypotheses that worry elicited following mild stress would predict increased negative emotionality and decreased positive emotionality following a subsequent, and more intense stressor and that emotional content following stress would significantly moderate vagal cardiac control following a 20-minute recovery. State worry following the presentation of the 3rd interstimulus interval PASAT (state worry-10) was regressed on both the PSWQ (trait worry) and baseline state worry (state worry-0) to control for the influence of trait worry and to test the stability of phasic variability in worry states. The emotional responses for the presence versus absence of anger, sadness, contentment, and happiness following the 2nd interstimulus interval PASAT were regressed on state worry-10 (emotion parameters were likewise regressed on trait worry). Finally, HR following recovery was regressed on RSA, anger, sadness, contentment, and happiness, and the relationship between RSA and HR was allowed to be moderated by anger, sadness, contentment, and happiness.

This model provided an excellent fit to the data ($\chi^2 = 38.69$, $df = 43$, $p = .66$, $CFI = 1.00$, $RMSEA < .001$). Table 1 presents the complete parameter results for the fomenting effect of worry on emotional response and the moderating effect of emotional content following stress on

vagal cardiac control following a 20-minute recovery. In addition, figure 1 depicts the path diagram for this model.

Effect of baseline and trait worry. Worry remained highly stable from baseline to the 3rd PASAT presentation ($d = 1.17$). Additionally, it was found that trait worry significantly predicted worried responses to the 3rd PASAT ($d = .35$). Trait worry did not significantly affect the presence versus absence of happiness, sadness, or anger, following the 2nd PASAT. However, trait worry significantly predicted the expression of contentment following the stress period ($d = .40$). Thus, those scoring higher on the PSWQ were more likely to report the presence of contentment following the stressor.

Fomenting effect of state worry following the 3rd PASAT on emotional content following the 2nd PASAT. The degree of worry elicited following the 3rd interstimulus interval PASAT significantly predicted increased incidence of negative emotionality and decreased incidence of positive emotionality. Greater levels of state worry predicted increased anger and sadness ($ds = .58, .56$) and decreased happiness and contentment ($d = -.42, -.33$).

Effects of emotional content following stress on HR and vagal cardiac control following recovery. The presence of anger ($d = .44$), sadness ($-.44$), and contentment ($-.55$) following the 2nd interstimulus interval PASAT all significantly moderated the relationship between RSA and HR following 20-minute recovery. The presence of both contentment and sadness predicted greater vagal cardiac control, whereas the presence of anger predicted decreased vagal cardiac control. Only anger had a significant direct effect on HR ($d = .65$). Thus, the presence of anger following the stressor predicted higher HR level following recovery.

Effect of GAD versus healthy control status on vagal cardiac control following recovery in GAD participants who did, and did not, express anger after the stressor.

Given the negative effect of the expression of anger following stress on vagal cardiac control after recovery, we sought to examine whether the expression of anger was the mechanism by which previously reported (Chapter 2) group differences in vagal cardiac control following recovery were expressed. Thus, we separated the GAD group into those who did ($n=57$) and did not ($n=18$) express anger and compared each of these groups to the healthy control group. State worry was held as a control variable in both models. Results of these analyses are presented in table 3.

Individuals with GAD who expressed anger following the stress procedure exhibited significantly impaired vagal cardiac control ($d = .74$) compared to healthy controls. However, GAD participants who did not express anger following the stressor did not statistically differ from healthy controls in vagal cardiac control ($d = .17$).

Discussion

Although a common perception of GAD is that it is a relatively innocuous disorder (Persons, Mennin, & Tucker, 2001), recent data have shown GAD to be a risk factor for CVD such as cardiac ischemia, sudden cardiac death, and myocardial infarction (Frasure-Smith & Lesperance, 2008; Martens et al., 2010). Additionally, GAD has been shown to be related to decreased parasympathetic cardiac control (Thayer, 1996; Lyonfields, 1995; Chapter 2), increased adrenergic sympathetic tone (Fisher, Granger, & Newman, 2010; Chapter 2), and HR (Thayer 1996; Hoehn-Saric, 2004), all independent risk factors for cardiac morbidity and mortality (Singh, Larson, Tsuji, et al., 1998; Gillum, Makuc, & Feldman, 1991). Recently, individuals with GAD were shown to exhibit decreased vagal cardiac control compared to

controls (Chapter 2). Additionally, these authors found that within the GAD group, worry was shown to paradoxically predict increased vagal cardiac control following the completion of a laboratory stressor. The present study sought to demonstrate that the paradoxical and apparently cardioprotective function of worry within GAD would be a function of expressed sadness. Additionally, we hypothesized that the expression of anger would lead to impaired vagal cardiac control and that – in the absence of expressed anger – individuals with GAD would exhibit cardiovascular functioning on par with healthy controls.

A longitudinal path model was constructed wherein levels of worry elicited in response to an initial, mild laboratory stressor were regressed upon baseline levels of phasic worry and allowed to predict emotional responses following the presentation of a successive, cognitively demanding stressor. These emotional responses were then allowed to predict HR level and vagal cardiac control – a measure of parasympathetic inhibitory control of HR – following a 20-minute recovery period. Results indicated that increases in worry following the initial stressor predicted increased expression of anger and sadness and decreased expression of contentment and happiness. The presence of sadness and contentment both predicted improved vagal cardiac control following recovery, whereas the presence of anger predicted poorer vagal cardiac control. Moreover, only anger significantly predicted HR level, wherein the presence of anger predicted higher HR. Happiness had no significant effect on vagal cardiac control or HR.

Finally, two regression models were run to assess the potentially mechanistic role of anger in impaired vagal cardiac control in GAD. Healthy control participants were compared to GAD participants who expressed anger (n=57) and those who did not (n=18). Results indicated that only those GAD participants who expressed anger exhibited impaired vagal cardiac control

following recovery. Individuals with GAD who did not express anger did not differ significantly from healthy controls in HR or vagal cardiac control.

Theories of worry predict that worry will be elicited in response to even mild environmental stressors in order to avoid the processing of emotional stimuli (Borkovec, et al., 2004) as well as to reduce the likelihood of a negative emotional contrast (Newman & Llera, 2011). Despite this, worry promotes increased incidence and experience of negative emotionality (McLaughlin, et al., 2007; Newman & Llera, 2011), promoting a cycle of elevated worry and negative emotionality and reinforcing the avoidance of a negative emotional contrast. The present study found evidence for the fomenting role of worry in promoting negative emotionality and decreasing positive emotionality. These findings support the role of worry as an avoidant response to emotional stimuli (Borkovec, et al., 2004; Newman & Llera, 2011) and an attempt to reduce the likelihood of a negative emotional contrast (Newman & Llera, 2011).

The present study provides important elaborations on previously reported results from Chapter 2. Although Chapter 2 revealed that levels of state worry paradoxically predicted higher levels of vagal cardiac control *within GAD participants*, the present data indicate that this apparently cardioprotective effect of state worry is a function of expressed sadness, as levels of state worry were temporally related to the expression of sadness. This finding is consistent with empirical work that has demonstrated that transient expressions of sadness can be cardioprotective by promoting increased parasympathetic tone (Gross, et al., 1994; Llera & Newman, 2010). Additionally, decrements in vagal cardiac control in GAD were fully accounted for by the expression of anger. Thus, those GAD participants who did not express anger following the stress procedure exhibited vagal cardiac control on par with healthy controls.

Importantly, although the expression of sadness appeared to play a cardioprotective role in the present study, it should be noted that this effect is likely better understood as *counteractive* rather than protective. Of the 35 GAD participants who expressed sadness following the stress procedure, 32 (91%) also expressed anger. Thus, only three GAD participants were able to benefit from the potentially cardioprotective effects of sadness. A similar conclusion can be drawn about the potentially protective effects of contentment. Of the 38 GAD participants who expressed contentment, 28 (74%) also expressed anger, whereas 10 (26%) did not. Table 3 provides the complete cross-tabulation of emotion endorsements following the stress procedure.

The present study provided further support for the growing empirical position that positive emotionality is protective against the development of disease processes (Kubzansky & Thurston, 2007; Richman, et al., 2005). The presence of contentment following the stress procedure provided a counteractive effect larger than the deleterious effect of anger. Of the 57 individuals with GAD who expressed anger, 29 expressed contentment as did an additional 10 individuals who did not express anger. Of note, the expression of happiness, although negatively predicted by level of state worry, did not have any significant impact on HR or vagal cardiac control. Future research should investigate the underlying differences in the relative effects of contentment and happiness reported here.

Anger played the most important and certainly most deleterious role in the present findings. The expression of anger by individuals with GAD predicted both decreased vagal cardiac control and increased HR, both prospective predictors of cardiovascular morbidity and mortality. These findings are consistent with prospective epidemiological work that has shown anger to be a predictor of CHD (Iribarren, et al., 2000; Miller, Smith, Turner, Guisjarro, & Hallet, 1996; Mittleman, et al., 1995), as well as data indicating a dose-response relationship between

level of anger and frequency of cardiac symptoms (Kawachi, Sparrow, Spiro Iii, Vokonas, & Weiss, 1996). Moreover, these data may suggest a temperamental disposition toward anger or hostility in individuals with GAD. To wit, Suarez and Blumenthal (1991) demonstrated that, compared to non-hostile individuals, hostile participants exhibited greater HR and blood pressure responses to mental tasks and of the 75 GAD participants in the present study, 76% (n=57) expressed anger following the presentation of a mental arithmetic task. Given these findings, and the recent finding that GAD diagnosis was significantly predictive of recent anger experiences (Hawkins & Cogle, 2011), future research should continue to assess the possibly dispositional role of anger within GAD.

One possible weakness of the current study is the method of recruitment and its impact on the generalizability of the present findings. GAD participants were screened and recruited from a large undergraduate subject pool. Although GAD status required agreement between a self-report diagnostic inventory and a structured clinical interview, these individuals were not treatment-seeking and represented a limited age range. However, it should be noted that the present study was able to identify decreased vagal cardiac control – a strong prospective predictor of cardiovascular morbidity and mortality – in relatively healthy, college-aged participants. Suls and Bunde (2005) note that anxiety may play a more important role in cardiopathogenesis than in the progression of already established cardiovascular disease. Thus, it could be argued that the population is a strength of the present study as research on younger, premorbid participants may facilitate the delineation of potential mechanisms by which to identify possible at risk populations. Moreover, Iribarren et al. (2000) demonstrated a significant prospective risk of coronary artery calcification in young adults aged 18-30 exhibiting anger and hostility. Nevertheless, further research should seek to replicate these findings with treatment-

seeking clinical populations from a community sample. Additionally, the present sample was predominantly Caucasian. Replication with more diverse populations would further strengthen the generalizability of the present findings.

Table 1.1: Means (standard deviations) for baseline variables by group.

	Healthy Control	High-Worry	GAD
GADQ-IV	1.27 (1.44)*	6.42 (2.56) ⁺	11.14 (3.35)
PSWQ	33.03 (8.23)*	55.47 (8.34) ⁺	62.55 (10.55)
State Worry (0-8 scale)	.81 (1.31)*	.89 (1.33) ⁺	2.87 (2.38)
BMI	23.52 (4.26)	23.11 (3.10)	22.42 (3.68)
Heart Rate	79.55 (11.68)	82.62 (13.47)	77.25 (11.94)
RSA	.57 (.14)	.59 (.24)	.65 (.27)
sAA	74.01 (52.11)	103.21 (67.77)	115.86 (92.42)
Cortisol	.20 (.13)	.18 (.12)	.18 (.13)

High-Worry = high-worry control group, GAD = generalized anxiety disorder; GADQ-IV = Generalized Anxiety Disorder Questionnaire-IV; PSWQ = Penn State Worry Questionnaire; sAA = salivary alpha-amylase; RSA = respiratory sinus arrhythmia. * Denotes significant differences between Controls and both GAD groups. ⁺ Denotes significant difference between GAD and high-worry control groups.

Table 1.2: Moderating effect of available cortisol on sympathetic stress response for healthy controls in worry and relaxation conditions.

	β	SE	t	p	d
Healthy Controls: Worry					
<i>Intercept</i>	3.85	.300	12.83	< .001	4.93
<i>Linear Time</i>	-.025	.029	-.87	.39	.27
<i>Quadratic Time</i>	.0007	.0009	.84	.41	.26
<i>Cortisol</i>	.54	.789	.69	.50	.21
<i>Lin. Time x Cortisol</i>	.226	.139	1.62	.12	.50
<i>Quad. Time x Cortisol</i>	-.006	.004	1.41	.17	.44
Healthy Controls: Relaxation					
<i>Intercept</i>	4.18	.114	36.67	< .001	11.33
<i>Linear Time</i>	.018	.009	1.91	.07	.59
<i>Quadratic Time</i>	-.0005	.0003	-1.96	.06	.61
<i>Cortisol</i>	.153	.084	1.83	.08	.57
<i>Lin. Time x Cortisol</i>	-.031	.010	-3.09	.006	.95
<i>Quad. Time x Cortisol</i>	.0008	.0003	3.05	.006	.94

Table 1.3: Moderating effect of available cortisol on sympathetic stress response for GAD participants in worry and relaxation conditions.

	β	SE	t	p	d
GAD: Worry					
<i>Intercept</i>	4.29	.117	36.67	< .001	9.46
<i>Linear Time</i>	.004	.009	.46	.65	.12
<i>Quadratic Time</i>	-.0002	.0003	-.95	.35	.25
<i>Cortisol</i>	-.086	.072	-1.20	.24	.31
<i>Lin. Time x Cortisol</i>	.031	.009	3.43	.002	.88
<i>Quad. Time x Cortisol</i>	-.0007	.0003	-2.42	.02	.62
GAD: Relaxation					
<i>Intercept</i>	4.49	.158	28.49	< .001	6.30
<i>Linear Time</i>	-.001	.003	-.47	.64	.10
<i>Cortisol</i>	.116	.080	1.45	.15	.32
<i>Lin. Time x Cortisol</i>	-.008	.003	2.50	.02	.55

Table 2.1: Moderating effect of trait and state moderators on vagal cardiac control at baseline.

	β	SE	t	p	d
Step 1: Trait Moderators					
<i>Intercept</i>	77.17	1.27	60.79	< .001	6.99
<i>RSA – Baseline</i>	-15.08	1.95	-7.72	< .001	.89
<i>GAD</i>	1.26	1.57	.81	.42	.13
<i>High-worry controls</i>	3.69	1.92	1.92	.06	.47
<i>RSA x GAD</i>	7.47	2.11	3.54	< .001	.57
<i>RSA x High-worry</i>	4.33	2.46	1.76	.08	.43
Step 2: + State Worry					
<i>Intercept</i>	77.26	1.30	59.64	< .001	6.86
<i>RSA – Baseline</i>	-15.39	1.97	-7.81	< .001	.90
<i>GAD</i>	1.35	1.72	.79	.43	.13
<i>High-worry controls</i>	3.76	1.92	1.95	.05	.48
<i>State Worry – Baseline</i>	-.06	.35	-.18	.86	.02
<i>RSA x GAD</i>	6.06	2.35	2.58	.01	.42
<i>RSA x High-worry</i>	4.48	2.47	1.81	.07	.45
<i>RSA x State Worry</i>	.41	.30	1.37	.17	.16

RSA = respiratory sinus arrhythmia.

Table 2.2: Moderating effect of trait and state moderators on vagal cardiac control following recovery period.

	β	SE	t	p	d
Step 1: Trait Moderators					
<i>Intercept</i>	79.01	1.80	43.86	< .001	5.04
<i>RSA – Baseline</i>	-11.24	2.30	-4.88	< .001	.56
<i>GAD</i>	-.35	2.24	-.16	.88	.03
<i>High-worry controls</i>	1.22	2.77	.44	.66	.11
<i>RSA x GAD</i>	5.93	2.59	2.29	.02	.37
<i>RSA x High-worry</i>	.85	3.24	.26	.79	.06
Step 2: + State Worry					
<i>Intercept</i>	79.05	1.81	43.68	< .001	5.02
<i>RSA – Recovery</i>	-11.09	2.30	-4.82	< .001	.55
<i>GAD</i>	.009	2.61	.003	1.00	0
<i>High-worry controls</i>	1.42	2.81	.51	.61	.13
<i>State Worry – Recovery</i>	-.30	.57	-.52	.60	.06
<i>RSA x GAD</i>	9.87	2.84	3.48	< .001	.56
<i>RSA x High-worry</i>	1.81	3.26	.56	.58	.14
<i>RSA x State Worry</i>	-1.28	.42	-3.07	.002	.35

RSA = respiratory sinus arrhythmia.

Table 3.1: Complete parameter estimates for the path model examining the fomenting effect of worry on emotional response and the moderating effect of emotional content following stress on vagal cardiac control following a 20-minute recovery.

	β	SE	t	p	d
State Worry-10 min ON					
PSWQ (trait worry)	.043	.020	2.14	.032	.35
State Worry-0 min	.564	.078	7.20	< .001	1.17
Anger-15 min ON					
State Worry-10 min	.068	.019	3.53	< .001	.58
PSWQ	-.005	.005	-1.13	.26	-.18
Sadness-15 min ON					
State Worry-10 min	.073	.021	3.44	.001	.56
PSWQ	.003	.006	.49	.63	.08
Contentment-15 min ON					
State Worry-10 min	-.048	.024	-2.02	.04	-.33
PSWQ	.013	.005	2.48	.01	.40
Happiness-15 min ON					
State Worry-10 min	-.058	.022	-2.60	.009	.42
WQ	.010	.005	1.78	.07	.29
Heart Rate-35 min ON					
Anger-15	.079	.020	3.96	< .001	.65
Sadness-15	-.042	.024	-1.75	.08	-.29
Contentment-15	.002	.033	.07	.94	.01
Happiness-15	-.058	.032	-1.82	.07	-.30
RSA-35	-.313	.152	-2.05	.04	-.33
Anger-15 x RSA-35	.434	.162	2.68	.007	.44
Sad-15 x RSA-35	-.493	.184	-2.68	.007	-.44
Content-15 x RSA-35	-.649	.192	-3.38	.001	-.55
Happy-15 x RSA-35	-.022	.175	-.12	.90	-.02

d = Cohen's d (calculated as $d = t(2/n)^{1/2}$); 0 min, 10 min, 15 min, 35 min = elapsed time in minutes, PSWQ = Penn State Worry Questionnaire; RSA = respiratory sinus arrhythmia.

Table 3.2: Effect of GAD status (versus healthy controls) on vagal cardiac control following a 20-minute recovery in GAD participants who did, and did not, express anger after the stressor.

	β	SE	t	p	d
GAD with Anger (n = 57)					
<i>Intercept</i>	78.93	1.93	40.82	< .001	5.76
<i>RSA</i>	-10.80	2.44	-4.43	< .001	-.62
<i>GAD</i>	3.35	2.63	1.28	.21	.24
<i>State Worry</i>	-.78	.48	-1.62	.11	-.23
<i>RSA x GAD</i>	11.59	2.93	3.96	< .001	.74
<i>RSA x State Worry</i>	-1.41	.38	-3.72	.003	-.52
GAD without Anger (n = 18)					
<i>Intercept</i>	80.83	1.39	58.19	< .001	10.47
<i>RSA</i>	-8.29	1.23	-6.72	< .001	-1.21
<i>GAD</i>	-3.89	2.14	-1.82	.07	-.61
<i>State Worry</i>	-.93	.53	-1.76	.08	-.32
<i>Anger</i>	-.83	3.61	-.23	.82	-.04
<i>RSA x GAD</i>	-1.12	2.26	-.50	.62	-.17
<i>RSA x State Worry</i>	-.81	.52	-1.53	.13	-.28

RSA = respiratory sinus arrhythmia.

Table 3.3: Cross-tabulation of the presence versus absence of discrete emotions following stress.

	Anger		Sadness		Contentment		Happiness	
	0	1	0	1	0	1	0	1
Anger								
0	18	0						
1	0	57						
Sadness								
0	15	25	40	0				
1	3	32	0	35				
Contentment								
0	8	29	21	16	37	0		
1	10	28	19	19	0	38		
Happiness								
0	8	28	22	14	29	7	36	0
1	10	29	18	21	8	31	0	39

Figure 1.1: The moderating effect of induced worry versus relaxation on trajectory of worry across study period (total sample).

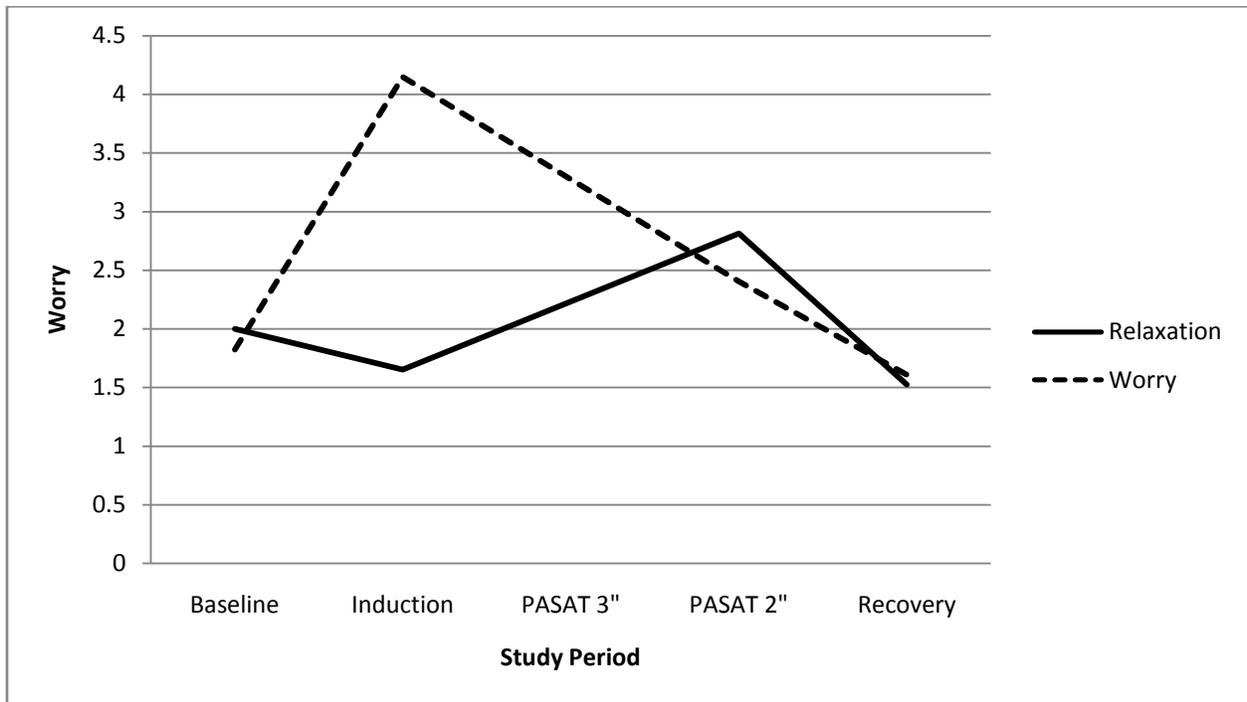


Figure 1.2: The moderating effect of induced worry versus relaxation on trajectory of heart rate in high-worry controls and individuals with GAD.

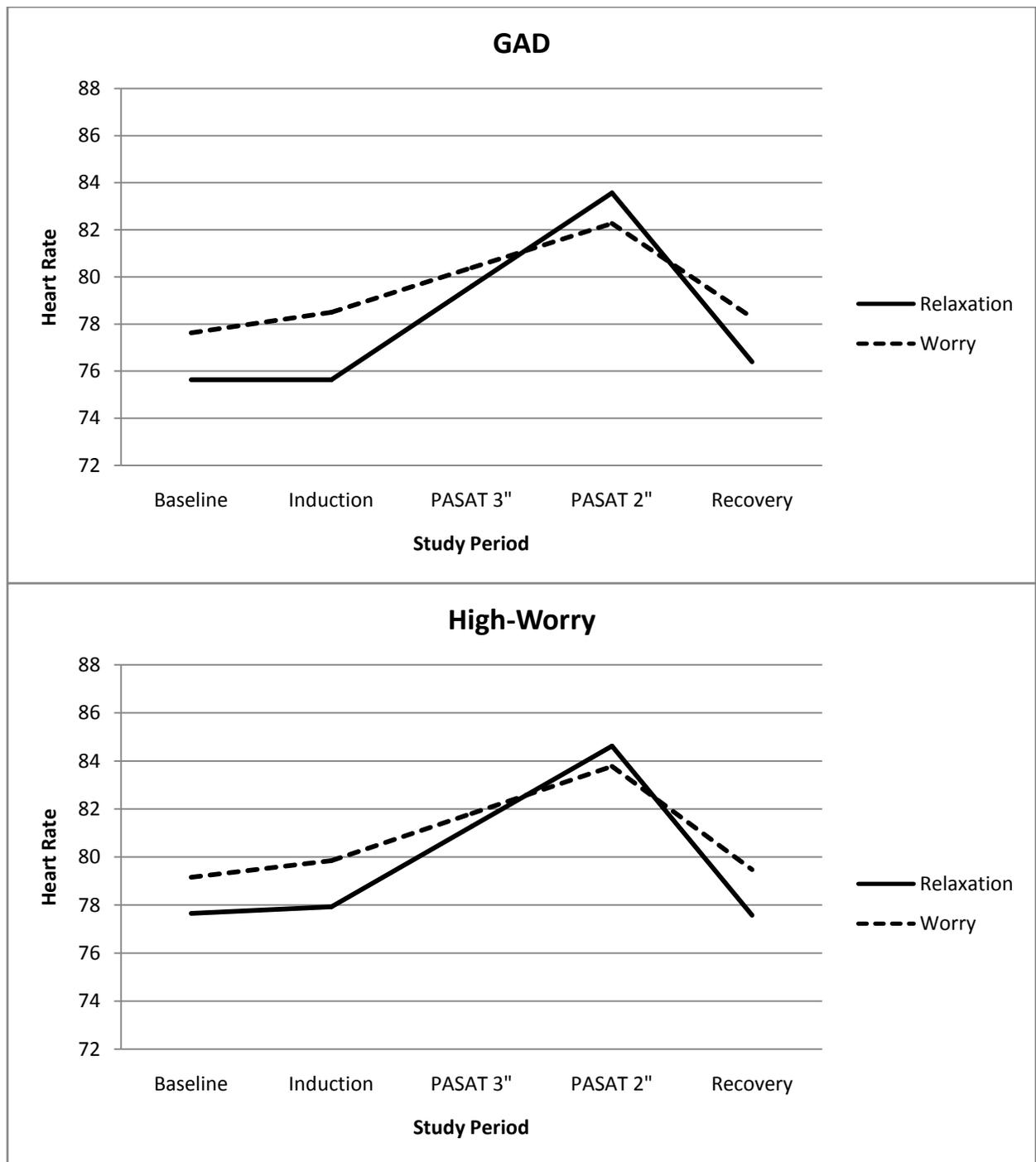
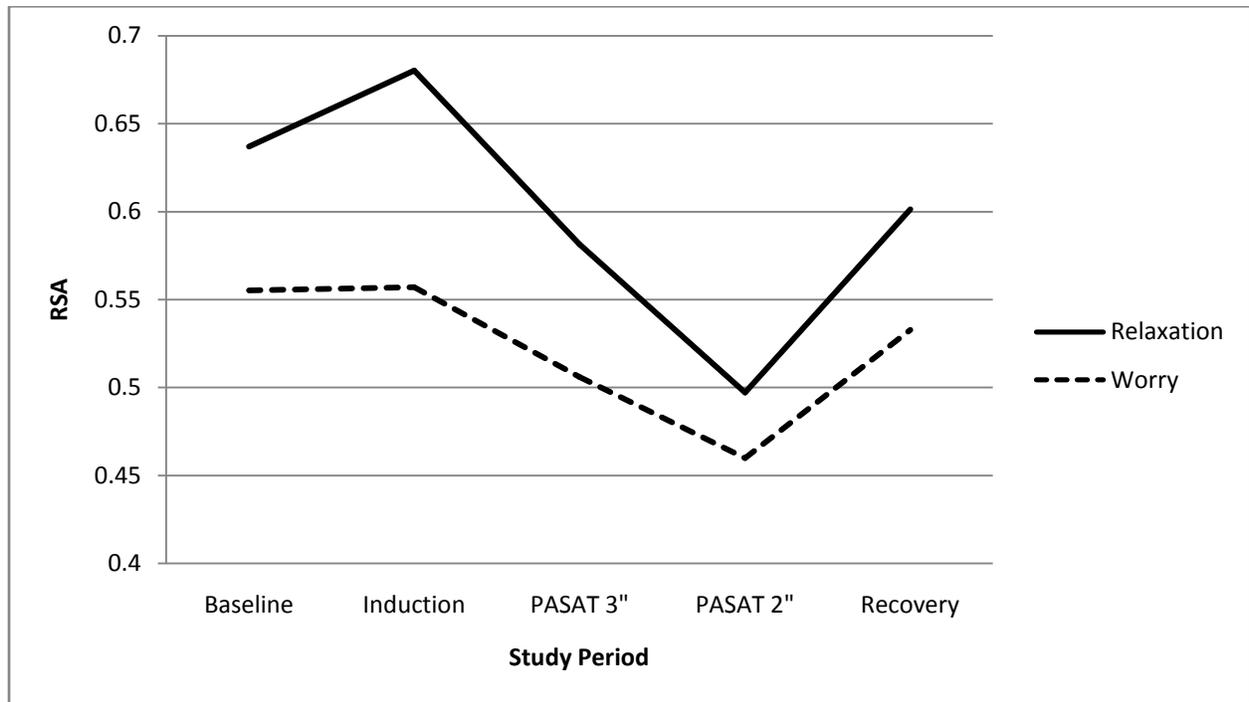


Figure 1.3: Moderating effect of induced worry versus relaxation on trajectory for respiratory sinus arrhythmia within individuals with GAD.



RSA = respiratory sinus arrhythmia.

Figure 1.4: Moderating effect of available cortisol on sympathetic stress response in healthy controls, within relaxation and worry conditions.

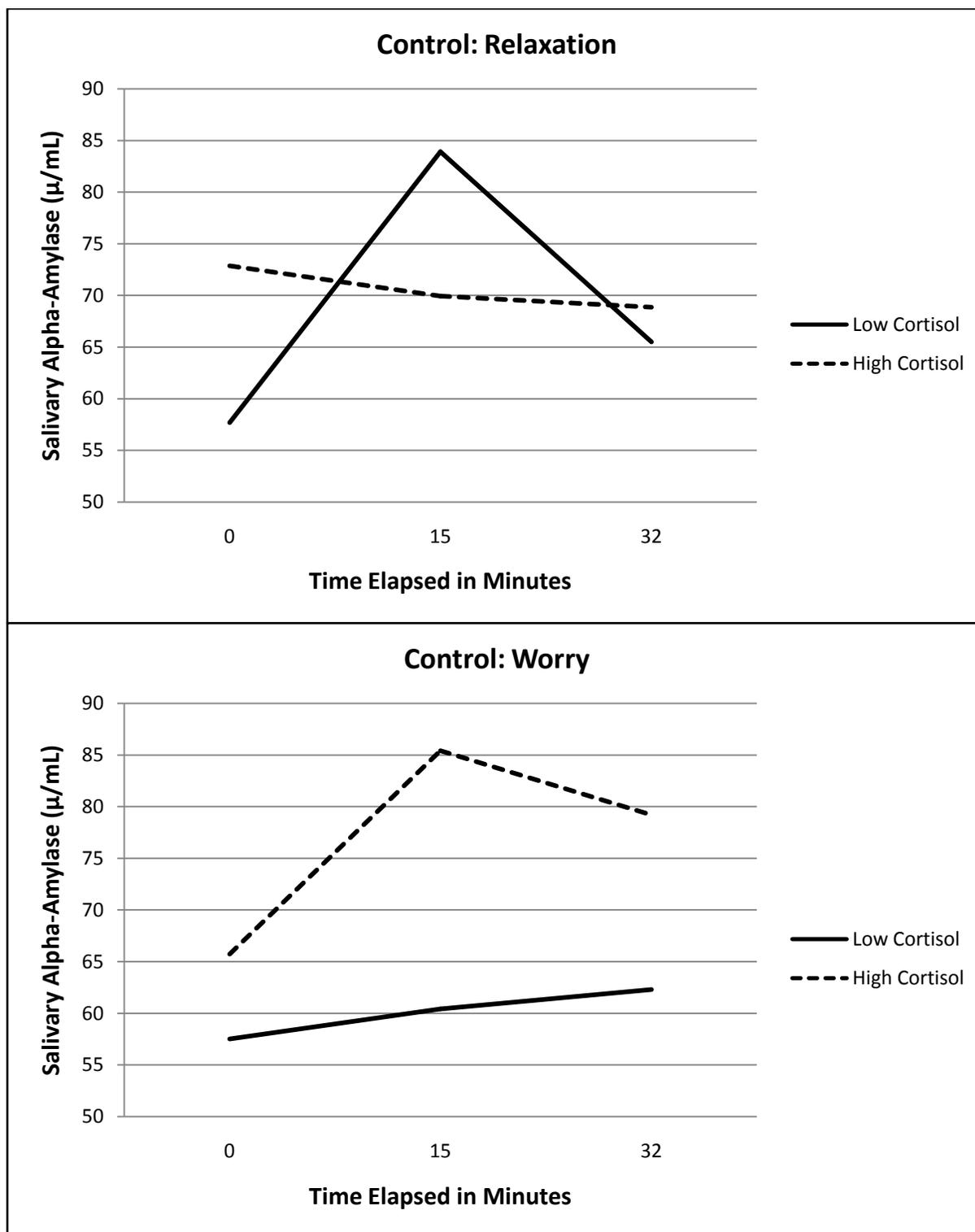
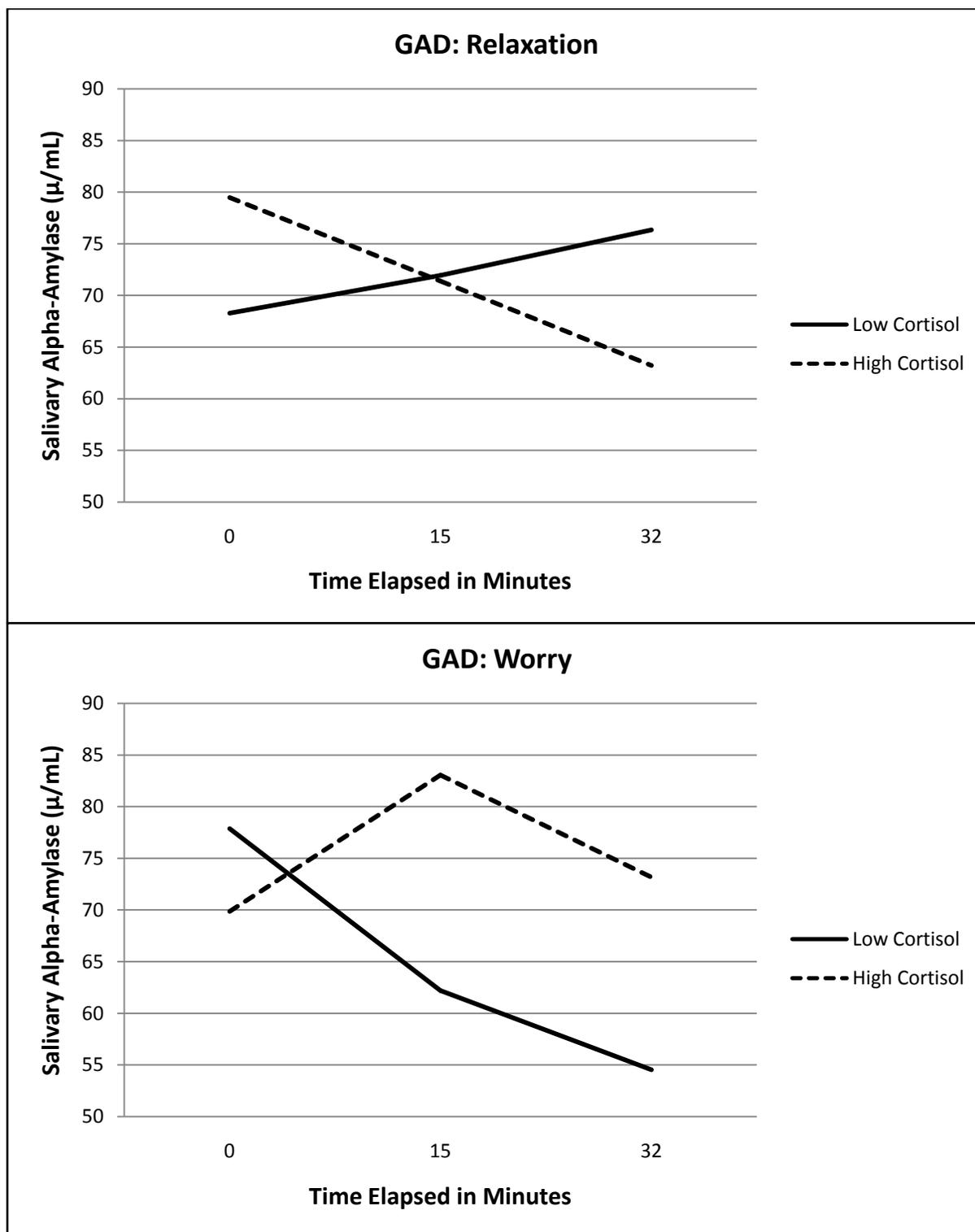


Figure 1.5: Moderating effect of available cortisol on sympathetic stress response in GAD participants, within the relaxation and worry conditions.



GAD = generalized anxiety disorder.

Figure 2.1: Vagal cardiac control, by group, at baseline.

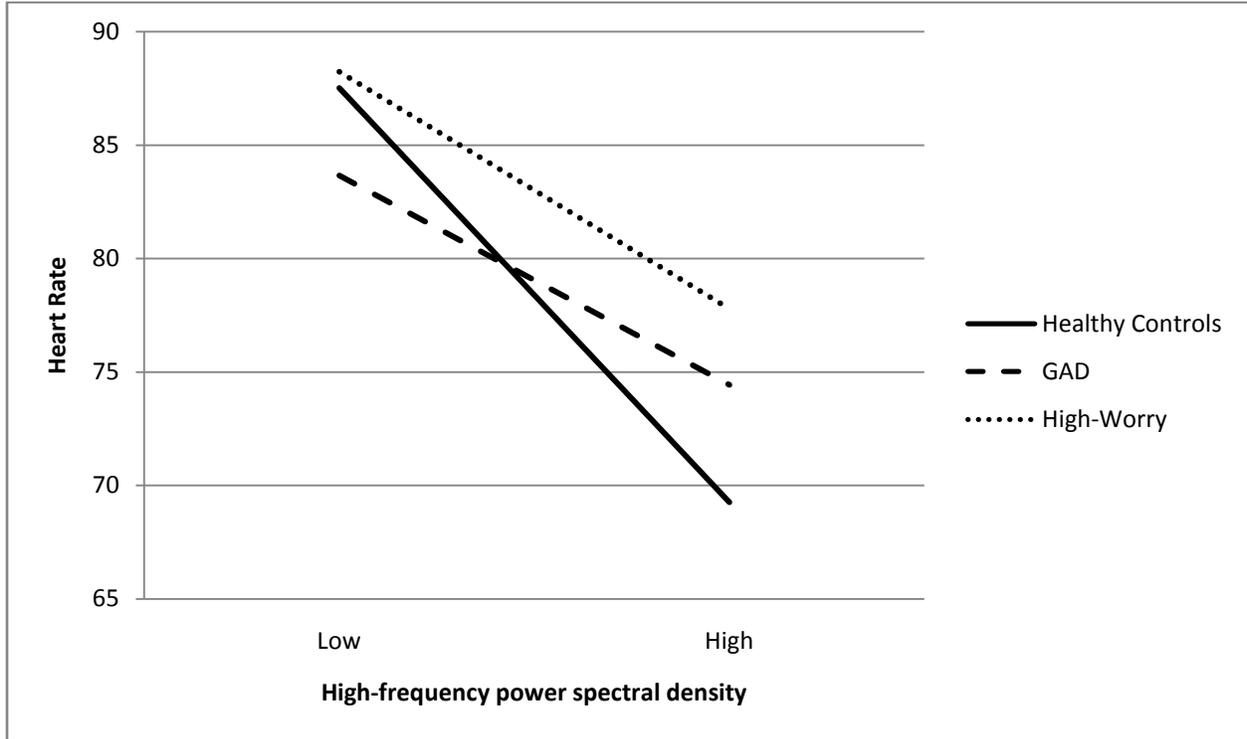


Figure 2.2: Moderating effect of salivary alpha-amylase on vagal cardiac control in GAD group following recovery period.

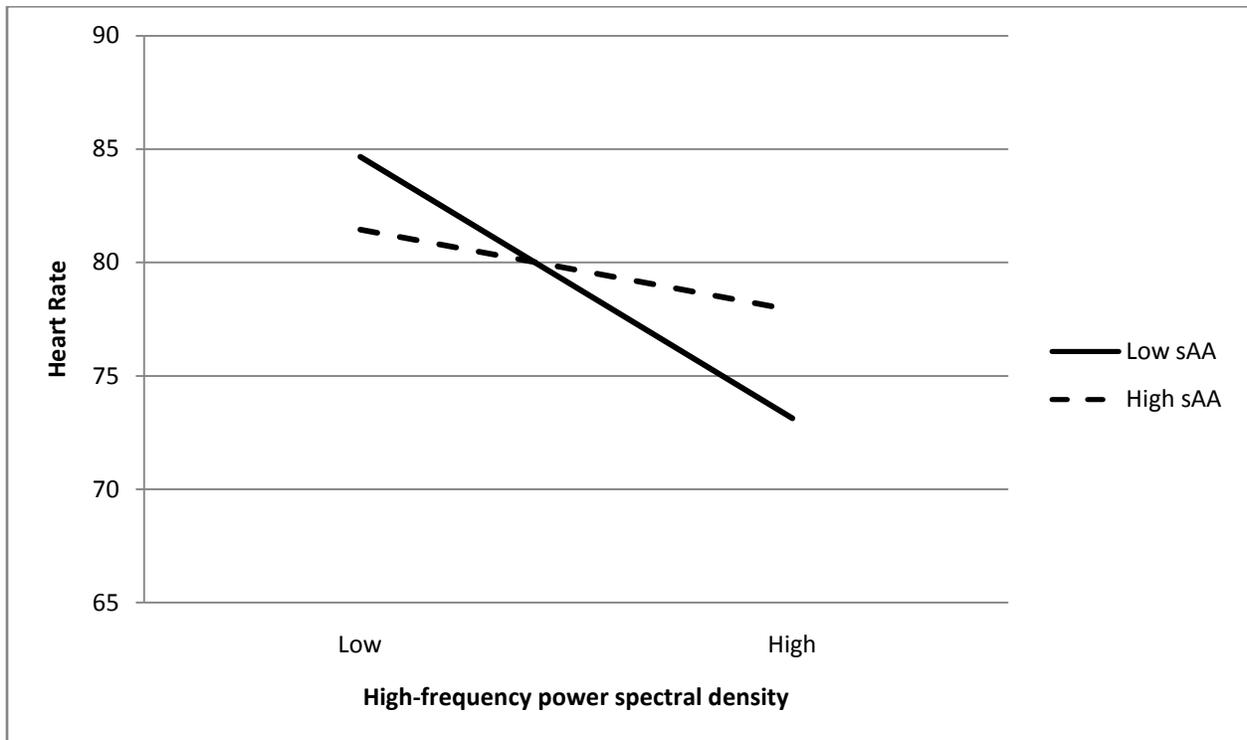
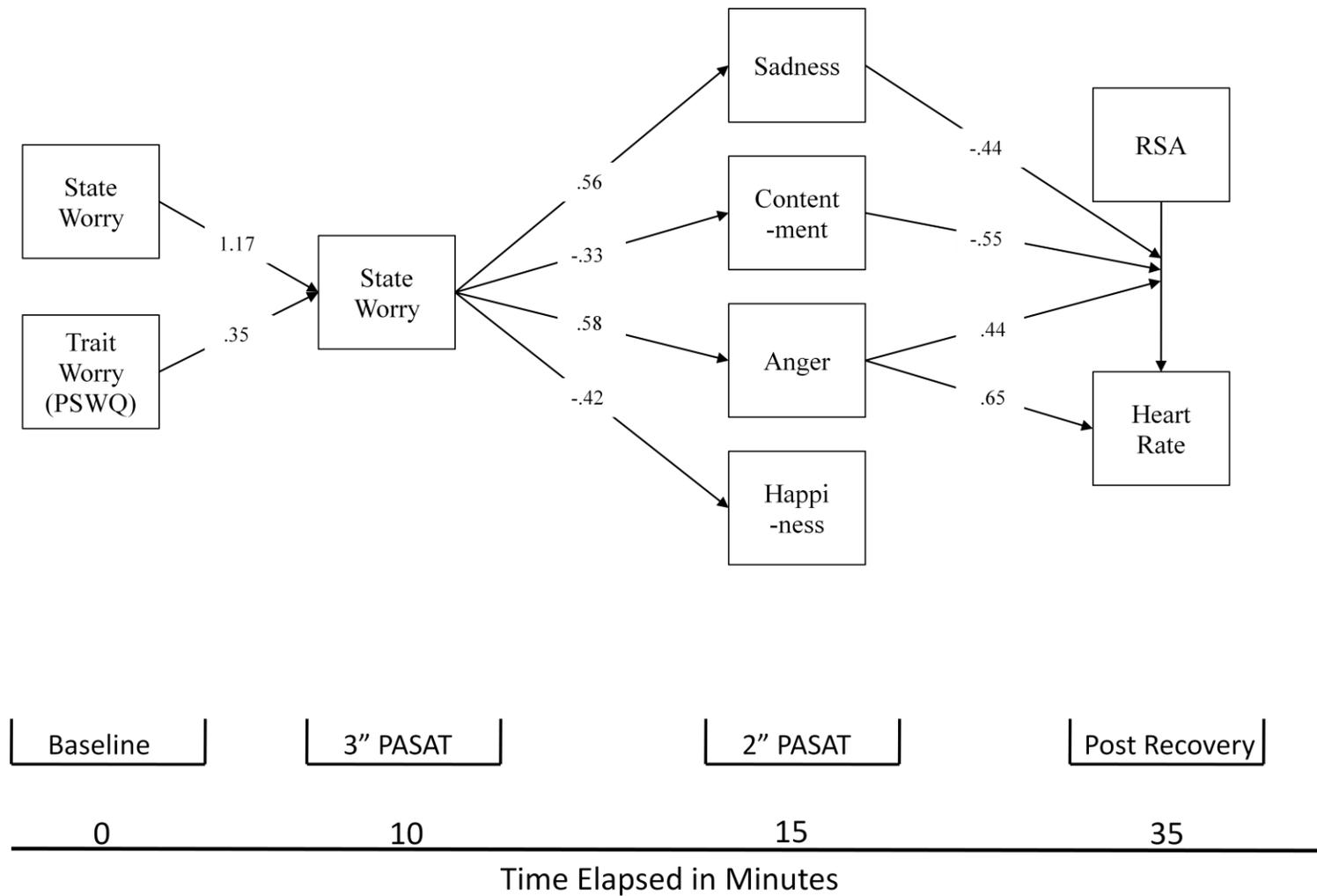


Figure 3.1: Diagram for path model (see table 2 for parameter estimates). Loadings presented as effect size (Cohen's d).



PSWQ = Penn State Worry Questionnaire; RSA = respiratory sinus arrhythmia; PASAT = Paced Auditory Serial Addition Task; 2'' = 2 second interstimulus interval; 3'' = 3 second interstimulus interval.

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27-32.

AARON J. FISHER

Stanford University School of Medicine
Psychiatry and Behavioral Sciences
401 Quarry Road
Palo Alto, CA 94304
Email: ajfisher@stanford.edu

EDUCATION

- 2012 **Ph.D., Clinical Psychology**
The Pennsylvania State University
- 2012 **Predoctoral Internship in Clinical Psychology**
VA Palo Alto Health Care System (APA Accredited)
- 2008 **M.S., Psychology**
The Pennsylvania State University
- 2006 **Post Baccalaureate Certificate, Psychology**
Columbia University
- 2000 **B.A., Music**
Wesleyan University

ACADEMIC HONORS AND AWARDS

- 2011 *Penn State Department of Psychology, Outstanding Publication Award (\$500)*
- 2011 *Society for Psychotherapy Research, Hans H. Strupp Memorial Award (\$850)*
- 2010 *American Psychological Foundation, Graduate Research Scholarship (\$1,000)*
- 2010 *Salimetrics, Graduate Student Research Award (\$5,700)*
- 2010 *Penn State College of the Liberal Arts, Dissertation Support Grant (\$3,250)*
- 2010 *Penn State Department of Psychology, Outstanding Publication Award (\$500)*
- 2008 *Society for Psychotherapy Research, Laura N. Rice Memorial Award (\$500)*
- 2008 *Psi Chi National Honor Society in Psychology, Outstanding Teaching Assistant Award*
- 2007 *Penn State Department of Psychology, H. Goffberg Memorial Research Award (\$350)*
- 2007 *Penn State College of the Liberal Arts, Nagle Family Graduate Fellowship*
- 2006 *Penn State Research and Graduate Studies Office, Travel Award (\$500)*
- 2006 *Penn State Department of Psychology, Five-Year Graduate Scholar Award*