THE IMPACT OF TRAIT ANXIETY AND PSYCHOSOCIAL STRESS ON SYMPATHETIC NEURAL CONTROL IN HUMANS

by

Jeremy Andrew Bigalke

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Psychological Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2023

©COPYRIGHT

by

Jeremy Andrew Bigalke

2023

All Rights Reserved

ACKNOWLEDGEMENTS

Throughout my academic career, I am lucky to have received mentorship from not only outstanding scientists, but also incredible, kind, and caring individuals. I would first like to thank my advisor, Dr. Jason Carter. Dr. Carter's drive and persistence have served as an inspiration to me during my time in his lab. I am forever grateful to Dr. Erica Wehrwein, without whom I would not be where I am today. Dr. Zhiying (Jenny) Shan first introduced me to research in a graduate setting during my M.S. degree. Her patience and knowledge further increased my desire to understand the physiology of cardiovascular regulation. I would not be where I am without the constant support of my parents, as well as my siblings, Alec, Mandy, and Karen, and I will forever be thankful to have been blessed with the family I have. My fiancé, Jenny, has been a constant source of support throughout the past few years. She always pushes me to become a better person in all facets of life, and I look forward to sharing the rest of our lives together!

I would also like to thank all of my committee members including Dr. Erica Wehrwein, Dr. Cara Palmer, Dr. Neha John-Henderson, and Dr. Brandon Scott for their willingness to serve on my dissertation committee. Last, I would like to thank all the friends and collaborators I have had the honor of working with including the sleep lab team including Dr. Ian Greenlund, Anne Tikkanen, and Elyse Barkstrom, as well as Dr. Josh Gonzalez, who has been an amazing friend and colleague since day one at MTU. Additionally, I would like to thank Dr. Manda Keller-Ross and Dr. John Durocher, who were instrumental in completing the current dissertation. Lastly, I would like to thank the numerous undergraduate researchers that have helped over the years.

TABLE OF CONTENTS

1. Literature Review 1

Stress and adaptation 1
Allostatic overload 2
Anxiety and cardiovascular health 5
Role of the SNS 6
Regulation of the SNS 7
Direct measures of sympathetic activity 7
Regulation of the SNS at rest 8
Regulation of the SNS during stress 10
Anxiety and resting muscle sympathetic nerve activity 12
Healthy adults 12
Anxiety disorders 15
Sympathetic reactivity to mental stress 18
Healthy adults 18
Anxiety disorders 22
Summary 24
Purpose and hypotheses 26

2. Blood Pressure and Muscle Sympathetic Nerve Activity
are Associated with Trait Anxiety in Humans 29

Contribution of authors & co-authors 29
Manuscript information 31
Introduction 32
Methods 34
Participants 34
Study design 35
Measurements 36
Data analysis 38
Statistical methods 38
Results 40
Subject characteristics 40
Correlational analyses 41
Trait anxiety, blood pressure, and MSNA 42
Discussion 47
Conclusion 57

3. Sympathetic neural reactivity to the trier social stress test 58

Introduction 58

TABLE OF CONTENTS CONTINUED

Methods 61
Participants 61
Study design 62
Trier Social Stress Test 63
Measurements 65
Data analysis 66
Statistical methods 68
Results 70
Subject characteristics 70
Perceived stress reactivity 71
Baroreflex sensitivity and HRV during anticipatory stress 72
Cardiovascular reactivity 74
Sympathetic reactivity 76
Relationship between DAP and MSNA 79
Relationship between MSNA reactivity during preparation, speech, and math 80
Threat versus challenge appraisal and cardiovascular reactivity 82
Relationship between trait anxiety and TSST reactivity 84
Discussion 84
Conclusion 91

4. Summary and Future Directions 92

Summary of findings 92
Future directions 94

REFERENCES CITED 97

APPENDICES 114

APPENDIX A: Chapter 2 raw data 115
APPENDIX B: Chapter 3 raw data 119
APPENDIX C: Chapter 2 statistical output 134
APPENDIX D: Chapter 3 statistical output 141

LIST OF TABLES

Table Page

2.1 Participant characteristics 40

2.2 Resting cardiovascular measures 41

2.3 Correlation matrix 42

2.4 Regression coefficients 44

3.1 Participant characteristics 70

3.2 Baseline vs. preparation period baroreflex sensitivity and HRV 73

3.3 Sex stratified cardiovascular reactivity 75

3.4 Sex stratified MSNA reactivity 78

LIST OF FIGURES

Figure Page

1.1. Types of allostatic load and overload 4

1.2. Representative autonomic recording 12

1.3. Influence of anxiety/mood disturbance on resting sympathetic activity in individuals with metabolic syndrome 14

1.4. Relationship between sympathetic and blood pressure responses to mental stress 20

1.5. Summarized relationship between clinical anxiety and MSNA 25

2.1. Associations between trait anxiety and blood pressure 45

2.2. Associations between trait anxiety and resting MSNA 46

3.1. Perceived stress reactivity to the TSST between sexes 72

3.2. Cardiovascular reactivity to the TSST 74

3.3. MSNA responses to the TSST 77

3.4. Relationship between changes in DAP and MSNA 79

3.5. Relationship between changes in MSNA during the preparation compared to speech and math phases 81

3.6. Relationship between threat vs. challenge appraisal and MAP reactivity 82

3.7. Relationship between trait anxiety and MAP, HR, MSNA reactivity 83

GLOSSARY or NOMENCLATURE

BF – MSNA burst frequency

BI – MSNA burst incidence

BMI – Body mass index

CO – Cardiac output

cvBRS – Cardiovagal baroreflex sensitivity

CVLM – Caudal Ventrolateral Medulla

DAP – Diastolic arterial pressure

ECG - Electrocardiogram

GAD – Generalized anxiety syndrome

HF – High frequency HRV

HR – Heart rate

HRV – Heart rate variability

LF – Low frequency HRV

MAP – Mean arterial pressure

MDD – Major depressive disorder

MetSyn – Metabolic syndrome

MSNA – Muscle sympathetic nerve activity

NE – Norepinephrine

NTS – Nucleus Tractus Solitarius

PD – Panic disorder

pNN50 - % of RRI varying by 50ms or more

PTSD – Post-traumatic stress disorder

RMSSD – Root mean squared of successive differences

RRI – R-R interval

RVLM – Rostral Ventrolateral Medulla

SAP – Systolic arterial pressure

sBRS – Sympathetic baroreflex sensitivity

SNS – Sympathetic nervous system

STAI – Spielberger trait anxiety inventory

SV – Stroke volume

TSST – Trier social stress test

ABSTRACT

Anxiety is highly prevalent, and while it is often adaptive, excessive stress and anxiety may predispose individuals to a heightened risk of cardiovascular disease. While excessive activity of the sympathetic nervous system (SNS) may underlie this association, direct measures of muscle sympathetic nerve activity (MSNA) indicate little, if any, alterations in resting sympathetic outflow in individuals with anxiety disorders. Assessment of the relationship between trait anxiety, MSNA, and blood pressure using a large cohort of healthy adults has not yet been conducted. Further, utilization of stress tasks within microneurographic settings that minimize the potential influence of breathing alterations, muscle movement, and other variables on the typically observed inter-individual variability in MSNA responsiveness to mental stress are needed to adequately assess the sole contribution of psychological stress on sympathetic neural activity. In **Study 1,** the association between trait anxiety, MSNA, and resting blood pressure was assessed in a population of 88 healthy adults, representing the largest study to date pairing trait anxiety with directly recorded sympathetic outflow to the periphery. Our findings indicate an independent relationship between trait anxiety, MSNA, and blood pressure when controlling for both age and sex. In **Study 2**, we utilized the trier social stress test (TSST) to assess the impact of anticipatory stress on MSNA and blood pressure in 28 healthy adults. Our findings showed that anticipatory stress is associated with increased blood pressure and reduced MSNA. Additionally, this appears to be baroreflex mediated as the magnitude of changes in blood pressure were directly proportional to reductions in MSNA, a relationship that was weakened or nonexistent during the active speech portion of the task. Lastly, anticipatory MSNA responsiveness accurately predicted reactivity to subsequent stress tasks. Together, these studies highlight a key relationship between both chronic, and acute psychological stress and anxiety on sympathoneural function in healthy adults.

CHAPTER ONE

LITERATURE REVIEW

Stress and Adaptation

Stress has become a ubiquitous term utilized in common vernacular across the globe to define numerous circumstances in which external pressures mount and elicit physical, emotional, and/or psychological strain. However, the concept of stress within the field of physiology was only introduced within the last century. Specifically, Hans Selye is credited with introducing the idea of stress into the realm of physiological research (H. Selye, 1950; Hans Selye, 1976; H. Selye, 1998). Initially repurposed from its use in physical science, Selye utilized the term stress to encompass the physiological reaction to environmental perturbations, which he further outlined as a generalized, nonspecific physiological response. Although some concepts surrounding the use of stress in physiological research, such as the theory of nonspecificity have been refuted (Pacak et al., 1998), the fundamental role of stress in our understanding of physiological function is nonetheless paramount to how the body, and human health, is viewed today as it pertains to reactivity to environmental pressure.

Although the ideas of physiologists such as Walter Cannon and Selye provided a foundation upon which our understanding of the human body was formed, the ideas of homeostasis and stress responsiveness were somewhat rigid. For instance, the idea of homeostatic preservation developed by Cannon (1932) outlined that the body responds to external stimuli in order to make its way back towards functioning at a homeostatic

setpoint. However, the problem with the rigidity of this proposed system was that it did not allow room for a dynamic capability of the physiological system to respond to stress in a manner which allows adjustment of constantly shifting set points. To improve upon these potential shortcomings, the concept of allostasis was subsequently developed to account for the innumerable means by which the human body responds to challenges and alterations to normal function in an active and dynamic manner (Sterling & Eyer, 1988). Effectively, allostasis outlines the means by which physiological occurrences counteract environmental perturbations, ultimately allowing for stability through change. Allostasis encompasses the robustness of the physiological system against insult and change, highlighting its innate ability to maintain a healthy level of function while operating at varying levels and set points which are most beneficial in response to a given stressor. While this mechanism of allostatic preservation is always active, there are occurrences whereby its efficiency in counteracting environmental pressure is dampened, resulting in an inability to adequately respond to stimuli, leading to detrimental consequences for the health and wellbeing of the organism.

Allostatic Overload

Stress and anxiety in response to environmental factors are a normal occurrence in everyday life. In all actuality, acute stress is an adaptive response that is necessary to maintain homeostatic balance in the face of environmental perturbations. However, there are instances where misplaced or excessive stress and anxiety can be detrimental to the physiological system. McEwen (1998) outlines four means by which allostatic preservation fails, and allostatic overload ensues, ultimately leading to chronic ailment

(**Figure 1.1**). First, repeated exposure to stress in excess of what the body can adequately handle results in chronic activity of the stress response system. In the second instance of allostatic overload, adaptation to the same stress over time becomes impaired, resulting in repetitive responsiveness when adjustment and adaptation should have occurred. The third type occurs when individuals are unable to adequately recover from a stressor which has subsided, resulting in prolonged activation of the stress system after cessation of the stress itself. The final form of allostatic overload occurs when responsiveness to stress is blunted, indicating an inability of the body to respond in a sufficient manner to any given stressor. Through these mechanisms, allostatic load and overload may result in chronic conditions, such as anxiety disorders, which can be detrimental to numerous physiological systems.

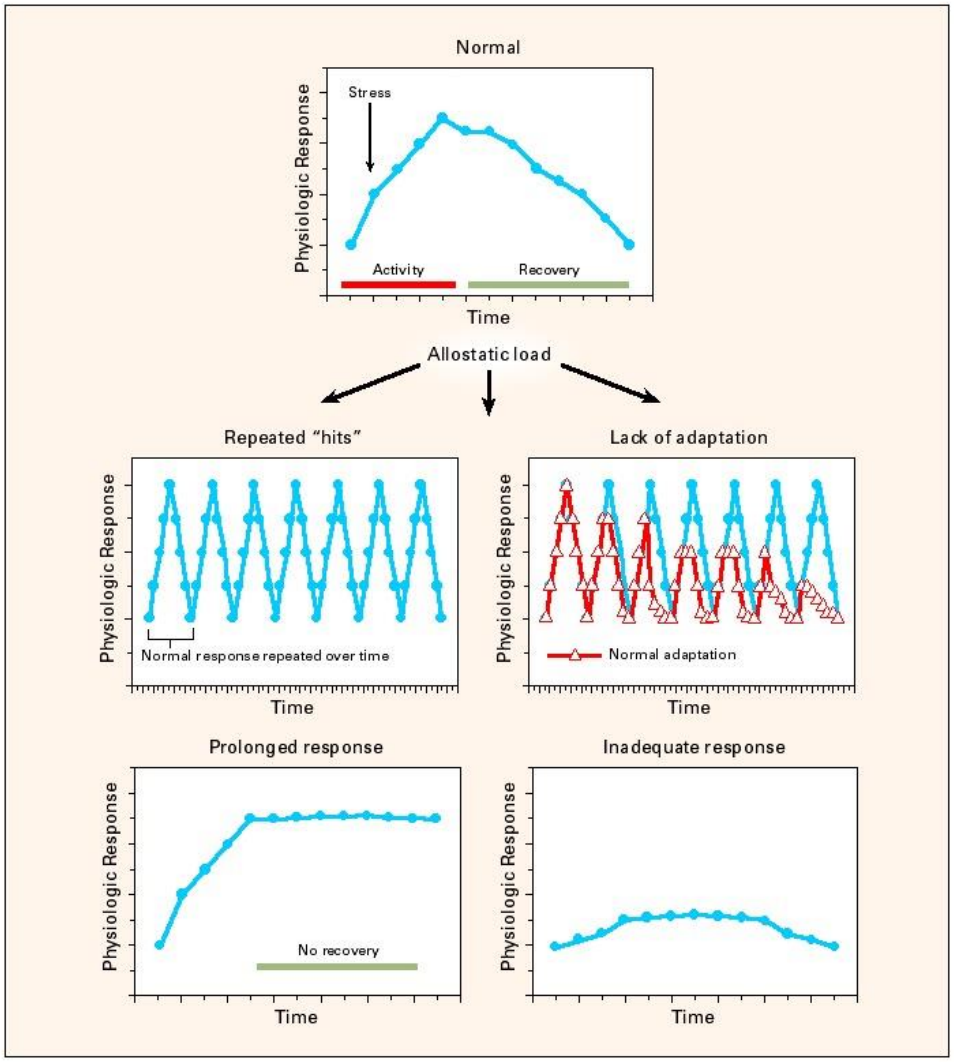


Figure 1.1: Types of allostatic load and overload.
The top panel shows normal reactivity to stress, whereby an active stress response is followed by adequate recovery to baseline. Below the top panel, 4 types of allostatic overload are shown including 1) excessive presentation of stress (repeated hits); 2) Lack of adaptation over time; 3) A prolonged recovery response; 4) or general inadequate responsiveness. Reproduced with permission from (McEwen BS (1998). Protective and Damaging Effects of Stress Mediators. New England Journal of Medicine, 338:171-179), Copyright © Massachusetts Medical Society.

Anxiety disorders are highly prevalent, occurring in over a third of the population (Kessler et al., 2005; Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). Anxiety disorders occur at young ages, and continue throughout the lifespan, indicating a lifelong impact of psychological disturbance on those individuals effected. Anxiety disorders are heavily comorbid with other physiological processes. Of relevance to the current dissertation, and as is discussed in the following section, anxiety disorders are heavily intertwined with chronic cardiovascular dysfunction (Lim, Solmi, & Cortese, 2021; Pan et al., 2015; Roest, Martens, de Jonge, & Denollet, 2010; Roest, Zuidersma, & de Jonge, 2012). The high prevalence of anxiety combined with associations with cardiovascular disease highlight the necessity for further research assessing the intersect of stress, anxiety, and cardiovascular function, as well as physiological systems that might mediate these associations.

Anxiety and Cardiovascular Health

Evidence supporting the association between stress, anxiety, and cardiovascular disease comes from large, epidemiological analyses. Numerous studies have shown significant associations between anxiety disorders and the development of cardiovascular diseases, including hypertension (Jonas, Franks, & Ingram, 1997), coronary heart disease (Roest et al., 2010; Smaardijk et al., 2019; Watkins et al., 2013), and acute myocardial infarction (Roest et al., 2012). A recent systematic review and meta-analysis by Lim et al. (2021) utilized both cross sectional and prospective analyses to assess this interaction in over 4 million individuals. Across 47 cross-sectional studies, the authors found that anxiety disorders were associated with a 37%

increase in the odds of hypertension diagnosis (Lim et al., 2021). While this offers a strong associative relationship, data from 14 prospective studies indicated a 40% increase in the odds of hypertension development in individuals with an anxiety disorder at baseline (Lim et al., 2021), indicating a causal relationship between anxiety disorders and hypertension development. Furthermore, in individuals with cardiovascular disease, anxiety may be associated with a heightened risk of mortality (Watkins et al., 2013), indicating that psychological factors may exacerbate chronic cardiovascular dysfunction. The strength of evidence linking anxiety and cardiovascular disease highlights the need for a better understanding of mechanisms that contribute to this relationship, as well as potential therapeutic interventions geared towards reducing the risks associated with chronic stress and anxiety.

Role of the sympathetic nervous system (SNS)

One such system that may be underlying the association between anxiety and cardiovascular dysfunction is the sympathetic nervous system (SNS). The SNS has been established as a key mediator in stress responsiveness reaching back to the initial findings of Walter Cannon, and subsequent studies which have highlighted the key roles for epinephrine and norepinephrine (NE) in the stress response (Cannon, 1932; V. Euler, 1946). Since the discovery of the role for these catecholamines in the proficiency of physiological stress responsiveness, regulation of the SNS has become a staple for researchers interested in the reactivity to both acute and chronic psychological stress. Furthermore, hyperactivity of the SNS is associated with numerous cardiovascular diseases including hypertension (Grassi, Cattaneo, Seravalle, Lanfranchi, & Mancia,

1998; Grassi et al., 2018), heart failure (Grassi et al., 1995), as well as conditions such as obstructive sleep apnea (Narkiewicz et al., 1999; Narkiewicz, van de Borne, Cooley, Dyken, & Somers, 1998; Somers, Dyken, Clary, & Abboud, 1995). The key role for the SNS in mediating the stress response, as well as numerous associations between overactivity of the SNS and cardiovascular disease, indicates a key mediating role for the SNS between excessive stress exposure, anxiety, and the development of cardiovascular dysfunction.

Regulation of the SNS

Direct sympathetic measures Only two procedures allow direct measurement of sympathetic nerve activity in humans. The first technique is NE spillover, which utilizes radioisotope dilution methodologies (Eisenhofer et al., 1996; Esler et al., 1988; Esler et al., 1990; Wallin et al., 1992; Wallin, Thompson, Jennings, & Esler, 1996). This technique allows assessment of NE spillover into plasma circulation, as well as its clearance through numerous means including reuptake and degradation, and further allows assessment of regional, organ-specific sympathetic activity, and has been used to quantify sympathetic activity at the level of the heart (Eisenhofer et al., 1996; Esler et al., 1988; Esler et al., 1990; Wallin et al., 1992), and kidneys (Wallin et al., 1996). However, NE spillover is an invasive protocol that requires catheterization and is a time-consuming technique to perform. The highly specialized procedure and the training required to carry out NE spillover measurement limits its usage in most laboratories.

The second technique to directly quantify peripheral sympathetic activity is through a procedure called microneurography (**Figure 1.2**) (Carter, 2019; Hagbarth &

Vallbo, 1968). Microneurography consists of the insertion of a tungsten recording electrode into superficial peripheral nerves at various anatomical locations. Percutaneous insertion of the electrode into the efferent peripheral sympathetic postganglionic nerve fascicle allows direct, real-time recording of action potentials of C-fibers within the nerve (Carter, 2019; Shoemaker, Klassen, Badrov, & Fadel, 2018; Vallbo, Hagbarth, & Wallin, 2004). While this technique allows assessment of nerve activity that innervates the skin vasculature, piloerector cells, and sweat glands (skin sympathetic nerve activity), most studies have monitored muscle sympathetic nerve activity (MSNA), which directly innervates the peripheral vasculature of skeletal muscle to modulate blood pressure. MSNA allows assessment of acute changes in peripheral sympathetic outflow over time at rest and in response to stressors, and is highly correlated with sympathetic outflow to the heart (Wallin et al., 1992) and kidneys (Wallin et al., 1996) at rest. The utilization of direct MSNA recordings allows a reproducible (Fagius & Wallin, 1993; Fonkoue & Carter, 2015; Sundlof & Wallin, 1977) methodology that accurately characterizes autonomic function in various populations both at rest and in response to stress. The remainder of this dissertation will focus on direct MSNA measurements, and will supplement with other sympathetic measures (i.e., NE spillover) as necessary.

Regulation of the SNS at rest A key manner in which the SNS responds to acute stress is through the release of NE at postganglionic varicosities, as well as the adrenal medulla (Bigalke & Carter, 2021; Tank & Lee Wong, 2015). However, it should be noted that the majority of NE is released from postganglionic varicosities, as only ~20% of

chromaffin cells within the adrenal medulla that are stimulated by sympathetic preganglionic neurons release NE, whereas the other 80% of chromaffin cells contain phenylethanolamine-N-methyltransferase which serves to convert NE into epinephrine (Tank & Lee Wong, 2015). While epinephrine plays a key role in stress reactivity, the remainder of the dissertation will focus primarily on the actions of NE. The readers are referred to other reviews, including some from our own laboratory (Bigalke & Carter, 2021; Carter & Goldstein, 2015), which cover the impact of epinephrine on organ systems (Tank & Lee Wong, 2015; Wehrwein, Orer, & Barman, 2016)

Upon release from sympathetic varicosities, NE binds to two α-adrenergic G-protein coupled receptors (α-1 and α-2) located throughout the body. NE carries out its primary cardiovascular regulatory role through interaction with α-1 receptors on the peripheral vasculature. Upon binding to the peripheral vasculature, NE causes vasoconstriction, and increases in total peripheral resistance, which subsequently increases blood pressure. NE can also alter the structure of the vasculature as discussed in a recent comprehensive review from our laboratory (Bigalke & Carter, 2021).

Activity of peripheral sympathetic nerve traffic is primarily regulated by the arterial baroreflex as shown in **figure 1.2**. Baroreceptors are mechano-sensory receptors located within the vessels of the aorta and carotid sinus (Wehrwein & Joyner, 2013). Increases in pulsatile pressure (i.e., increased blood pressure) is sensed by the baroreceptors. These receptors then send excitatory input to the nucleus tractus solitarius (NTS) via the glossopharyngeal and vagus nerves. The NTS then sends

glutamatergic projections to the caudal ventrolateral medulla (CVLM), which inhibits sympathetic premotor neurons within the rostral ventrolateral medulla (RVLM), effectively inhibiting sympathetic outflow to the periphery and reducing blood pressure. Once blood pressure is reduced sufficiently, disinhibition of the RVLM occurs through a cessation of baroreceptor activation, allowing premotor sympathetic nerves in the RVLM to fire once more at their intrinsic rate. Similarly, in response to increased blood pressure, excitatory synapses from the NTS interact with neurons within the nucleus ambiguous and dorsal motor nucleus of the vagus, causing downstream activation of postganglionic parasympathetic nerves at the level of heart, resulting in bradycardia through the release and binding of acetylcholine.

Regulation of the SNS During Stress In response to stress, the baroreflex generally remains functional, although it may begin to operate at a higher blood pressure, a process called baroreflex “resetting.” This is beneficial in instances where it is more proactive and physiologically relevant to operate at heightened blood pressure levels, such as during exercise (Ogoh, Fisher, Raven, & Fadel, 2007; Papelier, Escourrou, Gauthier, & Rowell, 1994; Potts, Shi, & Raven, 1993). Only a handful of studies have assessed sympathetic baroreflex regulation during psychological stress in humans (Bigalke, Greenlund, Nicevski, Tikkanen, & Carter, 2022; Durocher, Klein, & Carter, 2011; Incognito et al., 2019), with studies showing increased (Incognito et al., 2019), decreased (Durocher, Klein, et al., 2011), or unchanged (Bigalke et al., 2022) baroreflex sensitivity in response to psychogenic stress. However, alterations in baroreflex sensitivity primarily occur during active alterations in blood pressure during

the first 2 minutes of mental stress tasks (Durocher, Klein, et al., 2011; Incognito et al., 2019) but remain unchanged compared to baseline once blood pressure levels plateau (Bigalke et al., 2022; Durocher, Klein, et al., 2011), indicating resetting to a higher blood pressure level. Conversely, cardiovagal baroreflex sensitivity, or the regulatory efficiency of the vagus nerve in maintaining blood pressure, appear to be blunted in response to psychological stress (Bigalke et al., 2022), indicative of a potential dichotomous response in sympathetic versus parasympathetic regulation. Alterations in baroreflex function may underly chronic conditions such as heart failure (Grassi et al., 1995) and obstructive sleep apnea (Narkiewicz, Pesek, et al., 1998; Toschi-Dias, Trombetta, Dias da Silva, et al., 2013), highlighting the importance of baroreflex regulation of sympathetic nerve activity in proper physiological functioning both acutely and chronically.

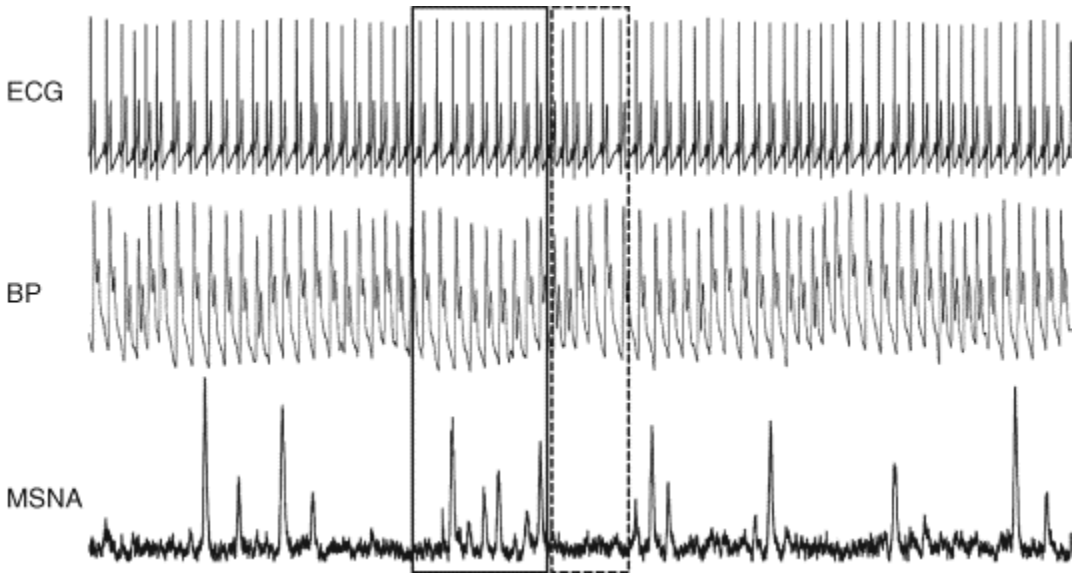


Figure 1.2: Representative autonomic recording.
An exemplary recording of muscle sympathetic nerve activity (MSNA) from the peroneal nerve in tandem with beat-by-beat blood pressure (BP) and electrocardiogram (ECG). When blood pressure falls (solid box), MSNA activity increases to counteract the BP reduction. Conversely, when blood pressure increases (dotted box), MSNA is silenced through baroreflex inhibition. Reproduced with permission from (Bigalke JA, Carter JR (2021). Sympathetic Neural Control in Humans with Anxiety-Related Disorders. Comprehensive Physiology, 12(1):3085-3117), Copyright © American Physiological Society.

Anxiety and Resting Muscle Sympathetic Nerve Activity

Healthy Adults

While various studies have highlighted a key role for increased MSNA as a mediator of cardiovascular risk (Grassi et al., 2019; Grassi et al., 1998; Grassi et al., 2018; Grassi et al., 1995; Grassi et al., 2000; Quarti Trevano et al., 2020), as outlined in our recent comprehensive review (Bigalke & Carter, 2021), MSNA has been characterized in only a handful of studies which assessed the influence of trait anxiety. In a population of individuals with metabolic syndrome, Toschi-Dias et al. (2013) found

that anxiety symptoms and mood disturbance were associated with a significant increase in resting MSNA, independent of the metabolic sequelae characteristic of metabolic syndrome (**Figure 1.3**). Similarly, Lambert et al. (2010) observed a significant relationship between trait anxiety and the firing of single vasoconstrictor neurons (i.e., single-unit MSNA) in individuals with both metabolic syndrome and elevated blood pressure. The combination of these findings indicate that anxiety symptom severity may lead to further sympathoexcitation in populations characterized by heightened sympathetic outflow and blood pressure, and may partially explain the proposed additive role for anxiety on increasing mortality associated with pre-existing cardiovascular disease states (Watkins et al., 2013). However, the underlying cardiometabolic dysfunction of the individuals within these studies make elucidation of the independent effects of anxiety severity on neural-cardiovascular control difficult to ascertain. For instance, in the study by Toschi-Dias et al. (2013), the participants within the cohort of adults with both metabolic disorder and anxiety/mood disturbance also exhibited the highest level of circulating triglycerides. A recent meta-analysis reported a significant independent association between triglycerides and resting MSNA, particularly in obese individuals (Grassi et al., 2019). These findings indicate that the underlying metabolic disturbance within the study population may be impacting the reported association between anxiety and MSNA. While the findings of sympathoexcitation in individuals with concurrent metabolic syndrome and anxiety/mood disturbance are informative, substantiation of these findings in healthy populations is necessary to solidify an independent role for anxiety severity in sympathetic outflow.

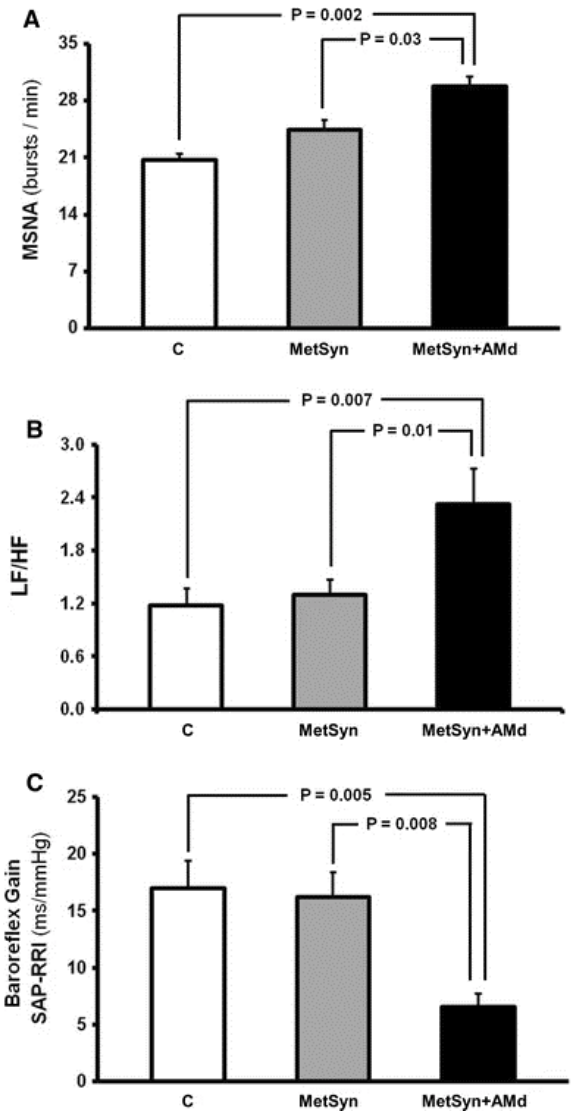


Figure 1.3: Influence of anxiety/mood disturbance on resting sympathetic activity in individuals with metabolic syndrome.
Resting muscle sympathetic nerve activity (MSNA), low-frequency to high-frequency heart rate variability ratio (LF/HF), and cardiovagal baroreflex sensitivity in healthy adults (C), individuals with metabolic syndrome (MetSyn), and concurrent MetSyn and anxiety/mood disorder (MetSyn+AMd). Individuals with MetSyn+AMd had elevated resting MSNA when compared to both controls and individuals with MetSyn alone, indicating a potential independent effect of excessive anxiety on resting sympathetic activity. Reproduced with permission from (Toschi-Dias, E., Trombetta, I. C., da Silva,

V. J., Maki-Nunes, C., Alves, M. J., Angelo, L. F., Cepeda, F. X., Martinez, D. G., Negrão, C. E., & Rondon, M. U. (2013). Symptoms of anxiety and mood disturbance alter cardiac and peripheral autonomic control in patients with metabolic syndrome. European journal of applied physiology, 113(3), 671–679.), Copyright © Springer Nature.

Unfortunately, studies to date assessing the impact of trait anxiety on directly measured sympathetic outflow are limited. One other study assessed the influence of personality type on MSNA at rest and in response to numerous stressors (Schroeder et al., 2000). Although anxiety severity was not measured, Schroeder et al. (2000) assessed MSNA in a population of individuals with varying levels of “type A” personality which is associated with stress, hostility, aggression, among other factors that may be tangentially related to anxiety. The authors did not observe any differences in MSNA at rest or in response to mental or physical stress when separating the population into tertiles of type A personality levels (Schroeder et al., 2000), opposing the view of a role for personality and psychological characteristics in modulating peripheral sympathetic outflow.

Anxiety Disorders

While evidence of excessive sympathoexcitation as a function of anxiety symptom severity using directly measured MSNA is limited to populations with concurrent cardiometabolic disturbance (E. Lambert et al., 2010; Toschi-Dias, Trombetta, da Silva, et al., 2013), further studies have been conducted in individuals with established anxiety disorders such as generalized anxiety disorder (GAD) (Holwerda et al., 2018), panic disorder (PD) (Barton et al., 2007; E. Lambert et al., 2006; E. A. Lambert et al., 2011; E. A. Lambert et al., 2002; Wilkinson et al., 1998),

specific phobias (Donadio et al., 2007), and trauma related conditions such as post-traumatic stress disorder (PTSD) (Fonkoue, Hu, et al., 2020; Fonkoue, Marvar, et al., 2020; Fonkoue et al., 2018; Park et al., 2017; Yoo et al., 2020). However, even within these study populations, there is little evidence of altered sympathetic outflow at baseline. A recent review from our laboratory highlighted this lack of evidence in baseline sympathoexcitation in adults with anxiety disorders (Bigalke & Carter, 2021). Interestingly, the only evidence of altered multiunit MSNA comes from studies performed in individuals with PTSD (Fonkoue, Marvar, et al., 2020; Park et al., 2017; Yoo et al., 2020). Specifically, individuals with diagnosed PTSD were observed to have augmented MSNA relative to noncombat experienced controls (Yoo et al., 2020), but not when compared to combat veterans who did not have PTSD (Fonkoue, Marvar, et al., 2020; Park et al., 2017). Conversely, compared to healthy controls, individuals with GAD (Holwerda et al., 2018), PD (Barton et al., 2007; E. Lambert et al., 2006; E. A. Lambert et al., 2011; E. A. Lambert et al., 2002; Wilkinson et al., 1998), and social phobia (Fonkoue, Marvar, et al., 2020; Park et al., 2017) do not exhibit any alterations in baseline MSNA outflow compared to healthy populations. When using directly measured NE spillover, similar null findings were observed. For example, individuals with PD had unchanged whole body and cardiac NE spillover at rest compared to healthy controls (Alvarenga, Richards, Lambert, & Esler, 2006; Villacres, Hollifield, Katon, Wilkinson, & Veith, 1987; Wilkinson et al., 1998), although clearance of NE at the end-organ may be reduced resulting in prolonged responsiveness.

Multiunit MSNA and NE spillover recordings highlight largely unaltered sympathetic outflow in populations with clinical anxiety disorders. However, the utilization of techniques including single-unit MSNA (Macefield & Wallin, 2018; Macefield, Wallin, & Vallbo, 1994) recording and action potential clustering (Salmanpour et al., 2011; Salmanpour & Shoemaker, 2012) have allowed further interrogation of underlying sympathetic recruitment patterns in individuals with clinical anxiety. In individuals with panic disorder, a higher likelihood of a single sympathetic fiber firing multiple times within one integrated sympathetic burst is present (E. Lambert et al., 2006), a phenotype associated with exaggerated NE release at the level of the heart (E. A. Lambert et al., 2011) and other conditions such as OSA, hypertension, and heart failure (Elam, McKenzie, & Macefield, 2002; Greenwood, Stoker, & Mary, 1999; Macefield, Rundqvist, Sverrisdottir, Wallin, & Elam, 1999). Importantly, these subtle recruitment strategy alterations are not readily accessible using multiunit MSNA recordings. A similar phenotype was observed in individuals with metabolic disorder and elevated blood pressure, whose single-unit multiple firing prevalence was directly correlated to underlying trait anxiety scores (E. Lambert et al., 2010), potentially indicating a role for clinical anxiety in altering the recruitment patterns of sympathetic nerves despite unchanged multiunit integrated burst occurrence.

The combination of these findings indicates relatively unchanged resting sympathetic outflow in individuals with clinical anxiety disorders, opposing the hypothesis of sympathoexcitation as a trademark of clinical anxiety. However, many of these studies were performed using limited sample sizes. As inter-individual differences

in MSNA are present and quite pronounced, the utilization of limited sample sizes in addition to the presence of comorbid conditions such as metabolic syndrome make conclusions regarding sympathetic outflow in clinical anxiety disorders difficult to ascertain.

Sympathetic Reactivity to Mental Stress

The lack of studies assessing direct measures of sympathetic activity in conditions associated with increased anxiety makes a consensus regarding underlying sympathetic alterations in these populations difficult. Further, causal assessment of the effect of anxiety on sympathoneural control is difficult to establish due to practical and ethical constraints. Nonetheless, numerous studies have assessed sympathetic reactivity to laboratory mental stress as a proxy for understanding the effects of stress on sympathetic outflow, in the hopes of better understanding how alterations in sympathetic activity in response to acute stress may translate to populations who experience chronic stress. Further, studies in individuals with anxiety disorders have allowed elucidation of underlying differences in sympathetic recruitment in response to stress in individuals with anxiety disorders, allowing a better understanding of the effects of chronic anxiety on MSNA and cardiovascular control (Bigalke & Carter, 2021).

Healthy Adults

Most studies reporting MSNA reactivity to laboratory mental stress have been conducted in healthy populations (Bigalke & Carter, 2021; Carter & Goldstein, 2015). Laboratory mental stress elicits a reproducible pressor response across studies

(Fonkoue & Carter, 2015). While some studies indicate a tandem increase in sympathetic reactivity (Anderson, Sinkey, & Mark, 1991; Anderson, Wallin, & Mark, 1987; Callister, Suwarno, & Seals, 1992; Carter & Lawrence, 2007; Carter & Ray, 2009; Matsukawa, Sugiyama, & Mano, 1995), many others report unchanged (Carter, Durocher, & Kern, 2008; Incognito et al., 2019; Kuipers, Sauder, Carter, & Ray, 2008; Wasmund, Westerholm, Watenpaugh, Wasmund, & Smith, 2002) or reduced (Carter & Ray, 2009; Halliwill et al., 1997; Jones et al., 1996) MSNA in response to mental stress. Carter et al. (2009) have indicated that while the majority of individuals present either unchanged or increased MSNA, some respond with reductions in sympathetic outflow, highlighting considerable variation in sympathetic responsiveness to mental stress between participants. Furthermore, the sympathetic responses to mental stress have been shown to be unrelated to blood pressure reactivity (Carter & Ray, 2009) (**Figure 1.4**), indicating that sympathetic outflow is not preceding the large increases in blood pressure that are observed in most cases. Still others have indicated that although sympathetic activity is not associated with overall blood pressure maintenance during mental stress, the rate at which blood pressure increases is related to subsequent MSNA responsiveness (El Sayed, Macefield, Hissen, Joyner, & Taylor, 2016, 2018), indicating a responding, rather than leading role for vascular sympathetic responsiveness to mental stress.

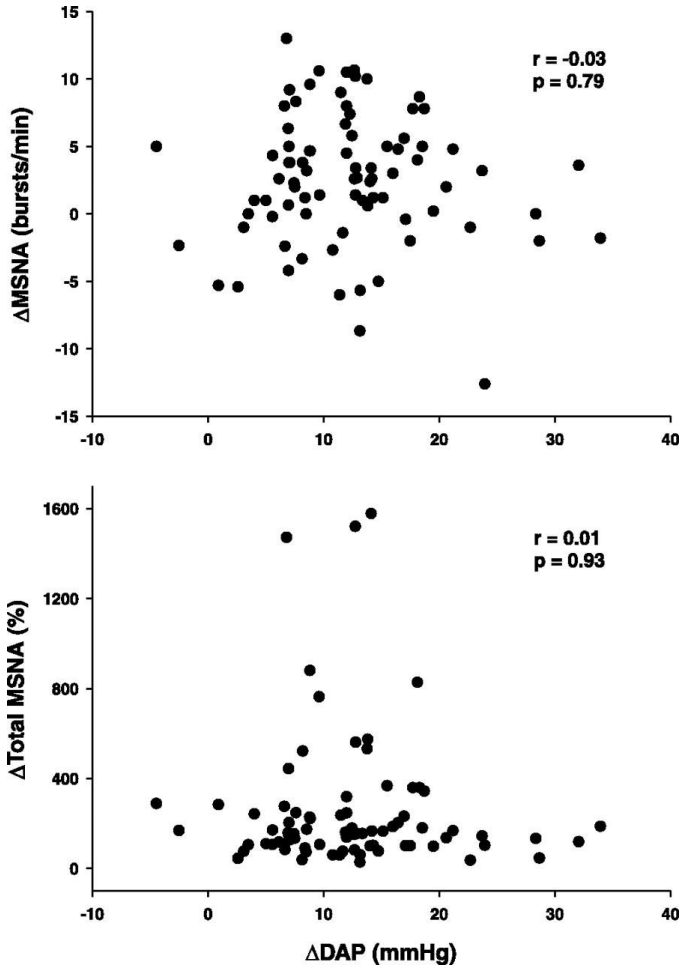


Figure 1.4: Relationship between sympathetic and blood pressure responses to mental stress.
Changes in diastolic arterial pressure (DAP) have been observed to be unrelated to changes in muscle sympathetic nerve activity (MSNA). Reproduced with permission from (Carter, J. R., & Ray, C. A. (2009). Sympathetic neural responses to mental stress: responders, nonresponders and sex differences. American journal of physiology. Heart and circulatory physiology, 296(3), H847–H853), Copyright © American Physiological Society.

The reason underlying the inter-individual variability in sympathetic responsiveness is unknown, but has been discussed in a recent in-depth review of the topic (Bigalke & Carter, 2021). Although sympathetic reactivity to mental stress between individuals is highly variable, it is reproducible within subjects (Fonkoue & Carter, 2015),

indicating individual characteristics which may be impacting responsiveness. Some evidence points to an impact of perceived stress on sympathetic outflow responses to mental stress. Callister et al. (1992) performed MSNA assessment in a group of healthy adults in response to varying difficulties of the stroop color word task. Importantly, the group performed testing in the absence of talking and used a computer to make the task non-verbal. Their findings indicate that sympathetic responsiveness is proportional to the perceived stress of participants, as well the absolute difficulty level of the tasks (Callister et al., 1992). These findings indicate that divergence in sympathetic responsiveness may be partially attributed to an individual's assessment and appraisal of the stress task. This finding may be particularly relevant to individuals with heightened anxiety symptomology who may perceive events and occurrences as more stressful and threatening (Bishop, 2007; O'Donovan, Slavich, Epel, & Neylan, 2013).

However, numerous other studies have challenged this concept (Carter et al., 2008; Carter & Ray, 2009; Durocher, Lufkin, King, & Carter, 2011; Freyschuss et al., 1990). Exposure of participants to emotional stress led to a significant increase in perceived stress, but unchanged cardiovascular and sympathetic reactivity (Carter et al., 2008). Similarly, social technology removal increased self-perceived levels of state anxiety, but had no impact on sympathetic reactivity (Durocher, Lufkin, et al., 2011). Lastly, in a large population of healthy adults, sympathetic reactivity to mental stress was observed to have no relationship to perceived stress, indicating the absence of an association between changes in perceived stress and sympathetic neural responsiveness to laboratory stress (Carter & Ray, 2009). The combination of these

findings both support and refute a role of perceived stress, making a consensus regarding the impact of perceived stress on sympathoneural reactivity difficult to establish in healthy adults.

Anxiety Disorders

A handful of studies have observed the sympathetic response to mental stress in populations experiencing chronic anxiety, adding further understanding of the influence of clinical anxiety disorders on mental stress reactivity (Bigalke & Carter, 2021). While baseline MSNA in individuals with anxiety disorders has indicated little to no alterations in baseline integrative MSNA, findings from mental stress exposure have shed more light on the impact of anxiety symptom severity on reactivity to stress (Bigalke & Carter, 2021).

In individuals with GAD, MSNA amplitude, or the strength of a sympathetic burst, is significantly increased compared to healthy controls in response to mental stress (Holwerda et al., 2018). Importantly, this difference began during anticipation of the stress, indicating excessive sympathoexcitation during anticipation of ensuing threat in individuals with GAD (Holwerda et al., 2018). This increase in sympathetic responsiveness was positively correlated to trait anxiety severity within the study population (Holwerda et al., 2018). In individuals with PD, while reactivity to laboratory mental stress was unchanged, MSNA amplitude was significantly increased during spontaneous panic attacks, and increased further with the severity and length of the attack (Wilkinson et al., 1998). In individuals with PTSD, combat simulation stress evoked an exaggerated MSNA response to combat and mental stress, but not cold

pressor stress (Park et al., 2017). Lastly, in individuals with specific phobia, greater sympathoinhibition was observed following startling stimuli when compared to non-phobic individuals susceptible to syncope following orthostatic stress (Donadio et al., 2007).

These reports of altered sympathetic neural control in the face of stress indicate that autonomic abnormalities associated with clinical anxiety and stress may be more pronounced during stress, rather than at rest. These alterations in sympathetic outflow are remarkably specific to each condition and are highly dependent on the stressor type and presentation method. Individuals with GAD respond to anticipatory stress (Holwerda et al., 2018). Those with PD only respond to panic attacks, and not mental arithmetic (Wilkinson et al., 1998). Individuals with specific phobias respond specifically to startling stimuli (Donadio et al., 2007), and individuals with PTSD respond with augmented MSNA during combat simulation, but not cold stress (Park et al., 2017). This presentation of stressor specificity indicates the importance of stressor type and presentation when assessing psychological stress and its impact on sympathetic reactivity in different populations. However, most studies have utilized either forced mental arithmetic or the stroop color word task to elicit psychological stress. Utilization of new stress tasks is necessary to disentangle the complex manner by which acute and repeated stress, as well as chronic anxiety symptomology elicit sympathetic alterations and abnormal cardiovascular regulation.

Summary

Anxiety is a natural and beneficial occurrence necessary for homeostatic function and maintenance. However, when present in excess, anxiety is associated with a plethora of physiological abnormalities. Relevant to the current study, anxiety is causally related to hypertension, heart disease, and the exacerbation of pre-existing cardiovascular diseases. Despite this association, the role of the SNS as a mediator of this relationship remains unknown.

Studies utilizing direct measures of SNS activity such as microneurography (i.e., MSNA), remain inconsistent. Studies performed in individuals with clinical anxiety disorders have indicated little, if any alterations in global sympathetic outflow, particularly as measured via multi-unit MSNA (**Figure 1.5**). In those studies that have indicated an influence of anxiety severity on MSNA, comorbid cardiometabolic dysfunction makes assessment of the independent effects of anxiety on sympathetic activity difficult to pinpoint. Lastly, those studies that have performed analysis between anxiety and MSNA have limited sample sizes, which may not offer adequate assessment of the subtle relationships between trait anxiety severity, blood pressure, and directly measured sympathetic outflow.

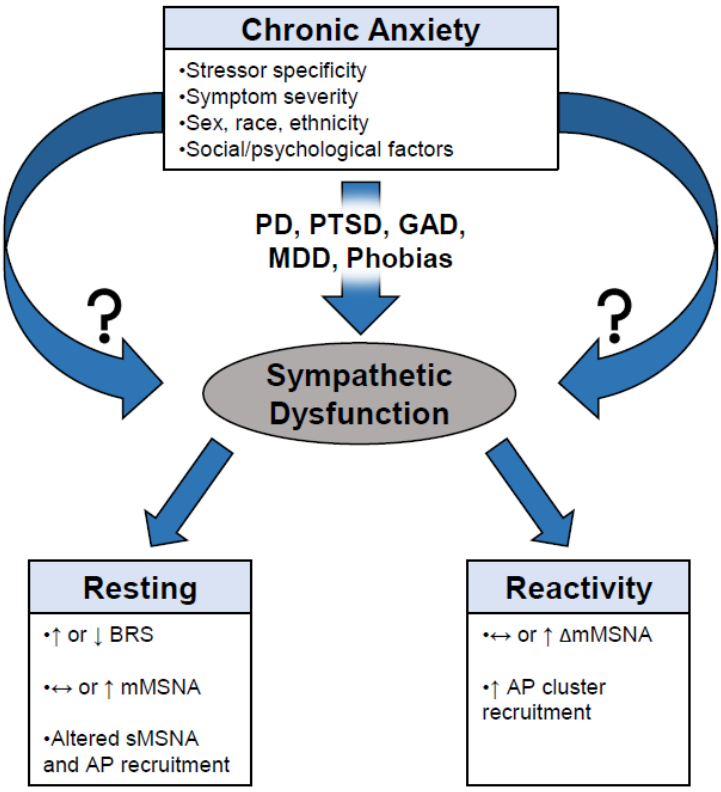


Figure 1.5: Summarized relationship between clinical anxiety and muscle sympathetic nerve activity.
Clinical anxiety has been shown to have little impact on baseline muscle sympathetic nerve activity (MSNA). Conversely, individuals with clinical anxiety disorders have presented altered sympathetic recruitment strategies in response to stress. Nonetheless, our current understanding of how chronic stress and anxiety impact sympathetic and cardiovascular function at rest and in response to stress is limited. Reproduced with permission from (Bigalke JA, Carter JR (2021). Sympathetic Neural Control in Humans with Anxiety-Related Disorders. Comprehensive Physiology, 12(1):3085-3117), Copyright © American Physiological Society.

The utilization of laboratory mental stress tasks has been used to assess and characterize sympathetic responsiveness to stress in both healthy individuals as well as populations with clinical anxiety disorders, with studies indicating altered sympathetic reactivity in clinicalally anxious populations (**Figure 1.5**). However, these results are

highly variable between individuals, and studies performed in individuals with anxiety disorders have offered mixed findings, with interesting relationships between sympathetic reactivity and stressor specificity (Bigalke & Carter, 2021). Additionally, the ecological validity of laboratory stressors to what is experienced in real-life is difficult to establish, with current stress tasks offering a cognitive challenge, rather than a psychosocial stressor. The additive effects of active speech, muscle movement, and irregular breathing patterns during typical mental stress study paradigms (i.e. mental arithmetic) may have a significant impact on sympathetic reactivity, and have not been adequately addressed in studies to date. The utilization of a new stress task devoid of these common confounders with improved ecological validity is warranted, and may offer evidence of a sympathetic response to mental stress which more accurately characterizes what may be experienced in the real world.

Purpose & Hypotheses

Project 1: The purpose of the first project is to assess the independent relationship between trait anxiety and MSNA and resting blood pressure in a large, healthy population of adults devoid of cardiometabolic abnormalities. We **hypothesize** that trait anxiety will be independently associated with resting MSNA and blood pressure when controlling for the effects of age and sex, which have known influences on sympathetic control (Carter, Fu, Minson, & Joyner, 2013; Hart et al., 2011; Hart et al., 2009; Keir et al., 2020).

Project 2: The main purpose of the second project is to implement the Trier Social Stress Test (TSST) (Kirschbaum, Pirke, & Hellhammer, 1993) to characterize the

impact of psychosocial stress on sympathetic and cardiovascular function in young healthy adults. Importantly, the TSST consists of an anticipatory stress phase prior to socially evaluated speech and math portions, allowing for assessment of anticipatory stress alone on MSNA and cardiovascular reactivity. The TSST utilizes elements of uncontrollability and social threat which increase the ecological validity of real-world stressors. We **hypothesize** that the TSST will lead to increases in cardiovascular variables, as well as MSNA. Additionally, we **hypothesize** that the commonly observed inter-individual variability observed in MSNA reactivity to mental stress (Carter & Ray, 2009) will be dampened during the anticipatory stage due to the removal of potential confounds such as active speech, muscle movement, and respiratory alterations.

Project 2A: As a secondary aim of project 2, we will characterize the differential impact of the TSST on MSNA and cardiovascular reactivity between sexes. Susceptibility to the development of clinical anxiety disorders is elevated in females (McLean, Asnaani, Litz, & Hofmann, 2011). Additionally, sympathetic control of cardiovascular function differs between sexes (Carter et al., 2013; Hart et al., 2009; Joyner, Wallin, & Charkoudian, 2016; Keir et al., 2020). Carter et al. (2009) have highlighted sex-differences in responsiveness to mental stress, with males exhibiting heightened blood pressure reactivity, and females exhibiting exacerbated perceived stress. Others have observed no sex differences in sympathetic reactivity to mental stress (Jones et al., 1996). In response to the TSST, Childs et al. (2010) observed a greater NE release in males compared to females. However, direct recordings of MSNA in response to the TSST have not yet been characterized between sexes. Due to these

discrepant findings, the aim of this analysis is to characterize any sex differences in sympathetic and cardiovascular reactivity to the TSST.

Project 2B: Lastly, the present study aims to assess any potential relationships between trait anxiety severity and MSNA reactivity to the TSST. Holwerda et al. (2018) reported exaggerated MSNA reactivity to anticipatory stress in individuals with GAD. Similarly, the present study **hypothesizes** that elevated trait anxiety symptoms within healthy adults will be associated with augmented MSNA reactivity to the TSST, particularly during the anticipatory phase of the task.

CHAPTER 2

BLOOD PRESSURE AND MUSCLE SYMPATHETIC NERVE ACTVITY ARE ASSOCIATED WITH TRAIT ANXIETY IN HUMANS[1]

Contribution of Authors and Co-Authors

Manuscript(s) in Chapter(s) 2:

Author: Jeremy A. Bigalke

Contributions: Conceived and designed the research, performed experiments/data collection, analyzed data, interpreted results, prepared figures, drafted manuscript, edited and revised manuscript, approved of final manuscript version.

Co-Author: John J. Durocher

Contributions: Performed experiments/data collection, interpreted results, edited and revised manuscript, approved of final manuscript version.

Co-Author: Ian M. Greenlund

Contributions: Performed experiments/data collection, interpreted results, edited and revised manuscript, approved of final manuscript version.

Co-Author: Manda Keller-Ross

---

[1] The current chapter was previously published in the American Journal of Physiology – Heart and Circulatory Physiology ("Copyright (2023) The American Physiological Society. Used with permission from (Bigalke JA, Durocher JJ, Greenlund IM, Keller-Ross M, Carter JR. “Blood pressure and muscle sympathetic nerve activity are associated with trait anxiety in humans.” *American Journal of Physiology – Heart and Circulatory Physiology*. Copyright 2023, The American Physiological Society).

Contributions: Performed experiments/data collection, interpreted results, edited and revised manuscript, approved of final manuscript version.

Co-Author: Jason R. Carter

Contributions: Conceived and designed the research, performed experiments/data collection, interpreted results, edited and revised manuscript, approved of final manuscript version.

Manuscript Information

Authors: JA Bigalke, JJ Durocher, IM Greenlund, M Keller-Ross, JR Carter

Journal: American Journal of Physiology – Heart and Circulatory Physiology

Status of Manuscript:
____ Prepared for submission to a peer-reviewed journal
____ Officially submitted to a peer-reviewed journal
____ Accepted by a peer-reviewed journal
__X_ Published in a peer-reviewed journal

Publisher: American Physiological Society
Issue: Online Ahead of Print
DOI: 10.1152/ajpheart.00026.2023

Introduction

Anxiety is common within the general population, with an estimated lifetime prevalence for an anxiety disorder of ~30% (Kessler et al., 2012). Anxiety is associated with several negative health outcomes, including the development of cardiovascular disease (Lim et al., 2021; Pan et al., 2015; Roest et al., 2010; Roest et al., 2012). Two recent meta-analyses reported significant associations between anxiety disorders and hypertension, and highlighted a causal role for anxiety in the development of hypertension by integrating prospective studies within the analyses (Lim et al., 2021; Pan et al., 2015). Anxiety can also exacerbate adverse cardiovascular conditions (Watkins et al., 2013), supporting a critical role for anxiety in cardiovascular disease pathology and management.

A suspected mediator of the relationship between anxiety and hypertension (Lim et al., 2021; Pan et al., 2015) is hyperactivity of the sympathetic nervous system (SNS). Measurable increases in sympathetic outflow via direct recording of muscle sympathetic nerve activity (MSNA) have been observed in the pathogenesis of numerous cardiovascular diseases, including hypertension (Grassi et al., 2018). While MSNA has been recorded in anxiety disorders such as generalized anxiety disorder (GAD) (Holwerda et al., 2018) and panic disorder (PD) (Bechir et al., 2007; E. Lambert et al., 2006; E. A. Lambert et al., 2011; Wilkinson et al., 1998), these studies surprisingly indicate little, if any, alterations in multiunit MSNA at rest compared to healthy controls, and instead primarily highlight altered sympathetic neural responses to stress (Bigalke & Carter, 2021). Although exaggerated pressor responses to laboratory stress are

predictive of future cardiovascular dysfunction (Chida & Steptoe, 2010), the impact of exaggerated MSNA reactivity on future cardiovascular health has not yet been characterized. Conversely, longitudinal changes in resting MSNA are associated with changes in blood pressure (Hering, Kara, Kucharska, Somers, & Narkiewicz, 2016), and elevated resting MSNA is observed in numerous cardiovascular disease states (Grassi et al., 1998; Grassi et al., 2018; Grassi et al., 1995), supporting the utility of understanding differences in resting sympathetic outflow when investigating propensity for future cardiovascular risk in individuals with chronic anxiety.

Despite the lack of evidence supporting altered levels of resting MSNA in diagnosed anxiety disorders, levels of trait anxiety, which assess an individual's propensity for anxiousness (Julian, 2011; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), have been shown to correspond to heightened resting multiunit MSNA in individuals with metabolic syndrome (Toschi-Dias, Trombetta, da Silva, et al., 2013). However, these findings are complicated by the presence of comorbid metabolic and cardiovascular dysfunction (Toschi-Dias, Trombetta, da Silva, et al., 2013). To our knowledge, a relationship between trait anxiety, blood pressure, and resting MSNA has not been established in adults free of overt cardiometabolic disease.

The present study leverages the largest study population to date with concurrent trait anxiety measures, resting blood pressure, and direct recordings of sympathetic neural activity (i.e., MSNA). Importantly, the utilization of trait anxiety assessment helps to circumvent the limitations of viewing anxiety disorders as dichotomous variables, rather than a spectrum of symptom severity, when assessing associations with

cardiovascular function. We hypothesized that heightened trait anxiety would be associated with increased resting MSNA and blood pressure in the current sample of healthy adults.

Methods

Participants

Subjects were analyzed from retrospective (Bigalke et al., 2022; Carter, Durocher, Larson, DellaValla, & Yang, 2012; Greenlund et al., 2021) and ongoing studies at Montana State University, Purdue University Northwest, University of Minnesota, and Michigan Technological University. All studies were approved by the corresponding institutional review boards, and all participants provided informed consent to participate in each respective study.

Participants were healthy adults between the ages of 18-47 years. Participants were required to have a body mass index (BMI) < 35kg/m^2 and all self-reported as non-smokers. All participants reported the absence of diabetes, as well as any known cardiovascular or autonomic disorders. Participants with stage II hypertension or greater (i.e., systolic arterial pressure (SAP) > 140 mmHg or diastolic arterial pressure (DAP) > 90mmHg) were excluded. Participants reported no usage of cardiovascular or autonomic medications. All females studied were premenopausal and reported regular menstrual cycles (i.e., 26-30 days in length). Females were tested in the early follicular phase of the menstrual cycle, or low hormone phase if using hormonal contraceptives. On the day of autonomic testing, participants were instructed to fast for at least three hours prior to testing, and refrain from caffeine, alcohol, and exercise for at least 12

hours. One participant's blood pressure was a significant outlier (96/44 mmHg) with a standardized residual that exceeded three standard deviations, thus was removed from analysis. A total of 88 participants (52 males, 36 females, 25 ± 1 years, 25 ± 1 kg/m^2) were included in the final analyses.

Study Design

Participants arrived at the laboratories of Montana State University, Purdue University Northwest, University of Minnesota, or Michigan Technological University to undergo autonomic testing. All participants completed the trait anxiety portion of the Spielberger state/trait anxiety inventory (STAI) (Spielberger et al., 1983) prior to the autonomic function test at either the initial orientation session or on the morning of the autonomic testing session. Upon arrival to the respective laboratory on the scheduled day of testing, participants were instructed to sit quietly for at least five minutes, after which three seated brachial blood pressure measurements were taken, separated by one minute each (Omron HEM-907XL, Kyoto, Japan). In six participants, supine brachial blood pressure measurements were taken instead of seated and used for resting blood pressure quantification in these individuals. Findings were not different with or without these six participants and given that MSNA was the primary outcome variable we included these individuals in the final blood pressure analyses. Immediately following resting blood pressure measurements, participants were brought into the autonomic testing laboratory and positioned supine or semi-recumbent on a cushioned tilt-table. Measurement of participants' heart rate (HR, electrocardiogram), beat-to-beat blood pressure via finger plethysmography (NOVA, Finapres Medical Systems,

Amsterdam, The Netherlands and Human NIBP Controller, AD Instruments, Colorado Springs, CO), and muscle sympathetic nerve activity (MSNA; 662C-3 Nerve Traffic Analysis System, University of Iowa Bioengineering and NeuroAmp EX, ADInstruments) were obtained. Participants were then asked to sit quietly for 10 minutes of non-recorded rest prior to data collection to ensure the accuracy of all measured cardiovascular and autonomic parameters. After the 10 minute period of nonrecorded testing, blood pressures were taken to ensure baseline levels had been reached. Next, three additional brachial blood pressure measures were taken, and the average was used to calibrate the finger plethysmography prior to recording. Lastly, 10 minutes of quiet, resting autonomic and cardiovascular measurements were collected. In three participants, we obtained 5 minutes of quality MSNA and heart rate.

Measurements

Spielberger state/trait anxiety inventory (STAI).The STAI (Spielberger et al., 1983) is a 40-question assessment used to evaluate both state and trait anxiety, with each section consisting of 20 questions. For the current study, only the trait anxiety portion of the assessment was used to quantify chronic anxiety symptoms and overall propensity for anxiousness. The trait anxiety scale serves as a valid metric of chronic anxiety severity with adequate psychometric properties (Bergua et al., 2012; Santangelo et al., 2016; Spielberger et al., 1983), and has shown intra-individual reliability over time (Spielberger et al., 1983). We report on the trait anxiety percentile scores provided by Spielberger et al. (Spielberger et al., 1983), which provides a normative percentile rank

based on participant age and biological sex. The results of the current study were unchanged when using the raw STAI scores rather than the percentile rankings.

Blood pressure and heart rate The average of the three resting brachial blood pressure measurements taken before the autonomic function tests were used to quantify average systolic (SAP), diastolic (DAP), and mean arterial pressure (MAP) in each participant. Beat-to-beat blood pressure was only used to assess sympathetic baroreflex sensitivity. Average HR from the 5-10-minute autonomic session was used to quantify average resting HR.

Muscle sympathetic nerve activity Multifiber MSNA was obtained and recorded using microneurography (662C-3 Nerve Traffic Analysis System, University of Iowa Bioengineering and NeuroAmp Ex, ADInstruments) as previously described by our laboratory (Bigalke et al., 2022; Greenlund et al., 2021) and a recent guidelines paper (Hart et al., 2017). Briefly, the location of the common peroneal nerve was mapped using external electrical stimulation at the level of the popliteal fossa and fibular head to evoke involuntary muscle activation resulting in lateral dorsiflexion. Next, a tungsten recording electrode was inserted percutaneously into the peroneal nerve of the right leg at the level of the popliteal fossa or head of the fibula. A reference electrode was inserted 2-3 cm away from the recording electrode. The electrodes were then connected to a differential preamplifier and then to an amplifier (total gain 80,000) where the nerve signal was band-pass filtered (700-2000 Hz) and integrated (time constant 0.1). The recording of MSNA was confirmed by assessing the nerve response to end-expiratory apnea, as well as the absence of a response to startle (i.e.,

shout/clap). Once a recording site was obtained, it was not adjusted throughout the duration of the recording. MSNA is reproducible (Fagius & Wallin, 1993; Fonkoue & Carter, 2015) and does not appear to be impacted by state anxiety changes at rest (Durocher, Lufkin, et al., 2011).

Data Analysis

All data were imported and analyzed in either WinCPRS (Absolute Aliens, Turku, Finland) or Ensemble (Elucimed Ltd., Wellington, New Zealand). Regardless of analysis software, R-waves of the ECG were automatically detected, manually confirmed, and used to quantify heart rate. Bursts of MSNA were detected automatically based on a signal-to-noise ratio of 3:1 and a burst peak latency of ~1.3 seconds from the previous R-wave. All bursts marked by the software were manually reviewed and edited by a trained investigator. MSNA was expressed as both burst frequency (BF; bursts/minute) and burst incidence (BI; bursts/100 heartbeats (hb)).

The slope of the weighted linear regression of DAP and MSNA during the autonomic baseline was used to quantify spontaneous sympathetic baroreflex sensitivity as previously described (Bigalke et al., 2022; Greenlund et al., 2021). DAP values on a beat-to-beat basis were binned into 3 mmHg intervals, and were subsequently plotted against MSNA burst incidence. All slopes were $r > 0.7$ based on recent recommendations (Holwerda et al., 2021).

Statistical Methods

All data were analyzed using SPSS statistics version 28.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were performed to characterize participant

characteristics (i.e., anthropometric, trait anxiety, cardiovascular, and autonomic variables).

Multiple linear regression was used to examine the independent relationship between anxiety and outcome variables of interest. The primary regression models all included age, sex, and trait anxiety as predictor variables, and either average SAP, DAP, MAP, resting HR, MSNA BF, or MSNA BI as dependent variables. BMI was not included as a predictor variable as it was not correlated with the main outcome variables (MSNA BF, $R = 0.069$, $P = 0.525$; MSNA BI, $R = 0.089$, $P = 0.410$) and did not contribute significantly when included in the regression models. Multicollinearity between predictor variables was assessed, and none of the predictor variables exhibited significant multicollinearity (i.e., all $r < 0.5$). All regression models were explored for outliers with standardized residuals exceeding 3 standard deviations. Lastly, all residuals were determined to be normally distributed.

Since a portion of the presented findings were taken from previous studies (Bigalke et al., 2022; Carter et al., 2012; Greenlund et al., 2021), an a priori power analysis was not conducted. However, a post hoc sensitivity analysis was performed to find the minimum effect size that the dataset could accurately detect. Multiple regression using 3 predictors (i.e., age, sex, and trait anxiety), with a total sample size of 88, and a power $(1 - \beta)$ of 0.8 at an $\alpha = 0.05$ resulted in a minimum detectable effect size of $R^2 = 0.115$. Although sex differences were initially explored, post-hoc sensitivity analysis indicated insufficient power to probe for these differences with adequate confidence. For this reason, sex differences were not included in the current dataset,

but this remains an interest for future work. All data are presented as mean ± standard error of the mean. A significance level of $\alpha < 0.05$ was used for all statistical analyses.

Results

Subject Characteristics

Table 2.1. Participant Characteristics

Variable	Mean ± SEM [Range]
N (M/F)	88 (52/36)
Age (years)	25 ± 1 [18-47]
BMI (kg/m^2)	25 ± 1 [18-34]
STAI-T (Raw)	36 ± 1 [20-67]
STAI-T (%)	51 ± 3 [3-100]

Note: BMI, body mass index; M, male; F, female; STAI-T, Trait Anxiety form of the Spielberger State-Trait Anxiety Inventory expressed as raw values and percentile.

Demographics, seated cardiovascular, and resting autonomic measures are presented in **Table 2.1** and **Table 2.2**. sBRS was assessed in 58 (31 males, 27 females) participants due to DAP-MSNA correlations that were positive or below the a priori cutoff (i.e., $r < 0.7$). sBRS was found to be unrelated to trait anxiety ($r = -0.028$, $p = 0.835$) and was not explored in the regression analyses.

Table 2.2 Resting Cardiovascular Measures

Variable	Mean ± SEM
SAP (mmHg)	115 ± 1
DAP (mmHg)	70 ± 1
MAP (mmHg)	85 ± 1
HR (beats/min)	63 ± 1
MSNA BF (bursts/min)	20 ± 1
MSNA BI (bursts/100hb)	31 ± 1
sBRS (bursts/100hb/mmHg)	-2.1 ± 0.2

Note: Sympathetic baroreflex sensitivity (sBRS) was measured in 58 participants (31M, 27F). SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; MSNA, muscle sympathetic nerve activity; BF, MSNA burst frequency; BI, MSNA burst incidence.

Correlational Analyses

Table 2.3 includes correlation coefficients between independent (age, sex, trait anxiety) and dependent variables (SAP, DAP, MAP, HR, MSNA BF, and MSNA BI). Trait anxiety was significantly correlated with SAP ($r = 0.251$, $p = 0.018$), DAP ($r = 0.291$, $p = 0.006$), MAP ($r = 0.328$, $p = 0.002$), MSNA BF ($r = 0.237$, $p = 0.026$), and MSNA BI ($r = 0.225$, $p = 0.035$).

Table 2.3. Correlation Matrix

		1	2	3	4	5	6	7	8	9	10
Age	**R** **Sig.**	1.00	---	---	---	---	---	---	---	---	---
Sex	**R** **Sig.**	.167 .120	1.00	---	---	---	---	---	---	---	---
STAI (au)	**R** **Sig.**	-.086 .424	-.108 .316	1.00	---	---	---	---	---	---	---
STAI (%)	**R** **Sig.**	-.059 .583	-.111 .305	.957 <.001	1.00	---	---	---	---	---	---
SAP	**R** **Sig.**	.057 .600	-.480 <.001	.269 .011	.251 .018	1.00	---	---	---	---	---
DAP	**R** **Sig.**	.316 .003	.080 .458	.268 .012	.291 .006	.377 <.001	1.00	---	---	---	---
MAP	**R** **Sig.**	.262 .014	-.175 .104	.321 .002	.328 .002	.754 <.001	.892 <.001	1.00	---	---	---
HR	**R** **Sig.**	-.104 .336	-.020 .853	.101 .350	.127 .239	.140 .194	.305 .004	.283 .007	1.00	---	---
MSNA BF	**R** **Sig.**	.162 .133	-.195 .069	.224 .036	.237 .026	.293 .006	.279 .008	.343 .001	.410 <.001	1.00	---
MSNA BI	**R** **Sig.**	.191 .074	-.208 .052	.210 .050	.225 .035	.273 .010	.216 .043	.289 .006	.138 .199	.949 <.001	1.00

Note: All values presented are Pearson's correlation coefficient and two-tailed significance level. STAI, Trait form of the Spielberger state trait anxiety inventory; SAP, systolic blood pressure (mmHg); DAP, diastolic arterial pressure (mmHg); MAP, mean arterial pressure (mmHg); HR, heart rate (beat/min); MSNA, muscle sympathetic nerve activity quantified as either burst frequency (BF, bursts/min) or burst incidence (BI, bursts/100heartbeats).

Trait anxiety, blood pressure, and MSNA

Multiple regression was performed to predict SAP, DAP, MAP, HR, MSNA BF, and MSNA BI with age, sex, and trait anxiety as predictors. These variables significantly predicted SAP ($F(3,84) = 11.521$, $R^2 = 0.292$, $p < 0.001$, DAP ($F(3,84) = 6.996$, $R^2 = 0.200$, $p < 0.001$), MAP ($F(3,84) = 8.005$, $R^2 = 0.222$, $p < .001$), MSNA BF ($F(3,84) =$

4.085, R^2 = 0.127, p = 0.009), and MSNA BI (F(3,84) = 4.592, R^2 = 0.141, p =0 .005), but not HR (F(3,84) = 0.731, R^2 = 0.025, p = 0.536).

Table 2.4 demonstrates the independent associations between each of the predictor variables and the outcome variables of interest. Notably, when controlling for the effects of age and sex, trait anxiety was independently associated with SAP (β = 0.206, p = 0.028, **Figure 1A**), DAP (β = 0.317, p = 0.002, **Figure 1B**), and MAP (β = 0.325, p = 0.001, **Figure 1C**).

Table 2.4. Regression Coefficients

Outcome	Predictor	B	SEM	β	t	p
SAP	**Age**	.248	.154	.149	1.602	.113
	Sex	-10.430	2.026	-.482	-5.147	<.001
	STAI-T%	.079	.036	.206	2.232	.028
DAP	**Age**	.384	.117	.325	3.279	.002
	Sex	.942	1.537	.061	.613	.542
	STAI-T%	.087	.027	.317	3.226	.002
MAP	**Age**	.351	.109	.314	3.210	.002
	Sex	-2.791	1.433	-.191	-1.947	.055
	STAI-T%	.084	.025	.325	3.358	.001
HR	**Age**	-.132	.147	-.098	-.898	.372
	Sex	.174	1.930	.010	.090	.929
	STAI-T%	.038	.034	.122	1.124	.264
MSNA BF	**Age**	.297	.147	.209	2.021	.046
	Sex	-3.789	1.926	-.205	-1.968	.052
	STAI-T%	.075	.034	.227	2.209	.030
MSNA BI	**Age**	.524	.223	.242	2.353	.021
	Sex	-6.355	2.923	-.224	-2.174	.032
	STAI-T%	.108	.051	.214	2.103	.038

Note: N = 88 in all analyses. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; MSNA, muscle sympathetic nerve activity expressed as bursts/minute (BF) and bursts/100 heart beats (BI).

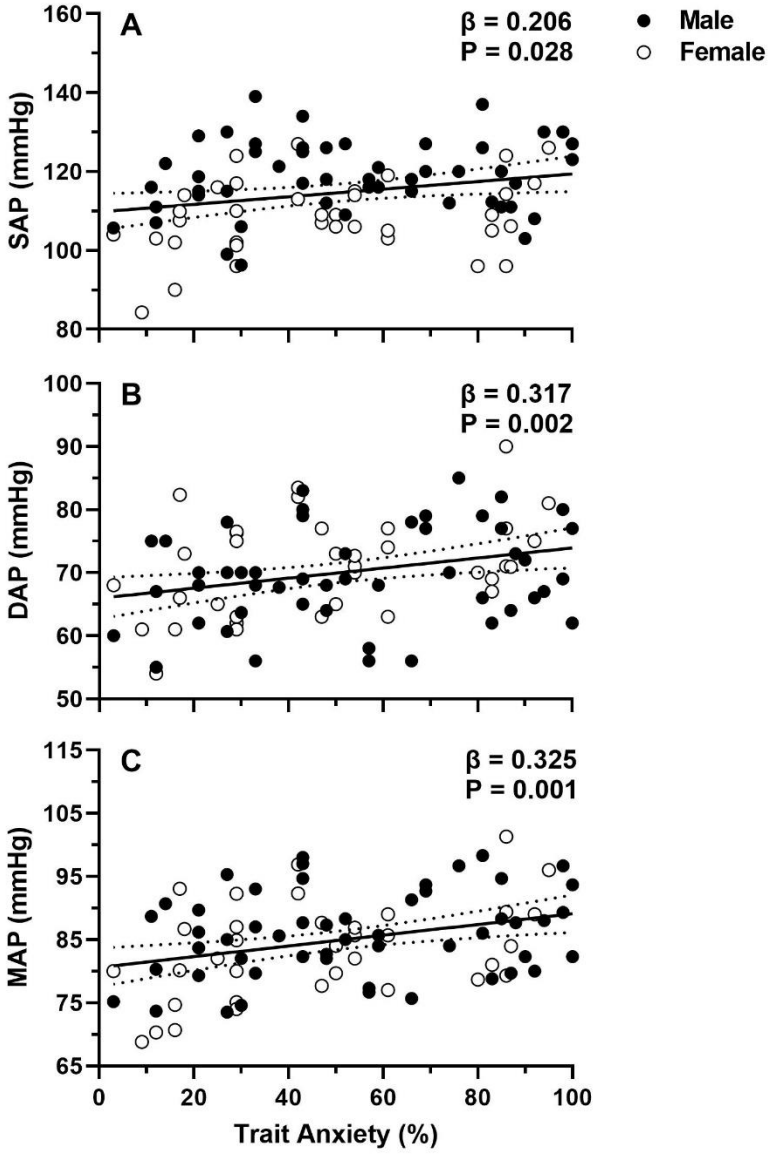


Figure 2.1: Associations between trait anxiety and blood pressure.
Trait anxiety severity was independently associated with systolic (SAP, Panel A), diastolic (DAP, Panel B), and mean arterial pressure (MAP, Panel C) when controlling for the effects of age and sex. Dotted lines indicate the 95% confidence bands of the best fit line. N = 88.

Trait anxiety was additionally independently associated with MSNA BF (β = 0.227, p = 0.030, **Figure 2A**) and MSNA BI (β = 0.214, p = 0.038, **Figure 2B**).

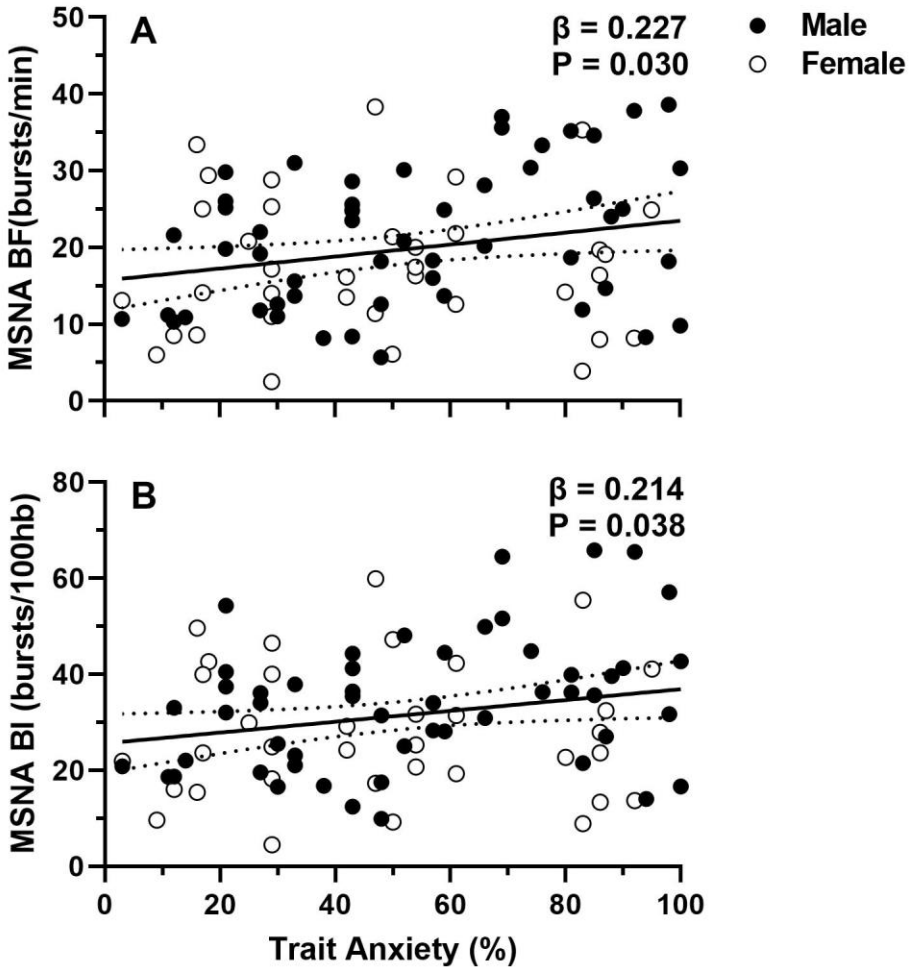


Figure 2.2: Associations between trait anxiety and resting muscle sympathetic nerve activity (MSNA).
Trait anxiety severity was independently associated with resting MSNA burst frequency (BF, Panel A) and burst incidence (BI, Panel B) when controlling for the effects of age and sex. Dotted lines indicate the 95% confidence bands of the best fit line. N = 88.

Discussion

Chronic anxiety is highly prevalent and contributes to cardiovascular diseases such as hypertension (Lim et al., 2021; Pan et al., 2015) and coronary heart disease (Roest et al., 2010), although the role of the SNS in this relationship remains equivocal. The present study examined the independent relationship between trait anxiety, blood pressure, and MSNA within a robust population of healthy adults, and we report two key findings. First, trait anxiety was independently associated with resting blood pressure. Second, trait anxiety was independently associated with resting MSNA. To our knowledge, the present study represents the largest sample size to date assessing the link between trait anxiety and resting cardiovascular function in tandem with direct measurement of MSNA via microneurography. These findings offer evidence of a link between heightened trait anxiety, augmented sympathetic outflow, and elevated blood pressure in otherwise healthy adults, highlighting a tangible relationship between chronic anxiety and autonomic function.

Previous studies have investigated the influence of clinical anxiety on blood pressure and MSNA using clinical populations such as GAD (Holwerda et al., 2018), PD (Bechir et al., 2007; E. Lambert et al., 2006; E. A. Lambert et al., 2011; Wilkinson et al., 1998), specific phobias (Donadio et al., 2007), as well as trauma related conditions such as post-traumatic stress disorder (PTSD) (Fonkoue, Marvar, et al., 2020; Park et al., 2017; Yoo et al., 2020). Despite the prevailing notion that these conditions are associated with global sympathoexcitation, few studies have reported augmented resting multiunit MSNA in these populations. In fact, the only significant elevations in

MSNA were reported in individuals with PTSD (Yoo et al., 2020), a condition categorized as a “trauma and stressor-related disorder” rather than an anxiety disorder in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (American Psychiatric Association, 2013). Nevertheless, significant overlap between PTSD and anxiety disorders has been reported (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995; Scherrer et al., 2019). Further, these elevations in MSNA were present when compared to healthy, noncombat experienced controls (Yoo et al., 2020), but not apparent when compared to combat experienced veterans without PTSD (Fonkoue, Marvar, et al., 2020; Park et al., 2017).

In contrast, Toschi-Dias et al. (Toschi-Dias, Trombetta, da Silva, et al., 2013) explored the impact of clinically diagnosed anxiety/mood disturbance comorbid with metabolic syndrome on MSNA, and reported that individuals who had anxiety/mood disturbance in addition to metabolic syndrome had heightened MSNA at rest compared to healthy controls and individuals with metabolic syndrome alone. While these findings are informative, the underlying metabolic and cardiovascular characteristics of this cohort make assessment of the independent effects of trait anxiety on MSNA difficult to disentangle. For instance, the individuals diagnosed with comorbid anxiety and mood disturbance had the highest levels of circulating triglycerides (Toschi-Dias, Trombetta, da Silva, et al., 2013), a metabolic characteristic that was found to be associated with resting MSNA in a recent meta-analysis, particularly in obese individuals (Grassi et al., 2019).

The present study addresses an important scientific gap by investigating the influence of trait anxiety on neural cardiovascular function in healthy adults while controlling for age and cardiometabolic health through study inclusion/exclusion criteria and statistical analyses. Our findings demonstrate significant and independent associations between trait anxiety, sympathoexcitation, and blood pressure. With 88 healthy participants, this is the largest study to date assessing the relationship between trait anxiety, blood pressure, and directly measured peripheral sympathetic activity (i.e., MSNA). The observed elevations in both blood pressure and MSNA in the absence of sBRS alterations suggest a resetting of baroreflex control of peripheral sympathetic outflow to a higher blood pressure set point in individuals with heightened trait anxiety. While the central brain regions underlying this resetting are not characterized in their entirety, hypothalamic structures such as the paraventricular nucleus and dorsomedial hypothalamus may play a key role (R. A. Dampney, 2015; R. A. L. Dampney, 2017; DiMicco, Samuels, Zaretskaia, & Zaretsky, 2002). Evidence in humans demonstrates a role for the dorsomedial hypothalamus in regulation of MSNA (James, Macefield, & Henderson, 2013). The hypothalamus receives input from structures such as the prefrontal cortex and the amygdala (R. A. Dampney, 2015; R. A. L. Dampney, 2017; Ulrich-Lai & Herman, 2009), the activity of which are negatively and positively related to trait anxiety in humans (Bishop, 2009; Indovina, Robbins, Nunez-Elizalde, Dunn, & Bishop, 2011). Whereas activation of amygdaloid structures is associated with heightened cardiovascular activity (Gianaros et al., 2008; Soltis, Cook, Gregg, & Sanders, 1997), the prefrontal cortex reduces cardiovascular reactivity to stress (Muller-

Ribeiro et al., 2012). Chronic hypoactivity of areas such as the prefrontal cortex, and hyperactivity of limbic regions including the amygdala in individuals with elevated trait anxiety (Bishop, 2009; Indovina et al., 2011) may directly and indirectly interact with hypothalamic and brainstem regions to modulate resting autonomic and cardiovascular activity. Whether these central alterations are responsible for the present relationship between trait anxiety, MSNA, and blood pressure remains unknown.

Leveraging a large dataset allowed assessment of subtle interactions between trait anxiety and sympathetic activity that may not have been detectable in previous studies. Further, the current study viewed trait anxiety along a spectrum, as opposed to a dichotomous variable whereby participants are separated based on anxiety disorder diagnosis. This approach is responsive to the need for assessment of psychological variables across complex spectrums within physiological research (Wehrwein & Carter, 2016), and offers evidence of a quantifiable relationship between subjective, self-perceived trait anxiety and objective, clinically relevant measures of autonomic and cardiovascular function. The selected approach to trait anxiety evaluation within the current study may also underly discrepant findings between the present study and those performed in clinically diagnosed anxiety disorders (Bechir et al., 2007; Holwerda et al., 2018; E. Lambert et al., 2006; E. A. Lambert et al., 2011; Wilkinson et al., 1998). The accumulation of physiological abnormalities within anxiety and mood disorders has been shown to be impacted by symptom severity in numerous studies (Fonkoue, Marvar, et al., 2020; E. A. Lambert et al., 2002; Scalco et al., 2009; Toschi-Dias, Trombetta, da Silva, et al., 2013), supporting the necessity for psychological

characteristics to be included as continuous variables within physiological experimentation and analyses.

Although previous studies on participants with anxiety disorders have suggested little to no alterations in multiunit MSNA recordings, considerable evidence supports an underlying difference in sympathetic recruitment patterns (E. Lambert et al., 2010; E. Lambert et al., 2006; Yoo et al., 2020). Utilization of single-unit MSNA recordings, which allow direct assessment of single vasoconstrictor nerve fibers, have reported a greater probability of single nerve fibers firing, as well as a greater likelihood of single nerve fibers firing multiple times within an integrated burst of MSNA in individuals with PD (E. Lambert et al., 2006). This augmented MSNA firing pattern has been associated with greater cardiac NE spillover to plasma (E. A. Lambert et al., 2011), indicative of potential regional differences in sympathoexcitation unique to PD that may be shared with general symptoms of anxiety. Similarly, trait anxiety has been shown to be correlated with alterations in the firing pattern of single-unit nerve fibers within individuals with metabolic syndrome (E. Lambert et al., 2010). It is possible that the healthy adults utilized in the current study have augmented single-unit firing rates and different sympathetic recruitment strategies as a function of trait anxiety, but we did not have the ability to analyze this given the MSNA data acquisition process within our respective laboratories. Future work investigating sympathetic recruitment patterns may be warranted.

Chronic anxiety and stress are difficult to study within a laboratory for various practical and ethical reasons, making conclusions regarding mechanisms leading to the

presently observed relationship difficult to establish. Numerous studies have assessed the cardiovascular and sympathetic response to acute laboratory mental stress in healthy adults to better understand the underlying sympathetic response to psychological stress (Bigalke & Carter, 2021; Carter & Goldstein, 2015). Mental stressors, such as forced mental arithmetic and the Stroop Color Word task, have been utilized to evoke a repeatable pressor response (Fonkoue & Carter, 2015) across multiple studies (for reviews (Bigalke & Carter, 2021; Carter & Goldstein, 2015)). While the pressor response to mental stress has been thought to be partially mediated by sympathetic activity, considerable variations in sympathetic responsiveness have been observed (Carter & Ray, 2009), and these changes in sympathetic activity are generally not associated with MAP reactivity (Carter & Ray, 2009).

A recent study from our laboratory characterized the sympathetic and cardiovascular response to the trier social stress test (TSST) (Kirschbaum et al., 1993), which utilizes a period of social stress anticipation devoid of alterations in respiration, muscle movement, and speech prior to socially-evaluated speech and mental arithmetic phases (Bigalke et al., 2022). Our findings demonstrated a significant pressor response to anticipatory stress, and a reduction in MSNA in healthy adults (Bigalke et al., 2022). These findings are particularly relevant to individuals with chronic anxiety, who often experience general anticipatory stress and anxiety prior to any stressor presentation. Whether the acute sympathetic and cardiovascular response to the anticipatory stress phase of the TSST observed in our recent study (Bigalke et al., 2022) is present across the spectrum of trait anxiety remains unknown. Holwerda et al. (2018) reported that

individuals with GAD showed augmented MSNA burst amplitude responsiveness during the anticipation of mental arithmetic compared to controls, indicating an exaggerated sympathetic response to anticipatory stress in this population (Holwerda et al., 2018). Similarly, Callister et al. (Callister et al., 1992) observed a positive relationship between perceived stress and MSNA reactivity to mental stress. Individuals who experience chronic anxiety exhibit selective attention and greater sensitivity to threat (Bishop, 2007; O'Donovan et al., 2013), ultimately impacting stress interpretation and likely sympathetic responsiveness. In line with the theory of allostasis and allostatic overload (McEwen, 1998), exaggerated threat perception to everyday events, as well as frequent stress presentation may lead to increases in cardiovascular activity, in turn resulting in chronic activation of the SNS and elevated blood pressure as is observed in the present study.

Whether alterations in sympathetic neural reactivity to laboratory mental stress translate to exaggerated blood pressure reactivity or augmented likelihood of hypertension development remains equivocal. However, Fonkoue et al. (Fonkoue, Wang, & Carter, 2016) reported an augmented MSNA reactivity to mental stress in participants with a family history of hypertension when compared to controls without a family history of hypertension. The translation of laboratory mental stress protocols to real-world applications remains an area that requires further substantiation before definitive conclusions can be drawn. Nevertheless, elevated resting MSNA is associated with hypertension development (Grassi et al., 2018), and increases in parallel with blood pressure over time (Hering et al., 2016). The association between trait anxiety and elevated resting MSNA in the current study may lend insight into baseline

sympathoexcitation as a mediating factor between anxiety proneness and hypertension, although longitudinal data are necessary to confirm this.

The current study included sex as a predictor variable within the regression analyses. Anxiety disorders disproportionately impact females (McLean et al., 2011) and sex-differences in neural control of cardiovascular regulation are well documented (Carter et al., 2013; Hart et al., 2009; Joyner et al., 2016; Keir et al., 2020). Sex differences with respect to psychological variables and cardiovascular outcomes have been noted. For example, young females with a history of myocardial ischemia have increased susceptibility to mental stress induced myocardial ischemia when compared to young males (Vaccarino et al., 2014). Experimental evidence from Greaney et al. (Greaney, Darling, Mogle, & Saunders, 2022) has supported an adverse role of psychological factors, namely major depressive disorder, on vascular β-adrenergic receptor function leading to impaired vasodilation, an effect that may be exacerbated with chronic psychosocial stress (Greaney, Koffer, Saunders, Almeida, & Alexander, 2019; Greaney, Surachman, Saunders, Alexander, & Almeida, 2020). This is particularly relevant in young females whose β-adrenergic receptor activity opposes the vasoconstrictor effects of NE released from sympathetic varicosities (Hart et al., 2011; Hart et al., 2009; Joyner et al., 2016). Our laboratory remains attentive to the impacts of sex and sex hormones on psychological and sympathetic regulation in response to stress (Bigalke, Greenlund, & Carter, 2020; Carter et al., 2012; Carter et al., 2019; Carter et al., 2013; Carter & Lawrence, 2007; Carter & Ray, 2009; Keller-Ross, Cunningham, & Carter, 2020; Yang, Drummer, & Carter, 2013). Unfortunately,

sensitivity analysis conducted on the present data set indicated that we did not have adequate statistical power to assess the relationship between trait anxiety, MSNA, and blood pressure stratified between biological sexes (i.e., male vs. female). Nonetheless, this is an area of active investigation within our laboratory and will be explored in future work.

We acknowledge the following limitations in our study. First, we included females with and without hormonal contraceptive use. To minimize confounding factors, females in whom contraceptive use was reported were assessed during the low-hormone phase of contraceptive use. Prior work has demonstrated similar MSNA between low-hormone phase and early follicular phase (Carter, 2019; Carter, Klein, & Schwartz, 2010; Harvey et al., 2015). More importantly, a separate assessment including only naturally menstruating females revealed similar results as those reported in the current study (data not shown). Second, brachial blood pressures were taken in the supine position in six participants, while seated blood pressure recordings were taken in the vast majority of the study population (i.e., 82 of 88 participants). Analyses were performed with and without these six participants, and the results were unchanged (data not shown). The current study did not include the state form of the STAI, which measures acute anxiety levels, so we cannot rule out the acute impact of state anxiety on the current findings. However, all participants quietly rested for at least 10 minutes following MSNA signal acquisition and prior to any active recording, which allowed for accurate recording of MSNA in a relaxed state. Additionally, previous studies which have manipulated state anxiety found no impact on MSNA at rest (Durocher, Lufkin, et al., 2011). Since the

present study included retrospective data, we were unable to exclude individuals based on diagnosed anxiety disorders. It is possible that some individuals had trait anxiety levels which may indicate an undiagnosed clinical anxiety disorder. The presence of undiagnosed anxiety disorders likely would not impact the interpretation of the findings, and instead may reinforce the necessity for accurate screening, diagnosis, and attentiveness to undiagnosed anxiety disorders in otherwise healthy individuals. Previous studies have indicated that severe major depressive disorder is associated with elevated baseline MSNA (Scalco et al., 2009). Conversely, others have shown unchanged MSNA in individuals with MDD compared to healthy controls and observed elevated plasma NE spillover only in those individuals with MDD and comorbid PD (Barton et al., 2007). Since anxiety and depressive symptoms often overlap (Saha et al., 2021), it is possible that individuals with heightened trait anxiety in the current dataset additionally had elevated depressive symptoms. Future work disentangling the unique effects of trait anxiety and depression on sympathetic activity are warranted. While cardiovascular and autonomic medication usage was excluded across all studies, the use of anxiolytics and anti-depressants were not assessed in all participants, so we cannot account for their potential impact on resting MSNA (Bechir et al., 2007; Scalco et al., 2009). In the current dataset, all participants self-reported the absence of any known cardiometabolic diseases. However, we did not assess plasma lipid levels, glucose tolerance, and other metabolic parameters that may impact sympathetic outflow at rest and cannot rule out the effects of alterations of these variables in the present study. Lastly, the present findings cannot apply causation to the relationship between trait

anxiety, MAP, and MSNA. The directionality between these relationships is difficult to assess given various practical and ethical constraints. Anxiety symptoms and the development of anxiety disorders may be exacerbated following acute cardiovascular events (Feng et al., 2016; Grace, Abbey, Irvine, Shnek, & Stewart, 2004; Hanssen, Nordrehaug, Eide, Bjelland, & Rokne, 2009). Though it is unlikely that this relationship exists in the current study population of individuals with no known cardiovascular or autonomic diseases, future prospective investigations following natural changes in trait anxiety across the lifespan may be appropriate and could provide key insights into potential causal relationships between anxiety, blood pressure, and MSNA.

Conclusion

The present study assessed the relationship between trait anxiety, blood pressure, and direct assessment of MSNA in a robust sample of healthy adults. Our findings indicate a direct and independent association between trait anxiety severity, resting blood pressure, and MSNA in healthy adults without comorbid cardiovascular or metabolic disorders. These findings signify an important role for trait anxiety on sympathetic neural control and cardiovascular function at rest and highlight the necessity to consider psychological characteristics when assessing physiological function.

CHAPTER 3

SYMPATHETIC NEURAL REACTIVITY TO THE TRIER SOCIAL STRESS TEST[2]

Introduction

Heightened cardiovascular reactivity to laboratory mental stress is associated with the development of cardiovascular disease (Chida & Steptoe, 2010). While this responsiveness is thought, at least in part, to be mediated by the SNS, studies utilizing direct measures of peripheral sympathetic activity (i.e., MSNA) indicate the absence of an association between sympathetic and cardiovascular reactivity in healthy adults (Carter & Ray, 2009). However, most mental stress tasks utilized in the laboratory setting (i.e., mental arithmetic, stroop color word task, etc.) consist of a stressor associated with changes in respiratory patterns and depth, muscle movement, active speech, among other factors which may influence MSNA responsiveness and ultimately impact conclusions drawn from these studies. The utilization of a stress task devoid of these potential variables may allow unique assessment of the direct effects of mental stress on sympathoneural control.

---

[2]A portion of the material contained in this chapter was previously published in the Journal of Physiology ("Copyright (2022) Wiley. Used with permission from (Bigalke JA, Greenlund IM, Nicevski JR, Tikkanen AL, Carter JR. “Sympathetic Neural Reactivity to the Trier Social Stress Test.” *Journal of Physiology*. Copyright 2022, The Physiological Society). However, the format and wording throughout the chapter has been changed from its original publication.

Few studies have assessed sex differences in sympathetic and cardiovascular reactivity to mental stress (Carter & Ray, 2009; Jones et al., 1996; Yang et al., 2013), with differing conclusions reached. Well-established sex differences in sympathetic regulation exist across the lifespan (Carter et al., 2013; Hart et al., 2009; Joyner et al., 2016; Keir et al., 2020). Further, sympathetic transduction to blood pressure differs between sexes, likely due to the vasodilatory response of β-adrenergic receptor activation in premenopausal females actively opposing sympathetically mediated vasoconstriction (Hart et al., 2009; Joyner et al., 2016). This is shown particularly in response to mental stress, whereby females exhibit exaggerated vasodilatory responsiveness to mental arithmetic compared to males, an occurrence which was observed to be independent of peripheral sympathetic outflow (Yang et al., 2013). The small number of studies which have assessed, and found opposing results regarding the influence of sex on sympathetic reactivity to mental stress (Carter & Ray, 2009; Jones et al., 1996; Yang et al., 2013) and the well-known sex related changes in neurovascular function at rest and in response to stress (Carter et al., 2013; Hart et al., 2009; Joyner et al., 2016; Keir et al., 2020), warrant further investigation to discern susceptibility to mental stress related cardiovascular changes in males and females.

Lastly, few studies have assessed the impact of trait levels of stress and anxiety on sympathetic reactivity to mental stress. While some studies have observed an impact of perceived stress and task difficulty on sympathetic responsiveness (Callister et al., 1992), more recent studies have observed no relationship between state anxiety, perceived stress, and MSNA reactivity to mental stress (Carter et al., 2008; Durocher,

Lufkin, et al., 2011). In contrast, a study performed in individuals with GAD observed significantly elevated MSNA in response to mental arithmetic when compared to healthy controls (Holwerda et al., 2018). This sympathoexcitation in GAD was observed during anticipation of the mental stress task (Holwerda et al., 2018), indicating a potential susceptibility of individuals with clinical anxiety to exacerbated sympathetic outflow during anticipation of coming stressors.

The present series of experiments and analyses sought to improve upon previous findings in the following ways:

The first analysis aimed to characterize the sympathetic and cardiovascular response to the Trier Social Stress Test (TSST), a commonly used psychological stress task which includes an anticipatory stress phase, followed by socially evaluated spontaneous speech and mental arithmetic. The anticipatory phase is devoid of major respiratory alterations, muscle movement, and speech, variables which may be partially responsible for the inter-individual variation observed in sympathetic responsiveness in previous studies (Carter & Ray, 2009). Notably, this is the first study to our knowledge to directly assess MSNA and continuous blood pressure via finger plethysmography in response to the TSST in healthy adults. We hypothesized that the MSNA variability previously reported would be reduced during the preparation phase of the TSST, and will be re-introduced during the speech and math portions of the task. We also hypothesized that baroreflex regulation of MSNA would remain unimpacted during the anticipatory phase, but would be disrupted during the speech and mental math portions (Durocher, Klein, et al., 2011).

Next, the current study aims to characterize sex-specific responses to the TSST. Previous studies have observed sex-specific responses in blood pressure and perceived stress (Carter & Ray, 2009) during mental stress, while others have observed no differences (Jones et al., 1996). In line with the findings of Childs et al. (2010) and Carter et al. (2009), we hypothesized that males would exhibit augmented MSNA and blood pressure reactivity, while females would exhibit augmented perceived stress in response to stress.

Finally, the current study explores the relationship between trait anxiety severity and sympathetic reactivity to the TSST in the current sample of healthy young adults, particularly during the anticipatory stress phase. Holwerda et al. (2018) observed a significantly augmented sympathetic response during the anticipation of mental stress in individuals with GAD. We hypothesized that trait anxiety severity within our dataset would be associated with augmented sympathetic reactivity to the TSST, particularly during the anticipatory phase of the stressor.

Methods

Participants

28 healthy adults between the ages of 18-40 participated in the current study. All participants underwent an in-depth orientation of the experimental study, expectations, risks, etc., and offered written informed consent to participate. Participants were nondiabetic, nonsmokers, and were required to have a BMI < 35kg/m^2. All participants were devoid of any known cardiovascular or autonomic disorders. Participants reported no usage of any cardiovascular, hypnotic, stimulant or analgesic medications. Females

reported regular menstrual cycles, and birth control and contraceptive usage were not exclusionary criteria. Females were tested during the early follicular phase of the menstrual cycle (2-5 days following menstruation, or during the placebo pill if on oral contraceptives). Lastly, participants were screened for sleep disordered breathing using a one night, at-home sleep apnea test (ApneaLink, Resmed, San Diego, CA, USA).

Study Design

A detailed experimental design for the current study was recently published elsewhere (Bigalke et al., 2022), and is identical to that presently utilized. Briefly, interested participants were screened and provided a detailed orientation process. After informed consent, all participants completed the trait form of the Spielberger state trait anxiety inventory (STAI) (Spielberger et al., 1983). After scheduling a laboratory testing date, participants were asked to arrive to the Montana State University Sleep Research Laboratory between 7-9am after an overnight fast from eating, alcohol, and caffeine consumption. Additionally, participants were asked not to exercise for 12 hours prior to arrival to the laboratory.

Upon arrival to the laboratory, participants were given an opportunity to void their bladder. Participants were then asked to sit quietly for 5 minutes, after which 3 seated brachial blood pressure recordings were obtained separated by one-minute (Omron HEM-907XL, Kyoto, Japan). After seated blood pressure measurements, participants were brought into the autonomic laboratory where they were asked to sit semi-recumbently on a cushioned tilt-table bed. Once seated, recordings of participants' heart rate (HR; electrocardiography), beat-to-beat blood pressure via finger plethysmography

(NOVA; Finapres Medical Systems, Amsterdam, The Netherlands), respiration (pneumobelt), and muscle sympathetic nerve activity (MSNA; microneurography) were established. Upon determination of adequate signal for each autonomic and cardiovascular measurement, participants were asked to rest quietly for 10 additional minutes. After the 10-minute period, three more brachial blood pressure recordings were taken and used to calibrate the finger plethysmography device. Next, baseline autonomic and cardiovascular measurements were recorded for 10 minutes while the participants once again rested quietly. After the baseline, participants underwent the Trier Social Stress Test (TSST). At the conclusion of the TSST, participants were unhooked from all recording equipment, concluding the study.

Trier Social Stress Test

The detailed TSST protocol used in the current study can be found in our laboratory's recent publication (Bigalke et al., 2022). Briefly, the TSST consists of a preparation, speech, mental math, and recovery phases, each lasting 5-minutes.

During the preparation phase, participants were randomly provided a speech task. The tasks were to 1) develop a speech regarding why they were the best candidate for their dream job, or (2) defend themselves against shoplifting allegations in front of store managers. These two topics were observed to be the most often utilized in a recent review on TSST methodologies (Narvaez Linares, Charron, Ouimet, Labelle, & Plamondon, 2020). After being given the speech task, participants were asked to mentally prepare a speech, and were informed that they would subsequently provide this speech to a panel of two judges, who would videotape and record the speech for

later assessment. Participants were reminded to remain quiet and still during this portion of the task.

At the completion of the preparation phase, two judges (laboratory staff) dressed in lab coats entered the laboratory, and sat at a table at the foot of the tilt-table upon which the participant sat, and subsequently set up a camera in front of the participant. The judges always consisted of one male and one female. During the speech and subsequent math portion, the judges read all prompts to the participants. After being reminded of the speech prompt, as well as the necessity to limit limb movements, participants provided their 5-minute speech to the judges. Importantly, judges were instructed to not offer any feedback to the participants and maintained a straight face throughout the study. However, if the participant stopped talking for 15-20 seconds, the judges would inform the participant that they still have time remaining, and to continue their speech.

Upon completion of the speech portion of the task, participants were then instructed by judges to continually subtract either 13 or 17 from 2043 as fast and accurately as possible for the remaining 5-minutes. If at any point the participants stated an incorrect answer, they were asked to start from the beginning. Again, judges were instructed to not offer any feedback, unless the participants were silent for 15-20 seconds, at which point they would once again be reminded that time remained and instructed to keep going.

After completion of the math task, the judges left the room, and participants were instructed to remain silent for a 5-minute resting recovery portion.

Measurements

Hemodynamic variables Blood pressure was recorded via automated brachial blood pressure measurements (Omron HEM-907XL, Omron Health Care), as well as by beat-to-beat finger plethysmography (NOVA; Finapres Medical Systems, Amsterdam, The Netherlands). Brachial blood pressure measurements were used to determine seated blood pressure, and once again prior to baseline and each phase of the TSST to calibrate the continuous finger plethysmography recording. Beat-to-beat blood pressure was monitored continuously throughout the TSST. A towel was placed over the participants' recording hand to maintain warmth and blood flow to the finger throughout the recording session. Systolic (SAP), diastolic (DAP), and mean arterial pressure (MAP) were quantified from these measures. Heart rate was monitored continuously throughout the protocol using a 3-lead electrocardiogram. Lastly, from the beat-to-beat arterial pressure waveform, stroke volume (SV) and cardiac output (CO) were estimated using the pulse contour method (Jansen, Wesseling, Settels, & Schreuder, 1990).

Muscle sympathetic nerve activity Direct multifiber muscle sympathetic nerve recordings were obtained via percutaneous insertion of a tungsten recording electrode into the peroneal nerve at the level of the popliteal fossa or fibular head of the right leg. A reference electrode was inserted approximately 2-3cm away from the recording site. Both electrodes were connected to a differential preamplifier and then to an amplifier (total gain of 80,000) where the nerve signal was band-pass filtered (700−2000 Hz) and integrated (time constant 0.1). MSNA recordings were required to be pulse-synchronous

and increased in response to an end-expiratory apnea, but did not respond to startle stimuli such as clapping or yelling.

Respiratory rateRespiratory rate was monitored by a pneumobelt situated around the upper chest.

Subjective stressPerceived stress levels were monitored using the same 4-point stress scale used originally by Callister et al. (1992) and subsequently within studies performed in our laboratory. Participants were asked to report their stress level on a scale of 0 (not stressful at all) to 4 (very, very stressful) between each phase of the TSST.

Threat versus challenge appraisal In a subset of the population (N=8), participants were asked to rate their appraisal of the upcoming stress after the preparation phase on a scale from 1 (completely threatening, not at all challenging) to 7 (completely challenging, not at all threatening) as used in previous work (Williams, Veldhuijzen van Zanten, Trotman, Quinton, & Ginty, 2017).

Data analysis

All data was imported into a custom software (WinCPRS, Absolute Aliens, Turku, Finland) for analysis. R-waves were automatically detected and confirmed by a lab investigator to be used in subsequent heart rate variability (HRV) analysis. Bursts of MSNA were detected automatically based on amplitude using a signal-to-noise ratio of 3:1 within a 0.5 s search window, accounting for an expected burst peak latency of ~1.3s from the previous R-wave. Bursts were reviewed and edited by a trained

investigator. Burst area and amplitude were normalized to the mean area and amplitude observed during the initial 10-min resting baseline. MSNA was expressed as burst frequency (BF; bursts/min), burst incidence (BI; bursts/100 heart beats (hb)), total amplitude, and area (MSNA burst frequency (BF) multiplied by the averaged normalized). In some cases, shifts in baseline MSNA activity did not allow total MSNA assessment. In these individuals, only MSNA BF and BI were reported.

Baroreflex sensitivity Sympathetic (sBRS) and cardiovagal baroreflex sensitivity (cvBRS) were analyzed as outlined in our recent publication (Bigalke et al., 2022). Briefly, sBRS was determined using the weighted linear regression between DAP and MSNA during the baseline and preparation phases of the TSST. DAP values were binned in 3mmHg intervals and plotted against MSNA BI and total area. DAP-MSNA regression coefficients were required to be negative and have a strength of $R \geq 0.5$.

cvBRS was assessed using previously outlined techniques (Yang & Carter, 2013). Briefly, cvBRS was calculated by performing linear regression between changes in SAP and the R-R interval (RRI). The criteria for a sequence were set at a minimum change of 1 mmHg for SAP and 4 ms for RRI across 3 or more consecutive cardiac cycles, with the linear regression between the changes reaching $R \geq 0.7$. These sequences were then separated into those in which increases in SAP were accompanied by increases in the RRI (up-up sequence; vagal activation) and those in which decreases in SAP were compensated for with decreases in the RRI (down-down sequence; vagal withdrawal). These values were obtained only during the baseline and preparation periods.

Heart rate variability Heart rate variability was quantified in both the time and frequency domains. Using the RRI, time-domain analyses including the percentage of adjacent R–R intervals that varied by 50 ms or more (pNN50) and the root mean squared of successive differences of R–R interval (RMSSD) were determined. Frequency domain analysis were performed using a fast fourier transform of the RRI. A low pass filter was used with a cut-off of 0.5 Hz. The integrated area within the high frequency (HF; 0.15–0.4Hz) and low frequency (LF; 0.04–0.15Hz) ranges were used in the current analysis.

Statistical Methods

All statistical analyses were performed in SPSS (SPSS Statistics 28.0, IBM Corps., Armonk, NY, USA). Variables were assessed for normality using Shapiro–Wilk assessment, followed by assessment of skewness if significant (Ghasemi & Zahediasl, 2012). Those measures that did not meet normality assumptions are presented as median (25th–75th percentile). Descriptive statistics were used to characterize anthropometric variables and baseline trait anxiety levels. Two-tailed independent samples T-test were used to compare anthropometric measures, baseline trait anxiety, and seated cardiovascular parameters between sexes.

Due to a priori directional hypotheses, one-tailed paired samples T-tests were performed to assess differences in HRV, cvBRS, and sBRS between the baseline and preparation phases of the TSST. Wilcoxon’s signed rank test was performed in the cases of normality violations.

Cardiovascular and sympathetic variables were averaged across the 10-minute baseline, and during each 5-minute phase of the TSST in each participant. A repeated measures analysis of variance (ANOVA) was used to determine changes in cardiovascular and sympathetic measures across the stress task, with TSST phase (i.e., baseline, preparation, speech, math, recovery) as the within-subject variable and sex (i.e., male and female) as the between-subject factor. The main effects of phase as well as the interaction between the sex and TSST phase were assessed. In the case of sphericity violations, the Greenhouse-Geisser correction was applied. Significant differences between TSST phases were probed using post hoc analyses between conditions with a Bonferroni adjustment for multiple comparisons.

Bivariate linear regression analyses were used to investigate the relationship between changes in DAP and MSNA during each phase of the TSST. The association between changes in CO and MAP were similarly conducted using bivariate linear regression. Lastly, the relationship between changes in MSNA BF during the preparation phase and changes in MSNA BF during both the speech and math phases was interrogated with linear regression.

Exploratory Pearson correlations were conducted to explore the relationship between trait anxiety severity as well as cardiovascular and sympathetic reactivity to each phase of the TSST. Pearson’s correlations were also used to assess the relationship between threat vs. challenge appraisal on reactivity to the TSST. All data are presented as mean ± SEM. A significance level of $\alpha < 0.05$ was used for all analyses.

Results

Subject Characteristics

Participant characteristics are outlined in **table 3.1**. Females had a significantly lower resting seated SAP compared to males, while all other characteristics between sexes were similar.

Table 3.1. Subject Characteristics

Variable	Male	Female	P-value
N	13	15	--
Age (years)	25±1	24±2	.551
BMI (kg/m^2)	25±1	23±1	.070
Trait Anxiety Scores			
STAI-T (a.u.)	38±2	34±2	.209
STAI-T (%)	61±7	50±6	.266
Seated Cardiovascular Measures			
SAP (mmHg)	119±3	111±2	.021
DAP (mmHg)	72±2	72±2	.768
MAP (mmHg)	87±2	85±2	.425
HR (beat/min)	75±4	76±3	.738

Note: Values are mean ± SEM. Comparisons between sexes made with two-tailed independent samples T-test. BMI, body mass index; STAI-T, Spielberger Trait Anxiety Inventory; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate.

Perceived Stress

Perceived stress levels throughout the TSST were recorded in 21 individuals (11M, 10F) and are presented in **figure 3.1** stratified by sex. RM-ANOVA revealed a significant effect of TSST phase on perceived stress ($F(1.860,35.346) = 34.358$, $P<.001$). Perceived stress was greater during the preparation (1.3 ± 0.5 A.U., $P<0.001$), speech (2.2 ± 1.0 A.U., $P<0.001$), and math phases (2.1 ± 1.2 A.U., $P<0.001$) compared to baseline (0.4 ± 0.5 A.U.). Additionally, compared to the preparation period, perceived stress was significantly higher in response to the speech ($P<0.001$) and math ($P=0.007$) tasks. RM-ANOVA indicated no significant interaction between sex and TSST phase on perceived stress reactivity ($F(1.860, 35.346) = 0.562$, $P = 0.563$).

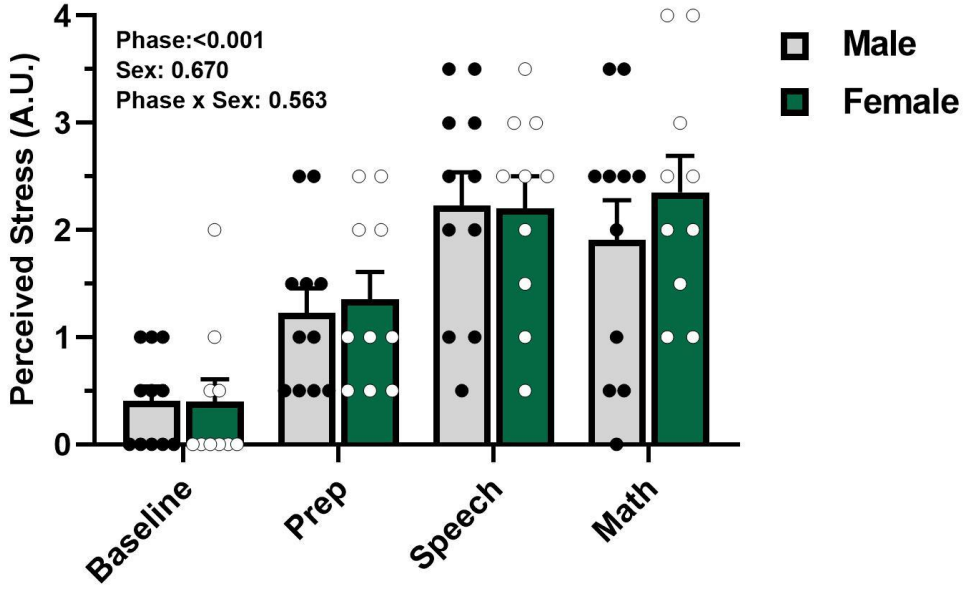


Figure 3.1: Perceived stress reactivity to the TSST between sexes.
Mean levels of perceived stress during each phase of the TSST between males (n=11) and females (n=10).

Baroreflex sensitivity and HRV during anticipatory stress

Table 3.2 shows changes in baroreflex sensitivity during the preparation phase of the TSST. While sBRS was unchanged, cvBRS up-up and cvBRS down-down were significantly reduced compared to baseline. Similarly, RRI, HF-HRV, pNN50, and RMSSD were significantly reduced during the preparation phase compared to baseline. Respiratory rate was additionally slightly elevated compared to baseline.

Table 3.2. Baseline vs. Preparation Period Baroreflex Sensitivity and HRV

Variable	Base	Prep	P-Value
sBRS-incidence (burst incidence/mmHg)	-1.7±0.1	-1.7±0.1	.495
sBRS-total (total MSNA/mmHg)	-569 [-888-(-514)]	-694 [-947-(-461)]	.474
cvBRS (Up) (ms/mmHg)	17±1	14±1	.037
cvBRS (Down) (ms/mmHg)	17±1	14±1	.005
Heart Rate Variability Measures			
RR (breath/min)	14±1	15±1	.004
RRI (ms)	881±20	795±21	<.001
LF (ms^2)	1392 [617-2177]	1205 [733-2243]	.437
HF (ms^2)	1036±155	832±137	.019
LF/HF (ms^2)	170 [119-240]	193 [142-303]	.181
pNN50 (%)	29±3	22±3	.003
RMSSD (ms)	55±4	45±4	.006

Note: All normally distributed values are mean ± SEM. Non-normal data expressed as median [25^{th}-75^{th} percentile]. One-tailed paired-samples T-tests performed on normally distributed data, while non-parametric Wilcoxon Signed Rank Test was performed on non-normal data. sBRS-incidence was assessed in N=19 participants, and sBRS-total was available in N=18 participants. All other measures were observed in all N=28 participants. sBRS-Incidence, sympathetic baroreflex sensitivity – MSNA burst incidence; sBRS-total, sympathetic baroreflex sensitivity – total MSNA; cvBRS (Up), cardiovagal baroreflex sensitivity – up-up sequence; cvBRS (Down), cardiovagal baroreflex sensitivity – down-down sequence; RR, respiratory rate; RRI, R-R interval; LF, low frequency heart rate variability; HF, high frequency heart rate variability; LF/HF, low frequency to high frequency heart rate variability ratio; pNN50, percentage of adjacent R-R intervals that varied by 50ms or more; RMSSD, root mean squared of successive differences of R-R interval.

Cardiovascular reactivity

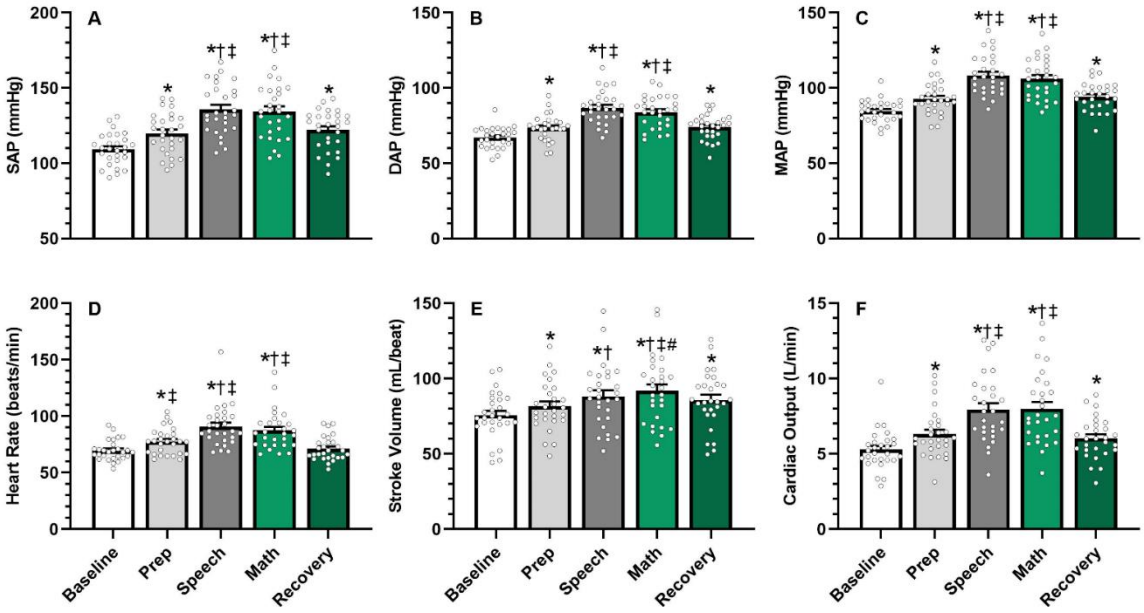


Figure 3.2: Cardiovascular reactivity to the TSST.
Mean levels of systolic (SAP, A), diastolic (DAP, B), and mean arterial pressure (MAP, C), as well as heart rate (D), stroke volume (E), and cardiac output (F) during each phase of the TSST. ∗P < 0.05 vs. baseline; †P < 0.05 vs. Prep; ‡P < 0.05 vs. recovery; #P < 0.05 vs. speech.

Cardiovascular reactivity to the TSST is presented in **table 3.3** and **figure 3.2**. TSST phase had a significant effect on SAP (F(1.910,49.651) = 80.788, P<0.001, **Figure 3.2A**), DAP (F(2.395,62.262) = 94.081, P<0.001, **Figure 3.2B**), MAP (F(2.070,53.814) = 97.408, P< 0.001, **Figure 3.2C**), HR (F(1.639,42.610) = 42.174, P<0.001, **Figure 3.2D**), SV (F(1.886,49.031) = 23.245, P<0.001, **Figure 3.2E**), and CO (F(1.780,46.273) = 50.735, P<0.001, **Figure 3.2F**).

Table 3.3 highlights raw values for each cardiovascular variable across each phase of the TSST. SAP, DAP, MAP, SV, and CO were all significantly elevated

throughout the preparation, speech, math, and recovery phases compared to baseline, while HR was elevated only during preparation, speech, and math phases (all P < 0.05). Compared to the preparation phase, SAP, DAP, MAP, HR, SV, and CO were significantly elevated during the speech and math phases (all P < 0.05). Compared to the recovery phase, SAP, DAP, MAP, HR, and CO were significantly elevated in both the speech and math phases, while SV was only elevated during the math phase (all P < 0.05). HR was additionally significantly reduced in the recovery phase when compared to the preparation phase (P < 0.05) (**figure 3.2**).

Table 3.3. Sex Stratified TSST Cardiovascular Reactivity

		TSST Phase					P-Value		
Variable	**Sex**	**Baseline**	**Prep**	**Speech**	**Math**	**Recovery**	**Ph.**	**Sex**	**Ph x Sex**
SAP (mmHg)	**M**	112±3	122±4	135±4	137±4	124±3	<.001	.564	.405
	F	107±2	118±4	136±5	132±5	120±4			
	All	109±2	120±3*	136±3*‡†	134±3*‡†	122±2*			
DAP (mmHg)	**M**	67±2	72±2	83±2	83±2	73±2	<.001	.379	.137
	F	67±2	74±2	90±3	85±3	75±2			
	All	67±1	73±2*	87±2*‡†	84±2*‡†	74±2*			
MAP (mmHg)	**M**	84±2	92±2	105±2	105±3	93±2	<.001	.432	.322
	F	85±2	94±3	111±4	107±4	95±3			
	All	85±1	93±2*	108±2*‡†	106±2*‡†	94±2*			
HR (bpm)	**M**	70±3	77±4	86±4	87±5	72±3	<.001	.689	.156
	F	70±2	78±2	95±5	88±5	71±2			
	All	70±2	78±2*†	91±3*‡†	87±3*‡†	71±1			
SV (mL/b)	**M**	75±5	81±6	88±7	92±7	85±6	<.001	.904	.855
	F	76±3	82±3	88±5	92±6	87±4			
	All	76±3	82±3*	88±4*‡†	92±4*‡†#	86±4*			
CO (L/min)	**M**	5.3±.5	6.3±6	7.7±0.7	8.1±0.8	6.0±0.5	<.001	.893	.513
	F	5.3±.2	6.4±2	8.2±0.5	7.9±0.5	6.0±0.2			
	All	5.3±.2	6.3±3*	7.9±0.4*‡†	8.0±0.5*‡†	6.0±0.3*			

Note: Presented are the mean ± SEM. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; M, male; F, female. *P < .05 vs. baseline; †P < .05 vs. Prep; ‡P < .05 vs. recovery; #P < .05 vs. speech. N = 28 (13M, 15F).

Cardiovascular responses stratified by sex are also presented in **table 3.3**. No significant interaction between sex and TSST phase on SAP (F(1.910,49.651) = 0.910, P=0.461), DAP (F(2.395,62.262) = 1.991, P=0.137), MAP (F(2.070,53.814) = 1.161, P=0.332), HR (F(1.639,42.610) = 1.991, P=0.156), SV (F(1.886,49.031) = 0.144, P=0.855), and CO (F(1.780,46.273) = 0.643, P=0.513) were observed.

Sympathetic reactivity

Sympathetic reactivity to the TSST is presented in **table 3.4** and **figure 3.3**. TSST phase had a significant effect on MSNA BF (F(4,76) = 4.855, P=0.002, **Figure 3.3A**), MSNA BI (F(2.794,53.081) = 8.022, P<0.001, **Figure 3.3B**), MSNA total amplitude (F(2.239,26.867) = 4.443, P=0.004, **Figure 3.3C**), and MSNA total area (F(2.254,27.048) = 5.263, P=0.001, **Figure 3.3D**).

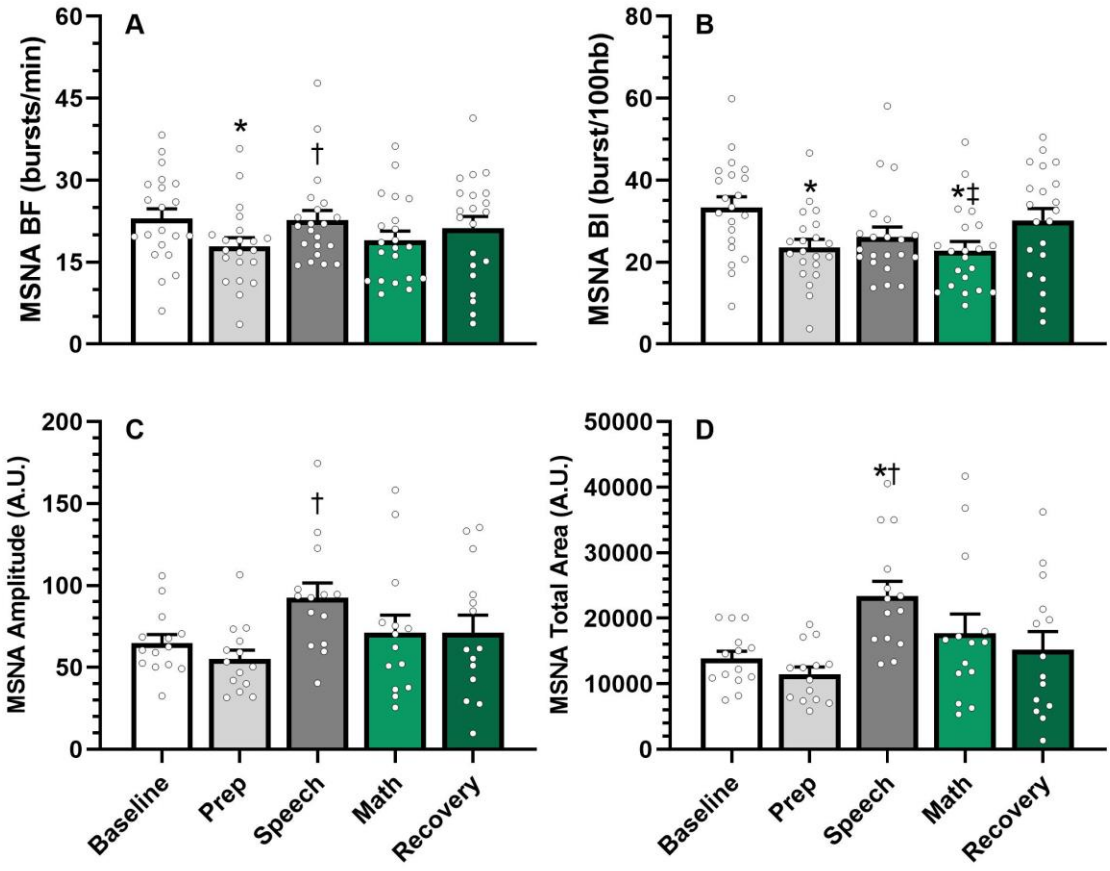


Figure 3.3: Muscle sympathetic nerve activity (MSNA) responses to the TSST. Mean levels of MSNA represented as burst frequency (BF, A) and incidence (BI, B) (N=21), as well as total amplitude (C) and area (D) (N=14) during each phase of the TSST. ∗P < 0.05 vs. baseline; †P < 0.05 vs. Prep; ‡P < 0.05 vs. recovery.

Table 3.4 highlights raw values for MSNA BF, BI, total area, and total amplitude across each phase of the TSST. During the preparation phase, MSNA BF and BI were significantly reduced compared to baseline (P<0.05). During the speech phase, MSNA BF, total amplitude, and total area were significantly elevated compared to the preparation phase, while MSNA total area was additionally elevated compared to baseline (all P < 0.05). During the math phase, MSNA BI was once again significantly

reduced compared to baseline (P < 0.05). During recovery, MSNA BI was elevated compared to the math phase (P < 0.05) (**figure 3.3B**).

Table 3.4. Sex-Stratified MSNA Reactivity

		TSST Phase					P-Value		
Variable	**Sex**	**Baseline**	**Prep**	**Speech**	**Math**	**Rec**	**Phase**	**Sex**	**Int.**
MSNA BF (b/min)	**M**	26±2	21±2	25±3	20±3	22±3	.002	.173	.470
	F	20±3	15±2	20±1	18±2	20±3			
	All	23±2	18±2*	23±2†	19±2	21±2			
MSNA BI (b/100hb)	**M**	38±2	28±3	31±4	24±3	32±4	<.001	.097	.365
	F	29±4	20±2	22±2	21±3	29±4			
	All	33±3	24±2*	26±2	23±2*‡	30±3			
Total Amp. (A.U.)	**M**	75±8	63±9	92±16	67±16	65±14	.018	.854	.307
	F	55±5	47±6	93±10	75±15	78±17			
	All	65±5	55±6	93±9†	71±11	71±11			
Total Area (A.U.)	**M**	16487±1447	13337±1452	22184±3437	16909±4472	13832±3513	.010	.860	.424
	F	11180±1084	9502±1500	24566±3180	18484±4153	16608±4477			
	All	13833±1139	11420±1135	23375±2274*†	17697±2940	15220±2761			

Note: Presented are the mean ± SEM. MSNA, muscle sympathetic nerve activity quantified as burst frequency (BF) and burst incidence (BI); A.U., arbitrary units; M, male; F, female. *P < .05 vs. baseline; †P < .05 vs. Prep; ‡P < .05 vs. recovery. N = 21 (10M, 11F) for MSNA BF and BI. N = 14 (7M, 7F) for total amplitude and area.

MSNA responses stratified by sex are presented in **table 3.4**. No significant interaction between sex and TSST phase on MSNA BF ($F(4,76) = 0.898$, $P=0.447$), MSNA BI ($F(2.794,53.081) = 1.074$, $P=0.365$), MSNA total amplitude ($F(2.239,26.867) = 1.245$, $P=0.307$), and MSNA total area ($F(2.254,27.048) = 0.912$, $P=0.424$) were found.

Relationship between DAP and MSNA

Linear regression was used to assess the relationship between changes in DAP and MSNA BF and BI during each phase of the TSST. During the preparation phase, changes in DAP were significantly associated with changes in MSNA BF (R^2 = 0.473, P = 0.001, **figure 3.4A**. Changes in DAP were significantly associated with changes in MSNA BF (R^2 = 0.194, P = 0.046, **figure 3.4C**) during the math phase, albeit to a lesser extent. Conversely, changes in DAP were unrelated to changes in MSNA BF during the speech portion of the TSST (R^2 = 0.052, P = 0.318, **figure 3.4B**).

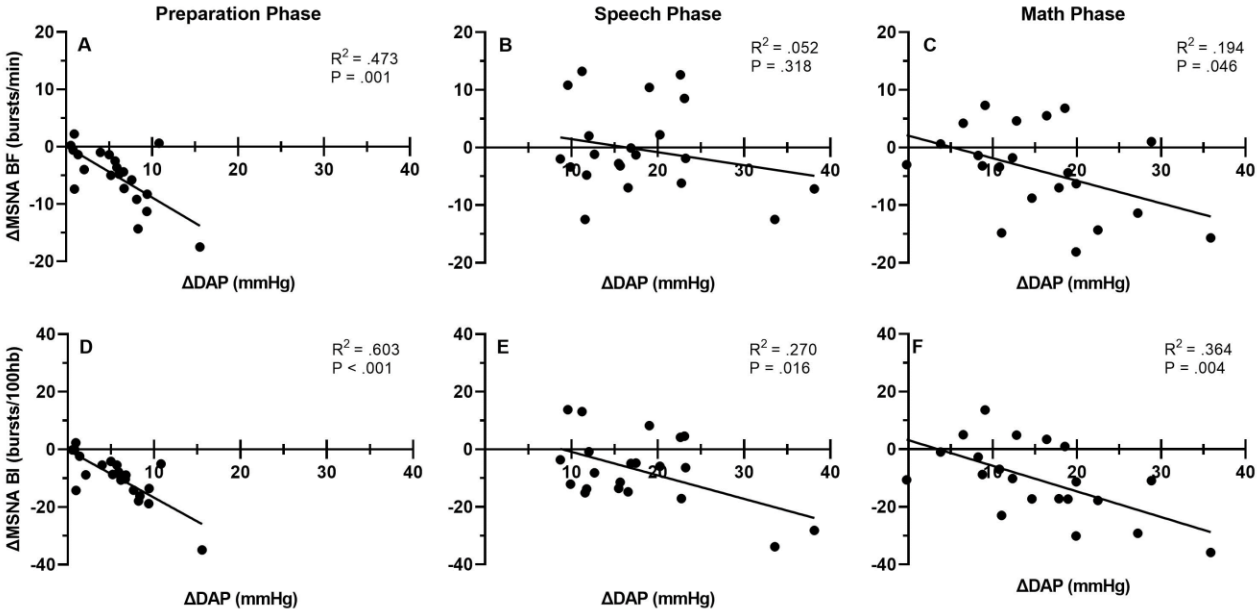


Figure 3.4: Relationship between changes in DAP and MSNA.
Linear regression was used to assess the relationship between changes in diastolic arterial pressure (DAP) and muscle sympathetic nerve activity burst frequency (MSNA BF) and incidence (MSNA BI) during the preparation (A, D), speech (B, E), and math (C, F) phases of the TSST (N = 21).

Similarly, changes in DAP were significantly associated with changes in MSNA BI during the preparation (($R^2 = 0.603$, $P < 0.001$, **figure 3.4D**) and math (($R^2 = 0.364$, $P = 0.004$, **figure 3.4F**) phases. Unlike the relationship with MSNA BF, changes in DAP were significantly associated with changes in MSNA BI during the speech phase (($R^2 = 0.270$, $P = 0.016$, **figure 3.4E**), although the strength of this relationship was the weakest out of all TSST phases.

Relationship between MSNA reactivity during preparation, speech, and math

Linear regression was used to assess whether MSNA reactivity during anticipatory stress predicts MSNA reactivity to subsequent stress tasks. Changes in MSNA BF during the preparation phase of the TSST significantly predicted change in MSNA BF during the speech ($R^2 = 0.547$, $P < 0.001$, **figure 3.5A**) and math ($R^2 = 0.427$, $P = 0.001$, **figure 3.5B**) phases of the TSST. Similarly, changes in MSNA BI during the preparation phase predicted MSNA reactivity to subsequent speech ($R^2 = 0.610$, $P < 0.001$, **figure 3.5C**) and math ($R^2 = 0.470$, $P < 0.001$, **figure 3.5D**) tasks.

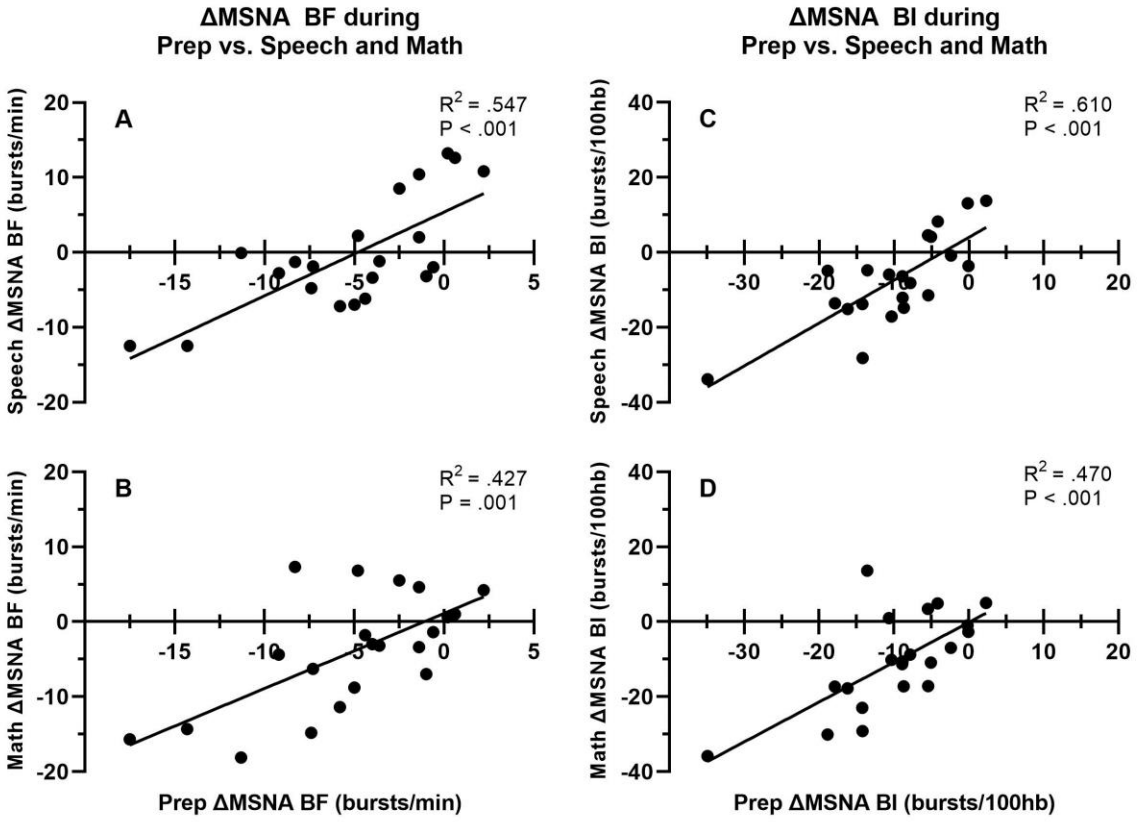


Figure 3.5: Relationship between changes in MSNA during the preparation compared to speech and math phases.
Linear regression was used to assess the relationship between changes in muscle sympathetic burst frequency (MSNA BF) and incidence (MSNA BI) during the preparation phase and subsequent speech (A, C) and math (B, D) phases (N=21).

Threat versus challenge appraisal and cardiovascular reactivity

Figure 3.6 shows the association between individual appraisal of the upcoming stress task (i.e., threatening vs. challenging) and MAP reactivity in a small subset of the study population (N = 8). Appraisal of the upcoming stress task as more challenging was associated with augmented SAP (R = 0.768, P = 0.016), DAP (R = 0.830, P = 0.006), and MAP (R = 0.873, P = 0.002, **figure 3.6A**) reactivity during the preparation phase of the TSST. During the speech period, appraisal was not significantly associated with SAP (R = 0.635, P = 0.066), DAP (R = 0.496, P = 0.174), or MAP (R = 0.555, P = 0.121, **figure 3.6B)** reactivity. Similarly, appraisal was not significantly associated with SAP (R = 0.614, P = 0.079), DAP (R = 0.591, P = 0.094), or MAP (R = 0.595, P = 0.091, **figure 3.7C**) responsiveness.

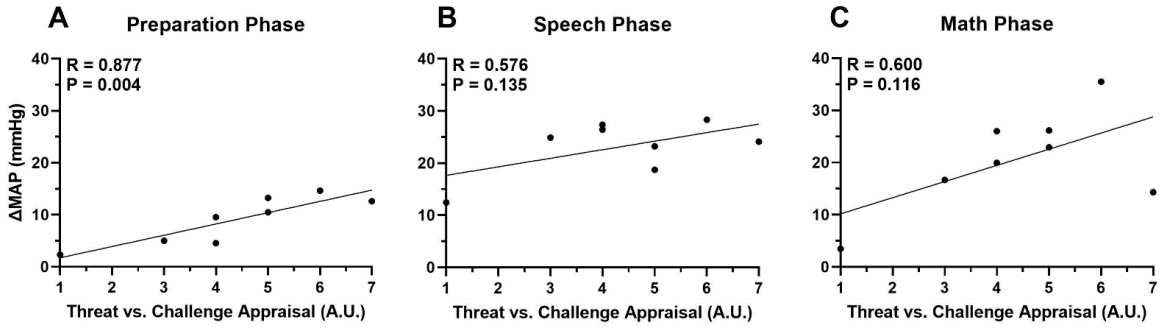


Figure 3.6: Relationship between threat vs. challenge appraisal and MAP reactivity. Bivariate correlations were used to assess the relationship between threat vs. challenge appraisal and mean arterial pressure (MAP) reactivity to each phase of the TSST (N = 8). Participants appraised the coming stress on a scale from 1 (completely threatening, not at all challenging) to 7 (completely challenging, not at all threatening).

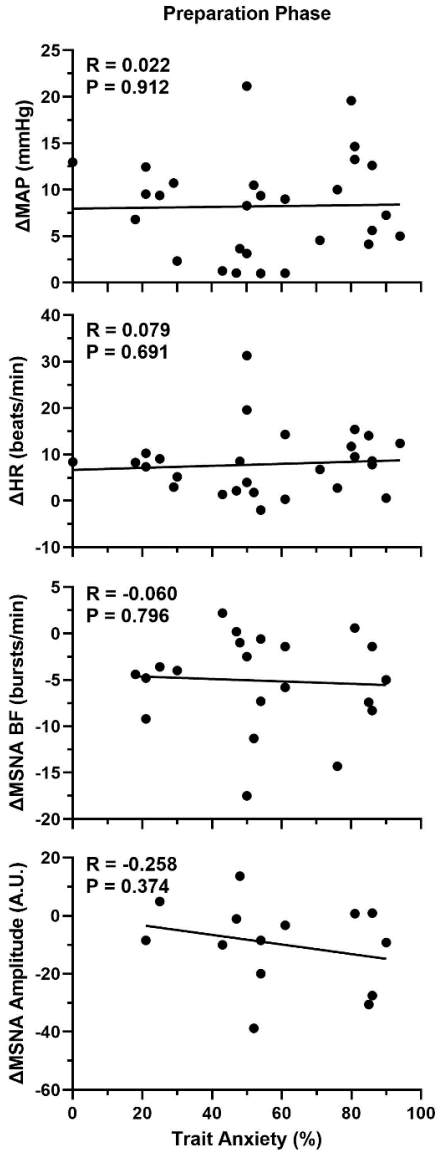


Figure 3.7: Relationship between trait anxiety and MAP, HR, and MSNA reactivity. Bivariate correlations were used to assess the relationship between trait anxiety (%) and changes in mean arterial pressure (MAP) and heart rate (HR) (N = 28), muscle sympathetic nerve activity burst frequency (BF, N = 21) and amplitude (N = 14).

Relationship between trait anxiety and TSST reactivity

Bivariate Pearson correlations were used to assess the relationship between individuals trait anxiety levels and cardiovascular as well as sympathetic reactivity to the TSST. There were no significant correlations between trait anxiety and any of the cardiovascular and sympathetic changes during the TSST (**Appendix D**). Since we were particularly interested in the sympathetic response to anticipatory stress as it relates to trait anxiety, **figure 3.7** plots the relationship between trait anxiety and MAP, HR, MSNA BF, and MSNA total amplitude. No significant relationships were found.

Discussion

The current study is the first to directly assess sympathetic reactivity to the TSST, and we report five novel findings. First, continuously recorded blood pressure was significantly elevated throughout the TSST and recovery, while heart rate recovered quickly following cessation of the stress protocol, suggesting expedited parasympathetic recovery and prolonged sympathetic activation. Next, anticipatory stress is marked by significant reductions in cvBRS and HRV, but no change in sBRS. Third, MSNA was significantly reduced during anticipatory stress and was observed in most participants, while classic interindividual variability of MSNA responsiveness was re-introduced during the speech task. Fourth, MSNA was inversely related to changes in blood pressure during the preparation and math phases of the TSST, but this relationship was blunted or absent during the speech phase. Fifth, changes in MSNA during anticipatory stress predicted sympathetic reactivity to subsequent stress tasks. Although a thorough discussion of these findings was recently published elsewhere

(Bigalke et al., 2022), the current discussion will seek to elaborate upon that published work, and add further insight into the findings.

Traditionally used laboratory mental stress tasks such as forced mental arithmetic and the Stroop Color Word Task are marked by significant and consistent elevations in blood pressure. However, the MSNA responses to these tasks harbor considerable interindividual variability with some studies showing increased (Anderson et al., 1991; Anderson et al., 1987; Callister et al., 1992; Carter & Lawrence, 2007; Carter & Ray, 2009; Matsukawa et al., 1995), unchanged (Carter et al., 2008; Incognito et al., 2019; Kuipers et al., 2008; Wasmund et al., 2002), or reduced (Carter & Ray, 2009; Halliwill et al., 1997; Jones et al., 1996) MSNA during mental stress. These variable sympathetic responses appear to be disassociated from blood pressure responses (Carter & Ray, 2009). Some factors which may play a role in altered inter-individual sympathetic responses to mental stress include abrupt changes in respiratory patterns, active speech, muscle movement, among others (Bigalke et al., 2022). The current study utilized the TSST which includes an anticipatory stress phase largely devoid of these experimental confounders, allowing a potentially cleaner assessment of the impact of psychosocial stress on sympathetic reactivity.

During the anticipatory stress phase, most participants responded with reductions in MSNA BF (18/21, 90%) and MSNA BI (20/21, 95%) likely through baroreflex inhibition. Conversely, during the speech phase when respiratory alterations, active speech, and muscle movement were re-introduced, inter-individual variability in responsiveness was once again observed with 14/21 (67%) of subjects responding with

reductions in MSNA BF, and 16/21 (76%) with reductions in MSNA BI. Further, MSNA was unchanged from baseline during the speech portion, while MSNA was significantly reduced compared to baseline during the preparation phase, likely in-part due to variability.

While previous studies have indicated that MSNA changes in response to mental stress are independent of changes in blood pressure (Carter & Ray, 2009), our findings highlight a strong inverse relationship between changes in DAP and MSNA particularly during the anticipatory stress period. This inverse association is likely to be due to maintained baroreflex function during the preparatory phase. Indeed, sBRS was found to be unchanged during the preparation phase. Previous studies have indicated both blunted (Durocher, Klein, et al., 2011) and augmented (Incognito et al., 2019) sBRS in response to mental stress. However, these alterations were observed in the presence of potential confounders in respiration, movement, etc. that may further impact baroreflex function (Incognito et al., 2019; Nardone et al., 2020). In support of previous findings (Carter & Ray, 2009), the relationship between changes in DAP and MSNA BF was not observed during the speech phase of the TSST. However, changes in DAP and MSNA BI were significantly related during this phase, although the strength of this relationship was reduced compared to the preparation phase, with changes in DAP explaining ~60% of the variance in MSNA BI during the preparation phase, but only ~27% during the speech task. This may be due to baroreflex alterations, and blunted baroreflex function during the active speech portion of the task (Durocher, Klein, et al., 2011). However, upon resumption of somewhat regular breathing patterns, and less muscle movements

during the math phase, the inverse relationship was once again observed between changes in DAP and MSNA BF, albeit to a lesser degree than that observed in the preparation phase ($R^2 = 0.194$ vs. $R^2 = 0.473$) and was strengthened when compared to that observed during the speech task ($R^2 = 0.364$ vs $R^2 = 0.270$). The combination of maintained sBRS and baroreflex function, as well as largely homogenous sympathetic responses between individuals support the role for anticipatory stress as an alternative stress task which may be utilized to delineate the impact of psychosocial stress on sympathetic and cardiovascular dynamics.

The current findings suggest that it is unlikely that peripheral sympathetic activity, at least within the lower limbs, is driving the observed increases in blood pressure throughout the stress task. This is not to say, however, that sympathetic activity at other organ systems is not accounting for increases in BP. Esler et al. (1989) have shown a disproportionate impact of mental stress on cardiac NE release, indicating potential regional differences in sympathetic outflow. Similarly, Kuipers et al. (2008) found that mesenteric and renal blood flow was reduced, while limb blood flow was increased during mental stress, indicating differences in regional sympathetic outflow to visceral vs. peripheral vascular beds. The current study additionally supports a key role for increased CO in maintaining blood pressure responsiveness to laboratory stress, supporting previous findings (Freyschuss, Hjemdahl, Juhlin-Dannfelt, & Linde, 1988; Sherwood, Allen, Obrist, & Langer, 1986).

Challenge and threat are two approaches towards motivated tasks. A challenge approach indicates that personal resources are perceived to exceed environmental

demands. Conversely, if an event is appraised as threatening, personal resources are perceived to be insufficient. Research has shown dichotomous cardiovascular responses to mental stress tasks dependent upon which appraisal type is adopted (Seery, 2011). Specifically, when a stressor is approached as a challenge, activation of the sympathoadrenomedullary axis leads to increased CO and reduced peripheral resistance, while a threat appraisal leads to simultaneous activation of the HPA axis and reduced CO responsiveness with augmented TPR reactivity (Seery, 2011). Experimental data shows these dynamic relationships, whereby instructed appraisal of a laboratory stress task alters the cardiovascular reactivity to the subsequent task (Griffin & Howard, 2021; Turner, Jones, Sheffield, Barker, & Coffee, 2014). The challenge response more closely resembles the cardiovascular response to exercise, namely through increased peripheral blood flow to muscles, and increased CO, which allows the individual to prepare to actively engage with and overcome the stress task at hand. Conversely, threat perception is associated with peripheral resistance increases in tandem with elevated cardiac output. Recent research has hinted at a deleterious effect of peripheral resistance increases in response to mental stress on cardiovascular outcomes (Kim et al., 2019), alluding to a worsened health outcome in individuals who adopt threat appraisals.

In the current study, appraisal of challenge was significantly associated with an augmented blood pressure response, particularly during the anticipatory phase. Greater cardiovascular reactivity to stress is similarly associated with adverse cardiovascular outcomes (Chida & Steptoe, 2010), adding ambiguity regarding which appraisal type is

protective and beneficial. While previous studies have indicated no association between perceived stress and mental stress sympathetic reactivity (Carter et al., 2008; Durocher, Lufkin, et al., 2011), the appraisal of the stress experienced may be related to acute cardiovascular responses and long-term cardiovascular health. This is an exciting area of future research and will shed further light upon the inter-individual variability in sympathetic responses to mental stress.

Our recently published study highlights potential reasons for the lack of sex differences observed in the present study (Bigalke et al., 2022). Carter et al. (2009) reported that males responded to mental stress with augmented blood pressure reactivity, whereas females reported greater levels of perceived stress. However, MSNA responses did not differ between sexes (Carter & Ray, 2009). Jones et al. (1996) observed no differences in either cardiovascular or sympathetic reactivity, while Childs et al. (2010) found that females respond with blunted NE release. The differences between these studies are likely attributed at least in part to sympathetic measurements utilized. Whereas plasma catecholamine measurement is subject to variability dependent on catecholamine reuptake, degradation, etc. (Esler et al., 1990), MSNA circumvents these limitations. However, MSNA only offers a snapshot of sympathetic activity at the level of the skeletal muscle, and does not offer insight into differences in sympathetic outflow to other vascular beds. Nonetheless, sex and sex hormones have been shown to have a profound effect on sympathetic regulation of cardiovascular function throughout the lifespan (Carter et al., 2013; Hart et al., 2011; Hart et al., 2009; Joyner et al., 2016). While sympathetic responses to mental stress may not differ

between sexes, the relationship between MSNA and vascular changes may vary in a sex-dependent manner (Yang et al., 2013). Sympathetic vascular coupling was unfortunately not assessed in the current study, limiting inferences that may be made regarding vascular responsiveness.

We hypothesized that individuals with heightened trait anxiety severity in the current dataset would exhibit augmented MSNA reactivity based on the findings of Holwerda et al. (2018). We did not observe any relationship between any of the measured cardiovascular variables and trait anxiety levels. It is important to note that the individuals in the study performed by Holwerda et al. (2018) were diagnosed with GAD. The maximum trait anxiety score observed in the current dataset (52 a.u.) fell below the average trait anxiety of the individuals with GAD (57 a.u.) in the study by Holwerda et al. (2018). It is possible that the current dataset of healthy young adults did not include individuals with anxiety levels high enough to impact MSNA reactivity. Symptom severity has been shown to impact sympathetic outflow in other populations including individuals with PTSD (Fonkoue, Marvar, et al., 2020), major depressive disorder (Scalco et al., 2009), and metabolic syndrome (Toschi-Dias, Trombetta, da Silva, et al., 2013). The addition of individuals with severe anxiety symptoms to the current dataset may allow for improved assessment of the impact of trait anxiety on MSNA reactivity to the TSST.

Limitations of the current study were outlined in our recent publication (Bigalke et al., 2022). However, in addition to those listed, the following limitations refer to those findings which were not previously reported. First, we were unable to characterize the

appraisal of threat vs. challenge in the entirety of the sample. Due to the limited sample size, the findings surrounding an association between stress appraisal and blood pressure reactivity should be considered preliminary. We plan to continue collecting this data on further participants in order to delineate the impact of challenge vs. threat approach strategies on MSNA reactivity. Next, the current study lacked participant representation with heightened trait anxiety severity, thus limiting the conclusions that can be drawn regarding the impact of trait anxiety severity on sympathetic reactivity. Lastly, future studies may benefit from tandem assessment of vascular responsiveness to assess whether the changes in MSNA are observed concurrently with vascular changes.

Conclusion

The current study is the first to directly assess sympathetic and cardiovascular responsiveness to a commonly used psychosocial stress task, the Trier Social Stress Task. The findings highlight the potential utility of anticipatory stress as a novel and reliable stress task to advance our understanding of the dynamic stress response of the SNS and its interaction with cardiovascular health.

CHAPTER 4

SUMMARY AND FUTURE DIRECTIONS

Summary of Findings

Anxiety and anxiety disorders are prevalent (Kessler et al., 2005; Kessler et al., 2012) and associated with cardiovascular dysfunction (Lim et al., 2021; Pan et al., 2015; Roest et al., 2010; Roest et al., 2012). Although the SNS has been thought to mediate this relationship, little evidence exists that resting MSNA is altered in participants with clinical anxiety disorders. In those studies which have shown an association (Toschi-Dias, Trombetta, da Silva, et al., 2013), the presence of comorbid cardiometabolic disturbance makes the independent role of anxiety symptomology with excessive sympathoexcitation difficult to ascertain.

In **experiment 1**, we utilized a large sample of young, healthy adults to assess the independent relationship between trait anxiety and resting MSNA, blood pressure, and HR while controlling for sex and age. In **support of our hypothesis**, we observed a significant positive association between trait anxiety, resting MSNA, and blood pressure. These findings are the first to report an independent association between trait anxiety and gold-standard measurement of peripheral sympathetic activity in a healthy population. These findings are responsive to the need for assessment of psychological characteristics across a spectrum of severity that interact with physiological variables in a complex and dynamic manner (Wehrwein & Carter, 2016).

Although experiments performed in populations with clinical anxiety disorders are sparse, numerous studies have been performed assessing MSNA reactivity to laboratory mental stress (Bigalke & Carter, 2021; Carter & Goldstein, 2015). While mental stress leads to a reproducible pressor response, substantial inter-individual variability exists with regards to MSNA reactivity (Carter & Ray, 2009). Some of this inter-individual variability may be due to the effects of variables including respiration, muscle movement, and active speech that might interact with sympathetic outflow and cardiovascular alterations (Bigalke et al., 2022). Furthermore, the anticipation of stress may offer greater ecological validity for assessing sympathetic reactivity to psychosocial stress alone. The TSST elicits a stress task which includes an anticipatory phase during which participant movement and active speech are minimized.

Experiment 2 is the first study to directly record MSNA and beat-by-beat blood pressure in response to the TSST. This study characterized the sympathetic and cardiovascular response to the TSST with several key findings. First, in **contrast to our initial hypothesis**, MSNA during the anticipatory phase was largely reduced, likely through baroreflex inhibition, in nearly all participants (i.e., >90%). Interestingly, the strong relationship between changes in DAP and MSNA during the anticipatory phase were absent or greatly reduced during the speech task, supporting potential alterations in baroreflex control of sympathetic outflow during the active speech portion of the task (Durocher, Klein, et al., 2011). Next, the anticipatory response of MSNA was significantly related to reactivity to subsequent tasks, indicating the potential for anticipatory stress responsiveness as a predictor for the differing mental stress

responder phenotypes (Carter & Ray, 2009). Lastly, the current study did not observe any relationship between sex, anxiety, and sympathetic or cardiovascular reactivity.

Future Directions

The current series of investigations opens the door for numerous future studies surrounding stress, anxiety, and sympathoneural function. First, both projects are attentive to the largely overlooked influence of psychological characteristics on objective physiological function (Wehrwein & Carter, 2016). Studies assessing the influence of psychological factors within the field of physiological research, particularly those studies using directly measured sympathetic activity, are limited. While the field of physiology often accounts for changes in factors such as activity levels and anthropometric features over time, the assessment of alterations in psychological features and mental health are rarely accounted for (Wehrwein & Carter, 2016). Future research endeavors utilizing longitudinal assessment may benefit from monitoring changes in psychological characteristics using validated questionnaires and techniques. Incorporation of both psychology and physiology into integrative research studies will facilitate enhanced understanding of human cardiovascular health.

Experiment 1 observed a significant, independent association between trait anxiety, MSNA, and blood pressure. While informative, directionality cannot be inferred due to the retrospective nature of the study. Further, assessment of mechanisms underlying the impact of chronic stress and anxiety on autonomic and cardiovascular function are technically difficult due to experimental and ethical constraints. Recent research has improved upon this limitation through assessment of real-world stressor

presentation prior to laboratory testing (Greaney et al., 2019; Greaney et al., 2020). These findings have outlined a tangible relationship between daily stress exposure and neurovascular functioning (Greaney et al., 2019; Greaney et al., 2020), indicating a causal role for chronic stress in neural-vascular coupling. Naturalistic study designs assessing the impact of stress exposure on cardiovascular outcomes will offer directionality and better understanding of the complex role stress plays in physiological functioning over time.

Anxiety disorders disproportionately impact females (McLean et al., 2011) and sex-differences in neural control of cardiovascular regulation are well documented (Carter et al., 2013; Hart et al., 2009; Joyner et al., 2016; Keir et al., 2020). Greaney and colleagues have noted vascular dysfunction in response to psychosocial stress (Greaney et al., 2020), and in individuals with MDD (Greaney et al., 2022; Greaney et al., 2019) through β-receptor dysfunction (Greaney et al., 2022). This is particularly important in premenopausal females, in whom β-receptor activity is thought to offset the vasoconstricting action of NE within the vasculature (Hart et al., 2011). Though it was not reported in experiment 1 due to sample size constraints, sex differences in both anxiety and sympathetic regulation remain an active area of investigation within our laboratory.

It may be beneficial to carry out prospective analyses integrating the influence of anxiety during young adulthood on the trajectory of age-related increases in MSNA in order to apply causality to the associations presented in the current study. MSNA has been observed to be reproducible after 10 years (Fagius & Wallin, 1993), and changes

in MSNA correspond to observed changes in blood pressure (Hering et al., 2016). The mediating effect of anxiety warrants future attention in this relationship.

Integration of psychological models into physiological research may enhance our understanding of the complex associations between psychological stress and sympathetic/cardiovascular research. Experiment 2 may indirectly support the biopsychosocial model with preliminary evidence indicating augmented blood pressure reactivity in individuals who appraised the stressor as a challenge rather than a threat. Delineation of the benefits of either appraisal approach, or a moderate approach, is a potential area of future research. Microneurographic recordings of MSNA would allow direct insight into the relationship between psychological appraisal and peripheral sympathetic activity, and may shed further insight into the responder vs. non-responder mental stress phenotypes (Carter & Ray, 2009).

REFERENCES CITED

REFERENCES CITED

Alvarenga, M. E., Richards, J. C., Lambert, G., & Esler, M. D. (2006). Psychophysiological mechanisms in panic disorder: a correlative analysis of noradrenaline spillover, neuronal noradrenaline reuptake, power spectral analysis of heart rate variability, and psychological variables. *Psychosom Med, 68*(1), 8-16. doi:10.1097/01.psy.0000195872.00987.db

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders (5th ed.)*. Arlington: American Psychiatric Association.

Anderson, E. A., Sinkey, C. A., & Mark, A. L. (1991). Mental stress increases sympathetic nerve activity during sustained baroreceptor stimulation in humans. *Hypertension, 17*(4 Suppl), III43-49. doi:10.1161/01.hyp.17.4_suppl.iii43

Anderson, E. A., Wallin, B. G., & Mark, A. L. (1987). Dissociation of sympathetic nerve activity in arm and leg muscle during mental stress. *Hypertension, 9*(6 Pt 2), III114-119. doi:10.1161/01.hyp.9.6_pt_2.iii114

Barton, D. A., Dawood, T., Lambert, E. A., Esler, M. D., Haikerwal, D., Brenchley, C., . . . Lambert, G. W. (2007). Sympathetic activity in major depressive disorder: identifying those at increased cardiac risk? *J Hypertens, 25*(10), 2117-2124. doi:10.1097/HJH.0b013e32829baae7

Bechir, M., Schwegler, K., Chenevard, R., Binggeli, C., Caduff, C., Buchi, S., . . . Noll, G. (2007). Anxiolytic therapy with alprazolam increases muscle sympathetic activity in patients with panic disorders. *Auton Neurosci, 134*(1-2), 69-73. doi:10.1016/j.autneu.2007.01.007

Bergua, V., Meillon, C., Potvin, O., Bouisson, J., Le Goff, M., Rouaud, O., . . . Amieva, H. (2012). The STAI-Y trait scale: psychometric properties and normative data from a large population-based study of elderly people. *Int Psychogeriatr, 24*(7), 1163-1171. doi:10.1017/S1041610212000300

Bigalke, J. A., & Carter, J. R. (2021). Sympathetic Neural Control in Humans with Anxiety-Related Disorders. *Compr Physiol, 12*(1), 3085-3117. doi:10.1002/cphy.c210027

Bigalke, J. A., Greenlund, I. M., & Carter, J. R. (2020). Sex differences in self-report anxiety and sleep quality during COVID-19 stay-at-home orders. *Biol Sex Differ, 11*(1), 56. doi:10.1186/s13293-020-00333-4

Bigalke, J. A., Greenlund, I. M., Nicevski, J. R., Tikkanen, A. L., & Carter, J. R. (2022). Sympathetic neural reactivity to the Trier social stress test. *J Physiol, 600*(16), 3705-3724. doi:10.1113/JP283358

Bishop, S. J. (2007). Neurocognitive mechanisms of anxiety: an integrative account. *Trends Cogn Sci, 11*(7), 307-316. doi:10.1016/j.tics.2007.05.008

Bishop, S. J. (2009). Trait anxiety and impoverished prefrontal control of attention. *Nat Neurosci, 12*(1), 92-98. doi:10.1038/nn.2242

Callister, R., Suwarno, N. O., & Seals, D. R. (1992). Sympathetic activity is influenced by task difficulty and stress perception during mental challenge in humans. *J Physiol, 454*, 373-387. doi:10.1113/jphysiol.1992.sp019269

Cannon, W. (1932). *The Wisdom of the Body*: W.W. Norton and Company, Inc.

Carter, J. R. (2019). Microneurography and sympathetic nerve activity: a decade-by-decade journey across 50 years. *J Neurophysiol, 121*(4), 1183-1194. doi:10.1152/jn.00570.2018

Carter, J. R., Durocher, J. J., & Kern, R. P. (2008). Neural and cardiovascular responses to emotional stress in humans. *Am J Physiol Regul Integr Comp Physiol, 295*(6), R1898-1903. doi:10.1152/ajpregu.90646.2008

Carter, J. R., Durocher, J. J., Larson, R. A., DellaValla, J. P., & Yang, H. (2012). Sympathetic neural responses to 24-hour sleep deprivation in humans: sex differences. *Am J Physiol Heart Circ Physiol, 302*(10), H1991-1997. doi:10.1152/ajpheart.01132.2011

Carter, J. R., Fonkoue, I. T., Greenlund, I. M., Schwartz, C. E., Mokhlesi, B., & Smoot, C. A. (2019). Sympathetic neural responsiveness to sleep deprivation in older adults: sex differences. *Am J Physiol Heart Circ Physiol, 317*(2), H315-H322. doi:10.1152/ajpheart.00232.2019

Carter, J. R., Fu, Q., Minson, C. T., & Joyner, M. J. (2013). Ovarian cycle and sympathoexcitation in premenopausal women. *Hypertension, 61*(2), 395-399. doi:10.1161/HYPERTENSIONAHA.112.202598

Carter, J. R., & Goldstein, D. S. (2015). Sympathoneural and adrenomedullary responses to mental stress. *Compr Physiol, 5*(1), 119-146. doi:10.1002/cphy.c140030

Carter, J. R., Klein, J. C., & Schwartz, C. E. (2010). Effects of oral contraceptives on sympathetic nerve activity during orthostatic stress in young, healthy women. *Am J Physiol Regul Integr Comp Physiol, 298*(1), R9-R14. doi:10.1152/ajpregu.00554.2009

Carter, J. R., & Lawrence, J. E. (2007). Effects of the menstrual cycle on sympathetic neural responses to mental stress in humans. *J Physiol, 585*(Pt 2), 635-641. doi:10.1113/jphysiol.2007.141051

Carter, J. R., & Ray, C. A. (2009). Sympathetic neural responses to mental stress: responders, nonresponders and sex differences. *Am J Physiol Heart Circ Physiol, 296*(3), H847-853. doi:10.1152/ajpheart.01234.2008

Chida, Y., & Steptoe, A. (2010). Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: a meta-analysis of prospective evidence. *Hypertension, 55*(4), 1026-1032. doi:10.1161/HYPERTENSIONAHA.109.146621

Childs, E., Dlugos, A., & De Wit, H. (2010). Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. *Psychophysiology, 47*(3), 550-559. doi:10.1111/j.1469-8986.2009.00961.x

Dampney, R. A. (2015). Central mechanisms regulating coordinated cardiovascular and respiratory function during stress and arousal. *Am J Physiol Regul Integr Comp Physiol, 309*(5), R429-443. doi:10.1152/ajpregu.00051.2015

Dampney, R. A. L. (2017). Resetting of the Baroreflex Control of Sympathetic Vasomotor Activity during Natural Behaviors: Description and Conceptual Model of Central Mechanisms. *Front Neurosci, 11*, 461. doi:10.3389/fnins.2017.00461

DiMicco, J. A., Samuels, B. C., Zaretskaia, M. V., & Zaretsky, D. V. (2002). The dorsomedial hypothalamus and the response to stress: part renaissance, part revolution. *Pharmacol Biochem Behav, 71*(3), 469-480. doi:10.1016/s0091-3057(01)00689-x

Donadio, V., Liguori, R., Elam, M., Karlsson, T., Montagna, P., Cortelli, P., . . . Wallin, B. G. (2007). Arousal elicits exaggerated inhibition of sympathetic nerve activity in phobic syncope patients. *Brain, 130*(Pt 6), 1653-1662. doi:10.1093/brain/awm037

Durocher, J. J., Klein, J. C., & Carter, J. R. (2011). Attenuation of sympathetic baroreflex sensitivity during the onset of acute mental stress in humans. *Am J Physiol Heart Circ Physiol, 300*(5), H1788-1793. doi:10.1152/ajpheart.00942.2010

Durocher, J. J., Lufkin, K. M., King, M. E., & Carter, J. R. (2011). Social technology restriction alters state-anxiety but not autonomic activity in humans. *Am J Physiol Regul Integr Comp Physiol, 301*(6), R1773-1778. doi:10.1152/ajpregu.00418.2011

Eisenhofer, G., Friberg, P., Rundqvist, B., Quyyumi, A. A., Lambert, G., Kaye, D. M., . . . Esler, M. D. (1996). Cardiac sympathetic nerve function in congestive heart failure. *Circulation, 93*(9), 1667-1676. doi:10.1161/01.cir.93.9.1667

El Sayed, K., Macefield, V. G., Hissen, S. L., Joyner, M. J., & Taylor, C. E. (2016). Rate of rise in diastolic blood pressure influences vascular sympathetic response to mental stress. *J Physiol, 594*(24), 7465-7482. doi:10.1113/JP272963

El Sayed, K., Macefield, V. G., Hissen, S. L., Joyner, M. J., & Taylor, C. E. (2018). Blood pressure reactivity at onset of mental stress determines sympathetic vascular response in young adults. *Physiol Rep, 6*(24), e13944. doi:10.14814/phy2.13944

Elam, M., McKenzie, D., & Macefield, V. (2002). Mechanisms of sympathoexcitation: single-unit analysis of muscle vasoconstrictor neurons in awake OSAS subjects. *J Appl Physiol (1985), 93*(1), 297-303. doi:10.1152/japplphysiol.00899.2001

Esler, M., Jennings, G., Korner, P., Willett, I., Dudley, F., Hasking, G., . . . Lambert, G. (1988). Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. *Hypertension, 11*(1), 3-20. doi:10.1161/01.hyp.11.1.3

Esler, M., Jennings, G., & Lambert, G. (1989). Measurement of overall and cardiac norepinephrine release into plasma during cognitive challenge. *Psychoneuroendocrinology, 14*(6), 477-481. doi:10.1016/0306-4530(89)90047-4

Esler, M., Jennings, G., Lambert, G., Meredith, I., Horne, M., & Eisenhofer, G. (1990). Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol Rev, 70*(4), 963-985. doi:10.1152/physrev.1990.70.4.963

Fagius, J., & Wallin, B. G. (1993). Long-term variability and reproducibility of resting human muscle nerve sympathetic activity at rest, as reassessed after a decade. *Clin Auton Res, 3*(3), 201-205. doi:10.1007/BF01826234

Feng, H. P., Chien, W. C., Cheng, W. T., Chung, C. H., Cheng, S. M., & Tzeng, W. C. (2016). Risk of anxiety and depressive disorders in patients with myocardial infarction: A nationwide population-based cohort study. *Medicine (Baltimore), 95*(34), e4464. doi:10.1097/MD.0000000000004464

Fonkoue, I. T., & Carter, J. R. (2015). Sympathetic neural reactivity to mental stress in humans: test-retest reproducibility. *Am J Physiol Regul Integr Comp Physiol, 309*(11), R1380-1386. doi:10.1152/ajpregu.00344.2015

Fonkoue, I. T., Hu, Y., Jones, T., Vemulapalli, M., Sprick, J. D., Rothbaum, B., & Park, J. (2020). Eight weeks of device-guided slow breathing decreases sympathetic

nervous reactivity to stress in posttraumatic stress disorder. *Am J Physiol Regul Integr Comp Physiol, 319*(4), R466-R475. doi:10.1152/ajpregu.00079.2020

Fonkoue, I. T., Marvar, P. J., Norrholm, S., Li, Y., Kankam, M. L., Jones, T. N., . . . Park, J. (2020). Symptom severity impacts sympathetic dysregulation and inflammation in post-traumatic stress disorder (PTSD). *Brain Behav Immun, 83*, 260-269. doi:10.1016/j.bbi.2019.10.021

Fonkoue, I. T., Marvar, P. J., Norrholm, S. D., Kankam, M. L., Li, Y., DaCosta, D., . . . Park, J. (2018). Acute effects of device-guided slow breathing on sympathetic nerve activity and baroreflex sensitivity in posttraumatic stress disorder. *Am J Physiol Heart Circ Physiol, 315*(1), H141-H149. doi:10.1152/ajpheart.00098.2018

Fonkoue, I. T., Wang, M., & Carter, J. R. (2016). Sympathetic neural reactivity to mental stress in offspring of hypertensive parents: 20 years revisited. *Am J Physiol Heart Circ Physiol, 311*(2), H426-432. doi:10.1152/ajpheart.00378.2016

Freyschuss, U., Fagius, J., Wallin, B. G., Bohlin, G., Perski, A., & Hjemdahl, P. (1990). Cardiovascular and sympathoadrenal responses to mental stress: a study of sensory intake and rejection reactions. *Acta Physiol Scand, 139*(1), 173-183. doi:10.1111/j.1748-1716.1990.tb08910.x

Freyschuss, U., Hjemdahl, P., Juhlin-Dannfelt, A., & Linde, B. (1988). Cardiovascular and sympathoadrenal responses to mental stress: influence of beta-blockade. *Am J Physiol, 255*(6 Pt 2), H1443-1451. doi:10.1152/ajpheart.1988.255.6.H1443

Ghasemi, A., & Zahediasl, S. (2012). Normality tests for statistical analysis: a guide for non-statisticians. *Int J Endocrinol Metab, 10*(2), 486-489. doi:10.5812/ijem.3505

Gianaros, P. J., Sheu, L. K., Matthews, K. A., Jennings, J. R., Manuck, S. B., & Hariri, A. R. (2008). Individual differences in stressor-evoked blood pressure reactivity vary with activation, volume, and functional connectivity of the amygdala. *J Neurosci, 28*(4), 990-999. doi:10.1523/JNEUROSCI.3606-07.2008

Grace, S. L., Abbey, S. E., Irvine, J., Shnek, Z. M., & Stewart, D. E. (2004). Prospective examination of anxiety persistence and its relationship to cardiac symptoms and recurrent cardiac events. *Psychother Psychosom, 73*(6), 344-352. doi:10.1159/000080387

Grassi, G., Biffi, A., Seravalle, G., Trevano, F. Q., Dell'Oro, R., Corrao, G., & Mancia, G. (2019). Sympathetic Neural Overdrive in the Obese and Overweight State. *Hypertension, 74*(2), 349-358. doi:10.1161/HYPERTENSIONAHA.119.12885

Grassi, G., Cattaneo, B. M., Seravalle, G., Lanfranchi, A., & Mancia, G. (1998). Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. *Hypertension, 31*(1), 68-72. doi:10.1161/01.hyp.31.1.68

Grassi, G., Pisano, A., Bolignano, D., Seravalle, G., D'Arrigo, G., Quarti-Trevano, F., . . . Mancia, G. (2018). Sympathetic Nerve Traffic Activation in Essential Hypertension and Its Correlates: Systematic Reviews and Meta-Analyses. *Hypertension, 72*(2), 483-491. doi:10.1161/HYPERTENSIONAHA.118.11038

Grassi, G., Seravalle, G., Cattaneo, B. M., Lanfranchi, A., Vailati, S., Giannattasio, C., . . . Pozzi, M. (1995). Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. *Circulation, 92*(11), 3206-3211. doi:10.1161/01.cir.92.11.3206

Grassi, G., Seravalle, G., Dell'Oro, R., Turri, C., Bolla, G. B., & Mancia, G. (2000). Adrenergic and reflex abnormalities in obesity-related hypertension. *Hypertension, 36*(4), 538-542. doi:10.1161/01.hyp.36.4.538

Greaney, J. L., Darling, A. M., Mogle, J., & Saunders, E. F. H. (2022). Microvascular beta-Adrenergic Receptor-Mediated Vasodilation Is Attenuated in Adults With Major Depressive Disorder. *Hypertension, 79*(5), 1091-1100. doi:10.1161/HYPERTENSIONAHA.122.18985

Greaney, J. L., Koffer, R. E., Saunders, E. F. H., Almeida, D. M., & Alexander, L. M. (2019). Self-Reported Everyday Psychosocial Stressors Are Associated With Greater Impairments in Endothelial Function in Young Adults With Major Depressive Disorder. *J Am Heart Assoc, 8*(4), e010825. doi:10.1161/JAHA.118.010825

Greaney, J. L., Surachman, A., Saunders, E. F. H., Alexander, L. M., & Almeida, D. M. (2020). Greater Daily Psychosocial Stress Exposure is Associated With Increased Norepinephrine-Induced Vasoconstriction in Young Adults. *J Am Heart Assoc, 9*(9), e015697. doi:10.1161/JAHA.119.015697

Greenlund, I. M., Cunningham, H. A., Tikkanen, A. L., Bigalke, J. A., Smoot, C. A., Durocher, J. J., & Carter, J. R. (2021). Morning sympathetic activity after evening binge alcohol consumption. *Am J Physiol Heart Circ Physiol, 320*(1), H305-H315. doi:10.1152/ajpheart.00743.2020

Greenwood, J. P., Stoker, J. B., & Mary, D. A. (1999). Single-unit sympathetic discharge : quantitative assessment in human hypertensive disease. *Circulation, 100*(12), 1305-1310. doi:10.1161/01.cir.100.12.1305

Griffin, S. M., & Howard, S. (2021). Instructed reappraisal and cardiovascular habituation to recurrent stress. *Psychophysiology, 58*(5), e13783. doi:10.1111/psyp.13783

Hagbarth, K. E., & Vallbo, A. B. (1968). Pulse and respiratory grouping of sympathetic impulses in human muscle-nerves. *Acta Physiol Scand, 74*(1), 96-108. doi:10.1111/j.1748-1716.1968.tb04218.x

Halliwill, J. R., Lawler, L. A., Eickhoff, T. J., Dietz, N. M., Nauss, L. A., & Joyner, M. J. (1997). Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. *J Physiol, 504 (Pt 1)*, 211-220. doi:10.1111/j.1469-7793.1997.211bf.x

Hanssen, T. A., Nordrehaug, J. E., Eide, G. E., Bjelland, I., & Rokne, B. (2009). Anxiety and depression after acute myocardial infarction: an 18-month follow-up study with repeated measures and comparison with a reference population. *Eur J Cardiovasc Prev Rehabil, 16*(6), 651-659. doi:10.1097/HJR.0b013e32832e4206

Hart, E. C., Charkoudian, N., Wallin, B. G., Curry, T. B., Eisenach, J., & Joyner, M. J. (2011). Sex and ageing differences in resting arterial pressure regulation: the role of the beta-adrenergic receptors. *J Physiol, 589*(Pt 21), 5285-5297. doi:10.1113/jphysiol.2011.212753

Hart, E. C., Charkoudian, N., Wallin, B. G., Curry, T. B., Eisenach, J. H., & Joyner, M. J. (2009). Sex differences in sympathetic neural-hemodynamic balance: implications for human blood pressure regulation. *Hypertension, 53*(3), 571-576. doi:10.1161/HYPERTENSIONAHA.108.126391

Hart, E. C., Head, G. A., Carter, J. R., Wallin, B. G., May, C. N., Hamza, S. M., . . . Osborn, J. W. (2017). Recording sympathetic nerve activity in conscious humans and other mammals: guidelines and the road to standardization. *Am J Physiol Heart Circ Physiol, 312*(5), H1031-H1051. doi:10.1152/ajpheart.00703.2016

Harvey, R. E., Hart, E. C., Charkoudian, N., Curry, T. B., Carter, J. R., Fu, Q., . . . Barnes, J. N. (2015). Oral Contraceptive Use, Muscle Sympathetic Nerve Activity, and Systemic Hemodynamics in Young Women. *Hypertension, 66*(3), 590-597. doi:10.1161/HYPERTENSIONAHA.115.05179

Hering, D., Kara, T., Kucharska, W., Somers, V. K., & Narkiewicz, K. (2016). Longitudinal tracking of muscle sympathetic nerve activity and its relationship with blood pressure in subjects with prehypertension. *Blood Press, 25*(3), 184-192. doi:10.3109/08037051.2015.1121708

Holwerda, S. W., Carter, J. R., Yang, H., Wang, J., Pierce, G. L., & Fadel, P. J. (2021). CORP: Standardizing methodology for assessing spontaneous baroreflex control

of muscle sympathetic nerve activity in humans. *Am J Physiol Heart Circ Physiol, 320*(2), H762-H771. doi:10.1152/ajpheart.00704.2020

Holwerda, S. W., Luehrs, R. E., Gremaud, A. L., Wooldridge, N. A., Stroud, A. K., Fiedorowicz, J. G., . . . Pierce, G. L. (2018). Relative burst amplitude of muscle sympathetic nerve activity is an indicator of altered sympathetic outflow in chronic anxiety. *J Neurophysiol, 120*(1), 11-22. doi:10.1152/jn.00064.2018

Incognito, A. V., Duplea, S. G., Lee, J. B., Sussman, J., Shepherd, A. D., Doherty, C. J., . . . Millar, P. J. (2019). Arterial baroreflex regulation of muscle sympathetic nerve activity at rest and during stress. *J Physiol, 597*(18), 4729-4741. doi:10.1113/JP278376

Indovina, I., Robbins, T. W., Nunez-Elizalde, A. O., Dunn, B. D., & Bishop, S. J. (2011). Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron, 69*(3), 563-571. doi:10.1016/j.neuron.2010.12.034

James, C., Macefield, V. G., & Henderson, L. A. (2013). Real-time imaging of cortical and subcortical control of muscle sympathetic nerve activity in awake human subjects. *Neuroimage, 70*, 59-65. doi:10.1016/j.neuroimage.2012.12.047

Jansen, J. R., Wesseling, K. H., Settels, J. J., & Schreuder, J. J. (1990). Continuous cardiac output monitoring by pulse contour during cardiac surgery. *Eur Heart J, 11 Suppl I*, 26-32. doi:10.1093/eurheartj/11.suppl_i.26

Jonas, B. S., Franks, P., & Ingram, D. D. (1997). Are symptoms of anxiety and depression risk factors for hypertension? Longitudinal evidence from the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Arch Fam Med, 6*(1), 43-49. doi:10.1001/archfami.6.1.43

Jones, P. P., Spraul, M., Matt, K. S., Seals, D. R., Skinner, J. S., & Ravussin, E. (1996). Gender does not influence sympathetic neural reactivity to stress in healthy humans. *Am J Physiol, 270*(1 Pt 2), H350-357. doi:10.1152/ajpheart.1996.270.1.H350

Joyner, M. J., Wallin, B. G., & Charkoudian, N. (2016). Sex differences and blood pressure regulation in humans. *Exp Physiol, 101*(3), 349-355. doi:10.1113/EP085146

Julian, L. J. (2011). Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). *Arthritis Care Res (Hoboken), 63 Suppl 11*(0 11), S467-472. doi:10.1002/acr.20561

Keir, D. A., Badrov, M. B., Tomlinson, G., Notarius, C. F., Kimmerly, D. S., Millar, P. J., . . . Floras, J. S. (2020). Influence of Sex and Age on Muscle Sympathetic Nerve Activity of Healthy Normotensive Adults. *Hypertension, 76*(3), 997-1005. doi:10.1161/HYPERTENSIONAHA.120.15208

Keller-Ross, M. L., Cunningham, H. A., & Carter, J. R. (2020). Impact of age and sex on neural cardiovascular responsiveness to cold pressor test in humans. *Am J Physiol Regul Integr Comp Physiol, 319*(3), R288-R295. doi:10.1152/ajpregu.00045.2020

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry, 62*(6), 593-602. doi:10.1001/archpsyc.62.6.593

Kessler, R. C., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Wittchen, H. U. (2012). Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *Int J Methods Psychiatr Res, 21*(3), 169-184. doi:10.1002/mpr.1359

Kessler, R. C., Sonnega, A., Bromet, E., Hughes, M., & Nelson, C. B. (1995). Posttraumatic stress disorder in the National Comorbidity Survey. *Arch Gen Psychiatry, 52*(12), 1048-1060. doi:10.1001/archpsyc.1995.03950240066012

Kim, J. H., Almuwaqqat, Z., Hammadah, M., Liu, C., Ko, Y. A., Lima, B., . . . Quyyumi, A. A. (2019). Peripheral Vasoconstriction During Mental Stress and Adverse Cardiovascular Outcomes in Patients With Coronary Artery Disease. *Circ Res, 125*(10), 874-883. doi:10.1161/CIRCRESAHA.119.315005

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*(1-2), 76-81. doi:10.1159/000119004

Kuipers, N. T., Sauder, C. L., Carter, J. R., & Ray, C. A. (2008). Neurovascular responses to mental stress in the supine and upright postures. *J Appl Physiol (1985), 104*(4), 1129-1136. doi:10.1152/japplphysiol.01285.2007

Lambert, E., Dawood, T., Straznicky, N., Sari, C., Schlaich, M., Esler, M., & Lambert, G. (2010). Association between the sympathetic firing pattern and anxiety level in patients with the metabolic syndrome and elevated blood pressure. *J Hypertens, 28*(3), 543-550. doi:10.1097/HJH.0b013e3283350ea4

Lambert, E., Hotchkin, E., Alvarenga, M., Pier, C., Richards, J., Barton, D., . . . Lambert, G. (2006). Single-unit analysis of sympathetic nervous discharges in patients with panic disorder. *J Physiol, 570*(Pt 3), 637-643. doi:10.1113/jphysiol.2005.100040

Lambert, E. A., Schlaich, M. P., Dawood, T., Sari, C., Chopra, R., Barton, D. A., . . . Lambert, G. W. (2011). Single-unit muscle sympathetic nervous activity and its relation to cardiac noradrenaline spillover. *J Physiol, 589*(Pt 10), 2597-2605. doi:10.1113/jphysiol.2011.205351

Lambert, E. A., Thompson, J., Schlaich, M., Laude, D., Elghozi, J. L., Esler, M. D., & Lambert, G. W. (2002). Sympathetic and cardiac baroreflex function in panic disorder. *J Hypertens, 20*(12), 2445-2451. doi:10.1097/00004872-200212000-00025

Lim, L. F., Solmi, M., & Cortese, S. (2021). Association between anxiety and hypertension in adults: A systematic review and meta-analysis. *Neurosci Biobehav Rev, 131*, 96-119. doi:10.1016/j.neubiorev.2021.08.031

Macefield, V. G., Rundqvist, B., Sverrisdottir, Y. B., Wallin, B. G., & Elam, M. (1999). Firing properties of single muscle vasoconstrictor neurons in the sympathoexcitation associated with congestive heart failure. *Circulation, 100*(16), 1708-1713. doi:10.1161/01.cir.100.16.1708

Macefield, V. G., & Wallin, B. G. (2018). Physiological and pathophysiological firing properties of single postganglionic sympathetic neurons in humans. *J Neurophysiol, 119*(3), 944-956. doi:10.1152/jn.00004.2017

Macefield, V. G., Wallin, B. G., & Vallbo, A. B. (1994). The discharge behaviour of single vasoconstrictor motoneurones in human muscle nerves. *J Physiol, 481 (Pt 3)*, 799-809. doi:10.1113/jphysiol.1994.sp020482

Matsukawa, T., Sugiyama, Y., & Mano, T. (1995). Increased muscle sympathetic nerve activity during delayed auditory feedback in humans. *Jpn J Physiol, 45*(5), 905-911. doi:10.2170/jjphysiol.45.905

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *N Engl J Med, 338*(3), 171-179. doi:10.1056/NEJM199801153380307

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res, 45*(8), 1027-1035. doi:10.1016/j.jpsychires.2011.03.006

Muller-Ribeiro, F. C., Zaretsky, D. V., Zaretskaia, M. V., Santos, R. A., DiMicco, J. A., & Fontes, M. A. (2012). Contribution of infralimbic cortex in the cardiovascular response to acute stress. *Am J Physiol Regul Integr Comp Physiol, 303*(6), R639-650. doi:10.1152/ajpregu.00573.2011

Nardone, M., Teixeira, A. L., Incognito, A. V., Vermeulen, T. D., Shafer, B. M., Millar, P. J., & Foster, G. E. (2020). Within-breath sympathetic baroreflex sensitivity is modulated by lung volume but unaffected by acute intermittent hypercapnic hypoxia in men. *Am J Physiol Heart Circ Physiol, 319*(1), H213-H221. doi:10.1152/ajpheart.00296.2020

Narkiewicz, K., Kato, M., Phillips, B. G., Pesek, C. A., Davison, D. E., & Somers, V. K. (1999). Nocturnal continuous positive airway pressure decreases daytime sympathetic traffic in obstructive sleep apnea. *Circulation, 100*(23), 2332-2335. doi:10.1161/01.cir.100.23.2332

Narkiewicz, K., Pesek, C. A., Kato, M., Phillips, B. G., Davison, D. E., & Somers, V. K. (1998). Baroreflex control of sympathetic nerve activity and heart rate in obstructive sleep apnea. *Hypertension, 32*(6), 1039-1043. doi:10.1161/01.hyp.32.6.1039

Narkiewicz, K., van de Borne, P. J., Cooley, R. L., Dyken, M. E., & Somers, V. K. (1998). Sympathetic activity in obese subjects with and without obstructive sleep apnea. *Circulation, 98*(8), 772-776. doi:10.1161/01.cir.98.8.772

Narvaez Linares, N. F., Charron, V., Ouimet, A. J., Labelle, P. R., & Plamondon, H. (2020). A systematic review of the Trier Social Stress Test methodology: Issues in promoting study comparison and replicable research. *Neurobiol Stress, 13*, 100235. doi:10.1016/j.ynstr.2020.100235

O'Donovan, A., Slavich, G. M., Epel, E. S., & Neylan, T. C. (2013). Exaggerated neurobiological sensitivity to threat as a mechanism linking anxiety with increased risk for diseases of aging. *Neurosci Biobehav Rev, 37*(1), 96-108. doi:10.1016/j.neubiorev.2012.10.013

Ogoh, S., Fisher, J. P., Raven, P. B., & Fadel, P. J. (2007). Arterial baroreflex control of muscle sympathetic nerve activity in the transition from rest to steady-state dynamic exercise in humans. *Am J Physiol Heart Circ Physiol, 293*(4), H2202-2209. doi:10.1152/ajpheart.00708.2007

Pacak, K., Palkovits, M., Yadid, G., Kvetnansky, R., Kopin, I. J., & Goldstein, D. S. (1998). Heterogeneous neurochemical responses to different stressors: a test of Selye's doctrine of nonspecificity. *Am J Physiol, 275*(4), R1247-1255. doi:10.1152/ajpregu.1998.275.4.R1247

Pan, Y., Cai, W., Cheng, Q., Dong, W., An, T., & Yan, J. (2015). Association between anxiety and hypertension: a systematic review and meta-analysis of epidemiological studies. *Neuropsychiatr Dis Treat, 11*, 1121-1130. doi:10.2147/NDT.S77710

Papelier, Y., Escourrou, P., Gauthier, J. P., & Rowell, L. B. (1994). Carotid baroreflex control of blood pressure and heart rate in men during dynamic exercise. *J Appl Physiol (1985), 77*(2), 502-506. doi:10.1152/jappl.1994.77.2.502

Park, J., Marvar, P. J., Liao, P., Kankam, M. L., Norrholm, S. D., Downey, R. M., . . . Rothbaum, B. O. (2017). Baroreflex dysfunction and augmented sympathetic nerve responses during mental stress in veterans with post-traumatic stress disorder. *J Physiol, 595*(14), 4893-4908. doi:10.1113/JP274269

Potts, J. T., Shi, X. R., & Raven, P. B. (1993). Carotid baroreflex responsiveness during dynamic exercise in humans. *Am J Physiol, 265*(6 Pt 2), H1928-1938. doi:10.1152/ajpheart.1993.265.6.H1928

Quarti Trevano, F., Dell'Oro, R., Biffi, A., Seravalle, G., Corrao, G., Mancia, G., & Grassi, G. (2020). Sympathetic overdrive in the metabolic syndrome: meta-analysis of published studies. *J Hypertens, 38*(4), 565-572. doi:10.1097/HJH.0000000000002288

Roest, A. M., Martens, E. J., de Jonge, P., & Denollet, J. (2010). Anxiety and risk of incident coronary heart disease: a meta-analysis. *J Am Coll Cardiol, 56*(1), 38-46. doi:10.1016/j.jacc.2010.03.034

Roest, A. M., Zuidersma, M., & de Jonge, P. (2012). Myocardial infarction and generalised anxiety disorder: 10-year follow-up. *Br J Psychiatry, 200*(4), 324-329. doi:10.1192/bjp.bp.111.103549

Saha, S., Lim, C. C. W., Cannon, D. L., Burton, L., Bremner, M., Cosgrove, P., . . . J, J. M. (2021). Co-morbidity between mood and anxiety disorders: A systematic review and meta-analysis. *Depress Anxiety, 38*(3), 286-306. doi:10.1002/da.23113

Salmanpour, A., Brown, L. J., Steinback, C. D., Usselman, C. W., Goswami, R., & Shoemaker, J. K. (2011). Relationship between size and latency of action potentials in human muscle sympathetic nerve activity. *J Neurophysiol, 105*(6), 2830-2842. doi:10.1152/jn.00814.2010

Salmanpour, A., & Shoemaker, J. K. (2012). Baroreflex mechanisms regulating the occurrence of neural spikes in human muscle sympathetic nerve activity. *J Neurophysiol, 107*(12), 3409-3416. doi:10.1152/jn.00925.2011

Santangelo, G., Sacco, R., Siciliano, M., Bisecco, A., Muzzo, G., Docimo, R., . . . Gallo, A. (2016). Anxiety in Multiple Sclerosis: psychometric properties of the State-Trait Anxiety Inventory. *Acta Neurol Scand, 134*(6), 458-466. doi:10.1111/ane.12564

Scalco, A. Z., Rondon, M. U., Trombetta, I. C., Laterza, M. C., Azul, J. B., Pullenayegum, E. M., . . . Lotufo-Neto, F. (2009). Muscle sympathetic nervous activity in depressed patients before and after treatment with sertraline. *J Hypertens, 27*(12), 2429-2436. doi:10.1097/HJH.0b013e3283310ece

Scherrer, J. F., Salas, J., Cohen, B. E., Schnurr, P. P., Schneider, F. D., Chard, K. M., . . . Lustman, P. J. (2019). Comorbid Conditions Explain the Association Between Posttraumatic Stress Disorder and Incident Cardiovascular Disease. *J Am Heart Assoc, 8*(4), e011133. doi:10.1161/JAHA.118.011133

Schroeder, K. E., Narkiewicz, K., Kato, M., Pesek, C., Phillips, B., Davison, D., & Somers, V. K. (2000). Personality type and neural circulatory control. *Hypertension, 36*(5), 830-833. doi:10.1161/01.hyp.36.5.830

Seery, M. D. (2011). Challenge or threat? Cardiovascular indexes of resilience and vulnerability to potential stress in humans. *Neurosci Biobehav Rev, 35*(7), 1603-1610. doi:10.1016/j.neubiorev.2011.03.003

Selye, H. (1950). Stress and the general adaptation syndrome. *Br Med J, 1*(4667), 1383-1392. doi:10.1136/bmj.1.4667.1383

Selye, H. (1976). Stress without Distress. In G. Serban (Ed.), *Psychopathology of Human Adaptation* (pp. 137-146). Boston, MA: Springer US.

Selye, H. (1998). A syndrome produced by diverse nocuous agents. 1936. *J Neuropsychiatry Clin Neurosci, 10*(2), 230-231. doi:10.1176/jnp.10.2.230a

Sherwood, A., Allen, M. T., Obrist, P. A., & Langer, A. W. (1986). Evaluation of beta-adrenergic influences on cardiovascular and metabolic adjustments to physical and psychological stress. *Psychophysiology, 23*(1), 89-104. doi:10.1111/j.1469-8986.1986.tb00602.x

Shoemaker, J. K., Klassen, S. A., Badrov, M. B., & Fadel, P. J. (2018). Fifty years of microneurography: learning the language of the peripheral sympathetic nervous system in humans. *J Neurophysiol, 119*(5), 1731-1744. doi:10.1152/jn.00841.2017

Smaardijk, V. R., Lodder, P., Kop, W. J., van Gennep, B., Maas, A., & Mommersteeg, P. M. C. (2019). Sex- and Gender-Stratified Risks of Psychological Factors for Incident Ischemic Heart Disease: Systematic Review and Meta-Analysis. *J Am Heart Assoc, 8*(9), e010859. doi:10.1161/JAHA.118.010859

Soltis, R. P., Cook, J. C., Gregg, A. E., & Sanders, B. J. (1997). Interaction of GABA and excitatory amino acids in the basolateral amygdala: role in cardiovascular

regulation. *J Neurosci, 17*(23), 9367-9374. doi:10.1523/JNEUROSCI.17-23-09367.1997

Somers, V. K., Dyken, M. E., Clary, M. P., & Abboud, F. M. (1995). Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest, 96*(4), 1897-1904. doi:10.1172/JCI118235

Spielberger, C., Gorsuch, R., Lushene, R., Vagg, P., & Jacobs, G. (1983). Manual for the State-Trait Anxiety Inventory. *Palo Alto (CA): Consulting Psychologists Press*.

Sterling, P., & Eyer, J. (1988). Allostasis: A new paradigm to explain arousal pathology. In S. Fischer & J. Reason (Eds.), *Handbook of Life Stress, Cognition and Health* (pp. 629-649). New York: John Wiley & Sons.

Sundlof, G., & Wallin, B. G. (1977). The variability of muscle nerve sympathetic activity in resting recumbent man. *J Physiol, 272*(2), 383-397. doi:10.1113/jphysiol.1977.sp012050

Tank, A. W., & Lee Wong, D. (2015). Peripheral and central effects of circulating catecholamines. *Compr Physiol, 5*(1), 1-15. doi:10.1002/cphy.c140007

Toschi-Dias, E., Trombetta, I. C., da Silva, V. J., Maki-Nunes, C., Alves, M. J., Angelo, L. F., . . . Rondon, M. U. (2013). Symptoms of anxiety and mood disturbance alter cardiac and peripheral autonomic control in patients with metabolic syndrome. *Eur J Appl Physiol, 113*(3), 671-679. doi:10.1007/s00421-012-2476-8

Toschi-Dias, E., Trombetta, I. C., Dias da Silva, V. J., Maki-Nunes, C., Cepeda, F. X., Alves, M. J., . . . Rondon, M. U. (2013). Time delay of baroreflex control and oscillatory pattern of sympathetic activity in patients with metabolic syndrome and obstructive sleep apnea. *Am J Physiol Heart Circ Physiol, 304*(7), H1038-1044. doi:10.1152/ajpheart.00848.2012

Turner, M. J., Jones, M. V., Sheffield, D., Barker, J. B., & Coffee, P. (2014). Manipulating cardiovascular indices of challenge and threat using resource appraisals. *Int J Psychophysiol, 94*(1), 9-18. doi:10.1016/j.ijpsycho.2014.07.004

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci, 10*(6), 397-409. doi:10.1038/nrn2647

V. Euler, U. S. (1946). A specific sympathomimetic ergone in adrengergic nerve fibres (sympathin) and its relations to adrenaline and nor-adrenaline. *Acta Physiol Scand, 12*(1), 73-97.

Vaccarino, V., Shah, A. J., Rooks, C., Ibeanu, I., Nye, J. A., Pimple, P., . . . Raggi, P. (2014). Sex differences in mental stress-induced myocardial ischemia in young survivors of an acute myocardial infarction. *Psychosom Med, 76*(3), 171-180. doi:10.1097/PSY.0000000000000045

Vallbo, A. B., Hagbarth, K. E., & Wallin, B. G. (2004). Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol (1985), 96*(4), 1262-1269. doi:10.1152/japplphysiol.00470.2003

Villacres, E. C., Hollifield, M., Katon, W. J., Wilkinson, C. W., & Veith, R. C. (1987). Sympathetic nervous system activity in panic disorder. *Psychiatry Res, 21*(4), 313-321. doi:10.1016/0165-1781(87)90015-1

Wallin, B. G., Esler, M., Dorward, P., Eisenhofer, G., Ferrier, C., Westerman, R., & Jennings, G. (1992). Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J Physiol, 453*, 45-58. doi:10.1113/jphysiol.1992.sp019217

Wallin, B. G., Thompson, J. M., Jennings, G. L., & Esler, M. D. (1996). Renal noradrenaline spillover correlates with muscle sympathetic activity in humans. *J Physiol, 491 (Pt 3)*, 881-887. doi:10.1113/jphysiol.1996.sp021265

Wasmund, W. L., Westerholm, E. C., Watenpaugh, D. E., Wasmund, S. L., & Smith, M. L. (2002). Interactive effects of mental and physical stress on cardiovascular control. *J Appl Physiol (1985), 92*(5), 1828-1834. doi:10.1152/japplphysiol.00019.2001

Watkins, L. L., Koch, G. G., Sherwood, A., Blumenthal, J. A., Davidson, J. R., O'Connor, C., & Sketch, M. H. (2013). Association of anxiety and depression with all-cause mortality in individuals with coronary heart disease. *J Am Heart Assoc, 2*(2), e000068. doi:10.1161/JAHA.112.000068

Wehrwein, E. A., & Carter, J. R. (2016). The Mind Matters: Psychology as an Overlooked Variable Within Physiology Studies. *Physiology (Bethesda), 31*(2), 74-75. doi:10.1152/physiol.00059.2015

Wehrwein, E. A., & Joyner, M. J. (2013). Regulation of blood pressure by the arterial baroreflex and autonomic nervous system. *Handb Clin Neurol, 117*, 89-102. doi:10.1016/B978-0-444-53491-0.00008-0

Wehrwein, E. A., Orer, H. S., & Barman, S. M. (2016). Overview of the Anatomy, Physiology, and Pharmacology of the Autonomic Nervous System. *Compr Physiol, 6*(3), 1239-1278. doi:10.1002/cphy.c150037

Wilkinson, D. J., Thompson, J. M., Lambert, G. W., Jennings, G. L., Schwarz, R. G., Jefferys, D., . . . Esler, M. D. (1998). Sympathetic activity in patients with panic disorder at rest, under laboratory mental stress, and during panic attacks. *Arch Gen Psychiatry, 55*(6), 511-520. doi:10.1001/archpsyc.55.6.511

Williams, S. E., Veldhuijzen van Zanten, J., Trotman, G. P., Quinton, M. L., & Ginty, A. T. (2017). Challenge and threat imagery manipulates heart rate and anxiety responses to stress. *Int J Psychophysiol, 117*, 111-118. doi:10.1016/j.ijpsycho.2017.04.011

Yang, H., & Carter, J. R. (2013). Baroreflex sensitivity analysis: spontaneous methodology vs. Valsalva's maneuver. *Clin Auton Res, 23*(3), 133-139. doi:10.1007/s10286-013-0195-9

Yang, H., Drummer, T. D., & Carter, J. R. (2013). Sex differences in sympathetic neural and limb vascular reactivity to mental stress in humans. *Am J Physiol Heart Circ Physiol, 304*(3), H436-443. doi:10.1152/ajpheart.00688.2012

Yoo, J. K., Badrov, M. B., Huang, M., Bain, R. A., Dorn, R. P., Anderson, E. H., . . . Fu, Q. (2020). Abnormal sympathetic neural recruitment patterns and hemodynamic responses to cold pressor test in women with posttraumatic stress disorder. *Am J Physiol Heart Circ Physiol, 318*(5), H1198-H1207. doi:10.1152/ajpheart.00684.2019

APPENDICES

APPENDIX A

RAW DATA FOR CHAPTER 2

	Demographics			STAI		Seated CV Measures			Resting MSNA		
ID	Sex	Age	BMI	Raw	%	SAP	DAP	MAP	HR	BF	BI
1	M	21	28.2	26	21	118.7	70	86.2	67.9	25.2	37.4
2	M	18	28.09	20	3	105.7	60	75.2	54.1	10.7	20.82
3	M	18	21.05	28	27	99	60.7	73.5	60.9	11.8	19.58
4	M	19	21.55	29	30	96.3	63.7	74.6	67	11	16.54
5	M	20	26.73	45	83	112.3	62	78.8	56.2	11.9	21.51
6	M	19	26.1	31	38	121.3	67.7	85.6	49.2	8.2	16.74
7	F	25	20.72	30	29	102	76.3	84.9	69.5	17.2	24.92
8	M	19	23.39	35	57	116	58	77.3	57.9	16	28.33
9	F	18	24.2	30	29	101.3	62	75.1	56.9	2.5	4.52
10	F	28	33.17	45	86	114.3	77	89.4	60.8	8	13.35
11	F	23	22.29	24	9	84.3	61	68.8	62.9	6	9.65
12	F	19	19.53	30	29	96	63	74	61.9	11	18.21
13	F	24	31.63	37	61	103	77	85.67	69.8	21.8	31.4
14	M	22	23.04	39	69	127	77	93.67	81.5	37	64.5
15	M	23	21.6	29	30	106	70	82	50.3	12.6	25.5
16	M	21	32.72	38	66	118	78	91.33	56.8	28.1	49.9
17	M	22	24.16	33	48	118	64	82	58	5.7	9.9
18	F	22	22.6	21	3	104	68	80	60	13.1	21.9
19	F	21	25.56	44	83	109	67	81	50.2	3.9	8.9
20	M	26	30.37	46	85	120	82	94.67	53.2	34.6	65.8
21	F	22	23.74	34	50	109	65	79.67	45.7	21.4	47.2
22	F	38	23.51	32	42	113	82	92.33	56.1	13.5	24.2
23	F	22	29.3	43	80	96	70	78.67	63.4	14.2	22.7
24	M	36	27	32	43	126	79	94.67	66.7	23.5	35.4
25	M	25	25	23	11	116	75	88.67	60.7	11.2	18.6
26	M	22	27	24	12	107	67	80.33	55.8	10.3	18.7
27	M	22	25	36	59	116	68	84	49.2	13.7	28.1
28	F	22	21.49	25	12	103	54	70.33	53.6	8.5	16
29	M	22	28.69	34	52	109	73	85	83.65	20.8	25
30	M	31	28.68	39	69	120	79	92.67	69.25	35.6	51.6
31	F	24	20.01	26	16	90	61	70.67	64.35	33.4	49.6
32	M	27	34.33	28	27	115	70	85	56.8	19.2	34
33	M	20	32.35	30	33	139	70	93	82.25	31	37.9
34	F	29	24.1	34	50	106	73	84	68	6.1	9.2
35	F	20	21	35	54	106	70	82	79.3	16.3	20.7
36	F	18	17.9	27	18	114	73	86.67	69.8	29.4	42.6
37	M	37	25.4	32	43	117	65	82.33	64.8	28.6	44.3
38	F	34	24.3	33	47	107	63	77.67	64.5	38.3	59.9

39	F	33	20.4	37	61	105	63	77	69.8	29.2	42.3
40	M	31	25.2	33	48	126	68	87.33	58.3	18.2	31.4
41	M	26	30.3	42	76	120	85	96.67	92.1	33.3	36.3
42	M	32	26.9	46	85	111	77	88.33	74.7	26.4	35.6
43	F	22	25.8	45	86	96	71	79.33	69.8	16.4	23.6
44	F	18	21.5	33	47	109	77	87.67	66.1	11.4	17.3
45	F	19	23.9	35	54	115	71	85.67	79.5	20	25.3
46	M	23	31.3	49	90	103	72	82.33	61.2	25	41.3
47	F	21	21.3	26	16	102	61	74.67	56.6	8.6	15.4
48	F	19	21.5	37	61	119	74	89	66.3	12.6	19.3
49	F	20	24.1	29	25	116	65	82	70.6	20.8	29.9
50	M	23	23.6	34	52	127	69	88.33	62.9	30.1	48.1
51	F	27	26.4	45	86	124	90	101.33	71.1	19.7	27.9
52	M	21	21.3	44	81	137	79	98.33	88.5	35.2	39.9
53	M	25	28.6	26	21	115	68	83.67	62.7	19.8	32
54	M	18	25.8	26	21	114	62	79.33	65.1	26	40.5
55	M	45	29.05	25	14	122	75	90.67	49.8	10.9	22
56	M	25	29.12	28	27	130	78	95.33	61.1	22	36.1
57	M	21	26.08	48	88	117	73	87.67	61.3	24	39.6
58	M	20	27.53	30	33	125	68	87	67.9	15.6	23.1
59	F	19	21.13	57	95	126	81	96	61.3	24.9	41.1
60	M	22	29.42	32	43	125	69	87.67	62.6	25.6	41.2
61	M	21	24.35	62	100	123	62	82.33	59.9	9.8	16.6
62	M	25	29.75	51	94	130	67	88	59.7	8.3	14
63	M	23	23.5	38	66	115	56	75.67	65.9	20.2	30.9
64	M	24	24.96	32	43	125	83	97	68.6	24.8	36.4
65	M	20	27.58	44	81	126	66	86	52.3	18.7	36.2
66	M	20	19.1	67	100	127	77	93.67	71.3	30.3	42.7
67	M	24	26.96	33	48	112	68	82.67	72.3	12.6	17.5
68	M	22	20.48	36	59	121	68	85.67	56.5	24.9	44.5
69	M	26	23.53	47	87	111	64	79.67	56.1	14.7	27
70	M	22	25.97	30	33	127	56	79.67	65.7	13.7	21
71	M	21	26.67	35	57	118	56	76.67	54.4	18.3	34
72	M	27	28.04	26	21	129	70	89.67	55.3	29.8	54.3
73	M	26	26.26	32	43	134	80	98	68.4	8.4	12.4
74	M	23	27.17	59	98	130	69	89.33	57.6	18.2	31.7
75	M	25	21.15	41	74	112	70	84	68.3	30.4	44.8
76	M	28	21.84	50	92	108	66	80	58	37.8	65.5
77	M	23	22.86	57	98	130	80	96.67	67.8	38.6	57.1

78	M	21	25.4	24	12	111	55	73.67	66	21.6	33
79	F	35	28	30	29	123.98	76.53	92.29	62.2	28.8	46.5
80	F	41	29	26	17	107.63	66.04	80.16	59.73	14.1	23.6
81	F	36	31.5	35	54	114.05	72.69	86.89	55.32	17.45	31.72
82	F	39	24.2	32	42	126.99	83.49	96.89	55.64	16.15	29.19
83	F	41	22.3	46	87	106.16	70.94	83.97	58.99	19.09	32.41
84	F	47	24.5	26	17	109.88	82.34	93.07	62.7	25.02	39.97
85	F	27	23.6	30	29	117	61	80	63.6	25.3	40
86	F	26	33.8	44	83	105	69	81	64.2	35.3	55.4
87	F	20	23.1	49	92	117	75	89	60.5	8.2	13.7
88	F	20	22	30	29	110	75	87	57.4	14	24.9

Table A1: Raw data for participants from experiment 1.

APPENDIX B

RAW DATA FOR CHAPTER 3

ID	Age	Sex	BMI	STAI (au)	STAI (%)	BAI	SAP	DAP	MAP	HR
1	29	F	24.1	34	50	10	106	73	84	75
2	20	F	21	35	54	10	102	67	79	99
3	21	F	26.2	30	29	8	112	76	88	65
4	34	F	22.5	20	0	3	120	76	91	54
5	18	F	17.9	27	18	7	114	73	87	68
6	21	F	21.8	43	80	5	107	67	80	72
7	37	M	25.4	32	43	2	117	65	82	71
8	34	F	24.3	33	50	5	107	63	78	73
9	33	F	20.4	37	61	6	105	63	77	58
10	31	M	25.2	33	48	6	126	68	87	71
11	26	M	30.3	42	76	0	120	85	97	93
12	32	M	26.9	46	85	15	111	77	88	80
13	22	F	25.8	45	86	15	96	71	79	91
14	18	F	21.5	33	47	7	109	77	88	70
15	19	F	23.9	35	54	18	117	79	92	87
16	24	F	25.7	34	50	17	106	73	84	85
17	23	M	31.3	49	90	16	103	72	82	72
18	19	F	21.5	37	61	7	119	74	89	77
19	20	F	24.1	29	25	7	116	65	82	87
20	23	M	23.6	34	52	2	127	69	88	56
21	27	F	26.4	45	86	29	124	90	101	85
22	21	M	18.8	40	71	11	119	65	83	79
23	21	M	21.3	44	81	6	137	79	98	97
24	21	M	25.4	52	94	10	127	77	94	93
25	27	M	23	29	30	3	120	76	91	63
26	25	M	28.6	26	21	2	115	68	84	66
27	23	M	23	44	81	35	105	69	81	69
28	18	M	25.8	26	21	1	114	62	79	62

Table B1: Raw seated cardiovascular measures.

ID	Base	Prep	Speech	Math
1	1	2	1	1
2	---	---	---	---
3	---	---	---	---
4	---	---	---	---
5	---	---	---	---
6	---	---	---	---
7	---	---	---	---
8	2	2.5	2.5	3
9	0	0.5	0.5	1
10	0.5	0.5	1	0
11	1	2.5	3.5	3.5
12	0.5	1.5	3	2.5
13	0.5	1	2	2
14	0	1	3	4
15	0.5	0.5	2.5	2.5
16	0	2.5	3.5	2.5
17	---	---	---	---
18	0	1	2.5	1.5
19	0	0.5	1.5	2
20	0	0.5	2	0.5
21	0	2	3	4
22	1	1.5	2.5	2.5
23	0	1.5	3	2.5
24	0.5	1	0.5	1
25	0	1	2.5	3.5
26	0	0.5	1	0.5
27	1	2.5	3.5	2.5
28	0	0.5	2	2

Table B2: Raw perceived stress levels of participants.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	99.1	109.7	126.78	120.84	113.8
2	20	F	21	103.5	116.56	135.2	134.6	127.16
3	21	F	26.2	110.15	123.26	154.1	148.22	127.64
4	34	F	22.5	112.45	129.42	149.02	157.96	131.24
5	18	F	17.9	92.8	99.9	117.18	103.4	98.98
6	21	F	21.8	106.5	127.42	161.02	148.98	122.18
7	37	M	25.4	108.3	109.44	122.36	117.32	114.6
8	34	F	24.3	113	139.1	167.28	174.96	142.98
9	33	F	20.4	104	115.66	143.84	135.02	120.74
10	31	M	25.2	115.6	118.48	133.4	138.44	131.66
11	26	M	30.3	101.9	114.12	121.94	134.32	103.62
12	32	M	26.9	107.3	115.36	129.38	131.04	116.8
13	22	F	25.8	95.9	102.66	120.44	116.62	103.44
14	18	F	21.5	105.1	106.22	113.46	108	107.32
15	19	F	23.9	119.2	122.16	128.78	128.36	125.86
16	24	F	25.7	109.5	112.98	130.22	131	124.42
17	23	M	31.3	118.9	131.6	144.9	142.74	127.6
18	19	F	21.5	94.7	95.52	106.96	105.12	92.92
19	20	F	24.1	112.2	127.76	132.04	127.14	130.88
20	23	M	23.6	90.4	102.58	109.48	115.2	106.48
21	27	F	26.4	128.5	142.96	157.44	146.18	135.2
22	21	M	18.8	94.9	98.7	123.3	116.18	112.18
23	21	M	21.3	113.1	132.62	145.58	156.36	136.64
24	21	M	25.4	130.9	138.02	157.68	150.22	134.9
25	27	M	23	120	122.58	131.84	123.12	125.16
26	25	M	28.6	117.9	129.9	147.94	149.18	131.64
27	23	M	23	110.5	124.56	133.48	137.56	131.08
28	18	M	25.8	124.3	142.42	156.3	163.42	140.98

Table B3: Systolic arterial pressure during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	67.5	73.2	90.6	83.9	81.24
2	20	F	21	66	72.74	89.24	85.9	74.56
3	21	F	26.2	64.25	73.08	97.74	88.12	71.14
4	34	F	22.5	69.3	79.28	93.9	95.08	76.46
5	18	F	17.9	62.9	69.58	85.64	75.24	68
6	21	F	21.8	72.5	88.98	113.4	102.06	80.44
7	37	M	25.4	61.3	62.24	70.9	67.78	63.04
8	34	F	24.3	68.5	84.06	102.06	104.36	85.62
9	33	F	20.4	65.2	72.84	103.34	92.4	79.08
10	31	M	25.2	59.8	63.78	75.46	77.64	70.9
11	26	M	30.3	69.4	77.76	80.98	91.88	69.38
12	32	M	26.9	74.4	75.36	86.16	85.44	78.36
13	22	F	25.8	59.9	64.92	78.94	72.72	62.88
14	18	F	21.5	72.8	73.34	84.04	76.6	74.76
15	19	F	23.9	73.3	74.1	82.02	81.56	76.98
16	24	F	25.7	69.6	72.44	87.24	87.98	82.56
17	23	M	31.3	71.9	77.12	88.46	86.54	76.4
18	19	F	21.5	54.9	56.3	66.94	65.68	53.7
19	20	F	24.1	59.8	65.7	72.48	68.56	67.94
20	23	M	23.6	63.7	73.08	80.6	83.58	76.14
21	27	F	26.4	85.5	94.94	102.98	94.58	88.66
22	21	M	18.8	52.3	56.96	76.46	69.06	61.66
23	21	M	21.3	65.6	76.42	88.24	94.42	77.54
24	21	M	25.4	73	74.84	92.36	84.52	75.36
25	27	M	23	73.1	75.2	83	72.86	71.26
26	25	M	28.6	60.5	66.66	80.76	79.06	64.46
27	23	M	23	71.6	83.56	92.24	94.38	88.64
28	18	M	25.8	70.4	78.62	85.88	89.32	73.78

Table B4: Diastolic arterial pressure during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	81.1	89.38	107.4	101.32	96.42
2	20	F	21	82.3	91.66	110.06	107.22	96.72
3	21	F	26.2	85	95.72	126.48	118.24	96.92
4	34	F	22.5	89.85	102.82	119.34	123.7	101.84
5	18	F	17.9	78.8	85.6	102.36	90.28	84.72
6	21	F	21.8	89.35	108.94	138.02	126.38	100.7
7	37	M	25.4	78.9	80.16	91.2	87.6	82.7
8	34	F	24.3	88	109.16	131.12	136.22	111.36
9	33	F	20.4	83.5	92.48	121.04	111.08	98.18
10	31	M	25.2	79.9	83.56	96.96	100.26	92.46
11	26	M	30.3	83.9	93.92	100.5	111.76	84.9
12	32	M	26.9	86.5	90.64	103.12	103.24	92.72
13	22	F	25.8	76.9	82.52	99.4	94.6	82.66
14	18	F	21.5	88.2	89.24	98.14	91.54	90.82
15	19	F	23.9	91.4	92.38	100.04	99.92	96.22
16	24	F	25.7	87.7	90.84	107.24	108.28	102.1
17	23	M	31.3	88.5	95.76	109.08	107.04	94.46
18	19	F	21.5	73.1	74.12	85.88	83.88	71.54
19	20	F	24.1	79.4	88.78	96.1	92.1	90.6
20	23	M	23.6	74.1	84.58	92.8	97.02	88.38
21	27	F	26.4	104.6	117.22	128.72	118.9	110.1
22	21	M	18.8	69.8	74.36	97.16	89.76	82.6
23	21	M	21.3	84.3	98.96	112.6	119.78	101.3
24	21	M	25.4	94.7	99.7	119.58	111.38	98.16
25	27	M	23	91.7	94.02	104.14	95.18	92.84
26	25	M	28.6	80	89.54	106.42	106.02	87.82
27	23	M	23	88.4	101.66	111.62	114.54	107.92
28	18	M	25.8	92.1	104.54	116.9	121.28	100.3

Table B5: Mean arterial pressure during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	68	99.32	107.46	93.84	71.72
2	20	F	21	79.3	77.32	100.84	107.3	76.08
3	21	F	26.2	61.85	64.84	93.12	79.46	63.04
4	34	F	22.5	53.6	61.98	73.9	74.38	53
5	18	F	17.9	69.8	78.08	91.8	86.22	72.86
6	21	F	21.8	70.9	82.64	103.54	94.1	73.04
7	37	M	25.4	64.8	66.18	68.04	66.68	60.48
8	34	F	24.3	64.5	84.12	99.2	98.1	67.04
9	33	F	20.4	69.8	84.12	156.84	138.98	91.56
10	31	M	25.2	58.3	66.84	74.88	79.98	63.52
11	26	M	30.3	92.1	94.88	98.32	103.3	93.54
12	32	M	26.9	74.7	88.78	99.68	92.94	76.8
13	22	F	25.8	69.8	77.62	85.08	74.28	66.08
14	18	F	21.5	66.1	68.28	81.28	74.56	66.48
15	19	F	23.9	79.5	77.54	84.72	83.1	76.78
16	24	F	25.7	81.8	85.78	96.64	88.74	82.66
17	23	M	31.3	61.2	61.8	68.8	70.26	62.4
18	19	F	21.5	66.3	66.66	78.6	75.3	63.28
19	20	F	24.1	70.6	79.68	92.16	84.48	69.72
20	23	M	23.6	62.9	64.7	69.46	67.16	62
21	27	F	26.4	71.1	79.7	81.48	66.46	66.2
22	21	M	18.8	68.4	75.18	86.88	87.26	69.6
23	21	M	21.3	88.5	103.9	109.42	125.34	93.34
24	21	M	25.4	81.4	93.78	111.1	101.34	84.26
25	27	M	23	59.5	64.7	77.18	74.4	57.76
26	25	M	28.6	62.7	70.04	84.8	80.88	65.32
27	23	M	23	68.1	77.6	84.68	85.14	80.78
28	18	M	25.8	65.1	75.36	86.78	93.9	65.06

Table B6: Heart rate during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	49.7	65.02	61.24	66.46	56.28
2	20	F	21	68.3	78.58	86.34	95.38	95.28
3	21	F	26.2	95.5	99.56	132.66	142.44	121
4	34	F	22.5	86.9	94.3	92.8	102.44	105.86
5	18	F	17.9	80.6	73	73.68	67.44	83.38
6	21	F	21.8	71.2	85.8	101.92	105.62	85.96
7	37	M	25.4	70.8	71.94	85.48	85.26	81.22
8	34	F	24.3	73.9	89.92	94.58	101.02	86.1
9	33	F	20.4	65.8	70.14	59.56	64.7	65.82
10	31	M	25.2	86.1	83.6	86.74	89.22	84.4
11	26	M	30.3	52	56.14	67.76	68.32	56.42
12	32	M	26.9	44.3	56.28	60.32	62.04	52.16
13	22	F	25.8	78.9	81.22	101.3	115.54	94.74
14	18	F	21.5	69.6	72.32	62.64	69.88	75.94
15	19	F	23.9	75.2	75.04	73.26	74.18	79.48
16	24	F	25.7	76.2	77.4	81.06	82.1	79.34
17	23	M	31.3	70.6	75.98	79.16	82.04	75.76
18	19	F	21.5	87.1	83.1	92.34	85.68	83.4
19	20	F	24.1	90.5	104.46	104.62	107.8	101.6
20	23	M	23.6	45.7	48.52	51.94	55.76	49.74
21	27	F	26.4	73.7	86.62	103.26	98.02	85.06
22	21	M	18.8	83.5	80.66	90.28	93.76	96.24
23	21	M	21.3	77.7	93.56	96.2	101.04	95.42
24	21	M	25.4	104.7	108.9	108.82	113.58	100.86
25	27	M	23	73.7	74.06	87.74	99.1	93.02
26	25	M	28.6	90	103.02	104.78	112	106.08
27	23	M	23	71.1	76.68	84.14	88.8	84.08
28	18	M	25.8	105.9	121.32	144.86	145.68	125.94

Table B7: Estimated stroke volume during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	3.34	6.418	6.508	6.208	4.02
2	20	F	21	5.4	6.07	8.688	10.426	7.232
3	21	F	26.2	5.89	6.458	12.35	11.304	7.624
4	34	F	22.5	4.64	5.792	6.814	7.584	5.588
5	18	F	17.9	5.57	5.666	6.674	5.74	6.012
6	21	F	21.8	5.04	7.068	10.526	9.92	6.272
7	37	M	25.4	4.58	4.752	5.792	5.67	4.898
8	34	F	24.3	4.72	7.558	9.338	9.676	5.766
9	33	F	20.4	4.58	5.9	9.338	8.974	6.018
10	31	M	25.2	5.01	5.576	6.44	7.118	5.352
11	26	M	30.3	4.79	5.328	6.636	7.05	5.274
12	32	M	26.9	3.31	4.99	5.998	5.78	4.008
13	22	F	25.8	5.49	6.284	8.572	8.536	6.248
14	18	F	21.5	4.58	4.914	5.068	5.138	5.03
15	19	F	23.9	5.97	5.812	6.172	6.142	6.074
16	24	F	25.7	6.22	6.638	7.824	7.27	6.546
17	23	M	31.3	4.31	4.69	5.428	5.572	4.72
18	19	F	21.5	5.75	5.52	7.21	6.43	5.252
19	20	F	24.1	6.39	8.316	9.604	9.08	7.08
20	23	M	23.6	2.87	3.138	3.602	3.728	3.074
21	27	F	26.4	5.22	6.89	8.392	6.5	5.6
22	21	M	18.8	5.66	6.022	7.832	8.18	6.656
23	21	M	21.3	6.88	9.694	10.5	12.648	8.914
24	21	M	25.4	9.79	10.198	12.006	11.478	8.482
25	27	M	23	4.37	4.78	6.764	7.358	5.356
26	25	M	28.6	5.62	7.2	8.854	9.058	6.91
27	23	M	23	4.83	5.93	7.106	7.532	6.766
28	18	M	25.8	6.89	9.13	12.552	13.672	8.192

Table B8: Estimated cardiac output during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	6.1	3.6	14.6	11.6	3.8
2	20	F	21	16.3	9	14.4	10	12.6
3	21	F	26.2	---	---	---	---	---
4	34	F	22.5	---	---	---	---	---
5	18	F	17.9	29.4	25	23.2	27.6	31.4
6	21	F	21.8	---	---	---	---	---
7	37	M	25.4	28.6	30.8	39.4	32.8	30.4
8	34	F	24.3	38.3	20.8	25.8	22.6	24.8
9	33	F	20.4	29.2	23.4	22	17.8	27.2
10	31	M	25.2	18.2	17.2	15	11.2	7.8
11	26	M	30.3	33.3	19	20.8	19	27.6
12	32	M	26.9	26.4	19	21.6	11.6	16.6
13	22	F	25.8	16.4	15	26.8	21	25.8
14	18	F	21.5	11.4	11.6	24.6	12	5.4
15	19	F	23.9	20	19.4	18	18.6	24.4
16	24	F	25.7	---	---	---	---	---
17	23	M	31.3	25	20	18	16.2	27.6
18	19	F	21.5	12.6	11.2	14.6	9.2	14.4
19	20	F	24.1	20.8	17.2	19.6	17.6	23.2
20	23	M	23.6	30.1	18.8	30	12	15.2
21	27	F	26.4	19.7	11.4	18.4	27	31
22	21	M	18.8	---	---	---	---	---
23	21	M	21.3	35.2	35.8	47.8	36.2	41.4
24	21	M	25.4	---	---	---	---	---
25	27	M	23	19.8	15.8	16.4	16.8	9
26	25	M	28.6	19.8	15	22	26.6	24.2
27	23	M	23	---	---	---	---	---
28	18	M	25.8	26	16.8	23.2	21.6	22

Table B9: MSNA BF during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	9.2	3.74	13.74	12.6	5.4
2	20	F	21	20.7	11.82	14.34	9.4	16.98
3	21	F	26.2	---	---	---	---	---
4	34	F	22.5	---	---	---	---	---
5	18	F	17.9	42.6	32.28	25.52	32.44	43.5
6	21	F	21.8	---	---	---	---	---
7	37	M	25.4	44.3	46.6	58.08	49.3	50.52
8	34	F	24.3	59.9	25.02	26.08	24.04	37.94
9	33	F	20.4	42.3	28.1	14.12	13.12	29.86
10	31	M	25.2	31.4	25.98	19.96	14.26	12.3
11	26	M	30.3	36.3	20.1	21.22	18.5	29.98
12	32	M	26.9	35.6	21.38	21.84	12.62	21.76
13	22	F	25.8	23.6	19.46	31.84	28.5	39.12
14	18	F	21.5	17.3	17.14	30.4	16.3	8.34
15	19	F	23.9	25.3	25.26	21.66	22.62	32.62
16	24	F	25.7	---	---	---	---	---
17	23	M	31.3	41.3	32.6	26.56	24.04	44.42
18	19	F	21.5	19.3	16.9	18.44	12.34	23.06
19	20	F	24.1	29.9	22.08	21.7	21.16	34.04
20	23	M	23.6	48.1	29.24	43.18	17.98	24.66
21	27	F	26.4	27.9	14.36	23.12	41.52	47.38
22	21	M	18.8	---	---	---	---	---
23	21	M	21.3	39.9	34.86	44.06	29.02	44.48
24	21	M	25.4	---	---	---	---	---
25	27	M	23	33.4	24.56	21.28	22.74	15.92
26	25	M	28.6	32	21.36	26.08	32.98	37.26
27	23	M	23	---	---	---	---	---
28	18	M	25.8	40.5	22.6	26.94	23.18	34.64

Table B10: MSNA BI during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	---	---	---	---	---
2	20	F	21	10906.6	7076.04	16919.84	6293	7573.1
3	21	F	26.2	---	---	---	---	---
4	34	F	22.5	---	---	---	---	---
5	18	F	17.9	---	---	---	---	---
6	21	F	21.8	---	---	---	---	---
7	37	M	25.4	20071.8	12426.66	16090.36	16275.2	5813.24
8	34	F	24.3	---	---	---	---	---
9	33	F	20.4	---	---	---	---	---
10	31	M	25.2	11067.8	19076.9	16816.42	6976.7	6657.52
11	26	M	30.3	---	---	---	---	---
12	32	M	26.9	16300.5	10608.58	20820.7	11592.76	9978.48
13	22	F	25.8	10568.8	12463.4	35007.04	16722.4	14179.94
14	18	F	21.5	8184.1	7402.46	35006.7	17214.9	1350.86
15	19	F	23.9	14592.1	8984.62	13370.18	16349.8	26616.3
16	24	F	25.7	---	---	---	---	---
17	23	M	31.3	15494.6	12781.9	13037.18	11863.86	19790.1
18	19	F	21.5	7534.7	7615.16	27527	13154.06	11116.08
19	20	F	24.1	15050	17128.9	22972.94	17973.8	19205.68
20	23	M	23.6	20132.5	7944.36	24567.68	5368.16	4771.96
21	27	F	26.4	11422.7	5842.38	21157.36	41678.18	36210.56
22	21	M	18.8	---	---	---	---	---
23	21	M	21.3	20124.9	17550.78	40555.2	36810.62	21378.06
24	21	M	25.4	---	---	---	---	---
25	27	M	23	---	---	---	---	---
26	25	M	28.6	12217.6	12971.28	23399.78	29478.78	28436.1
27	23	M	23	---	---	---	---	---
28	18	M	25.8	---	---	---	---	---

Table B11: MSNA total area during each phase of the TSST.

ID	Age	Sex	BMI	Base	Prep	Speech	Math	Recovery
1	29	F	24.1	---	---	---	---	---
2	20	F	21	51.8	31.86	63.3	32.46	42.7
3	21	F	26.2	---	---	---	---	---
4	34	F	22.5	---	---	---	---	---
5	18	F	17.9	---	---	---	---	---
6	21	F	21.8	---	---	---	---	---
7	37	M	25.4	70.6	60.54	92.62	73.8	51.06
8	34	F	24.3	---	---	---	---	---
9	33	F	20.4	---	---	---	---	---
10	31	M	25.2	60.5	74.12	64.32	37.68	27.7
11	26	M	30.3	---	---	---	---	---
12	32	M	26.9	96.7	66.16	94.64	50.94	55.34
13	22	F	25.8	52.5	53.42	132.42	77.46	84.48
14	18	F	21.5	32.6	31.5	122.76	62.26	9.76
15	19	F	23.9	67.8	59.32	59.68	70.34	122.46
16	24	F	25.7	---	---	---	---	---
17	23	M	31.3	49.3	40.08	40.42	36.58	60.9
18	19	F	21.5	50.2	46.9	97.76	52.06	61.62
19	20	F	24.1	68.6	73.48	94.46	75.36	89.34
20	23	M	23.6	80.9	42.1	93.56	25.6	29.36
21	27	F	26.4	62.5	35.02	81.46	158.32	135.4
22	21	M	18.8	---	---	---	---	---
23	21	M	21.3	105.9	106.62	174.56	143.42	133.28
24	21	M	25.4	---	---	---	---	---
25	27	M	23	---	---	---	---	---
26	25	M	28.6	59	50.48	83.58	101.68	94.36
27	23	M	23	---	---	---	---	---
28	18	M	25.8	---	---	---	---	---

Table B12: MSNA total amplitude during each phase of the TSST.

ID	sBRS inc.	sBRS total	cvBRS (up)	cvBRS (down)	RR	RRI	LF	HF	LF/HF	pNN50	RMSSD
1	-0.7	-266	40.1	22.9	6	905	17135	2553	671.1	47	102
2	-1.3	-516	9.6	12.4	16	761	1331	400	332.8	13.7	36
3	---	---	16.9	17.6	18	974	503	1114	45.1	56.8	70
4	---	---	11.6	10.5	7	1124	3954	314	1258.8	28.6	49
5	-1.1	-408	15.3	15.1	10	867	1818	1553	117	35.6	58
6	---	---	13.7	18.7	14	850	826	596	138.5	21.2	42
7	-1.6	-705	7.1	13.9	16	929	425	470	90.4	24.8	42
8	-1.8	-871	19.5	19.9	12	938	1364	1312	104	41.7	65
9	-2.0	-896	18.8	25	10	867	1943	1553	125.1	33.9	53
10	-2.1	-777	11.5	12.9	11	1033	1419	590	240.4	16.9	40
11	-1.4	-543	4.7	12.5	15	653	166	34	491.2	0	9
12	---	---	20.6	22.2	18	809	1003	738	136	24.5	71
13	-2.0	-910	13	11.6	16	862	494	302	163.9	9.4	32
14	---	---	11.9	15.4	14	910	615	406	151.5	21	38
15	-1.2	-515	17.9	17.2	16	760	1821	1273	143	33.6	58
16	---	---	8.4	9.8	19	736	544	194	280.8	2.9	24
17	-2.6	-1040	15.7	18.7	16	987	2103	633	332.3	26.1	51
18	-2.5	-1009	29	29	11	916	1787	2686	66.5	47.3	88
19	-1.6	-529	28.3	21.9	13	861	4594	2173	211.4	44.5	84
20	-1.8	-885	11.7	12.8	15	957	822	473	173.7	29.8	48
21	-1.8	-510	21.6	24.4	14	849	1645	876	187.8	29.4	53
22	---	---	25.5	24.1	10	892	5979	3174	188.4	52.5	105
23	-0.9	-404	6.9	5.2	16	679	197	87	224.8	0.2	15
24	---	---	18.2	11.4	16	740	624	603	103.5	16.6	39
25	-2.3	---	11.8	23.7	13	1010	690	681	101.4	28.2	47
26	-1.7	-593	13.6	20	9	969	3765	1578	238.5	44.2	71
27	---	---	20.7	22.5	14	892	3078	1318	233.5	39.9	64
28	-2.0	-544	19.1	11.1	16	933	2201	1317	167	47.7	84

Table B13: Sympathetic baroreflex sensitivity, cardiovagal baroreflex sensitivity, and heart rate variability during baseline.

ID	sBRS inc.	sBRS total	cvBRS (up)	cvBRS (down)	RR	RRI	LF	HF	LF/HF	pNN50	RMSSD
1	-0.7	-200	15.8	12.8	12	610	1407	992	141.7	9.2	32
2	-1.6	-814	14.8	15	14	781	1291	710	181.9	25.7	47
3	---	---	16.6	15.1	18	933	725	853	85	43.9	69
4	---	---	11.6	10.7	8	978	5188	637	814.2	33.3	55
5	-0.7	-386	12	13.8	11	774	1597	986	162	23.3	43
6	---	---	17.7	16.7	16	732	878	705	124.6	21.3	43
7	-1.6	-649	12	14.1	17	909	637	483	132.1	28.6	44
8	-1.6	-628	9.8	11	16	718	806	456	176.8	11.5	34
9	-1.4	-823	13.8	15.4	10	717	561	785	71.4	12.7	31
10	-2.4	-1819	12.7	7.4	14	903	1983	482	411.6	11.2	37
11	-1.5	-556	5.4	3.8	15	633	170	36	473.5	0	10
12	-2.2	-901	10.3	7.6	19	678	579	188	308.1	3.4	22
13	-1.8	-886	11.7	14.9	18	777	820	261	261	5.7	28
14	-1.0	-443	10.1	15.8	15	881	233	406	57.3	17.7	37
15	-1.7	-465	19.5	20.3	15	780	2330	1625	143.4	41.1	65
16	---	---	7.8	12.2	17	702	1220	217	561.1	3.1	22
17	-2.4	-988	17	12.5	19	975	1429	568	251.5	30	51
18	-1.1	-448	32	30.5	16	905	756	1449	52.5	57.7	78
19	-2.5	-1470	22.2	16.5	10	766	6570	2294	286.4	37.9	73
20	-1.5	-365	10.4	22.5	16	931	866	506	171	32.1	50
21	-1.6	-482	13.6	13.9	15	756	1232	292	421.6	8.6	21
22	---	---	25.4	19.1	9	808	5917	3401	174	28.4	80
23	-3.3	-1612	6	4.8	19	579	202	71	284.1	0.4	13
24	---	---	11.8	6.9	15	644	2598	1271	204.4	5.8	44
25	-1.8	---	8.7	12.9	16	930	1020	254	401.2	23.3	43
26	-1.3	-738	13.3	18.6	9	864	2922	1200	243.6	31.1	56
27	---	---	18.1	15.3	15	780	1190	833	142.9	22.7	48
28	-2.0	-933	23.5	11.5	17	804	2903	1340	216.7	34.7	80

Table B14: Sympathetic baroreflex sensitivity, cardiovagal baroreflex sensitivity, and heart rate variability during preparation phase.

APPENDIX C

CHAPTER 2 STATISTICAL OUTPUT

Model Summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.540	.292	.266	9.16713

ANOVA						
Model		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Regression	2904.480	3	968.160	11.521	<.001
	Residual	7059.040	84	84.036		
	Total	9963.521	87			

Coefficients						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	119.187	5.019		23.749	<.001
	Age	.248	.154	.149	1.602	.113
	Sex	-10.430	2.026	-.482	-5.147	<.001
	STAI (%)	.079	.036	.206	2.232	.028

Table C1: Resting systolic arterial pressure multiple linear regression.
Predictors: Age, sex, and STAI (%)
Outcome Variable: Systolic arterial pressure

Model Summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.447	.200	.171	6.95316

ANOVA						
Model		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Regression	1014.695	3	338.232	6.996	<.001
	Residual	4061.096	84	48.346		
	Total	5075.791	87			

Coefficients						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	54.729	3.806		14.378	<.001
	Age	.384	.117	.325	3.279	.002
	Sex	.942	1.537	.061	.613	.542
	STAI (%)	.087	.027	.317	3.226	.002

Table C2: Resting diastolic arterial pressure multiple linear regression.
Predictors: Age, sex, and STAI (%)
Outcome Variable: Diastolic arterial pressure

Model Summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.472	.222	.195	6.48384

ANOVA						
Model		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Regression	1009.639	3	336.546	8.005	<.001
	Residual	3531.379	84	42.040		
	Total	4541.017	87			

Coefficients						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	75.869	3.550		21.374	<.001
	Age	.351	.109	.314	3.210	.002
	Sex	-2.791	1.433	-.191	-1.947	.055
	STAI (%)	.084	.025	.325	3.358	.001

Table C3: Resting mean arterial pressure multiple linear regression.
Predictors: Age, sex, and STAI (%)
Outcome Variable: Mean arterial pressure

Model Summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.160	.025	-.009	8.73106

ANOVA						
Model		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Regression	167.194	3	55.731	.731	.536
	Residual	6403.438	84	76.231		
	Total	6570.632	87			

Coefficients						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	64.298	4.780		13.452	<.001
	Age	-.132	.147	-.098	-.898	.372
	Sex	.174	1.930	.010	.090	.929
	STAI (%)	.038	.034	.122	1.124	.264

Table C4: Resting heart rate multiple linear regression.
Predictors: Age, sex, and STAI (%)
Outcome Variable: Heart rate

Model Summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.357	.127	.096	8.71087

ANOVA						
Model		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Regression	929.936	3	309.979	4.085	.009
	Residual	6373.854	84	75.879		
	Total	7303.790	87			

Coefficients						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	13.847	4.769		2.904	.005
	Age	.297	.147	.209	2.021	.046
	Sex	-3.789	1.926	-.205	-1.968	.052
	STAI (%)	.075	.034	.227	2.209	.030

Table C5: Resting MSNA BF multiple linear regression.
Predictors: Age, sex, and STAI (%)
Outcome Variable: MSNA BF

Model Summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.375	.141	.110	13.22258

ANOVA						
Model		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
1	Regression	2408.344	3	802.781	4.592	.005
	Residual	14686.271	84	174.837		
	Total	17094.616	87			

Coefficients						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	21.825	7.239		3.015	.003
	Age	.524	.223	.242	2.353	.021
	Sex	-6.355	2.923	-.224	-2.174	.032
	STAI (%)	.108	.051	.214	2.103	.038

Table C6: Resting MSNA BI multiple linear regression.
Predictors: Age, sex, and STAI (%)
Outcome Variable: MSNA BI

APPENDIX D

CHAPTER 3 STATISTICAL OUTPUT

Group Statistics					
		N	Mean	SD	SEM
Age	M	13	25.2308	5.34094	1.48131
	F	15	23.9333	5.92171	1.52898
BMI	M	13	25.2769	3.49336	.96888
	F	15	23.1400	2.45700	.63440
STAI_Raw	M	13	38.2308	8.74789	2.42623
	F	15	34.4667	6.69613	1.72893
STAI_Percent	M	13	61.0000	26.40391	7.32313
	F	15	50.0667	24.44372	6.31134
SAP	M	13	118.5385	9.40404	2.60821
	F	15	110.6667	7.61265	1.96558
DAP	M	13	71.6923	6.65062	1.84455
	F	15	72.4667	7.04948	1.82017
MAP	M	13	87.2308	6.20690	1.72149
	F	15	85.2667	6.54071	1.68881
HR	M	13	74.7692	12.92384	3.58443
	F	15	76.4000	12.56867	3.24522

Independent Samples T-Test – Sex Differences								
	F	Sig.	t	df	P-Value	Std. Error Difference	Lower	Upper
Age	0.557	0.462	0.605	26	0.551	2.14516	-3.11201	5.70688
BMI	0.620	0.438	1.892	26	0.070	1.12939	-0.18456	4.45841
STAI RAW	3.193	0.086	1.288	26	0.209	2.92204	-2.24223	9.77043
STAI Percent	1.150	0.293	1.137	26	0.266	9.61247	-8.82539	30.69205
SAP	0.188	0.668	2.448	26	0.021	3.21583	1.26156	14.48203
DAP	0.049	0.826	-0.298	26	0.768	2.60261	-6.12410	4.57538
MAP	0.022	0.883	0.811	26	0.425	2.42093	-3.01219	6.94039
HR	0.015	0.902	-0.338	26	0.738	4.82526	-11.5492	8.28769

Table D1: Sex differences – seated cardiovascular measures.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	0.4091	0.43693	11
	F	0.4000	0.65828	10
	Total	0.4048	0.53896	21
Prep	M	1.2273	0.75378	11
	F	1.3500	0.81820	10
	Total	1.2857	0.76765	21
Speech	M	2.2273	1.03353	11
	F	2.2000	0.94868	10
	Total	2.2143	0.96917	21
Math	M	1.9091	1.22103	11
	F	2.3500	1.08141	10
	Total	2.1190	1.15005	21

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.762	18.161	3.000	17.000	0.000	0.762
	Wilks' Lambda	0.238	18.161	3.000	17.000	0.000	0.762
	Hotelling's Trace	3.205	18.161	3.000	17.000	0.000	0.762
	Roy's Largest Root	3.205	18.161	3.000	17.000	0.000	0.762
Phase x sex	Pillai's Trace	0.127	.824	3.000	17.000	0.499	0.127
	Wilks' Lambda	0.873	.824	3.000	17.000	0.499	0.127
	Hotelling's Trace	0.145	.824	3.000	17.000	0.499	0.127
	Roy's Largest Root	0.145	.824	3.000	17.000	0.499	0.127

Mauchly's Test of Sphericity							
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.387	16.836	5	0.005	0.620	0.721	0.333

Within Subject							
Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	45.047	3	15.016	34.358	0.000	0.644
	Greenhouse-Geisser	45.047	1.860	24.215	34.358	0.000	0.644
	Huynh-Feldt	45.047	2.163	20.830	34.358	0.000	0.644
	Lower-bound	45.047	1.000	45.047	34.358	0.000	0.644
Phase * Sex	Sphericity Assumed	0.737	3	0.246	0.562	0.642	0.029
	Greenhouse-Geisser	0.737	1.860	0.396	0.562	0.563	0.029
	Huynh-Feldt	0.737	2.163	0.341	0.562	0.587	0.029
	Lower-bound	0.737	1.000	0.737	0.562	0.462	0.029
Error(Phase)	Sphericity Assumed	24.911	57	0.437			
	Greenhouse-Geisser	24.911	35.346	0.705			
	Huynh-Feldt	24.911	41.090	0.606			
	Lower-bound	24.911	19.000	1.311			

Between Subject						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	190.864	1	190.864	98.489	0.000	0.838
Sex	0.364	1	0.364	0.188	0.670	0.010
Error	36.820	19	1.938			

Pairwise Comparisons						
(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-.884*	0.141	0.000	-1.298	-0.470
	3	-1.809*	0.236	0.000	-2.505	-1.113
	4	-1.725*	0.266	0.000	-2.509	-0.941
2	1	.884*	0.141	0.000	0.470	1.298
	3	-.925*	0.174	0.000	-1.437	-0.413
	4	-.841*	0.222	0.007	-1.495	-0.187
3	1	1.809*	0.236	0.000	1.113	2.505
	2	.925*	0.174	0.000	0.413	1.437
	4	0.084	0.156	1.000	-0.375	0.543
4	1	1.725*	0.266	0.000	0.941	2.509

	2	.841*	0.222	0.007	0.187	1.495
	3	-0.084	0.156	1.000	-0.543	0.375

Table D2: Repeated measures ANOVA analysis of perceived stress with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	111.8462	11.43020	13
	F	107.1067	9.57487	15
	Total	109.3071	10.55449	28
Prep	M	121.5677	13.35972	13
	F	118.0853	13.98001	15
	Total	119.7021	13.55703	28
Speech	M	135.1985	14.46226	13
	F	136.2507	18.43194	15
	Total	135.7621	16.41353	28
Math	M	136.5462	15.70556	13
	F	132.4267	20.35501	15
	Total	134.3393	18.13400	28
Recovery	M	124.1031	12.07769	13
	F	120.3173	14.19194	15
	Total	122.0750	13.15156	28

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.858	34.745b	4.000	23.000	0.000	0.858
	Wilks' Lambda	0.142	34.745b	4.000	23.000	0.000	0.858
	Hotelling's Trace	6.043	34.745b	4.000	23.000	0.000	0.858
	Roy's Largest Root	6.043	34.745b	4.000	23.000	0.000	0.858
Phase x sex	Pillai's Trace	0.201	1.444b	4.000	23.000	0.252	0.201
	Wilks' Lambda	0.799	1.444b	4.000	23.000	0.252	0.201
	Hotelling's Trace	0.251	1.444b	4.000	23.000	0.252	0.201
	Roy's Largest Root	0.251	1.444b	4.000	23.000	0.252	0.201

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.132	49.367	9	0.000	0.477	0.534	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	13326.626	4	3331.657	80.788	0.000	0.757
	Greenhouse-Geisser	13326.626	1.910	6978.540	80.788	0.000	0.757
	Huynh-Feldt	13326.626	2.137	6237.426	80.788	0.000	0.757
	Lower-bound	13326.626	1.000	13326.626	80.788	0.000	0.757
Phase * Sex	Sphericity Assumed	150.069	4	37.517	0.910	0.461	0.034
	Greenhouse-Geisser	150.069	1.910	78.584	0.910	0.405	0.034
	Huynh-Feldt	150.069	2.137	70.239	0.910	0.414	0.034
	Lower-bound	150.069	1.000	150.069	0.910	0.349	0.034
Error(Phase)	Sphericity Assumed	4288.908	104	41.239			
	Greenhouse-Geisser	4288.908	49.651	86.381			
	Huynh-Feldt	4288.908	55.551	77.207			
	Lower-bound	4288.908	26.000	164.958			

Between Subject

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared

Intercept	2153584.790	1	2153584.790	2329.432	0.000	0.989
Sex	316.529	1	316.529	0.342	0.564	0.013
Error	24037.278	26	924.511			

Pairwise Comparisons						
(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-10.350*	1.280	0.000	-14.277	-6.423
	3	-26.248*	2.199	0.000	-32.992	-19.504
	4	-25.010*	2.656	0.000	-33.155	-16.865
	5	-12.734*	1.469	0.000	-17.240	-8.227
2	1	10.350*	1.280	0.000	6.423	14.277
	3	-15.898*	1.394	0.000	-20.175	-11.621
	4	-14.660*	1.697	0.000	-19.864	-9.456
	5	-2.384	1.109	0.411	-5.786	1.019
3	1	26.248*	2.199	0.000	19.504	32.992
	2	15.898*	1.394	0.000	11.621	20.175
	4	1.238	1.282	1.000	-2.693	5.169
	5	13.514*	1.664	0.000	8.411	18.618
4	1	25.010*	2.656	0.000	16.865	33.155
	2	14.660*	1.697	0.000	9.456	19.864
	3	-1.238	1.282	1.000	-5.169	2.693
	5	12.276*	1.859	0.000	6.576	17.977
5	1	12.734*	1.469	0.000	8.227	17.240
	2	2.384	1.109	0.411	-1.019	5.786
	3	-13.514*	1.664	0.000	-18.618	-8.411
	4	-12.276*	1.859	0.000	-17.977	-6.576

Table D3: Repeated measures ANOVA analysis of systolic arterial pressure with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	66.6923	6.73764	13
	F	67.4633	7.24289	15
	Total	67.1054	6.89423	28
Prep	M	72.4308	7.65005	13
	F	74.3667	9.56710	15
	Total	73.4679	8.62767	28
Speech	M	83.1923	6.51247	13
	F	90.0373	12.54233	15
	Total	86.8593	10.60677	28
Math	M	82.8062	9.00188	13
	F	84.9827	11.66990	15
	Total	83.9721	10.38519	28
Recovery	M	72.8400	7.31714	13
	F	74.9347	9.11152	15
	Total	73.9621	8.24469	28

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.869	38.237b	4.000	23.000	0.000	0.869
	Wilks' Lambda	0.131	38.237b	4.000	23.000	0.000	0.869
	Hotelling's Trace	6.650	38.237b	4.000	23.000	0.000	0.869
	Roy's Largest Root	6.650	38.237b	4.000	23.000	0.000	0.869
Phase x sex	Pillai's Trace	0.229	1.703b	4.000	23.000	0.184	0.229
	Wilks' Lambda	0.771	1.703b	4.000	23.000	0.184	0.229
	Hotelling's Trace	0.296	1.703b	4.000	23.000	0.184	0.229
	Roy's Largest Root	0.296	1.703b	4.000	23.000	0.184	0.229

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.234	35.415	9	0.000	0.599	0.689	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	7272.999	4	1818.250	94.081	0.000	0.783
	Greenhouse-Geisser	7272.999	2.395	3037.143	94.081	0.000	0.783
	Huynh-Feldt	7272.999	2.756	2639.175	94.081	0.000	0.783
	Lower-bound	7272.999	1.000	7272.999	94.081	0.000	0.783
Phase * Sex	Sphericity Assumed	153.950	4	38.487	1.991	0.101	0.071
	Greenhouse-Geisser	153.950	2.395	64.288	1.991	0.137	0.071
	Huynh-Feldt	153.950	2.756	55.864	1.991	0.128	0.071
	Lower-bound	153.950	1.000	153.950	1.991	0.170	0.071
Error(Phase)	Sphericity Assumed	2009.939	104	19.326			
	Greenhouse-Geisser	2009.939	62.262	32.282			
	Huynh-Feldt	2009.939	71.650	28.052			

	Lower-bound	2009.939	26.000	77.305			

Between Subject						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	825280.700	1	825280.700	2481.186	0.000	0.990
Sex	266.146	1	266.146	0.800	0.379	0.030
Error	8648.002	26	332.615			

Pairwise Comparisons						
(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-6.321*	0.820	0.000	-8.836	-3.805
	3	-19.537*	1.528	0.000	-24.222	-14.852
	4	-16.817*	1.617	0.000	-21.777	-11.856
	5	-6.810*	1.019	0.000	-9.935	-3.684
2	1	6.321*	0.820	0.000	3.805	8.836
	3	-13.216*	1.097	0.000	-16.580	-9.853
	4	-10.496*	1.060	0.000	-13.747	-7.244
	5	-0.489	0.903	1.000	-3.259	2.282
3	1	19.537*	1.528	0.000	14.852	24.222
	2	13.216*	1.097	0.000	9.853	16.580
	4	2.720	1.005	0.119	-0.362	5.803
	5	12.727*	1.275	0.000	8.817	16.638
4	1	16.817*	1.617	0.000	11.856	21.777
	2	10.496*	1.060	0.000	7.244	13.747
	3	-2.720	1.005	0.119	-5.803	0.362
	5	10.007*	1.198	0.000	6.333	13.681
5	1	6.810*	1.019	0.000	3.684	9.935
	2	0.489	0.903	1.000	-2.282	3.259
	3	-12.727*	1.275	0.000	-16.638	-8.817
	4	-10.007*	1.198	0.000	-13.681	-6.333

Table D4: Repeated measures ANOVA analysis of diastolic arterial pressure with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	84.0615	7.32445	13
	F	85.2800	7.53416	15
	Total	84.7143	7.32525	28
Prep	M	91.6462	8.96345	13
	F	94.0573	11.21896	15
	Total	92.9379	10.12280	28
Speech	M	104.7754	8.96144	13
	F	111.4227	15.19379	15
	Total	108.3364	12.91472	28
Math	M	104.9892	10.75018	13
	F	106.9107	15.36626	15
	Total	106.0186	13.21926	28
Recovery	M	92.8123	7.64590	13
	F	95.3933	10.31104	15
	Total	94.1950	9.10099	28

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.873	39.626b	4.000	23.000	0.000	0.873
	Wilks' Lambda	0.127	39.626b	4.000	23.000	0.000	0.873
	Hotelling's Trace	6.892	39.626b	4.000	23.000	0.000	0.873
	Roy's Largest Root	6.892	39.626b	4.000	23.000	0.000	0.873
Phase x sex	Pillai's Trace	0.206	1.490b	4.000	23.000	0.238	0.206
	Wilks' Lambda	0.794	1.490b	4.000	23.000	0.238	0.206
	Hotelling's Trace	0.259	1.490b	4.000	23.000	0.238	0.206
	Roy's Largest Root	0.259	1.490b	4.000	23.000	0.238	0.206

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.158	45.052	9	0.000	0.517	0.585	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	10608.296	4	2652.074	97.408	0.000	0.789
	Greenhouse-Geisser	10608.296	2.070	5125.385	97.408	0.000	0.789
	Huynh-Feldt	10608.296	2.338	4536.992	97.408	0.000	0.789
	Lower-bound	10608.296	1.000	10608.296	97.408	0.000	0.789
Phase * Sex	Sphericity Assumed	126.418	4	31.605	1.161	0.332	0.043
	Greenhouse-Geisser	126.418	2.070	61.079	1.161	0.322	0.043
	Huynh-Feldt	126.418	2.338	54.067	1.161	0.325	0.043
	Lower-bound	126.418	1.000	126.418	1.161	0.291	0.043
Error(Phase)	Sphericity Assumed	2831.545	104	27.226			
	Greenhouse-Geisser	2831.545	53.814	52.618			
	Huynh-Feldt	2831.545	60.793	46.577			
	Lower-bound	2831.545	26.000	108.906			

Between Subject

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	1314185.970	1	1314185.970	2753.060	0.000	0.991
Sex	304.242	1	304.242	0.637	0.432	0.024
Error	12411.220	26	477.355			

Pairwise Comparisons

(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound

1	2	-8.181*	1.030	0.000	-11.341	-5.021
	3	-23.428*	1.837	0.000	-29.062	-17.795
	4	-21.279*	2.047	0.000	-27.557	-15.001
	5	-9.432*	1.163	0.000	-12.999	-5.866
2	1	8.181*	1.030	0.000	5.021	11.341
	3	-15.247*	1.201	0.000	-18.930	-11.564
	4	-13.098*	1.276	0.000	-17.013	-9.183
	5	-1.251	0.961	1.000	-4.198	1.696
3	1	23.428*	1.837	0.000	17.795	29.062
	2	15.247*	1.201	0.000	11.564	18.930
	4	2.149	1.097	0.608	-1.214	5.512
	5	13.996*	1.476	0.000	9.471	18.522
4	1	21.279*	2.047	0.000	15.001	27.557
	2	13.098*	1.276	0.000	9.183	17.013
	3	-2.149	1.097	0.608	-5.512	1.214
	5	11.847*	1.481	0.000	7.304	16.390
5	1	9.432*	1.163	0.000	5.866	12.999
	2	1.251	0.961	1.000	-1.696	4.198
	3	-13.996*	1.476	0.000	-18.522	-9.471
	4	-11.847*	1.481	0.000	-16.390	-7.304

Table D5: Repeated measures ANOVA analysis of mean arterial pressure with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	69.8231	11.05970	13
	F	69.5300	7.13992	15
	Total	69.6661	8.98991	28
Prep	M	77.2108	13.75919	13
	F	77.8453	9.52661	15
	Total	77.5507	11.45875	28
Speech	M	86.1554	14.76229	13
	F	95.1107	19.67185	15
	Total	90.9529	17.83812	28
Math	M	86.8138	16.73196	13
	F	87.9533	17.80335	15
	Total	87.4243	17.00324	28
Recovery	M	71.9123	12.47600	13
	F	70.6360	9.08685	15
	Total	71.2286	10.60249	28

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.814	25.101b	4.000	23.000	0.000	0.814
	Wilks' Lambda	0.186	25.101b	4.000	23.000	0.000	0.814
	Hotelling's Trace	4.365	25.101b	4.000	23.000	0.000	0.814
	Roy's Largest Root	4.365	25.101b	4.000	23.000	0.000	0.814
Phase x sex	Pillai's Trace	0.418	4.132b	4.000	23.000	0.011	0.418
	Wilks' Lambda	0.582	4.132b	4.000	23.000	0.011	0.418
	Hotelling's Trace	0.719	4.132b	4.000	23.000	0.011	0.418
	Roy's Largest Root	0.719	4.132b	4.000	23.000	0.011	0.418

Mauchly's Test of Sphericity							
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.049	73.747	9	0.000	0.410	0.450	0.250

Within Subject							
Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	9859.950	4	2464.987	42.174	0.000	0.619
	Greenhouse-Geisser	9859.950	1.639	6016.361	42.174	0.000	0.619
	Huynh-Feldt	9859.950	1.802	5473.018	42.174	0.000	0.619
	Lower-bound	9859.950	1.000	9859.950	42.174	0.000	0.619
Phase * Sex	Sphericity Assumed	465.438	4	116.359	1.991	0.101	0.071
	Greenhouse-Geisser	465.438	1.639	284.002	1.991	0.156	0.071
	Huynh-Feldt	465.438	1.802	258.353	1.991	0.152	0.071
	Lower-bound	465.438	1.000	465.438	1.991	0.170	0.071
Error(Phase)	Sphericity Assumed	6078.573	104	58.448			
	Greenhouse-Geisser	6078.573	42.610	142.655			
	Huynh-Feldt	6078.573	46.840	129.772			
	Lower-bound	6078.573	26.000	233.791			

Between Subject						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared

Intercept	875876.317	1	875876.317	1231.036	0.000	0.979
Sex	116.867	1	116.867	0.164	0.689	0.006
Error	18498.880	26	711.495			

Pairwise Comparisons						
(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-7.852*	1.367	0.000	-12.043	-3.660
	3	-20.956*	2.914	0.000	-29.892	-12.021
	4	-17.707*	2.714	0.000	-26.031	-9.384
	5	-1.598	1.024	1.000	-4.738	1.542
2	1	7.852*	1.367	0.000	3.660	12.043
	3	-13.105*	2.402	0.000	-20.471	-5.739
	4	-9.856*	2.334	0.003	-17.013	-2.698
	5	6.254*	1.301	0.001	2.265	10.243
3	1	20.956*	2.914	0.000	12.021	29.892
	2	13.105*	2.402	0.000	5.739	20.471
	4	3.249	1.250	0.152	-0.583	7.082
	5	19.359*	2.152	0.000	12.758	25.960
4	1	17.707*	2.714	0.000	9.384	26.031
	2	9.856*	2.334	0.003	2.698	17.013
	3	-3.249	1.250	0.152	-7.082	0.583
	5	16.109*	2.023	0.000	9.906	22.313
5	1	1.598	1.024	1.000	-1.542	4.738
	2	-6.254*	1.301	0.001	-10.243	-2.265
	3	-19.359*	2.152	0.000	-25.960	-12.758
	4	-16.109*	2.023	0.000	-22.313	-9.906

Table D6: Repeated measures ANOVA analysis of heart rate with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	75.0846	19.68171	13
	F	76.2067	11.31008	15
	Total	75.6857	15.45370	28
Prep	M	80.8200	21.43082	13
	F	82.4320	11.16949	15
	Total	81.6836	16.41596	28
Speech	M	88.3246	23.44565	13
	F	88.0840	20.00114	15
	Total	88.1957	21.25457	28
Math	M	92.0462	23.83401	13
	F	91.9133	21.90393	15
	Total	91.9750	22.38865	28
Recovery	M	84.7185	22.19162	13
	F	86.6160	15.84003	15
	Total	85.7350	18.70570	28

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.631	9.839b	4.000	23.000	0.000	0.631
	Wilks' Lambda	0.369	9.839b	4.000	23.000	0.000	0.631
	Hotelling's Trace	1.711	9.839b	4.000	23.000	0.000	0.631
	Roy's Largest Root	1.711	9.839b	4.000	23.000	0.000	0.631
Phase x sex	Pillai's Trace	0.030	.176b	4.000	23.000	0.948	0.030
	Wilks' Lambda	0.970	.176b	4.000	23.000	0.948	0.030
	Hotelling's Trace	0.031	.176b	4.000	23.000	0.948	0.030
	Roy's Largest Root	0.031	.176b	4.000	23.000	0.948	0.030
Mauchly's Test of Sphericity							

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.104	55.355	9	0.000	0.471	0.527	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	4390.643	4	1097.661	23.245	0.000	0.472
	Greenhouse-Geisser	4390.643	1.886	2328.246	23.245	0.000	0.472
	Huynh-Feldt	4390.643	2.107	2084.072	23.245	0.000	0.472
	Lower-bound	4390.643	1.000	4390.643	23.245	0.000	0.472
Phase * Sex	Sphericity Assumed	27.212	4	6.803	0.144	0.965	0.006
	Greenhouse-Geisser	27.212	1.886	14.430	0.144	0.855	0.006
	Huynh-Feldt	27.212	2.107	12.916	0.144	0.876	0.006
	Lower-bound	27.212	1.000	27.212	0.144	0.707	0.006
Error(Phase)	Sphericity Assumed	4911.014	104	47.221			
	Greenhouse-Geisser	4911.014	49.031	100.161			
	Huynh-Feldt	4911.014	54.776	89.657			
	Lower-bound	4911.014	26.000	188.885			

Between Subject

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	997469.616	1	997469.616	590.229	0.000	0.958
Sex	25.255	1	25.255	0.015	0.904	0.001
Error	43939.239	26	1689.971			

Pairwise Comparisons

(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound

1	2	-5.980*	1.320	0.001	-10.029	-1.932
	3	-12.559*	2.338	0.000	-19.728	-5.390
	4	-16.334*	2.672	0.000	-24.528	-8.140
	5	-10.022*	1.593	0.000	-14.906	-5.137
2	1	5.980*	1.320	0.001	1.932	10.029
	3	-6.578*	1.857	0.015	-12.272	-0.884
	4	-10.354*	2.138	0.001	-16.911	-3.797
	5	-4.041	1.541	0.144	-8.768	0.686
3	1	12.559*	2.338	0.000	5.390	19.728
	2	6.578*	1.857	0.015	0.884	12.272
	4	-3.775*	0.948	0.005	-6.684	-0.867
	5	2.537	1.692	1.000	-2.652	7.726
4	1	16.334*	2.672	0.000	8.140	24.528
	2	10.354*	2.138	0.001	3.797	16.911
	3	3.775*	0.948	0.005	0.867	6.684
	5	6.313*	1.693	0.009	1.121	11.504
5	1	10.022*	1.593	0.000	5.137	14.906
	2	4.041	1.541	0.144	-0.686	8.768
	3	-2.537	1.692	1.000	-7.726	2.652
	4	-6.313*	1.693	0.009	-11.504	-1.121

Table D7: Repeated measures ANOVA analysis of stroke volume with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	5.3008	1.78443	13
	F	5.2533	0.79484	15
	Total	5.2754	1.32036	28
Prep	M	6.2637	2.16433	13
	F	6.3536	0.85921	15
	Total	6.3119	1.57060	28
Speech	M	7.6546	2.64082	13
	F	8.2052	1.88534	15
	Total	7.9496	2.24072	28
Math	M	8.0649	2.94085	13
	F	7.9285	1.90874	15
	Total	7.9919	2.39535	28
Recovery	M	6.0463	1.77993	13
	F	6.0241	0.90860	15
	Total	6.0344	1.35509	28

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.747	16.995b	4.000	23.000	0.000	0.747
	Wilks' Lambda	0.253	16.995b	4.000	23.000	0.000	0.747
	Hotelling's Trace	2.956	16.995b	4.000	23.000	0.000	0.747
	Roy's Largest Root	2.956	16.995b	4.000	23.000	0.000	0.747
Phase x sex	Pillai's Trace	0.240	1.813b	4.000	23.000	0.161	0.240
	Wilks' Lambda	0.760	1.813b	4.000	23.000	0.161	0.240
	Hotelling's Trace	0.315	1.813b	4.000	23.000	0.161	0.240
	Roy's Largest Root	0.315	1.813b	4.000	23.000	0.161	0.240

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.087	59.597	9	0.000	0.445	0.494	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	161.950	4	40.488	50.735	0.000	0.661
	Greenhouse-Geisser	161.950	1.780	90.997	50.735	0.000	0.661
	Huynh-Feldt	161.950	1.975	82.004	50.735	0.000	0.661
	Lower-bound	161.950	1.000	161.950	50.735	0.000	0.661
Phase * Sex	Sphericity Assumed	2.053	4	0.513	0.643	0.633	0.024
	Greenhouse-Geisser	2.053	1.780	1.154	0.643	0.513	0.024
	Huynh-Feldt	2.053	1.975	1.040	0.643	0.528	0.024
	Lower-bound	2.053	1.000	2.053	0.643	0.430	0.024
Error(Phase)	Sphericity Assumed	82.994	104	0.798			
	Greenhouse-Geisser	82.994	46.273	1.794			
	Huynh-Feldt	82.994	51.347	1.616			
	Lower-bound	82.994	26.000	3.192			

Between Subject

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	6270.299	1	6270.299	442.501	0.000	0.945
Sex	0.263	1	0.263	0.019	0.893	0.001
Error	368.423	26	14.170			

Pairwise Comparisons

(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	-1.032*	0.180	0.000	-1.585	-0.478

	3	-2.653*	0.304	0.000	-3.584	-1.721
	4	-2.720*	0.349	0.000	-3.790	-1.649
	5	-.758*	0.140	0.000	-1.189	-0.327
2	1	1.032*	0.180	0.000	0.478	1.585
	3	-1.621*	0.235	0.000	-2.341	-0.902
	4	-1.688*	0.268	0.000	-2.509	-0.867
	5	0.273	0.169	1.000	-0.244	0.790
3	1	2.653*	0.304	0.000	1.721	3.584
	2	1.621*	0.235	0.000	0.902	2.341
	4	-0.067	0.145	1.000	-0.513	0.379
	5	1.895*	0.247	0.000	1.136	2.653
4	1	2.720*	0.349	0.000	1.649	3.790
	2	1.688*	0.268	0.000	0.867	2.509
	3	0.067	0.145	1.000	-0.379	0.513
	5	1.962*	0.263	0.000	1.154	2.769
5	1	.758*	0.140	0.000	0.327	1.189
	2	-0.273	0.169	1.000	-0.790	0.244
	3	-1.895*	0.247	0.000	-2.653	-1.136
	4	-1.962*	0.263	0.000	-2.769	-1.154

Table D8: Repeated measures ANOVA analysis of cardiac output with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	26.2400	5.77162	10
	F	20.0182	9.25849	11
	Total	22.9810	8.24552	21
Prep	M	20.8200	6.86145	10
	F	15.2364	6.57804	11
	Total	17.8952	7.14048	21
Speech	M	25.4200	10.61883	10
	F	20.1818	4.59692	11
	Total	22.6762	8.27610	21
Math	M	20.4000	8.87243	10
	F	17.7273	6.49201	11
	Total	19.0000	7.63990	21
Recovery	M	22.1800	10.32901	10
	F	20.3636	9.74344	11
	Total	21.2286	9.81535	21

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.719	10.256b	4.000	16.000	0.000	0.719
	Wilks' Lambda	0.281	10.256b	4.000	16.000	0.000	0.719
	Hotelling's Trace	2.564	10.256b	4.000	16.000	0.000	0.719
	Roy's Largest Root	2.564	10.256b	4.000	16.000	0.000	0.719
Phase x sex	Pillai's Trace	0.108	.483b	4.000	16.000	0.748	0.108
	Wilks' Lambda	0.892	.483b	4.000	16.000	0.748	0.108
	Hotelling's Trace	0.121	.483b	4.000	16.000	0.748	0.108
	Roy's Largest Root	0.121	.483b	4.000	16.000	0.748	0.108
Mauchly's Test of Sphericity							

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.408	15.618	9	0.076	0.736	0.931	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	425.996	4	106.499	4.855	0.002	0.204
	Greenhouse-Geisser	425.996	2.944	144.715	4.855	0.005	0.204
	Huynh-Feldt	425.996	3.725	114.347	4.855	0.002	0.204
	Lower-bound	425.996	1.000	425.996	4.855	0.040	0.204
Phase * Sex	Sphericity Assumed	78.768	4	19.692	0.898	0.470	0.045
	Greenhouse-Geisser	78.768	2.944	26.758	0.898	0.447	0.045
	Huynh-Feldt	78.768	3.725	21.143	0.898	0.464	0.045
	Lower-bound	78.768	1.000	78.768	0.898	0.355	0.045
Error(Phase)	Sphericity Assumed	1667.232	76	21.937			
	Greenhouse-Geisser	1667.232	55.930	29.809			
	Huynh-Feldt	1667.232	70.784	23.554			
	Lower-bound	1667.232	19.000	87.749			

Between Subject

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	45580.491	1	45580.491	187.785	0.000	0.908
Sex	485.737	1	485.737	2.001	0.173	0.095
Error	4611.825	19	242.728			

Pairwise Comparisons

(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	5.101*	1.122	0.002	1.539	8.663

	3	0.328	1.697	1.000	-5.059	5.716
	4	4.065	1.677	0.255	-1.258	9.389
	5	1.857	1.571	1.000	-3.129	6.843
2	1	-5.101*	1.122	0.002	-8.663	-1.539
	3	-4.773*	1.153	0.006	-8.431	-1.115
	4	-1.035	1.263	1.000	-5.043	2.972
	5	-3.244	1.431	0.353	-7.786	1.299
3	1	-0.328	1.697	1.000	-5.716	5.059
	2	4.773*	1.153	0.006	1.115	8.431
	4	3.737	1.330	0.112	-0.484	7.958
	5	1.529	1.814	1.000	-4.227	7.285
4	1	-4.065	1.677	0.255	-9.389	1.258
	2	1.035	1.263	1.000	-2.972	5.043
	3	-3.737	1.330	0.112	-7.958	0.484
	5	-2.208	1.219	0.860	-6.078	1.662
5	1	-1.857	1.571	1.000	-6.843	3.129
	2	3.244	1.431	0.353	-1.299	7.786
	3	-1.529	1.814	1.000	-7.285	4.227
	4	2.208	1.219	0.860	-1.662	6.078

Table D9: Repeated measures ANOVA analysis of MSNA BF with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	38.2800	5.48772	10
	F	28.9091	14.32951	11
	Total	33.3714	11.79907	21
Prep	M	27.9280	8.23990	10
	F	19.6509	8.08364	11
	Total	23.5924	9.00938	21
Speech	M	30.9200	12.94645	10
	F	21.9055	6.32025	11
	Total	26.1981	10.80187	21
Math	M	24.4620	10.73375	10
	F	21.2764	9.91739	11
	Total	22.7933	10.18240	21
Recovery	M	31.5940	12.89038	10
	F	28.9309	13.90383	11
	Total	30.1990	13.16391	21

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.714	9.991b	4.000	16.000	0.000	0.714
	Wilks' Lambda	0.286	9.991b	4.000	16.000	0.000	0.714
	Hotelling's Trace	2.498	9.991b	4.000	16.000	0.000	0.714
	Roy's Largest Root	2.498	9.991b	4.000	16.000	0.000	0.714
Phase x sex	Pillai's Trace	0.135	.623b	4.000	16.000	0.653	0.135
	Wilks' Lambda	0.865	.623b	4.000	16.000	0.653	0.135
	Hotelling's Trace	0.156	.623b	4.000	16.000	0.653	0.135
	Roy's Largest Root	0.156	.623b	4.000	16.000	0.653	0.135

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.337	18.955	9	0.026	0.698	0.874	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	1699.323	4	424.831	8.022	0.000	0.297
	Greenhouse-Geisser	1699.323	2.794	608.258	8.022	0.000	0.297
	Huynh-Feldt	1699.323	3.497	485.975	8.022	0.000	0.297
	Lower-bound	1699.323	1.000	1699.323	8.022	0.011	0.297
Phase * Sex	Sphericity Assumed	227.490	4	56.873	1.074	0.375	0.053
	Greenhouse-Geisser	227.490	2.794	81.428	1.074	0.365	0.053
	Huynh-Feldt	227.490	3.497	65.058	1.074	0.372	0.053
	Lower-bound	227.490	1.000	227.490	1.074	0.313	0.053
Error(Phase)	Sphericity Assumed	4024.791	76	52.958			
	Greenhouse-Geisser	4024.791	53.081	75.823			
	Huynh-Feldt	4024.791	66.438	60.580			
	Lower-bound	4024.791	19.000	211.831			

Between Subject

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	78568.817	1	78568.817	215.688	0.000	0.919
Sex	1107.315	1	1107.315	3.040	0.097	0.138
Error	6921.148	19	364.271			

Pairwise Comparisons

(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	9.805*	1.844	0.000	3.952	15.659

	3	7.182	2.706	0.157	-1.406	15.770
	4	10.725*	2.779	0.011	1.906	19.544
	5	3.332	2.549	1.000	-4.759	11.423
2	1	-9.805*	1.844	0.000	-15.659	-3.952
	3	-2.623	1.708	1.000	-8.044	2.797
	4	0.920	2.009	1.000	-5.454	7.295
	5	-6.473	2.274	0.103	-13.691	0.745
3	1	-7.182	2.706	0.157	-15.770	1.406
	2	2.623	1.708	1.000	-2.797	8.044
	4	3.544	1.860	0.721	-2.361	9.448
	5	-3.850	2.619	1.000	-12.161	4.462
4	1	-10.725*	2.779	0.011	-19.544	-1.906
	2	-0.920	2.009	1.000	-7.295	5.454
	3	-3.544	1.860	0.721	-9.448	2.361
	5	-7.393*	1.778	0.005	-13.037	-1.749
5	1	-3.332	2.549	1.000	-11.423	4.759
	2	6.473	2.274	0.103	-0.745	13.691
	3	3.850	2.619	1.000	-4.462	12.161
	4	7.393*	1.778	0.005	1.749	13.037

Table D10: Repeated measures ANOVA analysis of MSNA BI with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	16487.1000	3829.48914	7
	F	11179.8571	2869.09310	7
	Total	13833.4786	4260.40796	14
Prep	M	13337.2086	3840.52604	7
	F	9501.8514	3967.65063	7
	Total	11419.5300	4246.59344	14
Speech	M	22183.9029	9093.28788	7
	F	24565.8657	8413.85632	7
	Total	23374.8843	8506.75429	14
Math	M	16909.4400	11832.39174	7
	F	18483.7343	10988.84047	7
	Total	17696.5871	11000.82187	14
Recovery	M	13832.2086	9295.42298	7
	F	16607.5029	11843.70977	7
	Total	15219.8557	10329.30112	14

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.757	7.023b	4.000	9.000	0.008	0.757
	Wilks' Lambda	0.243	7.023b	4.000	9.000	0.008	0.757
	Hotelling's Trace	3.121	7.023b	4.000	9.000	0.008	0.757
	Roy's Largest Root	3.121	7.023b	4.000	9.000	0.008	0.757
Phase x sex	Pillai's Trace	0.375	1.353b	4.000	9.000	0.323	0.375
	Wilks' Lambda	0.625	1.353b	4.000	9.000	0.323	0.375
	Hotelling's Trace	0.601	1.353b	4.000	9.000	0.323	0.375
	Roy's Largest Root	0.601	1.353b	4.000	9.000	0.323	0.375

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.125	21.628	9	0.011	0.564	0.758	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	1163028681.209	4	290757170.302	5.263	0.001	0.305
	Greenhouse-Geisser	1163028681.209	2.254	515979735.120	5.263	0.010	0.305
	Huynh-Feldt	1163028681.209	3.033	383500690.112	5.263	0.004	0.305
	Lower-bound	1163028681.209	1.000	1163028681.209	5.263	0.041	0.305
Phase * Sex	Sphericity Assumed	201489988.134	4	50372497.034	0.912	0.465	0.071
	Greenhouse-Geisser	201489988.134	2.254	89391390.244	0.912	0.424	0.071
	Huynh-Feldt	201489988.134	3.033	66439934.585	0.912	0.446	0.071
	Lower-bound	201489988.134	1.000	201489988.134	0.912	0.358	0.071
Error(Phase)	Sphericity Assumed	2651958344.479	48	55249132.177			
	Greenhouse-Geisser	2651958344.479	27.048	98045501.531			
	Huynh-Feldt	2651958344.479	36.392	72872081.868			
	Lower-bound	2651958344.479	12.000	220996528.707			

Between Subject

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta

						Squared
Intercept	18618540323.836	1	18618540323.836	147.582	0.000	0.925
Sex	4069208.650	1	4069208.650	0.032	0.860	0.003
Error	1513889028.491	12	126157419.041			

Pairwise Comparisons

(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound
1	2	2413.949	1368.695	1.000	-2278.544	7106.441
	3	-9541.406*	2520.827	0.026	-18183.920	-898.891
	4	-3863.109	3072.037	1.000	-14395.418	6669.201
	5	-1386.377	2872.426	1.000	-11234.328	8461.574
2	1	-2413.949	1368.695	1.000	-7106.441	2278.544
	3	-11955.35*	2380.955	0.003	-20118.326	-3792.382
	4	-6277.057	3128.163	0.679	-17001.790	4447.675
	5	-3800.326	2933.492	1.000	-13857.640	6256.989
3	1	9541.406*	2520.827	0.026	898.891	18183.920
	2	11955.354*	2380.955	0.003	3792.382	20118.326
	4	5678.297	3061.530	0.884	-4817.988	16174.583
	5	8155.029	3907.247	0.589	-5240.751	21550.808
4	1	3863.109	3072.037	1.000	-6669.201	14395.418
	2	6277.057	3128.163	0.679	-4447.675	17001.790
	3	-5678.297	3061.530	0.884	-16174.583	4817.988
	5	2476.731	2076.516	1.000	-4642.488	9595.951
5	1	1386.377	2872.426	1.000	-8461.574	11234.328

	2	3800.326	2933.492	1.000	-6256.989	13857.640
	3	-8155.029	3907.247	0.589	-21550.808	5240.751
	4	-2476.731	2076.516	1.000	-9595.951	4642.488

Table D11: Repeated measures ANOVA analysis of MSNA total area with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Descriptive Statistics				
Sex		Mean	Std. Deviation	N
Baseline	M	74.7000	20.84059	7
	F	55.1429	12.55785	7
	Total	64.9214	19.39640	14
Prep	M	62.8714	22.95346	7
	F	47.3571	15.84454	7
	Total	55.1143	20.58732	14
Speech	M	91.9571	41.47992	7
	F	93.1200	27.65793	7
	Total	92.5386	33.87534	14
Math	M	67.1000	42.51629	7
	F	75.4657	39.72154	7
	Total	71.2829	39.76620	14
Recovery	M	64.5714	37.61390	7
	F	77.9657	43.99455	7
	Total	71.2686	39.93252	14

	Multivariate Tests						
		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Phase	Pillai's Trace	0.767	7.422b	4.000	9.000	0.006	0.767
	Wilks' Lambda	0.233	7.422b	4.000	9.000	0.006	0.767
	Hotelling's Trace	3.299	7.422b	4.000	9.000	0.006	0.767
	Roy's Largest Root	3.299	7.422b	4.000	9.000	0.006	0.767
Phase x sex	Pillai's Trace	0.348	1.201b	4.000	9.000	0.375	0.348
	Wilks' Lambda	0.652	1.201b	4.000	9.000	0.375	0.348
	Hotelling's Trace	0.534	1.201b	4.000	9.000	0.375	0.348
	Roy's Largest Root	0.534	1.201b	4.000	9.000	0.375	0.348
Mauchly's Test of Sphericity							

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Phase	0.126	21.541	9	0.011	0.560	0.752	0.250

Within Subject

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Phase	Sphericity Assumed	10547.096	4	2636.774	4.443	0.004	0.270
	Greenhouse-Geisser	10547.096	2.239	4710.782	4.443	0.018	0.270
	Huynh-Feldt	10547.096	3.006	3508.300	4.443	0.009	0.270
	Lower-bound	10547.096	1.000	10547.096	4.443	0.057	0.270
Phase * Sex	Sphericity Assumed	2955.406	4	738.851	1.245	0.305	0.094
	Greenhouse-Geisser	2955.406	2.239	1320.010	1.245	0.307	0.094
	Huynh-Feldt	2955.406	3.006	983.062	1.245	0.308	0.094
	Lower-bound	2955.406	1.000	2955.406	1.245	0.286	0.094
Error(Phase)	Sphericity Assumed	28484.552	48	593.428			
	Greenhouse-Geisser	28484.552	26.867	1060.201			
	Huynh-Feldt	28484.552	36.076	789.572			
	Lower-bound	28484.552	12.000	2373.713			

Between Subject

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	353119.964	1	353119.964	120.852	0.000	0.910
Sex	103.311	1	103.311	0.035	0.854	0.003
Error	35062.926	12	2921.911			

Pairwise Comparisons

(I) Phase		Mean Difference (I-J)	Std. Error	Sig.b	Lower Bound	Upper Bound

1	2	9.807	4.039	0.318	-4.040	23.655
	3	-27.617	8.411	0.065	-56.454	1.219
	4	-6.361	10.028	1.000	-40.740	28.017
	5	-6.347	9.156	1.000	-37.739	25.045
2	1	-9.807	4.039	0.318	-23.655	4.040
	3	-37.424*	7.889	0.005	-64.472	-10.377
	4	-16.169	10.124	1.000	-50.878	18.540
	5	-16.154	9.481	1.000	-48.659	16.351
3	1	27.617	8.411	0.065	-1.219	56.454
	2	37.424*	7.889	0.005	10.377	64.472
	4	21.256	10.495	0.657	-14.725	57.237
	5	21.270	12.862	1.000	-22.826	65.366
4	1	6.361	10.028	1.000	-28.017	40.740
	2	16.169	10.124	1.000	-18.540	50.878
	3	-21.256	10.495	0.657	-57.237	14.725
	5	0.014	6.791	1.000	-23.270	23.298
5	1	6.347	9.156	1.000	-25.045	37.739
	2	16.154	9.481	1.000	-16.351	48.659
	3	-21.270	12.862	1.000	-65.366	22.826
	4	-0.014	6.791	1.000	-23.298	23.270

Table D12: Repeated measures ANOVA analysis of MSNA total amplitude with pairwise comparisons between TSST phase. Note: Phase 1 = base; phase 2 = prep; phase 3 = speech; phase 4 = math; phase 5 = recovery.

Group Statistics					
	Condition	Mean	N	SD	SEM
DAP-MSNA BI	Base	-1.7079	19	0.52673	0.12084
	Prep	-1.7099	19	0.61947	0.14212
DAP-MSNA Total	Base	-662.4052	18	229.22496	54.02884
	Prep	-792.4053	18	445.80062	105.07621
SAPRRI Up	Base	16.5250	28	7.69515	1.45425
	Prep	14.4143	28	6.01632	1.13698
SAPRRI Down	Base	16.8500	28	6.17495	1.16696
	Prep	13.9857	28	5.48653	1.03686
LF	Base	2244.5000	28	3246.32645	613.49803
	Prep	1715.3571	28	1662.21718	314.12952
HF	Base	1035.7500	28	818.94307	154.76569
	Prep	832.1786	28	724.04481	136.83161
LFHF	Base	239.9429	28	238.91668	45.15101
	Prep	248.4143	28	170.16690	32.15852
RRI	Base	880.8214	28	107.90876	20.39284
	Prep	794.5714	28	112.68357	21.29519
RMSSD	Base	54.9286	28	23.77663	4.49336
	Prep	44.8571	28	19.61993	3.70782
pNN50	Base	29.2143	28	15.65285	2.95811
	Prep	21.5857	28	14.73772	2.78517
Resp	Base	13.5591	28	3.33809	0.63084
	Prep	14.6830	28	3.24881	0.61397

Paired Samples T Test						
	Lower 95%	Upper 95%	t	df	One sided P-Value	Two-sided P-value
DAP-MSNA BI	-0.35774	0.36176	0.012	18	0.495	0.991
SAP_RRI_Up_	-0.21336	4.43479	1.863	27	0.037	0.073
SAP_RRI_Down_	0.77512	4.95345	2.813	27	0.005	0.009
RRI_	59.18066	113.31934	6.538	27	0.000	0.000
RMSSD_	3.07508	17.06778	2.954	27	0.003	0.006
pNN50	2.80361	12.45353	3.244	27	0.002	0.003
HF-HRV	11.9822	395.16066	2.180	27	.019	0.038
Respi_Rate	-1.93058	-0.31729	-2.859	27	0.004	0.008

Wilcoxon Signed Rank – sBRS Total	

Total N	18
Test Statistic	84.000
Standard Error	22.962
Standardized Test Statistic	-0.065
Asymptotic Sig.(2-sided test)	0.948
Wilcoxon Signed Rank – LF	
Total N	28
Test Statistic	196.000
Standard Error	43.915
Standardized Test Statistic	-0.159
Asymptotic Sig.(2-sided test)	0.873
Wilcoxon Signed Rank – LFHF	
Total N	28
Test Statistic	243.000
Standard Error	43.915
Standardized Test Statistic	0.911
Asymptotic Sig.(2-sided test)	0.362

Table D13: Baseline vs. Prep phase baroreflex sensitivity and HRV.

Descriptive					
	Mean		Std. Deviation		N
MSNA BF	-5.0857		5.01810		21
DAP	5.7886		3.88036		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.688	0.473	0.446		3.73615
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	238.408	1	238.408	17.079	<.001b
Residual	265.217	19	13.959		
Total	503.626	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	0.065	1.489		0.043	0.966
DAP Prep	-0.890	0.215	-0.688	-4.133	0.001

Table D14: Linear regression analysis of changes in diastolic pressure (predictor) on changes in MSNA BF (outcome) during the prep phase.

Descriptive					
	Mean		Std. Deviation		N
MSNA BF	-0.3048		7.58996		21
DAP	17.7295		7.67201		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.229a	0.052	0.003		7.58010
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	60.448	1	60.448	1.052	.318
Residual	1091.701	19	57.458		
Total	1152.150	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	3.713	4.252		0.873	0.393
DAP Prep	-0.227	0.221	-0.229	-1.026	0.318

Table D15: Linear regression analysis of changes in diastolic pressure (predictor) on changes in MSNA BF (outcome) during the speech phase.

Descriptive					
	Mean		Std. Deviation		N
MSNA BF	-3.9810		7.70134		21
DAP	15.4105		8.68453		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.441	0.194	0.152		7.09262
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	230.412	1	230.412	4.580	.046
Residual	955.801	19	50.305		
Total	1186.212	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	2.042	3.212		0.636	0.533
DAP Prep	-0.391	0.183	-0.441	-2.140	0.046

Table D16: Linear regression analysis of changes in diastolic pressure (predictor) on changes in MSNA BF (outcome) during the math phase.

Descriptive					
	Mean		Std. Deviation		N
MSNA BI	-9.7790		8.24767		21
DAP	5.7886		3.88036		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.777	.603	.583		5.32912
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	820.888	1	820.888	28.905	<.001
Residual	539.591	19	28.400		
Total	1360.480	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	-.222	2.124		-.104	.918
DAP Prep	-1.651	.307	-.777	-5.376	<.001

Table D17: Linear regression analysis of changes in diastolic pressure (predictor) on changes in MSNA BI (outcome) during the prep phase.

Descriptive					
	Mean		Std. Deviation		N
MSNA BI	-7.1733		12.07433		21
DAP	17.7295		7.67201		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.519	.270	.231		10.58695
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	786.200	1	786.200	7.014	.016
Residual	2129.586	19	112.083		
Total	2915.787	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	7.316	5.939		1.232	.233
DAP Speech	-.817	.309	-.519	-2.648	.016

Table D18: Linear regression analysis of changes in diastolic pressure (predictor) on changes in MSNA BI (outcome) during the speech phase.

Descriptive					
	Mean		Std. Deviation		N
MSNA BI	-10.5781		12.79525		21
DAP	15.4105		8.68453		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.603	.364	.331		10.46817
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	1192.301	1	1192.301	10.880	.004
Residual	2082.069	19	109.583		
Total	3274.370	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	3.123	4.740		.659	.518
DAP Math	-.889	.270	-.603	-3.299	.004

Table D19: Linear regression analysis of changes in diastolic pressure (predictor) on changes in MSNA BI (outcome) during the math phase.

Descriptive					
	Mean		Std. Deviation		N
MSNA BF Speech	-0.3048		7.58996		21
MSNA BF Prep	-5.0857		5.01810		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.739a	0.547	0.523		5.24350
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	629.757	1	629.757	22.905	<.001
Residual	522.392	19	27.494		
Total	1152.150	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	5.382	1.650		3.263	0.004
MSNA BF Prep	1.118	0.234	0.739	4.786	0.000

Table D20: Linear regression analysis of changes in MSNA BF during the prep phase (predictor) on changes in MSNA BF during the speech phase (outcome).

Descriptive					
	Mean		Std. Deviation		N
MSNA BF Math	-3.9810		7.70134		21
MSNA BF Prep	-5.0857		5.01810		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.654	0.427	0.397		5.97910
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	506.968	1	506.968	14.181	.001b
Residual	679.244	19	35.750		
Total	1186.212	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	1.122	1.881		0.596	0.558
MSNA BF Prep	1.003	0.266	0.654	3.766	0.001

Table D21: Linear regression analysis of changes in MSNA BF during the prep phase (predictor) on changes in MSNA BF during the math phase (outcome).

Descriptive					
	Mean		Std. Deviation		N
MSNA BI Speech	-7.1733		12.07433		21
MSNA BI Prep	-9.7790		8.24767		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.781	.610	.590		7.73247
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	1779.756	1	1779.756	29.766	<.001
Residual	1136.031	19	59.791		
Total	2915.787	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	4.012	2.655		1.511	.147
MSNA BI Prep	1.144	.210	.781	5.456	<.001

Table D22: Linear regression analysis of changes in MSNA BI during the prep phase (predictor) on changes in MSNA BI during the speech phase (outcome).

Descriptive					
	Mean		Std. Deviation		N
MSNA BI Math	-10.5781		12.79525		21
MSNA BI Prep	-9.7790		8.24767		21
Model Summary					
Model	R	R^2	R^2 Adjusted		SE of Estimate
	.685	.470	.442		9.55974
ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	1537.988	1	1537.988	16.829	<.001
Residual	1736.382	19	91.389		
Total	3274.370	20			
Coefficients					
	B	St. Error	Beta	t	Sig.
(Constant)	-.181	3.283		-.055	.957
MSNA BI Math	1.063	.259	.685	4.102	<.001

Table D23: Linear regression analysis of changes in MSNA BI during the prep phase (predictor) on changes in MSNA BI during the math phase (outcome).

SAP					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
Threat vs Challenge	Pearson Correlation	.826	.641	.617	.465
	Sig. (2-tailed)	.012	.087	.103	.245
	N	8	8	8	8

Table D24: Correlation between threat vs. challenge appraisal and systolic arterial pressure changes during each phase of the TSST.

DAP					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
Threat vs Challenge	Pearson Correlation	.829	.521	.595	.536
	Sig. (2-tailed)	.011	.185	.120	.171
	N	8	8	8	8

Table D25: Correlation between threat vs. challenge appraisal and diastolic arterial pressure changes during each phase of the TSST.

MAP					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
Threat vs Challenge	Pearson Correlation	.877	.576	.600	.543
	Sig. (2-tailed)	.004	.135	.116	.164
	N	8	8	8	8

Table D26: Correlation between threat vs. challenge appraisal and mean arterial pressure changes during each phase of the TSST.

HR					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
Threat vs Challenge	Pearson Correlation	.272	-.436	-.195	.042
	Sig. (2-tailed)	.514	.280	.643	.921
	N	8	8	8	8

Table D27: Correlation between threat vs. challenge appraisal and heart rate changes during each phase of the TSST.

SV					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
Threat vs Challenge	Pearson Correlation	.623	.552	.100	-.015
	Sig. (2-tailed)	.099	.156	.814	.972
	N	8	8	8	8

Table D28: Correlation between threat vs. challenge appraisal and stroke volume changes during each phase of the TSST.

CO					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
Threat vs Challenge	Pearson Correlation	.613	.253	.016	.234
	Sig. (2-tailed)	.106	.545	.970	.577
	N	8	8	8	8

Table D29: Correlation between threat vs. challenge appraisal and cardiac output changes during each phase of the TSST.

MSNA BF					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
Threat vs Challenge	Pearson Correlation	-.152	.440	.193	.629
	Sig. (2-tailed)	.807	.458	.756	.256
	N	5	5	5	5

Table D30: Correlation between threat vs. challenge appraisal and MSNA BF changes during each phase of the TSST.

MSNA BI					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
Threat vs Challenge	Pearson Correlation	-.186	.739	.289	.635
	Sig. (2-tailed)	.765	.153	.637	.250
	N	5	5	5	5

Table D31: Correlation between threat vs. challenge appraisal and MSNA BI changes during each phase of the TSST.

SAP					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	-0.001	0.003	-0.043	-0.214
	Sig. (2-tailed)	0.996	0.986	0.827	0.275
	N	28	28	28	28
STAI (%)	Pearson Correlation	-0.004	-0.009	-0.020	-0.180
	Sig. (2-tailed)	0.982	0.963	0.921	0.359
	N	28	28	28	28
DAP					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	-0.006	0.011	0.002	-0.018
	Sig. (2-tailed)	0.975	0.955	0.992	0.929
	N	28	28	28	28
STAI (%)	Pearson Correlation	0.024	0.024	0.044	0.032
	Sig. (2-tailed)	0.903	0.902	0.825	0.873
	N	28	28	28	28
MAP					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	0.007	0.023	-0.021	-0.094
	Sig. (2-tailed)	0.972	0.908	0.914	0.635
	N	28	28	28	28
STAI (%)	Pearson Correlation	0.022	0.026	0.007	-0.048
	Sig. (2-tailed)	0.912	0.897	0.970	0.808
	N	28	28	28	28
HR					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	0.079	-0.035	-0.080	0.126
	Sig. (2-tailed)	0.690	0.858	0.687	0.522
	N	28	28	28	28

STAI (%)	Pearson Correlation	0.079	-0.036	-0.068	0.128
	Sig. (2-tailed)	0.691	0.856	0.731	0.516
	N	28	28	28	28
SV					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	0.059	0.065	0.019	-0.296
	Sig. (2-tailed)	0.767	0.744	0.924	0.126
	N	28	28	28	28
STAI (%)	Pearson Correlation	0.064	0.065	0.019	-0.292
	Sig. (2-tailed)	0.747	0.743	0.925	0.132
	N	28	28	28	28
CO					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	-0.009	-0.065	-0.115	-0.216
	Sig. (2-tailed)	0.965	0.742	0.562	0.271
	N	28	28	28	28
STAI (%)	Pearson Correlation	0.010	-0.070	-0.103	-0.174
	Sig. (2-tailed)	0.958	0.722	0.601	0.375
	N	28	28	28	28
MSNA BF					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	-0.037	0.007	-0.140	0.275
	Sig. (2-tailed)	0.875	0.976	0.545	0.227
	N	21	21	21	21
STAI (%)	Pearson Correlation	-0.060	0.030	-0.153	0.230
	Sig. (2-tailed)	0.796	0.898	0.507	0.317
	N	21	21	21	21
MSNA BI					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery

STAI (a.u.)	Pearson Correlation	0.041	0.073	0.017	0.278
	Sig. (2-tailed)	0.859	0.753	0.943	0.222
	N	21	21	21	21
STAI (%)	Pearson Correlation	0.021	0.090	0.006	0.234
	Sig. (2-tailed)	0.928	0.697	0.981	0.307
	N	21	21	21	21
MSNA Amp					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	-0.233	0.000	0.074	0.173
	Sig. (2-tailed)	0.424	1.000	0.802	0.554
	N	14	14	14	14
STAI (%)	Pearson Correlation	-0.258	0.038	0.080	0.157
	Sig. (2-tailed)	0.374	0.899	0.786	0.593
	N	14	14	14	14
Total MSNA Area					
	Measure	ΔPrep	ΔSpeech	ΔMath	ΔRecovery
STAI (a.u.)	Pearson Correlation	-0.172	0.066	0.129	0.152
	Sig. (2-tailed)	0.556	0.823	0.660	0.604
	N	14	14	14	14
STAI (%)	Pearson Correlation	-0.199	0.107	0.133	0.128
	Sig. (2-tailed)	0.496	0.715	0.650	0.662
	N	14	14	14	14

Table D32: Correlation between trait anxiety (%) and cardiovascular and sympathetic reactivity to each phase of the TSST.