Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Joohee Kang March 30, 2018

Acute Effects of Device-Guided Slow Breathing on Neurocardiovascular Activity in Post-Traumatic Stress Disorder

by

Joo Hee Kang

Jeanie Park, MD
Adviser

Department of Biology

Jeanie Park, MD
Adviser

Victor Corces, PhD
Committee Member

Robert Jensen, PhD
Committee Member

2018

Acute Effects of Device-Guided Slow Breathing on Neurocardiovascular Activity in Post Traumatic Stress Disorder

By

Joohee Kang

Jeanie Park, MD
Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

2018

Abstract

Acute Effects of Device-Guided Slow Breathing on
Neurocardiovascular Activity in Post-Traumatic Stress Disorder

By Joohee Kang

Post-traumatic stress disorder (PTSD) is associated with an increased risk for developing hypertension and cardiovascular (CV) disease, but the mechanisms underlying this risk remain unknown. Patients with PTSD have higher resting heart rate and blood pressure (BP) and decreased heart rate variability (HRV), suggesting a state of increased sympathetic nervous system (SNS) and decreased parasympathetic nervous system (PNS) activity that could contribute to increased CV risk. Therefore, SNS overactivity and decreased PNS activity could serve as putative therapeutic targets to improve CV risk in PTSD. Prior studies have shown that device-guided slow breathing (DGB) may lower BP and SNS activity in hypertensive patients; however, the potential beneficial effects of DGB on PNS have not previously been explored in PTSD. We hypothesized that DGB will lower BP and improve autonomic function in PTSD patients. We recruited 27 Veterans with PTSD and 16 Veterans without PTSD (controls). The PTSD group was randomly assigned to the DGB (PTSD+DGB, N=16) during which respiratory rates were monitored and lowered using a biofeedback device to sub-physiologic levels (~5 breaths/min) versus an identical sham device (PTSD+SHAM, N=11) in which respiratory rates were held at a normal rate of 14 breaths/min. All control participants without PTSD were assigned to the DGB device (CON+DGB, N=16). Continuous EKG and beat-to-beat arterial blood pressure were monitored for 10 minutes at rest, followed by 10 minutes of breathing with either DGB or SHAM. From baseline to the end of 10 minutes of DGB, the PTSD+DGB group had a significant reduction in systolic blood pressure (SBP, -12±3 mmHg, $p<0.001$) and mean arterial pressure (MAP, -7±2 mmHg, $p=0.003$), and a trend towards reduction in diastolic blood pressure (DBP, -4±2 mmHg, p=0.0565). In the CON+DGB group, we observed a significant reduction in SBP (-11±4 mmHg, $p=0.0105$), and a trend towards reduced DBP and MAP. There was no significant reduction in SBP, DBP, and MAP with the sham device in PTSD patients. There was no significant change in HR, RMSSD, pNN50, and HF either with DGB or SHAM. DGB acutely lowers blood pressure in PTSD patients and Controls, but does not improve PNS measures. Long-term studies are needed to determine if DGB could represent a novel therapeutic intervention to improve hemodynamics and autonomic physiology in PTSD patients.

Acute Effects of Device-Guided Slow Breathing on Neurocardiovascular Activity in Post-Traumatic Stress Disorder

By

Joohee Kang

Jeanie Park, MD
Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

2018

Acknowledgements

I would like to thank Dr. Jeanie Park for her tremendous support and guidance throughout my time in the laboratory. I would like to thank Dr. Ida Fonkoue, Melanie Kankam, and Dana DaCosta for their care in teaching and explaining the necessary skills required to complete this study.

This work was supported by National Institutes of Health Grant K23 HL-098744 and R01 HL135183; Satellite Health Care, a not-for-profit renal care provider; the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Clinical Studies Center, Decatur, Georgia; the Atlanta Research and Education Foundation; and Public Health Service Grant UL1 RR-025008 from the Clinical and Translational Science Award program, National Institutes of Health, National Center for Research Resources.

Table of Contents

Acute Effects of Device-Guided Slow Breathing on Neurocardiovascular Activity in Post-Traumatic Stress Disorder

By Joohee Kang

Emory School of Medicine

Honors Thesis

Introduction

Epidemiology of Post-Traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) is a mental health disorder that involves psychiatric symptoms after exposure to a traumatic event. The American Psychiatric Association classifies PTSD under trauma and stress-related disorders in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), a standard book classifying all mental disorders diagnosed by mental health professionals[40]. Patients are diagnosed as having PTSD if they meet all diagnostic criteria that include re-experiencing the traumatic event, avoidance, negative thoughts, hyper-arousal, and functional impairment that lasts for more than one month after being exposed to a seriously traumatic event[40].

PTSD is highly prevalent among the general population and among military Veterans. According to the National Comorbidity Survey Replication (NCS-R), about 7-8% of the general US adult population will meet the diagnostic criteria for PTSD throughout their lifetime[39]. During a given year, the number of adults diagnosed as having PTSD is shown to be about half of the lifetime prevalence of PTSD, which suggests that about 8 million people are diagnosed as having PTSD each year[42].

Compared to the general population, the prevalence of PTSD increases drastically in

military service members[39, 42, 45]. A study including over 3,000 American Veterans from the Vietnam War estimated that 30.9% male and 26.9% female Veterans from the Vietnam era will develop PTSD throughout their lifetime[45]. At the time of the study on Vietnam War Veterans, 15.2% of males and 8.1% females were diagnosed with PTSD[45]. A larger sample of more than 11,000 Gulf War Veterans assessed the prevalence of PTSD to be greater than 10% using the PTSD Checklist (PCL) questionnaire [46]. A more recent study conducted among Operation Enduring Freedom/ Operation Iraqi Freedom/ Operation New Dawn (OEF/OIF/OND) Veterans, reported a 11.5-19.9% prevalence of PTSD post-deployment to Afghanistan and Iraq [1, 47]. These post 9/11 Veterans were exposed to multiple traumatic events, and studies suggest that the prevalence of PTSD increases with multiple event exposures[41]. These studies altogether highlight the growing prevalence of PTSD both in the general population and among Veterans. Given the tremendous detrimental impact of PTSD on mental health and quality of life, further studies to understand the mechanisms and effective treatments for PTSD are crucial.

Post-traumatic stress disorder and Cardiovascular Disease Risk

Hypertension, or chronic high blood pressure (BP), affects 1 out of 3 US adults and can cause serious health conditions such as heart disease or stroke, which are leading causes of death in the US[48, 51]. Some other consequences of high BP over time are coronary artery disease, renal or heart failure, and vision loss[51]. PTSD is independently associated with an increased risk of hypertension, cardiovascular (CV) disease, and mortality[2, 16, 18, 19, 36]. A study conducted between 2001 and 2008 on over 300,000 young OEF/OIF Veterans reported

that Veterans with PTSD have a 59% higher chance of developing hypertension[2]. The same study on OEF/OIF Veterans showed that about 35% of OEF/OIF Veterans with PTSD had hypertension after controlling for age, race, rank, and deployment numbers[2]. The number of combat exposures has a role in hypertension development as well; among military Veterans, those with multiple combat experiences were 1.33 times more likely to develop hypertension than those with no combat exposures[16]. However, a study comparing two groups of World War II Prisoners of War (POW) with and without PTSD showed that there are more factors other than exposure to trauma that increases risk of hypertension. This study on POWs with PTSD compared to POWs without PTSD shows that POWs with PTSD had 60% higher chance of developing vascular disease, 25% higher chance of hypertension, and 19% higher risk of developing chronic heart disease[18]. Based on over 4000 Vietnam Veterans without CV disease at baseline, those with PTSD were at 2.2 times greater risk of CV mortality[19]. Taken together, these studies lead to the conclusion that PTSD is significantly and independently associated with an increased risk of hypertension, CV disease, and premature death[16, 18, 19].

Mechanisms of Increased CV Risk in PTSD

Prior studies have established a link between PTSD and increased risk of CV disease, but they have not identified the mechanisms underlying this relationship[39]. A proposed mechanism between PTSD and increased CV risk is attributed to allostasis, the process by which the body makes physiologic adjustments in response to external stressors[36]. After being exposed to stress, the body attempts to re-achieve internal balance through physiological manipulations, particularly by the autonomic nervous system[78].

The nervous system activates and inhibits appropriate pathways to reach homeostasis, the stable points set by the body[78]. The nervous system consists of the central nervous system (CNS), which comprises the brain and the spinal cord, and the peripheral nervous system[78]. The CNS controls the body activities along with the peripheral nervous system that connects the CNS with sensory and motor divisions of our body[78]. Under motor divisions, a somatic nervous system and an autonomic nervous system (ANS) respectively control voluntary and involuntary movements[78]. The ANS controls involuntary body processes such as the BP, heart rate (HR), and respiration[78]. ANS includes the sympathetic nervous system (SNS) that controls the "fight-or-flight" response and the parasympathetic nervous system (PNS) that manages the "rest and digest" functions[78]. SNS activation increases BP and HR, while PNS activation decreases BP and HR to maintain homeostatic conditions[78]. The SNS is activated during stress, while the PNS counterbalances SNS to elicit the opposite reaction[78].

High allostatic load may lead to chronic physiologic changes in PTSD characterized by elevated SNS activity[36]. During recovery from stress, reduction of SNS and increase in PNS restores homeostasis; however, with chronic stress, as experienced by patients with PTSD, SNS remains chronically activated and PNS remains deactivated. In conjunction with the proposed allostatic mechanism, a prior study from our laboratory demonstrated that PTSD is associated with augmented SNS reactivity during stress and heightened SNS activity at rest[36]. We have shown that Veterans with PTSD greater SNS and BP reactivity during mental stress compared to Veterans without PTSD[36]. Elevated BP and SNS overactivity are known risk factors for CV disease, which may be a causal link between PTSD and CV risk[15, 53-55].

Blood Pressure and Relationship to Autonomic Modulation

While high BP has serious health consequences, it often has no warning symptoms[48, 51]. The diagnosis of hypertension is made by measurement of arterial BP indicate the pressure generated in the resistance vessels by the heart pumping blood into the vessels[78]. When the left ventricle of the heart ejects blood into the systemic (body) arteries, arterial BP is generated as a resistance to cardiac output (CO, L/min) [78]. CO represents the amount of blood pumped by the heart per minute, which is calculated as the product of the heart rate (beats/min) and stroke volume (SV, L/beat). SV is the volume of blood pumped from each ventricle during heart contraction[78]. SV is calculated by calculating the difference between blood pumped into the heart (end-diastolic volume (EDV) or preload) and blood ejected from the heart (end-systolic volume (ESV) or afterload) [78]. SV increases when preload increases or afterload decreases [78]. An increase in contractility of the cardiac muscles in the ventricle is also associated with an increase in sympathetic activity that causes SV to increase [78].

During heart contraction (ventricular systole), blood volume in the blood vessels increases as blood is ejected into arteries, and the wall of the aorta, the largest vessel connecting heart and body organs, stretches[78]. This is the maximum pressure exerted in the arteries, which is known as systolic blood pressure (SBP) [78]. As the heart relaxes (ventricular diastole), blood volume in the arteries decreases as blood in the arteries is distributed to body organs[78]. Diastolic blood pressure (DBP) is the minimum pressure in the arteries during heart relaxation[78]. Through changes in the vessel diameter, BP is controlled, and the blood flow is regulated systemically[78]. Mean arterial pressure (MAP) is commonly described as a general term for BP, as MAP combines both SBP and DBP into consideration[78]. MAP is calculated

by dividing the sum of SBP and twice of DBP by three (MAP= (2 DBP + SBP) / 3) [78]. This equation calculates MAP from systolic and diastolic pressure values[78]. As diastole lasts up to twice as long as systole, the longer duration of DBP is accounted into the equation for MAP[78]. Another equation calculating MAP considers hemodynamic variables[78]. In hemodynamic terms, MAP is the product of CO and total peripheral resistance (TPR) to flow[78]. TPR measures the sum of all vascular resistance within the systemic circulation[78]. Arteries (larger radius) and arterioles (smaller radius) will respectively decrease and increase BP during vasodilation and vasoconstriction[78]. Smooth muscle of blood vessels regulates the radius of these vessels to ultimately regulate blood flow to body organs[78]. DBP, SBP, CO, and TPR are all associated with determining MAP, so changes to these values will affect MAP measurements[78].

BP is commonly measured noninvasively using a sphygmomanometer, which gives systolic and diastolic pressure measurements in mmHg[48]. SBP 90-120 mmHg and/ DBP 60-80 mmHg are considered normal BP ranges[78]. When an individual has SBP (>140 mmHg) or DBP (>90 mmHg), this individual is considered to be hypertensive[3]. Population with SBP (120-139 mmHg) or DBP (80-89 mmHg) in between normal and hypertensive range are considered to be pre-hypertensive[51]. As the only way to know SBP and DBP is through the BP test, it is important to regularly check BP[48]. The gold standard method of measuring BP is by placing an intravenous catheter into the arteries, which measures beat-to-beat intra-arterial BP[80]. The intra-arterial method directly measures BP, but it is invasive and therefore is hard to use in a clinical setting[80]. A sphygmomanometer is a convenient method of measuring BP, as it has an inflatable cuff applied to the upper arm that easily measures SBP and DBP by

increasing and gradually releasing the pressure in the cuff[80]. This method allows the general population to regularly check BP, but it is not appropriate to use in a clinical setting in which beat-to-beat changes to BP is examined. Therefore, a plethysmography technique is commonly used in a research setting as it noninvasively measures beat-to-beat arterial BP by putting finger cuffs around fingers[80].

The autonomic nervous system plays a major role in maintaining hemodynamic stability in our body. PNS and SNS work together to control blood volume, cardiac output, and vascular resistance in a moment-by-moment fashion, to maintain arterial BP within a narrow range[78]. PNS activation lowers heart rate and blood volume in the arteries[78]. Conversely, SNS activation increases CO by increasing both SV and HR, and leads to vasoconstriction, thereby increasing TPR. These effects result in an increase in arterial BP. Chronic elevations of SNS activity have been described in multiple patient populations at increased CV risk such as hypertension, chronic kidney disease, and heart failure[22-24].

Hemodynamic and Sympathetic Hyperactivity in PTSD

Prior studies suggest that PTSD patients have increased BP and SNS reactivity[3, 53-55]. Sympathetic overactivity is hypothesized to play a role in the development of hypertension and CV disease[22-24]. Since SNS overactivity leads to serious health consequences, a better assessment of SNS activity and regulation in PTSD is necessary. Past studies have measured SNS activity at baseline through indirect methods, and have reported higher resting BP and heart rates and decreased heart rate variability (HRV) in PTSD patients [4, 39]. HRV is one of the most convenient ways to evaluate SNS and PNS activity to the heart[60]. HRV correlates

well with PNS activity, but less so with SNS activity [61, 62, 69]. When the autonomic nervous system is functioning properly, SNS and PNS counteract each other, as increases in SNS activity lead to decreases in PNS activity[78]. Therefore, assessing either SNS or PNS can be used to indirectly examine the activity of the other. There are different methods to assess SNS and PNS activity using HRV. There include the time domain and frequency domain methods. These two domains are shown to correlate with each other[62]. Time domain HRV variables measure the time interval between the peak points (R-wave to R-wave) of each heartbeat, while the frequency domain variables quantify the absolute number of rhythms within different frequency ranges observed[61]. Unlike frequency domain HRV values, the time domain HRV measurements are time sensitive, in which time range measurements can range from <1 min to >24 hours depending on the researcher[61, 62].

Under time domain HRV measurements, the measurements include the mean R-R interval (RRI, ms), the standard deviation of N-N intervals (SDNN, ms), the root mean square of successive differences between normal heartbeats (RMSSD, ms), and the percent of consecutive N-N intervals that differ by more than 50ms (pNN50, %) [62]. The highest point of each heartbeat is known as the R-wave[62]. The interval between the two peak points is known as the R-R interval or the N-N interval[60]. The difference between R-R interval and N-N interval is that N-N interval only accounts for every normal R-R interval, as there are premature ventricular contractions that cause R-waves to be abnormal[60]. RRI is inversely correlated with heart rate since the distance between the R-waves is greater when the heart rate is slow[61]. However, the fluctuations in heart rate and RRI do not always conform to one another[69]. While RRI increases have shown to result in decreases in heart rate, the extent of

heart rate reductions may be different for the same degree of increase in RRI[69]. The SDNN value is correlated with the PNS activity, and it is commonly calculated during at least 18 hours for accurate HRV measurements[61, 69]. RMSSD and pNN50 can be measured over relatively short time periods (>1 minute) [61-62]. RMSSD also reflects the beat-to-beat variance in PNS activity by calculating the time differences between heartbeats[61]. In RMSSD, the differences are squared and averaged before the square root of the average is measured[61]. The pNN50 also reflects the PNS activity, and it is the percent of adjacent N-N intervals that differ by more than 50ms divided by the total N-N intervals[61]. Increase in RMSSD and pNN50 indicate PNS activation.

Frequency domain HRV includes low frequency power (LF, ms^2), high frequency power (HF, ms^2), and the ratio of low frequency to high frequency (LF/HF) [61-62]. LF and HF power measures absolute power of the low and high frequency bands that fall within the range of 0.04-0.15 Hz for LF and 0.15-0.40 Hz for HF. As with time domain HRV measurements, different frequency domain HRV variables correlate with PNS and SNS activity. Previously believed to indicate SNS activity, LF is recently believed to show a mix of both PNS and SNS activities[61-62, 66]. The ratio of LF to HF also indicates the mix of both SNS and PNS activity, by estimating the ratio between sympathetic and parasympathetic activation under controlled conditions[66]. As LF and LF/HF values show a mix of PNS and SNS activity, these measurements do not distinguish PNS and SNS activity separately. Conversely, HF reflects PNS activity alone[66]. LF and HF can be measured from short-term (>1 min) recordings[66].

The relationship between the PNS and SNS branches is both linear and non-linear depending on which HRV measurement is studied. An increase in PNS activity is expected to

decrease SNS activity, but a rise in PNS activity has been shown to decrease, maintain, or increase SNS values[59, 60]. There is a consensus for some HRV measurements, while there is ongoing debate for HRV variables such as LF and whether it represents measures of PNS and SNS, PNS only, or SNS only[61]. In addition, some of these HRV measurements have shown contradictory responses in PTSD patients[2, 39]. In the normally functioning nervous system in which SNS and PNS activities are inversely related, mental stress is expected to increase hemodynamic responses through SNS activation and PNS deactivation[78]. However, some studies show exaggerated hemodynamic (heart rate and BP) responses during mental stress (arithmetic or recalling of traumatic events), while others demonstrate blunted hemodynamic responses during mental stress[2, 39]. The mixed results may be due to differences in methodology such as the exposure to mental stress, and differences in the PTSD study populations. However, alterations in responses in hemodynamic reactivity during stress in PTSD patients suggest that these patients may have altered autonomic modulation at rest and during mental stress. Studies have shown that PTSD patients have decreased HF of HRV measurements, which suggests that PTSD patients have decreases parasympathetic activity of the heart[2, 81-82].

Taken together, HRV measurements correlate well with cardiac PNS activity, but are less reliable for estimating SNS activity. To bypass the limitations of HRV on estimating SNS control, our laboratory directly measures SNS activity from the peroneal nerve in humans using the gold-standard technique of microneurography [2, 36]. In this technique, muscle sympathetic nerve activity (MSNA) is measured and quantified directly from a recording electrode placed inside the peripheral nerve[5, 56]. MSNA is a direct measure of sympathetic

nerve traffic innervating the blood vessels that supply blood flow to the lower limb. MSNA is the best way to directly measure baseline and changes in SNS in real-time, as MSNA correlates with cardiac, renal, and total central sympathetic activity[57, 58] We have shown that MSNA responses during combated-related and noncombat related mental stress are exaggerated in PTSD patients[36]. Such augmented SNS reactivity during stress can contribute to increased risk of hypertension and CV disease in this population. High SNS activity increases CO and TPR, thereby increasing BP, but has a number of deleterious effects that are independent of BP, including increased risk of cardiac arrhythmias, vascular stiffness, renal and cardiac fibrosis[70]. These effects accelerate disease progression in these patients, eventually increasing risks of CV mortality. PTSD is characterized by chronic elevation of SNS activity and reactivity, and low PNS activity that may be a mechanism for increased risk of hypertension and CV disease in this population[2, 81-82]. Therefore, therapeutic approaches to intervene on these autonomic imbalance has potential to improve future risk of hypertension and cardiovascular risk in PTSD [15, 53-55]. Pharmacological approaches, such as alpha-adrenoceptor and beta-adrenoceptor blocking agents, are commonly used to inhibit SNS activation in patients with high SNS [63]. These agents inhibit SNS activity through blocking the action of norepinephrine by binding the adrenoreceptors and preventing vasoconstriction and increases in cardiac output. However, these pharmacological approaches are associated with side effects such as dizziness, hypotension (low blood pressure), and bradycardia (abnormally slow heart beat) [63]. Therefore, there is a need for well-tolerated, alternative methods to treat SNS overactivity in PTSD.

Device-Guided Slow Breathing

Slow breathing is recognized by many ancient meditative practices to have beneficial effects. Prior studies performed on normal subjects and patients with hypertension and heart failure have examined beneficial physiologic effects of slow breathing[9, 10, 12]. Slow breathing decreases respiratory rate and elongates the breathing exhalation time of the breathing cycle over time[79]. Some studies have concluded that slow breathing has short-term benefits in reducing BP in normal subjects and patients with hypertension or heart failure[64-68]. Slow breathing is shown to lead to a reduction in BP and sympathetic activity and increased HRV[22]. Respiratory rate modulates autonomic function: SNS activity increases acutely during inspiration and decreases during expiration[79]. Although the precise mechanism underlying the BP-lowering effects of slow breathing is unclear, modulation of SNS and PNS activity has been implicated. Prior study has shown an acute reduction in MSNA during DGB in hypertensive individuals. Slow breathing leads to a compensatory increase in the tidal volume in order to maintain minute ventilation. This increase in tidal volume may activate thoracic stretch receptors that lead to a reflex decrease in SNS activity, thereby leading to a reduction in BP.

Slow breathing has been tested as a possible adjunctive therapy for hypertension and has shown mixed results[36]. Some prior studies have shown acute reduction in BP in hypertensive patients, but others have not observed a significant reduction[62-65, 68]. Studies have investigated sustained effects of slow breathing, and they demonstrated a beneficial effect after 8 weeks of slow breathing in hypertensive population[67]. However, some studies report that there is no significant benefit of slow breathing in BP after 8 to 9 weeks of daily slow breathing[72-73].

Device-guided slow breathing (DGB) method is used in our study to test acute effects of slow breathing. The slow breathing is clinically achieved using the RESPeRATE device, which is FDA-approved for the adjunctive treatment of hypertension and for relaxation[7]. The device monitors breathing rates and effortlessly lowers the rate to 5-6 breaths/min using musical tones that guide breathing rates to sub-physiologic levels. DGB aims to lower the respiratory rate from regular breathing rates from 12-20 breaths/min to 5-6 breaths/min. DGB is an easily usable electronic device that uses biofeedback to measure adherence and success of lowering the respiratory rate in participants using DGB. This device has a belt-type respiratory sensor that monitors breathing movements and uses headphones to guide with breathing tones.

The potential benefits of DGB have not previously been explored in PTSD. DGB may be particularly useful in PTSD given its ease of use, good side-effect profile, relaxation effect, and potential modulation of the autonomic nervous system.We hypothesized that DGB will acutely lower SNS activity and increase PNS activity in PTSD patients. If DGB has beneficial effects on BP and autonomic function, it could represent a novel therapeutic intervention to improve autonomic physiology and to decrease CV risk in PTSD patients.. To test our hypothesis, the effects of acute DGB will be tested in OEF/OIF/OND Veterans with and without PTSD. We hypothesize that DGB at 5 breaths/min will acutely reduce BP, heart rate, and SNS activity, and increase PNS activity in PTSD patients when compared to breathing with a sham device at a normal respiratory rate. We further hypothesize that DGB will have a greater hemodynamic and autonomic effect in PTSD participants compared to non-PTSD participants. An identical sham device (SHAM) that maintains participants'

respiratory rates at normal rates of ~14 breaths/min will be used in Veterans with PTSD to compare the beneficial effects of DGB in Veterans with PTSD. The SHAM device has identical features, musical tones, and respiratory sensor belt as the DGB, and the SHAM will work as a placebo to see if slow breathing alone may have beneficial effects in this population.

Methods

Study Participants

This study enrolled a total of 43 Veterans: 27 patients with PTSD and 16 age-matched Veterans without PTSD as Controls. Inclusion criteria for all 43 participants were OEF/OIF/OND veterans matched for age, gender, race, body mass index (BMI), BP, and family history of hypertension. Exclusion criteria for all participants included hypertension, cardiovascular disease, diabetes, illicit drug use, excessive alcohol use (>2 drinks per day), hyperlipidemia, autonomic dysfunction, and medications known to affect SNS (clonidine, beta blockers, Angiotensin-Converting Enzyme (ACE) inhibitors). Participants were recruited at the Atlanta VA Medical Center (VAMC) from mental health clinics. Veterans with previous PTSD diagnosis on medical records were recruited as PTSD participants and those without PTSD diagnosis were recruited as controls. The Clinician Administered PTSD Scale for DSM-IV (CAPS-IV) was used to confirm the clinical diagnosis of PTSD in the PTSD group.

Ethical Approval

This study conformed to the standards set by the Declaration of Helsinki and was approved by the Emory University Institutional Review Board and the Atlanta VA Medical Center Research and Development Committee. All study participants signed informed consent for study participation prior to study procedures.

Measurements

Blood Pressure

Baseline BP was measured for 10 minutes at rest while participants were rested in a seated position with the arm supported at heart level. To obtain resting BP, sphygmomanometer (Omron HEM-907XL, Omron Healthcare) was used to measure BP a total of 3 times, separated by 5 minutes. This process verified that participants were not hypertensive. During study procedures, appropriate sized cuffs were used on participant's upper arm and on two fingers to monitor continuous and noninvasive arterial BP using digital photoplethysmography (CNAP, CNSystems). This device has been validated to measure absolute and relative changes in BP[25, 26]. BP measurements during 10 minutes of DGB were monitored and recorded during the entire study procedure. These measurements were transmitted and recorded in LabChart 7 (PowerLab 16sp, ADInstruments) using a BioAmp system.

Heart Rate

Baseline heart rate was measured at rest while participants were rested in the same position as above. Continuous heart rate measurement was made using a 3-lead electrocardiography (ECG). Heart rate measurements were monitored and recorded in LabChart 7 (PowerLab 16sp, ADInstruments) during baseline and during 10 minutes of device use.

Muscle Sympathetic Nerve Activity (MSNA) by Microneurography

Microneurography technique was used to monitor muscle sympathetic nerve activity

(MSNA). This technique is performed by inserting a tungsten microelectrode (tip diameter 5-15 μm) in the peroneal nerve which is a large peripheral nerve innervating the lower leg and the vasculature. A reference microelectrode was inserted in close proximity. Microneurography is done on each patient's leg after positioning properly and cleansing the site using alcohol wipes. The signals were amplified (total gain 50,000-100,000), filtered (700-2000 Hz), rectified, and integrated (time constant 0.1 sec) for mean voltage display of sympathetic nerve activity (Nerve Traffic Analyzer, Model 662C-4; Bioengineering, University of Iowa). Based on the participant's leg twitch, location of the nerve was identified and verified. After locating the nerve, two sterile tungsten electrodes were put into their skin, one just under the skin and the other into the nerve. The wire needles were connected to the computer recorder to record nerve activity. The neurogram was recorded to LabChart 7 (PowerLab 16sp, ADInstruments) along with continuous BP and HR recordings using a bioamp. MSNA was expressed as burst frequency (bursts per minute) and burst incidence (bursts per 100 heart beats).

Device-Guided Slow Breathing (DGB)

Participants were instructed to use the RESPeRATE (InterCure, Ltd) device for device-guided slow breathing (DGB) to lower respiratory rates to 5-6 breaths per minute, or an identical sham looking device (SHAM) that maintains respiratory rates at a normal physiologic rate of 14 breaths/min[7]. All participants were guided to use the given device (DGB or SHAM) for 10 minutes during the study procedure. Participants were randomly assigned to DGB or SHAM. With an identical display, both devices included elastic belts

with respiration sensors that were placed around the upper abdomen for biofeedback and headphones that delivered musical tones to guide respiratory rates to subphysiologic rates (~5 breaths/min in DGB) or normal respiratory rates (~14 breaths/min in SHAM). Each device monitored the breathing rate of each participant and calculated inspiration and expiration times to generate a personalized melody to guide respiratory rates. Two distinct tones (ascending and descending) were personalized for inhalation and exhalation guidance. DGB slowly guided the participants to effortlessly lower their respiratory rates while listening to the musical tone while SHAM played the same musical tone to maintain participants' respiratory rates maintained at the same level. Adherence to the RESPeRATE device was checked during the study procedure, and continuous respiratory rates were measured and automatically stored to LabChart 7 (PowerLab 16sp, ADInstruments) using a belt type sensor.

Clinician Administered PTSD Scale (CAPS) IV and the PTSD Checklist

The diagnosis of PTSD was confirmed in all participants using the Clinician Administered PTSD Scale (CAPS) IV and the PTSD Checklist – Military Version (PCL-M). The CAPS is the standardized screening tool used to diagnose and assess the severity of PTSD symptoms. To clinically confirm the presence of PTSD, a severity score of 45 or higher was required on either survey [28, 29]. The diagnosis of PTSD was confirmed for participants in the PTSD group and was excluded for participants in the control group. The survey was administered during the first screening visit.

Experimental Protocol

Screening Visits (1 or 2 visits): 1 hour

1-2 screening visits were done on each patient to check for their qualification for further participation for the study. Each screening visit lasted 30 minutes to an hour. At each screening visit, vitals (BP, pulse, and respiratory rate) were measured and recorded. BP was taken a total of 3 times (separated by 5 minutes) and averaged during each screening visit to check participant's pre-hypertension status (SBP 120-129 mmHg, and/or DBP 80-89 mmHg) and to obtain baseline values. The Clinician Administered PTSD Scale (CAPS) or the PCL-M was tested to confirm the presence and the severity of PTSD in the PTSD group and to exclude the diagnosis of PTSD in the Control group. Participants were randomly assigned to a device-guided slow breathing (DGB; N=27) or an identical sham device (SHAM; N=16) and were instructed on how to breathe using the appropriate device.

Study Visit (Micro visits): ~2hours

Overview

Heart rate, blood pressure, respiratory rate, and nerve activity measured at baseline (10 minutes) and during Device-Guided Slow Breathing or Sham Device (10 minutes)

Qualified participants after screening visits were asked to come in for the study visit at the Atlanta VAMC Clinical Studies Center. Each participant was instructed to fast from food, caffeine and alcohol for at least 12 hours, and exercise and smoking for at least 24 hours before coming in. The experimental procedural room was quiet, semi-dark, and temperate (~21° C). Participants were placed on a comfortable stretcher in a supine position.

Appropriate sized finger cuffs were placed on the fingers of the dominant arm and on the upper arm for continuous beat-to-beat arterial BP measurements and occasional automatic calibrations of the finger cuffs. Continuous heart rate was recorded using 3-lead electrocardiography (ECG) patch electrodes. For monitoring and recording of continuous respiratory rate, the belt-type sensor was placed around the abdomen. Participants' legs were positioned for microneurography, and the sterile tungsten microelectrode was inserted to each patient's leg. The location of the peroneal nerve was identified and verified by the principle investigator, and wire needles were manipulated to obtain a satisfactory nerve recording. Wire needles were connected to the computer for nerve activity recordings. 10 minutes after the nerve recording began, each participant's continuous BP, heart rate, nerve activity, and respiratory rate were measured and recorded. The first 10 minutes of BP, heart rate, nerve activity, and respiratory rate measurements were used as baseline measurements. Following baseline recordings, participants began using the assigned device (DGB or SHAM) for 10 minutes. Patients were monitored for about 10 minutes to check their hemodynamic conditions before being sent home after the study.

Data Analysis

Heart Rate and Heart Rate Variability

During the study procedure, continuous ECG data were recorded using the Labchart 7 (PowerLab 16sp, ADInstruments), and the data was exported to WinCPRS (Absolute Aliens, Turku, Finland) for analysis. The WinCPRS program automatically detected and marked highest points (R-waves) of each heart rate from continuous ECG recordings. R-waves were manually inspected for accuracy of detected and were fixed for inaccurate detections by a single investigator (J. Kang). After all heart rate recordings were inspected, a R-R interval (RRI) channel displaying time and frequency domain HRV measurements was generated. Previous studies found that time and frequency domain of HRV measurements correlate strongly with each other[31]. For instance, a strong correlation was observed between HF (frequency domain) and RMSSD and pNN50 (time domain), and these measurements (HF, RMSSD, and pNN50) were shown to reflect parasympathetic activation[30, 32-34]. Other HRV variables were commonly believed to predict general HRV activity, a mixture of SNS and PNS activity, or measured over a long time period, so they were concluded to be inappropriate for our acute measurements of HRV. Heart rate, RMSSD, pNN50, and HF were recorded in 2-minute time blocks during 10 minutes of baseline and during 10 minutes of device use. Heart rate and HRV data from baseline and the last two minutes of DGB or SHAM were compared.

Blood Pressure

Continuous BP data were recorded with Labchart 7 during the study procedure and exported

to WinCPRS for data analysis. WinCPRS automatically marked appropriate SBP and DBP from corresponding ECG data. Detected SBP and DBP were inspected for accuracy and were fixed for inaccurate detections by a single investigator (J. Kang). After all SBP and DBP inspection, mean arterial pressure (MAP) channel was generated. SBP, DBP, MAP, and PP data were recorded at 10 minutes of baseline and during 10 minutes of DGB in 2-minute time blocks. BP data from baseline and the last two minutes of DGB or SHAM were compared.

Muscle Sympathetic Nerve Activity

Continuous MSNA data were exported from Labchart 7 into WinCPRS for analysis. MSNA bursts were automatically detected and marked in the WinCPRS program by using the following criteria: 3:1 burst to noise ratio within a 0.5-second search window, with an average latency in burst occurrence of 1.2-1.4 seconds from the previous R-wave. After automatic detection of MSNA bursts by the WinCPRS program, the MSNA neurograms were visually inspected for accuracy of detection and edited if needed, by a single investigator (J. Park). MSNA was expressed as burst frequency (bursts/min) and burst incidence (bursts/100 heartbeats).

Statistical analysis

Baseline characteristics were compared between the three groups using a one-way analysis of variance (ANOVA). Three groups (PTSD patients who used DGB (PTSD+DGB), PTSD patients who used SHAM (PTSD+SHAM), and Controls who used DGB (CON+DGB)) were tested. Changes in BP (SBP, DBP, MAP), heart rate, and autonomic variables HRV (RMSSD,

pNN50, HF) and MSNA (burst frequency and burst incidence) were compared among the three groups using repeated measures ANOVA. Statistical analysis was done using the IBM SPSS software (SPSS 22.0, IBM SPSS). We compared changes between 10 minutes of baseline and last 2 minutes of DGB or SHAM. Within each group, effects of assigned breathing intervention after 10 minutes were examined with time as a within factor. To measure between-group differences in PTSD+DGB versus PTSD+SHAM, the device was used as a between factor. The interaction term "time*device" compared the difference between the effect of both devices from baseline to the last 2 minutes of each device. To compare the between-group effects in PTSD+DGB versus CON+DGB, the group was used as a between factor. The interaction term "time*group" compared the difference between the presence of PTSD from baseline to the last 2 minutes of DGB use. Between PTSD+DGB and CON+DGB groups from baseline to last 2 minutes of DGB, effects of DGB were measured in all participants between two groups, and the interaction term "time" compared the effects of DGB in two groups. Two-tailed t-tests were conducted to compare MSNA at baseline and during the breathing intervention between the two PTSD groups. Significantly different baseline variables were accounted for in the analysis. A response to the breathing intervention in each group was calculated as a mean difference between baseline and after 10 minutes of DGB/SHAM. All results were expressed as mean ± standard error. All p-values are two-tailed, with values less than 0.05 were considered statistically significant.

Results

Baseline Characteristics (Table)

Table. Baseline Characteristics – 3 Groups Comparison

Characteristics	PTSD + DGB (n=16)	PTSD + Sham (n=11)	Control + DGB (n=16)	*p* value
Age (years)	34.9 ± 1.8	31.0 ± 1.5	34.3 ± 1.8	0.816
Sex (Male/Female)	12/1	7/0	10/1	-
Race (Black/White)	10/3	7/0	11/0	-
Body Mass Index (kg/m^2)	31.7 ± 1.7	28.3 ± 2.5	28.9 ± 1.6	0.436
CAPS Score	84.3 ± 9.9	69.5 ± 9.0	38.3 ± 4.1	<0.001*
Baseline Hemodynamics				
Heart Rate (bpm)	72.2 ± 4.1	78.8 ± 6.2	67.5 ± 3.8	0.067
Systolic blood pressure (mmHg)	122.7 ± 3.4	130.3 ± 4.2	128.6 ± 2.5	0.117
Diastolic blood pressure (mmHg)	79.9 ± 2.4	83.8 ± 3.1	82.0 ± 2.9	0.510
Mean Arterial Pressure (mmHg)	94.2 ± 2.5	99.3 ± 3.2	97.5 ± 2.5	0.284
MSNA (Burst/min)	26.6 ± 3.3	39.8 ± 4.1	-	.004*
MSNA (Burst/100hb)	14.2 ± 1.1	21.7 ± 1.7	-	.001*

The Table depicts the baseline characteristics for the study population. Values are mean ± SE or number of participants with that condition out of each group. Study participants were primarily comprised of young, African-American males with pre-hypertensive blood pressure. MSNA, muscle sympathetic nerve activity.

A total of 43 OEF/OIF/OND Veterans with PTSD (N=27) and without PTSD (N=16) were enrolled in the study. Veterans with PTSD were randomized to DGB (N=16) and SHAM (N=11). One-way ANOVA test was performed to compare the 3 groups. Among the three groups, there was no significant difference in age, gender distribution, race, or body mass index (BMI). Groups were well matched for age with a mean age of 34.9 ± 1.8 years in the PTSD + DGB group, 31.0 ± 1.5 years in the PTSD+ SHAM group, and 34.3 ± 1.8 years in

the CONTROL + DGB group. The majority of study participants were African-American males with pre-hypertension. Diagnosis of PTSD was confirmed in all participants using CAPS-IV, and as expected, mean CAPS-IV score was significantly higher among PTSD patients compared to controls without PTSD. PTSD+DGB group and PTSD+SHAM group had comparable CAPS-IV scores. Resting mean SBP, DBP, MAP, heart rate, and HRV (RMSSD, pNN50, and HF) were similar among the three groups ($p>0.05$). MSNA data was acquired only in the PTSD participants, in which they were randomized into the DGB and the SHAM groups. Both MSNA (burst incidence and burst frequency) were significantly higher at baseline in the PTSD+DGB group than in the PTSD+SHAM group both for burst frequency (26.6 ± 3.3 vs 14.2 ± 1.1) and burst incidence (39.8±4.1 vs 21.7 ± 1.7). The difference in baseline MSNA was accounted for when interpreting changes to MSNA after breathing intervention.

Heart Rate Variability at Baseline

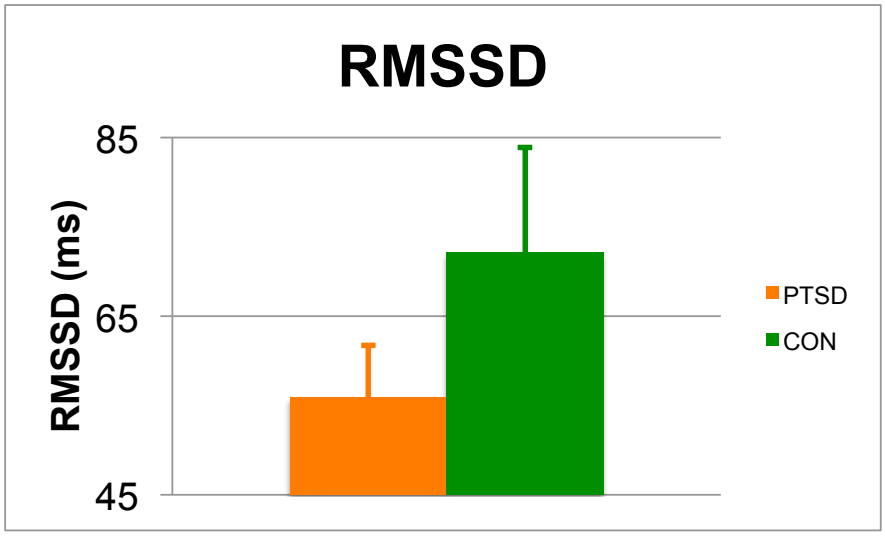

Figure 1A. RMSSD (root mean square of the successive differences) at Baseline

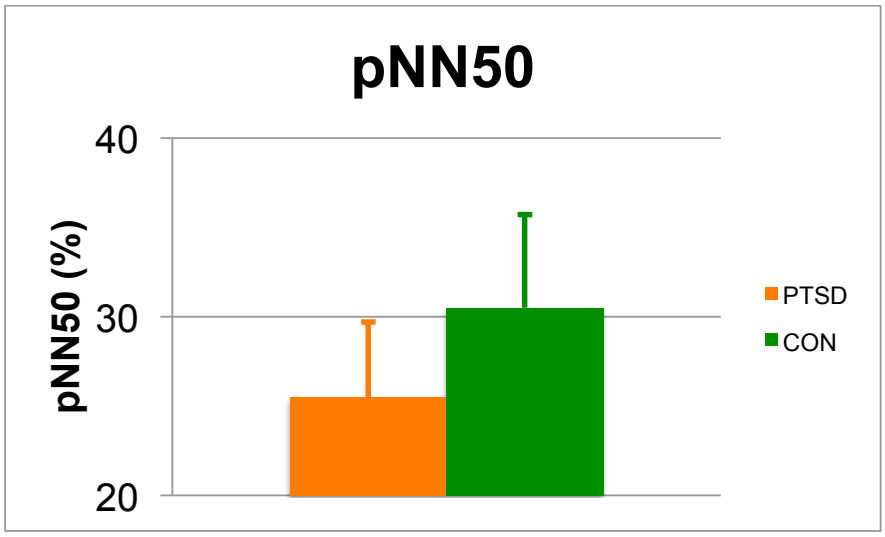

Figure 1B. pNN50 (proportion of NN > 50ms) at Baseline

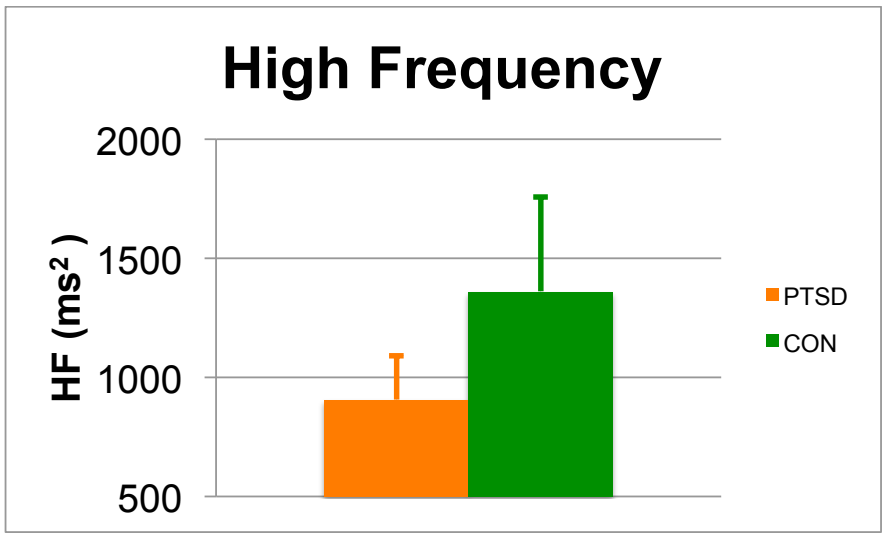

Figure 1C. High Frequency at Baseline

Figure 1A-C depict HRV variables (RMSSD, pNN50, and HF) at baseline. These figure show that HRV tend to be lower on PTSD patients compared to the Controls. However, the comparison between the two groups was not significant (Figure 1A-C).

At baseline, there is a trend lowers lower RMSSD, pNN50, and HF in PTSD than in non-PTSD **(Figure 1A-C)**. These measures of HRV indicate parasympathetic activity, and these figures suggest that parasympathetic activity may be reduced in PTSD compared to PNS in Controls **(Figure 1A-C)**.

Hemodynamic responses during breathing intervention

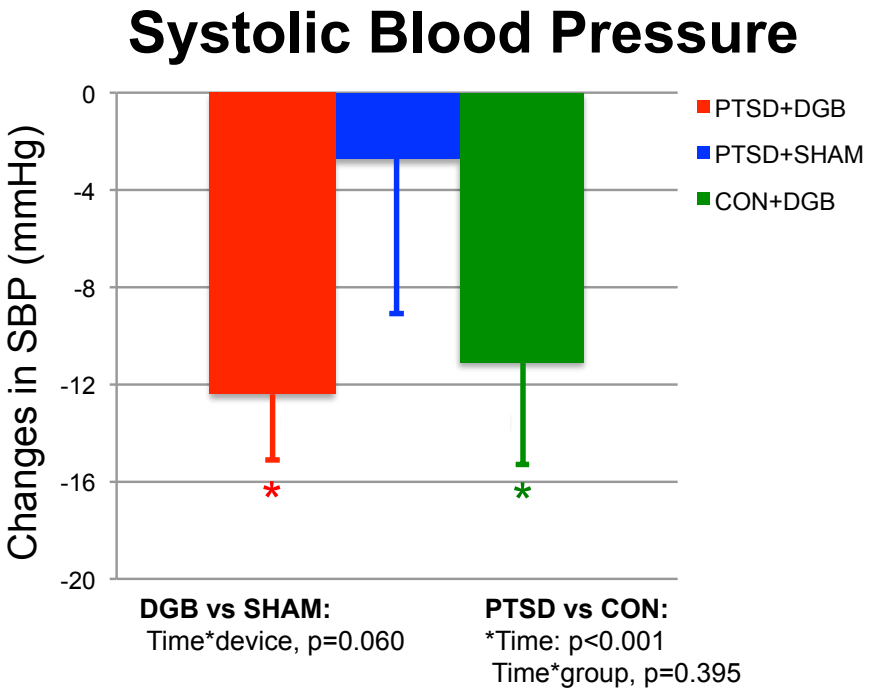

Figure 2A. Systolic Blood Pressure (SBP)

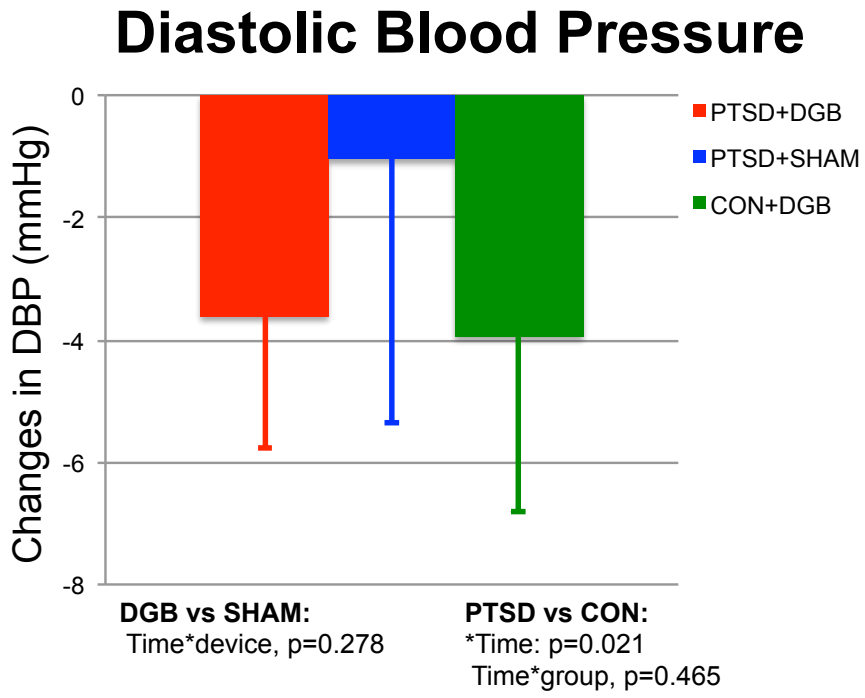

Figure 2B. Diastolic Blood Pressure (DBP)

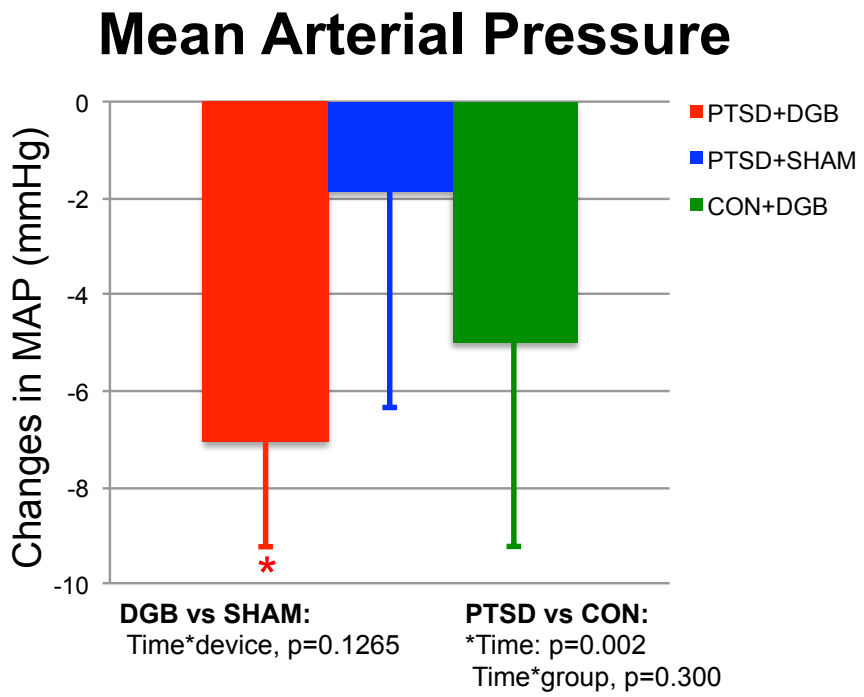

Figure 2C. Mean Arterial Pressure (MAP)

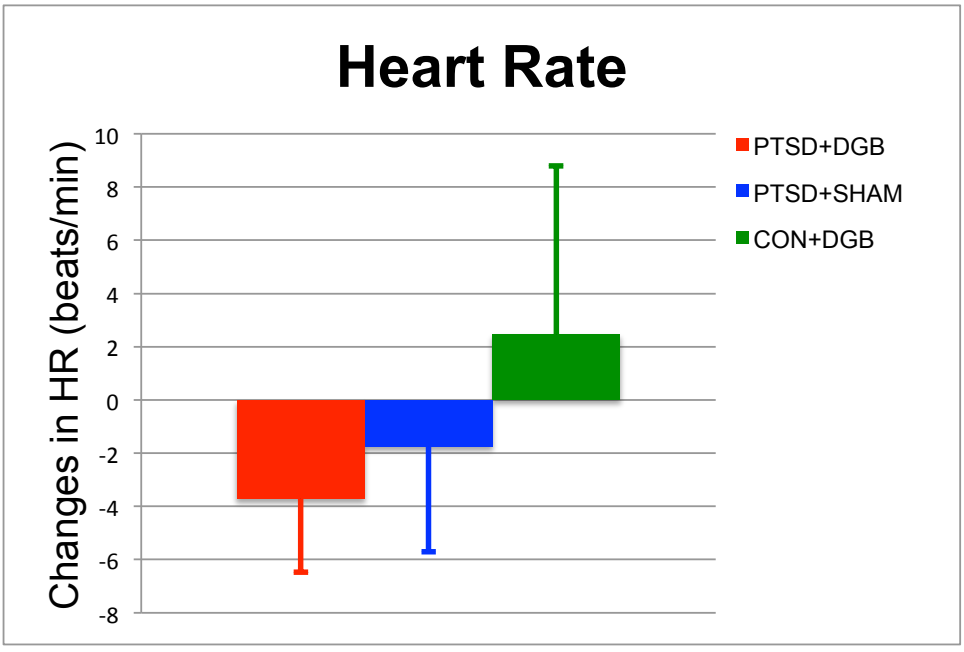

Figure 2D. Heart Rate

Figure 2A-D. Changes in SBP, DBP, MAP, and heart rate between 10 minutes of baseline and after 10 minutes of breathing intervention. Changes in BP and heart rate compared within each group, between PTSD+DGB and PTSD+SHAM groups, and between PTSD+DGB and CON+DGB groups. *Significant p-values < 0.05

After 10 minutes of breathing intervention, there was a greater reduction in SBP within PTSD+DGB group (time, $p<0.001$) than within CON+DGB group (time, $p=0.0105$) (**Figure 2A**). There was no significant difference in the change in SBP with intervention within PTSD+SHAM group (time, $p=0.3385$) **(Figure 2A)**. Between the two PTSD groups (PTSD+DGB versus PTSD+SHAM), there was a trend towards a significantly greater reduction in SBP with DGB versus SHAM (time*device, $p=0.0595$) **(Figure 2A)**. When measuring changes during 10 minutes of DGB use in PTSD+DGB and CON+DGB groups, there was a significant reduction in SBP (time, $p<0.001$) **(Figure 2A)**. However, DGB did not differentially affect SBP between the PTSD and Control groups (time*group, $p=0.395$) **(Figure 2A)**.

There was a trend towards reduction in DBP within the PTSD+DGB group (time, $p=0.0565$), but no significant change in DBP was observed with intervention in the PTSD+SHAM group (time, $p=0.4075$) or in the CON+DGB group (time, $p=0.0975$). There was no significant difference in the change in DBP between DGB and SHAM devices in the PTSD groups (time*device, $p=0.2775$) **(Figure 2B)**. In all study participants who used DGB (both PTSD+DGB and CON+DGB), DGB had a significant effect in reducing DBP (time, $p=0.021$), but there was no significant difference in the DBP-lowering effect of DGB between PTSD and Control groups (time*group, $p=0.4645$) **(Figure 2B)**.

10 minutes of DGB significantly reduced MAP within PTSD patients (time, $p=0.003$), and there was a trend towards reduced MAP with DGB within Controls (time, $p=0.077$) **(Figure 2C)**. There was no significant effect of SHAM on MAP after 10 minuteswithin PTSD patients (time, $p=0.3435$) **(Figure 2C).** In PTSD groups, there was no significant

difference in the change in MAP with intervention between the DGB and SHAMgroups (time*device, p=0.1265) **(Figure 2C).** In PTSD and Control participants, 10 minutes of DGB led to significant reduction in MAP (time, p=0.002). There was no significant difference in the MAP response to DGB between PTSD and Controls (time*group, p=0.3).

During 10 minutes of breathing intervention with DGB or SHAM, there was no significant difference in heart rate within each group: PTSD+DGB (time, p=0.201), PTSD+SHAM (time, p=0.671), and CON+DGB (time, p=0.263) **(Figure 2D).** In PTSD participants, there was no difference in heart rate response between the DGB and SHAM interventions (time*device, p=0.679) **(Figure 2D)**. There was no significant change in heart rate in all participants (PTSD and Controls) with 10 minutes of DGB use (time, p=0.088), and there was no significant difference in heart rate response to DGB between PTSD patients and Controls (time*group, p=0.919) **(Figure 2D).**

Autonomic response during breathing intervention

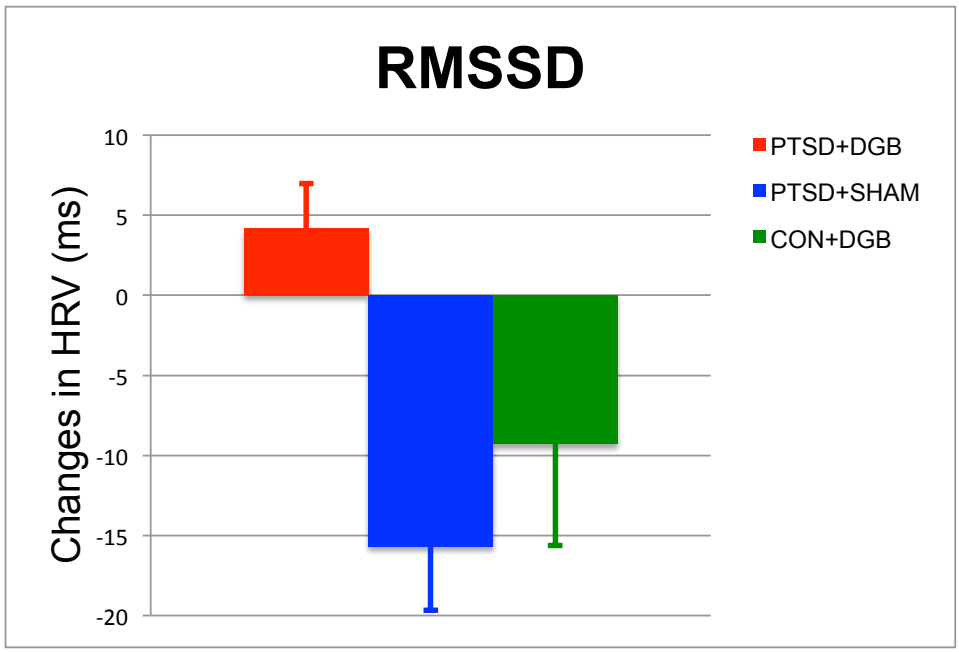

Figure 3A. RMSSD

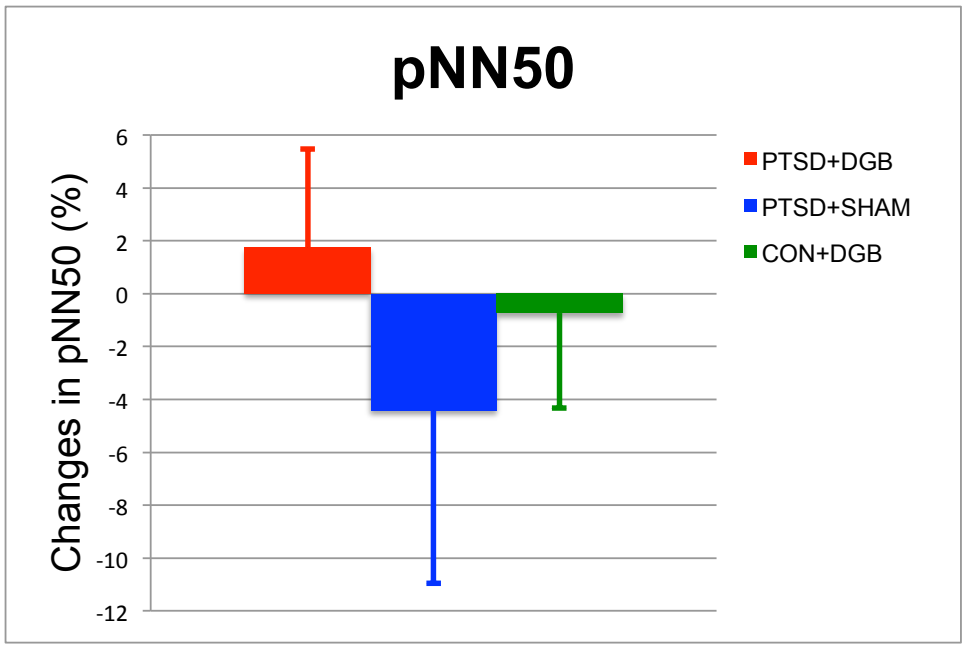

Figure 3B. pNN50

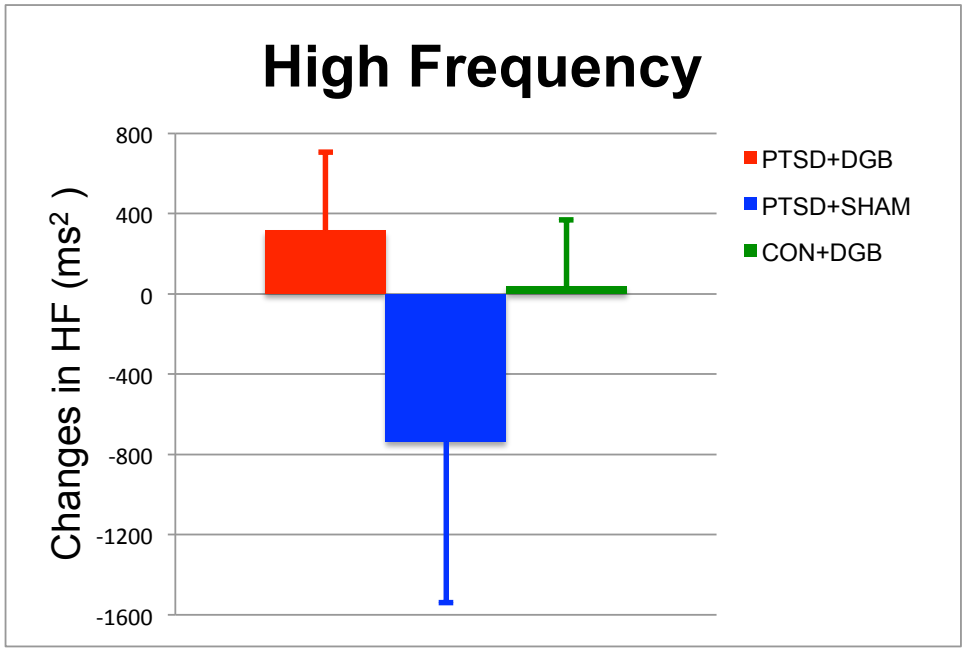

Figure 3C. High Frequency (HF)

Figure 3A-C Changes in RMSSD, pNN50, and HF between 10 minutes of baseline and after 10 minutes of breathing intervention. Changes in parasympathetic activity compared within each group, between PTSD+DGB and PTSD+SHAM groups, and between PTSD+DGB and CON+DGB groups. *Significant p-values < 0.05

PNS activity was measured using three HRV measurements: RMSSD, pNN50, and HF. Within all three groups (PTSD+DGB, PTSD+SHAM, CON+DGB), there was no significant difference in RMSSD (time, p=0.679, p=0.2335, p=0.1025), pNN50 (time, p=0.647, p=0.247, p=0.4215), and HF (time, p=0.435, p=0.383, p=0.911) with breathing intervention **(Figure 3A-C).** There was no significant difference in PTSD patients using DGB versus SHAM in the change in RMSSD (time*device, p=0.342), pNN50 (time*device, p=0.344), and HF (time*device, p=0.202) **(Figure 3A-C).** There was no significant difference in thechange in RMSSD (time, p=0.685), pNN50 (time, p=0.847), and HF (time, p=0.505)) with 10 minutes of DGB use between the PTSD and non-PTSD Controls **(Figure 3A-C).** There was no significant difference in the change in RMSSD (time*group, p=0.289), pNN50 (time*group, p=0.639), and HF (time*group, p=0.6) **(Figure 3A-C)** with DGB use between PTSD and non-PTSD controls.

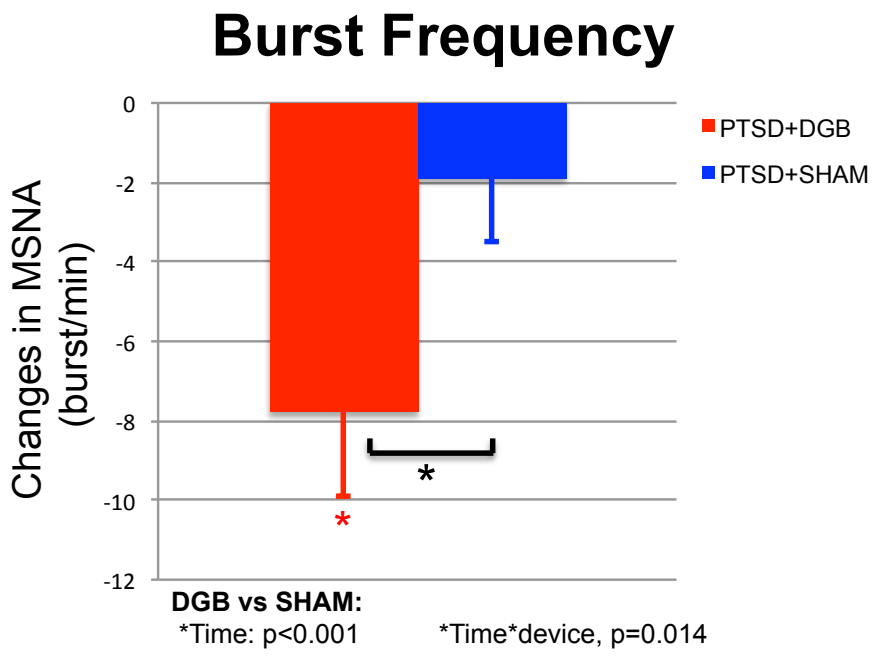

Figure 4A. Burst Frequency

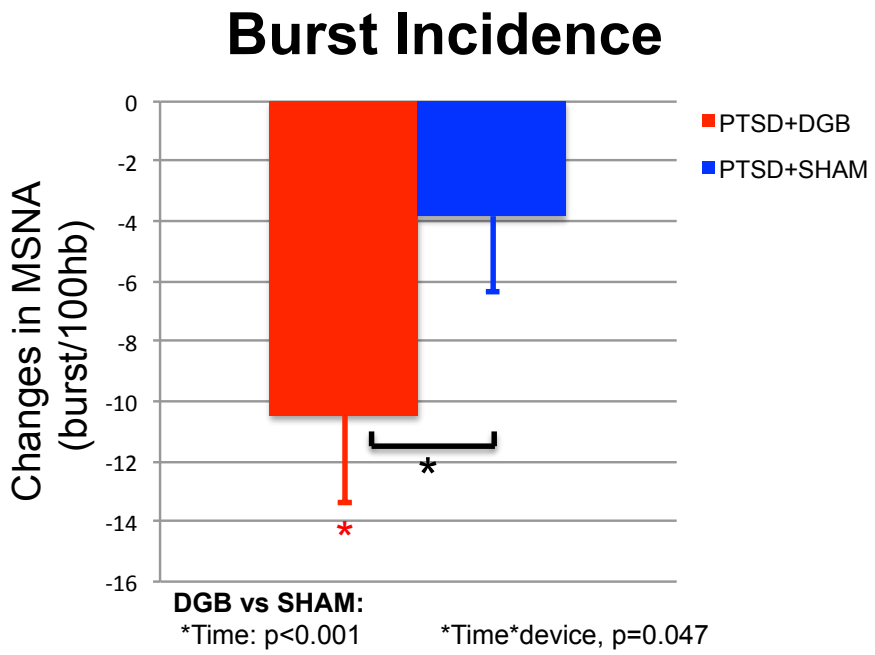

Figure 4B. Burst Incidence

Figure 4A-B. Changes in MSNA (Burst Frequency and Burst Incidence) between 10 minutes of baseline and after 10 minutes of breathing intervention. Changes in parasympathetic activity compared within each group, between PTSD+DGB and PTSD+SHAM groups, and between PTSD+DGB and CON+DGB groups. *Significant p-values < 0.05

Change in MSNA burst frequency (burst/min) and burst incidence (burst/100heartbeat) during 10 minutes of breathing intervention was used to measure changes in SNS activity. Similar to BP response, DGB significantly reduced in MSNA burst frequency (time, p=0.004) and MSNA burst incidence (time, p=0.003) within the PTSD+DGB group (Figure 3A, 3B). Within PTSD patients using the SHAM device, there was a significant change in MSNA burst incidence (time, p=0.033) and a tendency towards a reduction in MSNA burst frequency (time, p=0.060) **(Figure 4A-B).** MSNA significantly decreased after 10 minutes of breathing intervention (both DGB and SHAM) (time, p<0.001), mainly due to DGB having a

greater effect on PTSD patients. Between the PTSD groups using DGB or SHAM, the change in MSNA burst frequency (time x group, p=0.021) and burst incidence (time x group, p=0.037) were significantly different **(Figure 4A-B).** The PTSD+DGB group had a higher resting MSNA compared to the PTSD+SHAM group due to chance, as all PTSD participants were randomized between using DGB and SHAM **(Table).** The difference in baseline MSNA between groups was adjusted for during statistical analysis.

Discussion

This study focused on relatively young Veterans with combat exposure in Iraq and Afghanistan without hypertension or CV disease. The goal of this study was to identify the effects of slow breathing using DGB on BP, heart rate, sympathetic, and parasympathetic activity in PTSD. To our knowledge, this is the first study examining the effects of slow breathing using DGB compared to an identical SHAM device to assess BP, HRV, and MSNA responses in both PTSD and non-PTSD Control populations. Our new findings of this study are: 1) 10 minutes of DGB significantly lowers BP and MSNA, while there are no significant reductions in BP and MSNA with SHAM intervention; 2) DGB significantly lowers BP and MSNA both in PTSD and Controls, but there was no significant difference in the BP and MSNA-lower effects of DGB between the groups; 3) DGB acutely lowers sympathetic activity, but does not increase parasympathetic activity. Together, these findings suggest that DGB may have beneficial effects on BP and SNS activity in PTSD.

Over the past 15 years, over 2 million military service members were deployed as part of OEF, OIF, and OND. These service members are returning with high rates of PTSD, estimated at a 20% prevalence of PTSD post-deployment[1]. PTSD is also common in the non-military population. In the general adult population, 7-8% of the US adult population is diagnosed with PTSD during their lifetime[39]. Thus, PTSD is highly prevalent and represents a major public health problem.

PTSD is also independently associated with an increased risk of hypertension and CV diseases[39-43]. Studies in the past have identified long-term consequences of PTSD, including stroke, coronary artery disease, renal or heart failure, vision loss, and mortality[51]. In the

current study, we proposed that chronic stress in PTSD leads to increased allostatic load, leading to chronic maladaptive physiologic adjustments such as such as sympathetic hyperactivity[53-55]. SNS overactivity has been described in many patient populations at increased CV risk, and could lead up to hypertension, heart failure, and renal failure. Prior studies have shown that PTSD is characterized by chronic SNS overactivation, which could be a mechanistic link between PTSD and CV diseases[53-55].

At baseline, we did not observe a significantly elevated BP and heart rate or decreased HRV in PTSD patients compared to Controls. Prior studies found that PTSD patients have higher resting BP and heart rates and lower HRV, suggesting higher sympathetic activity and lower parasympathetic activity at rest[4, 39, 48, 51]. The lack of significant difference in BP and heart rate between the PTSD and non-PTSD controls at baseline may be attributed to the fact that most of our study patients were pre-hypertensive. Although HRV variables were not significantly different between PTSD and Controls, there was a trend towards reduced parasympathetic activity in PTSD patients compared to non-PTSD Controls. Previous studies largely measured sympathetic activity in PTSD patients using indirect measurements such as blood pressure and plasma norepinephrine levels[4, 39]. In this study, we measured sympathetic activity directly by measuring sympathetic nerve traffic in real-time with the microneurography technique, while simultaneously measure parasympathetic nerve activity using HRV measurements with continuous ECG. These concomitant measures at rest and during breathing interventions are unique features of this study.

In this study, we investigated the potential beneficial effects of slow breathing using DGB in PTSD. If DGB has a potential benefit in reducing BP or SNS activity, we expect that

DGB could represent a nonpharmacological and noninvasive intervention to lowering BP or SNS activity, as DGB does not have side effects that current pharmacological interventions such as alpha-blockers and beta-blockers do[61]. Since breathing therapies are often used as a part of cognitive-behavioral therapy in PTSD, DGB may be a clinical way to induce beneficial effects on lowering BP and SNS activity in this population[9, 10, 12]. Previous studies have shown mixed conclusions about the effects of DGB on hemodynamics and autonomic function[10, 13, 71-76]. Previous studies have shown that DGB may reduce arterial BP and lower SNS activity in patients with high BP after 10-15 minutes of daily DGB usage [8-10]. However, some studies report that DGB has no acute benefits to BP and sympathetic activity[73-74]. Studies investigating the effects of DGB in patients with hypertension and chronic heart failure reported acute reduction in SBP in those populations[9, 10, 12]. In the current study, 10 minutes of breathing intervention using DGB lowered BP in both the PTSD and non-PTSD control groups, while there was no significant reduction in BP with SHAM Both SBP (-12 mmHg) and MAP (-7 mmHg) were lowered significantly lowered with DGB within PTSD+DGB group, but only MAP (-5 mmHg) was significantly decreased with DGB within the CON+DGB group, suggesting that PTSD patients may benefit more from using DGB. Even though BP significantly decreased within the PTSD+DGB group, while there was no change in BP within the PTSD+SHAM group, there was no statistically significant difference in BP responses to intervention between the PTSD+DGB versus PTSD+SHAM groups. This suggests that guided breathing alone, whether participants use DGB or SHAM, may have a beneficial effect on reducing BP, perhaps through a relaxation effect. Further, the difference in BP changes were not statistically significant between PTSD+DGB and CON+DGB groups,

which suggests that DGB reduces BP PTSD patients and in Controls, and PTSD does not affect the BP-lowering effect of DGB.

Other studies in the past reported the effects of slow breathing by using calm music or spontaneous breathing[9, 72, 76]. They investigated HRV measurements in variables such as low-frequency, reporting that slow breathing increase low-frequency measurements[13]. In our study, we assessed HRV using RMSSD, pNN50, and HF as these correlate with PNS activity, rather than a mix of PNS and SNS activity[30, 32-34]. There were no significant differences in changes in heart rate, RMSSD, pNN50, and HF during 10 minutes of breathing intervention with DGB or SHAM, suggesting that heart rate and parasympathetic activity are not affected by slow breathing. Past studies also investigated the effects of slow breathing on sympathetic activity, and showed that slow breathing lowers MSNA in healthy participants, patients with hypertension and heart failure [9, 10, 12]. Consistent with previous findings in other patient population, we observed a significant reduction in MSNA with DGB in both Veterans with and without PTSD measured as MSNA burst frequency (-8 mmHg) and burst incidence (-10 mmHg). This finding suggests that DGB effectively acutely reduces sympathetic activity in PTSD patients. In addition, there is a significant difference in changes in burst frequency (-6 mmHg) and burst incidence (-9 mmHg) between PTSD+DGB and PTSD+SHAM groups, suggesting that lowering respiratory rates of DGB are more beneficial to lowering SNS activity in PTSD patients. Overall, there was a reduction in BP and in SNS activity, but PNS activity remained unchanged.

The mechanisms underlying the acute BP lowering effect of slow breathing remains unknown, but we propose that sympathetic activity was affected by DGB but not

parasympathetic activity because sympathetic activity plays a more diverse role in manipulating physiologic systems. While the sympathetic system has effects on controlling all aspects of blood pressure, parasympathetic system primarily affects blood volume and heart rate. As both heart rate and PNS were unaffected by slow breathing in our study, it suggests that DGB does not have a significant effect on PNS or heart rate. These findings suggest that instead of modulating PNS activity, DGB lowers BP in PTSD by lowering SNS activity in PTSD As SNS activity was lowered and heart rate remained unaffected after 10 minutes of DGB, the sympathetic system could have affected SV or TPR, both of which affect BP[78]. If TPR was affected due to changes in sympathetic activity, then DBP is also usually affected since DBP is affected by afterload, which controls blood returning to the heart[78]. Thus, MSNA correlates more with changes in DBP. The resistance within vessels affects blood returning back to the heart, but since DBP was unaffected by DGB, we speculate that TPR was not modulated by changes in sympathetic activity during DGB. Although not directly measured, we speculate that reduction of sympathetic activity during DGB may have lowered SV of study participants, which reduced cardiac output and ultimately MAP. SV is affected both by preload and contractility, and thus lowering of pre-load and contractility may also lower BP.

We found potential benefits of DGB acutely lowered SNS activity and BP in PTSD. Contrary to our hypothesis, we did not observe a greater SNS-lowering or BP-lowering effect of DGB in PTSD compared to the non-PTSD Controls. Instead, we observed that the reductions in BP and SNS activity with DGB were comparable between the PTSD and non-PTSD controls. The reason for this finding is unclear, but may be due to the presence of

prehypertension in both the PTSD and non-PTSD control populations. Prehypertensive patients are known to have a higher risk of CV disease compared to normotensive individuals, and also have higher SNS activity at rest. Therefore, prehypertensive PTSD and non-PTSD participants may have had a greater benefit of DGB compared to normotensive individuals. Thus, PTSD and prehypertension may be a phenotype that may respond well to autonomic modulation by DGB. In addition, DGB has a relaxation component. As PTSD patients chronically experience stress, slow breathing through DGB may be more beneficial in this population.. PTSD is also associated with sympathovagal imbalance, which is the imbalance between sympathetic and parasympathetic systems[3]. The sympathovagal imbalance between the two autonomic systems could result in increases in SNS activity not always ending up with decreased PNS activity as expected, which could explain why sympathetic activity is lowered while parasympathetic activity remains unaffected[3].

In summary, PTSD is a highly prevalent mental illness that is associated with an increased risk of hypertension and CV diseases[2, 16, 18, 19]. Increases in sympathetic activity in PTSD may play a role in increased risk of developing hypertension and CV disease[48, 51. 551]. We found that DGB acutely lowers BP and MSNA in PTSD. Therefore, DGB could represent a novel nonpharmacological intervention to lower SNS activity and improve CV risk in PTSD.

Limitations

We recognize several other limitations to our study. First, our study population was mainly comprised of African-American Veterans. Whether these findings can be generalized to females, other races and ethnicities, or non-Veterans are unclear. Second, we used a CAPS IV score of 45 and above to confirm the diagnosis of PTSD in patients, and patients with a score below a 45 were excluded to the Control group. The mean CAPS score in the Control group was a 38, which is not enough to be classified in the PTSD group but is enough to be considered to be the range for subthreshold PTSD (CAPS score 20-39). A To be considered for not having PTSD at all, a CAPS score between 0 and 19 is needed. As our study participants were all service members, they had combat exposures, which could be a reason why all Control population in our study had a CAPS score within the subthreshold PTSD range. According to recent studies, subthreshold range PTSD is also associated with physiologic changes[77]. We investigated the effect of DGB in PTSD to compare with the benefits in Controls, but our Controls were in the subthreshold range for PTSD. By investigating Controls without any PTSD symptoms (CAPS score 0-19) or any combat exposures, we could have observed a more significant difference between PTSD and Control groups with the use of DGB. Third, the DGB was supposed to lower respiratory rates at 5-6 breaths/min and the SHAM was supposed to maintain at 14 breaths/min. However, some patients had higher or lower respiratory rates than expected after 10 minutes of DGB/SHAM. Fourth, only SNS activity from the muscle was measured during breathing intervention. However, the effects of DGB on sympathetic and parasympathetic activity in other organs such as the heart remain unknown. Finally, we did not measure MSNA from Controls, so our

findings on reduced sympathetic activity may not be generalized to Controls. While we expect that Controls also acutely benefitted in their sympathetic activity after using DGB, we would like to study further to confirm this proposal. If Controls have a reduction in sympathetic activity as well, then we would be able to conclude that DGB reduces sympathetic activity in all our study participants.

Future Directions

The next steps of this study include collect data from additional subjects. We will enroll more participants to both device guided slow breathing and to the sham device. We will analyze MSNA data in Controls and in additional subjects to compare the effects of DGB and SHAM on SNS activity in in Controls as well. After enrolling enough pre-hypertensive participants, determining the effect of prehypertension on BP and SNS-lowering effects of DGB would also be interesting. We observed that DGB has a beneficial effect in reducing BP, so DGB may be especially beneficial to pre-hypertensive population. Further experiments can be done on Control patients using the SHAM device. Most importantly, the project will also extend to a clinical trial testing the long-term benefits of DGB versus SHAM on hemodynamic and autonomic parameters to determine whether 8 weeks of daily device use improves resting BP, heart rate, PNS, and SNS activity in Veterans with PTSD. Determining the long-term effects of DGB will be critical to understanding whether DGB may have a clinical impact of future CV risk in PTSD.

References

1. Hoge, C. W., Castro, C. A., Messer, S. C., McGurk, D., Cotting, D. I., & Koffman, R. L. (2004). Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. *N Engl J Med, 351*(1), 13-22. doi:10.1056/NEJMoa040603
2. Cohen, B. E., Marmar, C., Ren, L., Bertenthal, D., & Seal, K. H. (2009). Association of cardiovascular risk factors with mental health diagnoses in Iraq and Afghanistan war veterans using VA health care. *Jama, 302*(5), 489-492. doi:10.1001/jama.2009.1084
3. Matthews, K. A., Woodall, K. L., & Allen, M. T. (1993). Cardiovascular reactivity to stress predicts future blood pressure status. *Hypertension, 22*(4), 479-485.
4. Buckley, T. C., & Kaloupek, D. G. (2001). A meta-analytic examination of basal cardiovascular activity in posttraumatic stress disorder. *Psychosom Med, 63*(4), 585-594.
5. Mano, T., Iwase, S., & Toma, S. (2006). Microneurography as a tool in clinical neurophysiology to investigate peripheral neural traffic in humans. *Clin Neurophysiol, 117*(11), 2357-2384. doi:10.1016/j.clinph.2006.06.002
6. Matsukawa, T., Gotoh, E., Hasegawa, O., Shionoiri, H., Tochikubo, O., & Ishii, M. (1991). Reduced baroreflex changes in muscle sympathetic nerve activity during blood pressure elevation in essential hypertension. *J Hypertens, 9*(6), 537-542.
7. Sharma, M., Frishman, W. H., & Gandhi, K. (2011). RESPeRATE: nonpharmacological treatment of hypertension. *Cardiol Rev, 19*(2), 47-51. doi:10.1097/CRD.0b013e3181fc1ae6
8. Viskoper, R., Shapira, I., Priluck, R., Mindlin, R., Chornia, L., Laszt, A., . . . Alter, A. (2003). Nonpharmacologic treatment of resistant hypertensives by device-guided slow breathing exercises. *Am J Hypertens, 16*(6), 484-487.
9. Oneda B, Ortega KC, Gusmao JL, Araujo TG, Mion D, Jr. Sympathetic nerve activity is decreased during device-guided slow breathing. *Hypertens Res.* Jul 2010;33(7):708-712.
10. Joseph CN, Porta C, Casucci G, et al. Slow breathing improves arterial baroreflex sensitivity and decreases blood pressure in essential hypertension. *Hypertension.* Oct 2005;46(4):714-718.

11. Elliot WJ, Izzo JL, Jr., White WB, et al. Graded blood pressure reduction in hypertensive outpatients associated with use of a device to assist with slow breathing. *J Clin Hypertens (Greenwich).* Oct 2004;6(10):553-559; quiz 560-551.
12. Bernardi L, Porta C, Spicuzza L, et al. Slow breathing increases arterial baroreflex sensitivity in patients with chronic heart failure. *Circulation.* Jan 15 2002;105(2):143-145.
13. Anderson DE, McNeely JD, Windham BG. Device-guided slow-breathing effects on end-tidal CO(2) and heart-rate variability. *Psychol Health Med.* Dec 2009;14(6):667-679.
14. Wallin BG, Esler M, Dorward P, et al. Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J Physiol.* 1992;453:45-58.
15. McFarlane AC. The long-term costs of traumatic stress: intertwined physical and psychological consequences. World Psychiatry. 2010;9:3–10.
16. Granado NS, Smith TC, Swanson GM, Harris RB, Shahar E, Smith B, Boyko EJ, Wells TS, Ryan MA. Newly reported hypertension after military combat deployment in a large population-based study. *Hypertension.* 2009;54:966-973
17. Mancia G, Grassi G, Giannattasio C, Seravalle G. Sympathetic activation in the pathogenesis of hypertension and progression of organ damage. *Hypertension.* Oct 1999;34(4 Pt 2):724-728.
18. Boscarino JA. A prospective study of PTSD and early-age heart disease mortality among Vietnam veterans: implications for surveillance and prevention. *Psychosom Med.* Jul 2008;70(6):668-676.
19. Kang HK, Bullman TA, Taylor JW. Risk of selected cardiovascular diseases and posttraumatic stress disorder among former World War II prisoners of war. *Ann Epidemiol.* May 2006;16(5):381-386.
20. Grassi G, Seravalle G, Cattaneo BM, et al. Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. *Circulation.* Dec 1 1995;92(11):3206-3211.
21. Grassi G, Seravalle G, Colombo M, et al. Body weight reduction, sympathetic nerve traffic, and arterial baroreflex in obese normotensive humans. *Circulation.* May 26

1998;97(20):2037-2042.

22. Brook RD, Julius S. Autonomic imbalance, hypertension, and cardiovascular risk. Am J Hypertens. 2000;13:112S–122S.
23. Pitzalis MV, Mastropasqua F, Massari F, Passantino A, Colombo R, Mannarini A, Forleo C, Rizzon P. Effect of respiratory rate on the relationships between RR interval and systolic blood pressure fluctuations: a frequency-dependent phenomenon. Cardiovasc Res. 1998;38:332–339.
24. Lanfranchi PA, Somers VK. Arterial baroreflex function and cardiovascular variability: interactions and implications. Am J Physiol Regul Integr Comp Physiol. 2002;283:R815–R826.
25. Jeleazcov C, Krajinovic L, Munster T, Birkholz T, Fried R, Schuttler J & Fechner J. (2010). Precision and accuracy of a new device (CNAPTM) for continuous non-invasive arterial pressure monitoring: assessment during general anaesthesia. *Br J Anaesth* 105, 264-272.
26. Ilies C, Bauer M, Berg P, Rosenberg J, Hedderich J, Bein B, Hinz J & Hanss R. (2011). Investigation of the agreement of a continuous non-invasive arterial pressure device in comparison with invasive radial artery measurement. *Br J Anaesth*.
27. Rudas L, Crossman AA, Morillo CA, Halliwill JR, Tahvanainen KU, Kuusela TA, and Eckberg DL. Human sympathetic and vagal baroreflex responses to sequential nitroprusside and phenylephrine. *Am J Physiol* 276: H1691-1698, 1999.
28. Weathers FW, Keane TM & Davidson JR. (2001). Clinician-administered PTSD scale: a review of the first ten years of research. *Depression and anxiety* 13, 132-156.
29. Weathers FW & Ruscio AM. (1999). Psychometric Properties of Nine Scoring Rules for the Clinician-Administered Posttraumatic Stress Disorder Scale. *Psychological Assessment* 11, 124-133.
30. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation,* 93: 1043-1065, 1996.
31. Blacher, J, Guerin, AP, Pannier, B, Marchais, SJ, Safar, ME, London, GM: Impact of aortic stiffness on survival in end-stage renal disease. *Circulation,* 99: 2434-2439, 1999.

32. Malliani, A, Lombardi, F, Pagani, M: Power spectrum analysis of heart rate variability: a tool to explore neural regulatory mechanisms. *Br Heart J,* 71: 1-2, 1994.
33. Montano, N, Ruscone, TG, Porta, A, Lombardi, F, Pagani, M, Malliani, A: Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation,* 90: 1826-1831, 1994.
34. Kamath, MV, Fallen, EL: Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. *Crit Rev Biomed Eng,* 21: 245-311, 1993.
35. Dutoit AP, Hart EC, Charkoudian N, Wallin BG, Curry TB, and Joyner MJ. Cardiac baroreflex sensitivity is not correlated to sympathetic baroreflex sensitivity within healthy, young humans. *Hypertension* 56: 1118-1123, 2010.
36. Park J, Marvar PJ, Liao P, Kankam ML, Norrholm SD, Downey RM, McCullough SA, Le NA, and Rothbaum BO. Baroreflex dysfunction and augmented sympathetic nerve responses during mental stress in veterans with post-traumatic stress disorder. *The Journal of physiology* 595: 4893-4908, 2017.
37. Von Kanel R, Hepp U, Kraemer B, et al. Evidence for low-grade systemic proinflammatory activity in patients with posttraumatic stress disorder. *J Psychiatr Res.* Nov 2007;41(9):744-752.
38. Walczewska J, Rutkowski K, Wizner B, Cwynar M, Grodzicki T. Stiffness of large arteries and cardiovascular risk in patients with post-traumatic stress disorder. *Eur Heart J.* Mar 2011;32(6):730-736.
39. Bedi US, Arora R. Cardiovascular manifestations of posttraumatic stress disorder. *J Natl Med Assoc*. 2007;99:642-649
40. American Psychiatric Association. (2013) Diagnostic and statistical manual of mental disorders, (5th ed.). Washington, DC: Author.
41. Kilpatrick, D. G., Resnick, H. S., Milanak, M. E., Miller, M. W., Keyes, K. M., & Friedman, M. J. (2013). National estimates of exposure to traumatic events and PTSD prevalence using *DSM-IV* and *DSM-5* criteria. *Journal of Traumatic Stress, 26,* 537-547. doi:10.1002/jts.21848
42. Kessler, R.C., Berglund, P., Delmer, 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. *Archives of General Psychiatry, 62(6)*: 593-602.

43. National Comorbidity Survey. (2005). NCS-R appendix tables: Table 1. Lifetime prevalence of DSM-IV/WMH-CIDI disorders by sex and cohort. Table 2. Twelve-month prevalence of DSM-IV/WMH-CIDI disorders by sex and cohort.
44. Kessler, R.C., & Ustun, T. B. (Eds.). (2008). *The WHO World Mental Health Surveys: global perspectives on the epidemiology of mental disorders*. New York: Cambridge University Press, 1-580.
45. Kulka, R.A., Schlenger, W.A., Fairbanks, J.A., Hough, R.L., Jordan, B.K., Marmar, C.R., ... Cranston, A.S. (1990). *Trauma and the Vietnam War generation: Report of findings from the National Vietnam Veterans Readjustment Study*. New York: Brunner/Mazel.
46. Kang, H.K., Natelson, B.H., Mahan, C.M., Lee, K.Y., & Murphy, F.M. (2003). Post-Traumatic Stress Disorder and Chronic Fatigue Syndrome-like illness among Gulf War Veterans: A population-based survey of 30,000 Veterans. *American Journal of Epidemiology*, 157(2):141-148.
47. Tanielian, T. & Jaycox, L. (Eds.). (2008). *Invisible Wounds of War: Psychological and Cognitive Injuries, Their Consequences, and Services to Assist Recovery*. Santa Monica, CA: RAND Corporation.
48. Chobanian AV. Vascular effects of systemic hypertension. Am J Cardiol 1992;69:3–7.
49. Di Tullio M, Alli C, Avanzini F, Bettelli G, Colombo F, Devoto MA, Marchioli R, Mariotti G, Radice M, Taioli E, Tognoni G, Villella M, Zussino A, for the Gruppo di Studio Sulla Pressione Arteriosa Nell'Anziano. Prevalence of symptoms generally attributed to hypertension or its treatment: study on blood pressure in elderly outpatients (SPAA) J Hypertens. 1988;6:S87–S90.
50. Bidani AK, Griffin KA: Long-term renal consequences of hypertension for normal and diseased kidneys.Curr Opin Nephrol Hypertens 11: 73–80, 2002
51. Fasce E, Flores M, Fasce F. Prevalence of symptoms associated with blood pressure in normal and hypertensive population. Rev Med Chil. 2002;130:160–166.
52. Proietti R, Mapelli D, Volpe B, Bartoletti S, Sagone A, Dal Bianco L, Daliento L. Mental stress and ischemic heart disease: Evolving awareness of a complex association. *Future*

Cardiol. 2011;7:425-437

53. Grassi G. Assessment of sympathetic cardiovascular drive in human hypertension: Achievements and perspectives. *Hypertension*. 2009;54:690-697
54. Julius S. Corcoran lecture. Sympathetic hyperactivity and coronary risk in hypertension. *Hypertension*. 1993;21:886-893
55. Grassi G, Seravalle G, Dell'Oro R, Facchini A, Ilardo V, Mancia G. Sympathetic and baroreflex function in hypertensive or heart failure patients with ventricular arrhythmias. *Journal of hypertension*. 2004;22:1747-1753
56. Rosenthal T, Alter A, Peleg E, Gavish B. Device-guided breathing exercises reduce blood pressure: Ambulatory and home measurements. *Am J Hypertens*. 2001;14:74-76
57. Wallin BG, Esler M, Dorward P, Eisenhofer G, Ferrier C, Westerman R, Jennings G. Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *J Physiol*. 1992;453:45-58
58. Wallin BG, Thompson JM, Jennings GL, Esler MD. Renal noradrenaline spillover correlates with muscle sympathetic activity in humans. *J Physiol*. 1996;491 (Pt 3):881-887
59. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol (2013) 4:26.10.3389/fphys.2013.00026
60. Billman GE, Huikuri HV, Sacha J, Trimmel K. An introduction to heart rate variability: methodological considerations and clinical applications. Front Physiol (2015) 6:55.10.3389/fphys.2015.00055
61. Ko DT, Hebert PR, Coffey CS, Curtis JP, Foody JM, Sedrakyan A, Krumholz HM. Adverse effects of beta‐blocker therapy for patients with heart failure: a quantitative overview of randomized trials. Arch Intern Med 2004; 164: 1389–1394.
62. Barnes VA, Pendergrast RA, Harshfield GA, Treiber FA. Impact of breathing awareness meditation on ambulatory blood pressure and sodium handling in prehypertensive African American adolescents. *Ethn Dis* 18: 1–5, 2008.
63. Chen Y, Yang X, Wang L, Zhang X. A randomized controlled trial of the effects of brief mindfulness meditation on anxiety symptoms and systolic blood pressure in Chinese

nursing students. *Nurse Education Today* 33: 1166–1172, 2012.

64. Hughes JW, Fresco DM, Myerscough R, MHMvD, Carlson LE, Josephson R. Randomized controlled trial of mindfulness-based stress reduction for prehypertension. *Psychosomatic Med* 75: 721–728, 2013.
65. Kingston J, Chadwick P, Meron D, Skinner TC. A pilot randomized control trial investigating the effect of mindfulness practice on pain tolerance, psychological well-being, and physiological activity. *J Psycho- somatic Res* 62: 297–300, 2007.
66. Shaffer, F., & Ginsberg, J. P. (2017). An Overview of Heart Rate Variability Metrics and Norms. *Frontiers in Public Health*, *5*, 258. http://doi.org/10.3389/fpubh.2017.00258
67. Grossman E, Grossman A, Schein MH, Zimlichman R, and Gavish B. Breathing-control lowers blood pressure. *J Hum Hypertens* 15: 263-269, 2001.
68. Blom K, Baker B, How M, Dai M, Irvine J, Abbey S, Abramson BL, Myers MG, Kiss A, Perkins NJ, Tobe SW. Hypertension analysis of stress reduction using mindfulness meditation and yoga: results from the Harmony Randomized Controlled Trial. *Am J Hypertens* 2013.
69. Draghici, A. E., & Taylor, J. A. (2016). The physiological basis and measurement of heart rate variability in humans. *Journal of Physiological Anthropology*, *35*, 22.
70. Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G, Butler J (2009). "The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications". J. Am. Coll. Cardiol. 54 (19): 1747–62. doi:10.1016/j.jacc.2009.05.015. PMID 19874988.
71. de Barros S, da Silva GV, de Gusmao JL, de Araujo TG, de Souza DR, Cardoso CG, Jr., Oneda B, and Mion D, Jr. Effects of long term device-guided slow breathing on sympathetic nervous activity in hypertensive patients: a randomized open-label clinical trial. *Blood Press* 26: 359-365, 2017.
72. Hering D, Kucharska W, Kara T, Somers VK, Parati G, and Narkiewicz K. Effects of acute and long-term slow breathing exercise on muscle sympathetic nerve activity in untreated male patients with hypertension. *J Hypertens* 31: 739-746, 2013.
73. Landman GW, Drion I, van Hateren KJ, van Dijk PR, Logtenberg SJ, Lambert J, Groenier KH, Bilo HJ, and Kleefstra N. Device-guided breathing as treatment for

hypertension in type 2 diabetes mellitus: a randomized, double-blind, sham-controlled trial. *JAMA internal medicine* 173: 1346-1350, 2013.

74. Logtenberg SJ, Kleefstra N, Houweling ST, Groenier KH, and Bilo HJ. Effect of device-guided breathing exercises on blood pressure in hypertensive patients with type 2 diabetes mellitus: a randomized controlled trial. *J Hypertens* 25: 241-246, 2007.
75. Mahtani KR, Nunan D, and Heneghan CJ. Device-guided breathing exercises in the control of human blood pressure: systematic review and meta-analysis. *J Hypertens* 30: 852-860, 2012.
76. Harada D, Asanoi H, Takagawa J, Ishise H, Ueno H, Oda Y, Goso Y, Joho S, and Inoue H. Slow and deep respiration suppresses steady-state sympathetic nerve activity in patients with chronic heart failure: from modeling to clinical application. *American journal of physiology Heart and circulatory physiology* 307: H1159-1168, 2014.
77. Costanzo ME, Leaman S, Jovanovic T, Norrholm SD, Rizzo AA, Taylor P & Roy MJ. (2014). Psychophysiological response to virtual reality and subthreshold posttraumatic stress disorder symptoms in recently deployed military. *Psychosom Med* 76, 670-677.
78. Stanfield, C. L., Germann, W. J., Niles, M. J., & Cannon, J. G. (2009). *Principles of human physiology*. San Francisco: Pearson/Benjamin Cummings.
79. Anderson, D., McNeely, J., & Windham, B. (2009). DEVICE-GUIDED SLOW BREATHING EFFECTS ON END TIDAL CO_2 AND HEART RATE VARIABIALITY. *Psychology, Health & Medicine*, *14*(6), 667–679.
80. Holland WW, Humerfelt S. Measurement of blood-pressure: comparison of intra-arterial and cuff values. BMJ. 1964;2:1241–1243.
81. Chang HA, Chang CC, Tzeng NS, Kuo TB, Lu RB & Huang SY. (2013). Decreased cardiac vagal control in drug-naive patients with posttraumatic stress disorder. *Psychiatry investigation* 10, 121-130.
82. Shah AJ, Lampert R, Goldberg J, Veledar E, Bremner JD & Vaccarino V. (2013). Posttraumatic stress disorder and impaired autonomic modulation in male twins. *Biol Psychiatry* 73, 1103-1110.