

**The Acute Therapeutic Effect of Bhramari Pranayama on Autonomic Function and Self-Reported Anxiety**

by

**John Tyler Butler**

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This thesis was presented

by

**John Tyler Butler**

It was defended on

July 7, 2022

and approved by

Dr. Kelliann Davis, Associate Chairperson  
Department of Health and Human Development

Dr. Brian Galla, Associate Professor  
Department of Health and Human Development

Thesis Advisor: Dr. Christopher Kline  
Associate Professor, Department of Health and Human Development

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# **The Therapeutic Effect of Bhramari Pranayama on Stress**

John Tyler Butler, B.S.

University of Pittsburgh, 2022

The United States is in the midst of a mental health crisis. Individuals are navigating an increasingly complex world during a global pandemic that offers no shortages of uncertainty. Continuous daily stressors accumulated over time without adequate self-regulation measures are exhausting people and promoting disease, both psychological and physiological. One tool that may better equip individuals to manage their mental health and potentially provide numerous health benefits is breath modulation or breathing exercises. The evidence is becoming clear that breathing practices can provide a wide range of practically significant benefits, though evidence for specific methods and results is limited. **Purpose:** This study compared the changes in heart rate variability (SDNN, rMSSD, HF power, LF power), blood pressure (Systolic, Diastolic, MAP), and anxiety level (STAI) resulting from a five-minute practice of Bhramari Pranayama, a specific breathing exercise involving a hummed exhalation, and a five-minute slow breathing practice. **Methods:** Twenty healthy adults (10 females, 10 males) participated in a within-subject crossover study to measure effects from both breathing conditions. Data were gathered with a heart rate monitor, automated sphygmomanometer, and through self-report. Repeated measures analysis of variance models were used to examine mean-level differences in outcomes over time between breathing conditions. **Results:** Both breathing conditions improved HRV measures over time (SDNN [ $P < .001$ ], rMSSD [ $P = 0.03$ ], LF power [ $P < .001$ ]) with the exception of HF power ( $P \geq .362$ ). However, no significant time x breathing condition interaction effects were present for HRV outcomes (each  $P \geq .155$ ). No significant effects were observed between breathing conditions or

over time for blood pressure measures (each  $P \geq .108$ ). For self-reported anxiety (measured with the STAI), the time x breathing condition interaction effect ( $P = .062$ ) and time effect ( $P = .084$ ), trended towards statistical significance, indicating that Bhramari Pranayama may produce more potent anxiety-reducing effects compared to slow breathing. **Conclusion:** Both breathing methods examined may improve HRV indices during practice, while Bhramari Pranayama may more effectively reduce feelings of anxiety. Neither breathing method demonstrated efficacy in reducing blood pressure.

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## **1.0 Introduction**

In the 10 years between 2008 and 2018, reported anxiety increased from 5.1% to 6.7% among adults in the US (Goodwin et al., 2020). Young adults aged 18-25 years, in particular experienced the most significant increase from 8.0% to 14.7%. Stress and anxiety appear to be only increasing further as the Coronavirus disease 2019 (COVID-19) pandemic and the implemented prevention measures have led to an overall reduction in wellbeing along with widespread consequences for physical and mental health (Varma et al., 2021). Shortly following the onset of the COVID-19 pandemic, researchers collected cross-sectional survey data from more than 1,600 participants from around the world to assess anxiety, depression, sleep quality, loneliness, and resilience. Over 70% of respondents reported higher than moderate levels of stress. Nearly two-thirds of respondents met the criteria for clinically significant anxiety and over one-third reported moderate depressive symptoms (Varma et al., 2021). The American Psychological Association (APA) is sounding an alarm bell stating that “we are facing a national mental health crisis that could yield serious health and social consequences for years to come” (Stress in America, 2020). Additionally, data estimates suggest that the direct and indirect economic costs of depression and anxiety are \$210.5 billion and over \$42 billion, respectively (Shalit & Gettas, 2020). These data indicate that individuals need support and efficacious self-regulation strategies to mediate the distress and ultimately help address the secondary pandemic of global mental health decline. Self-regulation refers to the ability to be aware of and appropriately manage one’s energy states, thoughts, emotions, and behaviors in ways that produce beneficial results.

Despite living in a time of widespread comfort and safety, more people than ever are reporting heightened levels of stress, anxiety, and depression. Pharmacological medicine remains

the most common treatment option for these issues. However, medications only work while they're taken, they treat symptoms and almost never address the root cause while also typically having side effects. Non-pharmacological treatment of stress and other mood disorders entails, but is not limited to, individuals working to balance their autonomic nervous system (ANS) states and other homeostatic processes through physical activity, improving sleep, dietary changes, and improving pulmonary and cardiac function. The 2018 Physical Activity Guidelines Advisory Committee Scientific Report concludes that there is strong evidence demonstrating that physical activity reduces state anxiety and the risk of experiencing depression (PA Guidelines. 2018). The physical and mental health benefits of physical activity are well established, and the benefits of physical activity are widely accessible.

An even more accessible tool to improve quality of life may center around learning to breathe better. Of all the low-hanging fruit available to an individual that will improve their overall health and wellbeing, modulating the rate and depth of your breath is at the top of the list. Consciously or subconsciously, you will spend your entire life bringing air into your body and expelling air out of your body. Through the respiratory process, the human body works to bring the vital chemicals of the air inside to fuel organ systems, restore homeostasis, and expel metabolic waste. As research on the effects of specific breathing patterns continues to grow, it is becoming clear that breath modulation exercises are associated with increased wellbeing and positive health outcomes. Chronic shallow breathing leads to a weakened diaphragm and low vagal tone, a correlate of reduced heart rate variability (HRV).

As a noninvasive measure, HRV allows one to measure the influence of the parasympathetic nervous system (PNS) on cardiac regulation. HRV is an important metric to understand for improving quality of life, as an optimal magnitude of variation in HR is indicative

of an individual's capacity to positively adapt to changing situations. Higher HRV is correlated with improved self-regulation on many levels of health (Laborde et al., 2017), while low HRV is a predictor of future health issues (Dekker et al., 1997). Improving HRV is correlated with the ability to better self-regulate stress responses (Breit et al., 2018). People with enhanced self-regulation are better able to maintain internal stability and respond more effectively to challenges and threats; thus, enhanced self-regulation can reduce physical and mental pain, depression, and other neurodegenerative conditions (Sullivan et al., 2018). By modulating breathing to enhance the depth of inhalation and length of exhalation, it is possible to improve the function of the diaphragm and increase vagal tone. Thus, this study aims at investigating *Bhramari Pranayama* (Bhr. P.), a well-known breathing style emphasizing a hummed exhale. Like many breathing practices, Bhr. P. has had relatively few scientific investigations on its physiological or psychological effects.

An often-ignored aspect of maintaining homeostasis and overall health is a focus on preventative approaches to health decline. Yoga therapies and other mind-body therapies offer proven models to help people restore and maintain overall health and perceived well-being. Therefore, focusing a relatively short intervention on an enhanced breathing technique provides an opportunity to observe the potential therapeutic effects and may equip participants with a tool to increase their capacity to respond to perceived anxiety in real-time.

## **1.1 Significance and Rationale**

Stress increases blood pressure and vasoconstriction through the activation of the sympathetic nervous system (SNS). Repeated and chronic instances of stress may lead to the

development of hypertension (Kulkarni et al., 1998). Under normal circumstances, blood pressure (BP) rises and falls in a given day, but when BP is elevated at rest to certain thresholds, we classify it as prehypertension or hypertension. These chronic elevations in BP can have serious health consequences. Unfortunately, about 50 percent of the United States populace have hypertension, which is a leading risk factor for heart disease and stroke, two of the leading causes of death in the U.S. (Stierman, 2021). These data suggest that reducing BP will have a major impact on public health. Individuals dealing with repeated stress will benefit from accessible tools to better self-regulate their psychophysiological states.

Empirical evidence indicates that performing deliberate breathing practices can help promote reductions in stress and improve overall resilience. The literature suggests improvements in heart rate variability, vagal tone, and stress management through yogic breathing or pranayama practices. Most studies observe participants over a length of time to determine the longer-term implications of the often-generalized breath practices. Since the evidence indicates that prolonged practice leads to positive health benefits, it seems appropriate to determine whether short-term therapeutic effects of specific pranayama practices also exist.

The term *Prana* means vital force while *Yama* means control. Taken together, *Pranayama* refers to the vital act of breathing. In practice, Pranayama refers to a set of conscious breathing techniques that regulate inhalation and exhalation by modulating one or more parameters of breathing, such as frequency, depth, or timing. There are various methods of pranayama and each one produces specific physiological responses depending on the breathing parameters modulated. Most scientific investigations into the health effects of pranayama techniques identify benefits from the performance of multiple styles. While preliminary evidence suggests that pranayama

practices are beneficial to human health, there is limited documentation on the specific benefits of individual pranayama styles.

Similar to the differences in health outcomes between aerobic exercise and resistance exercise, specific breathing practices provide varied health effects. To reduce stress and SNS activity, it is appropriate to concentrate on slow breathing techniques. Slow breathing techniques are linked to increased psychophysiological flexibility, HRV, and relaxation, along with decreased anxiety (Zaccaro et al., 2018). This study aims to measure the impact of a single slow breathing practice that incorporates an endogenous vibration through a hummed exhale and compare those results with a similar length session of slow breathing without the hummed exhale.

The hummed exhale of Bhr. P. is shown to increase production of endogenous nitric oxide (NO) levels by 15-fold compared with quiet breathing (Weitzberg & Lundberg, 2002). Nitric oxide is an essential molecule for an array of biological processes that sustain vascular homeostasis. Declining NO release is associated with increased risk factors for CVD such as hypertension and hypercholesterolemia, while the reduced bioavailability of NO is linked with vascular inflammation and constriction of heart vessels (Cannon, 1998). Bhr. P. may then offer practitioners a multi-factored boost to their health with increases in the production of NO.

In 2017, the American College of Cardiology released guidelines to help prevent and manage high blood pressure in adults (Rubenfire, 2018). These guidelines included non-pharmacological interventions such as weight loss, dietary modification, and increased physical activity. There is no mention of breathing techniques nor is there any discussion around supplementing pharmacological treatment with known stress-reducing mindfulness practices. The lack of inclusion around breathwork is a catalyst for the current study, as more evidence is needed to demonstrate the efficacy of pranayama and bring its use into the mainstream. It is well

established that high blood pressure is related to a dysfunctional or overactive SNS and to elevated heart rate. The current scientific literature is indicative that slower breathing is linked to both reductions in SNS activity and heart rate. Slower breathing promotes activity in the parasympathetic nervous system (PNS) (Zaccaro et al., 2018) and reductions in blood pressure (Chaddha et al., 2019).

Given the mental health crisis society is currently facing, the COVID-19 pandemic and the accompanying distress, and the high prevalence of hypertension, it is urgent that individuals are alerted to effective tools that they may use to help reduce anxiety and SNS activity. This study examined the efficacy of a short Bhr. P. intervention to improve HRV and reduce BP and anxiety. We hypothesized that healthy adults who perform five minutes of hummed breaths will improve HRV and reduce BP and anxiety more than those who perform five minutes of slow breathing without the hummed exhale. If this study's hypotheses are supported, it will provide evidence for an accessible and timely tool that individuals can use to improve their cardiovascular health and perceived anxiety.

## **1.2 Primary Aims and Hypotheses**

1. To examine the effect of a single Bhr. P. practice on HRV in healthy adults.

Hypothesis: The group practicing Bhr. P. will have improved measures of HRV when compared to the slow breathing control group.

2. To examine the effect of Bhr. P. practice on BP in healthy adults.

Hypothesis: The group practicing Bhr. P. will have reduced measures of blood pressure when compared to the slow breathing control group.

3. To examine the effect of Bhr. P. practice on anxiety level in healthy adults.

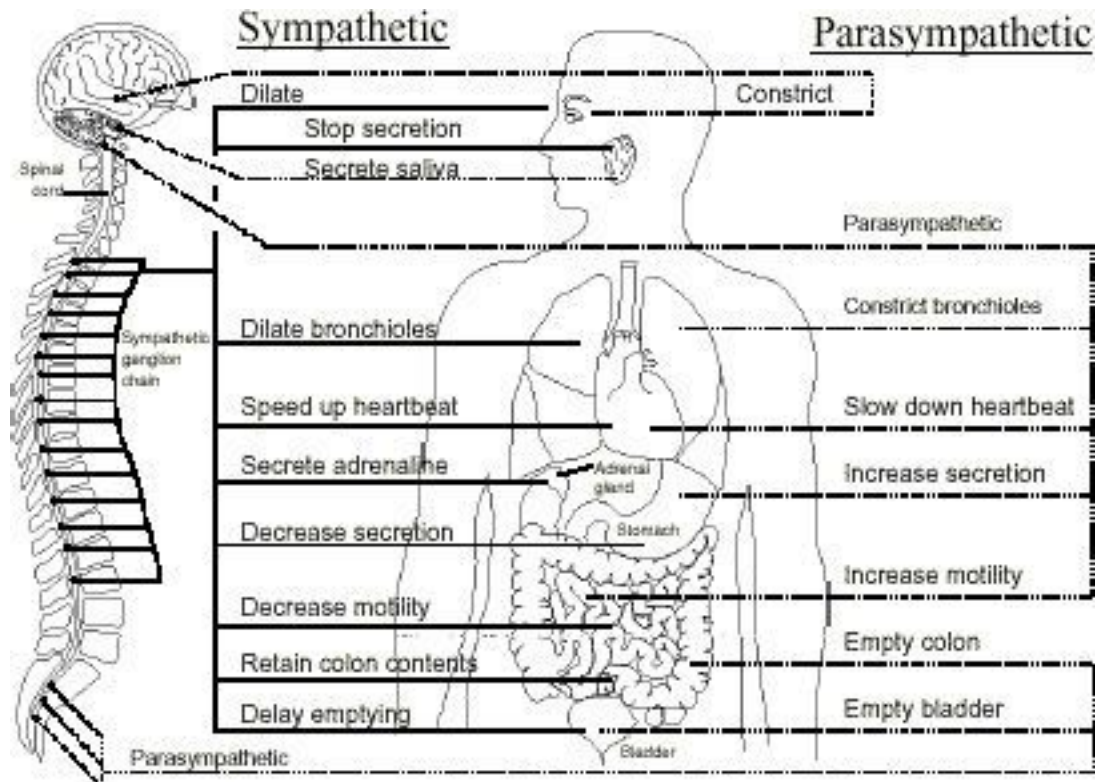
Hypothesis: The group practicing Bhr. P. will have reduced feelings of anxiety when compared to the slow breathing control group.

## 2.0 Literature Review

Stress, as defined by Kenneth Hambly, is a “maladaptive state in which the sympathetic nervous system is overactivated, causing acute or chronic physical, psychological, and behavioral impairment” (Kim et al., 2018). The Yale School of Medicine defines chronic stress as feeling consistently overwhelmed across time (Chronic Stress, n.d.). In 2020, a systematic review and meta-analysis sampling over 9,000 people found that the prevalence of stress in the general population during the pandemic was approximately 30% (Salari et al., 2020). Sympathetic nervous system (SNS) overactivation is directly linked to both psychological and physical conditions such as anxiety, depression, addiction, hypertension, heart disease, and obesity. As a result, the impact that SNS overactivation has on the health of individuals and our society at large is substantial.

The etiology of chronic stress is correlated with an imbalance in the autonomic nervous system (ANS). The ANS is divided into two branches: the SNS and the parasympathetic nervous system (PNS). The SNS classically governs the ‘fight or flight’ response as it works to prepare the body for action. In contrast, the PNS is responsible for ‘rest and digest’ as it acts to preserve energy. These two branches of the ANS work jointly upon the function of most of the internal organs as shown in Figure 1. In the heart, the interaction of the SNS and the PNS affect the sinoatrial (SA) node, often called the pacemaker of the heart, and the atrioventricular node (AV). While at rest, a time of PNS predominance, the SA node maintains HR between 60 to 80 beats per minute. During typical resting conditions, HR varies synchronously with breathing, a phenomenon known as respiratory sinus arrhythmia (RSA). RSA is an index of PNS-mediated cardiac control that can serve as a reliable metric of emotion regulation (Beauchaine, 2015). During inhalation, SNS activity increases and vagal input is inhibited, leading to an increase in HR. Vagal input to the

heart is restored during exhalation, slowing HR (Shaffer et al., 2014). Under normal conditions when stressors are low, the PNS works to bring back physiological homeostasis (McCorry, 2007).

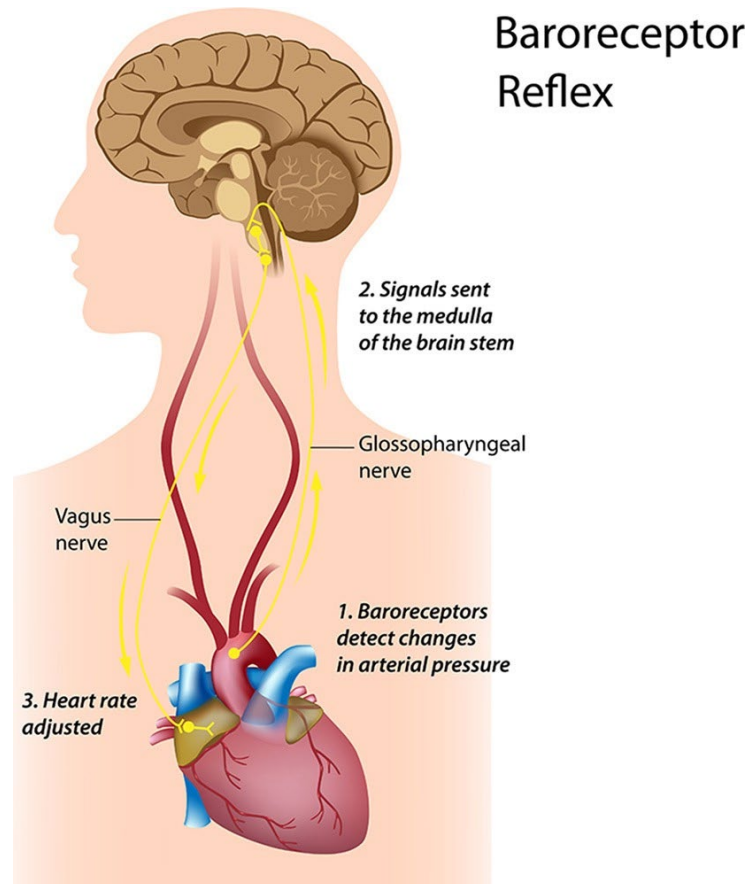


**Figure 1. ANS Innervation to Body Organs**  
Figure from Heart Math Institute<sup>1</sup>

A key cardiovascular mechanism responsible for PNS stimulation is the baroreceptor system. Baroreceptors are located at different sites around the body including the carotid sinuses, aortic arch, and work to maintain a relatively constant blood pressure through the body as it moves. Baroreceptors within the heart make breath-induced heart rate acceleration and deceleration, or RSA, possible. This baroreceptor reflex, or baroreflex, connects HR, BP, and vascular tone

<sup>1</sup>[https://www.researchgate.net/publication/293944391\\_Science\\_of\\_the\\_Heart\\_Volume\\_2\\_Exploring\\_the\\_Role\\_of\\_the\\_Heart\\_in\\_Human\\_Performance\\_An\\_Overview\\_of\\_Research\\_Conducted\\_by\\_the\\_HeartMath\\_Institute](https://www.researchgate.net/publication/293944391_Science_of_the_Heart_Volume_2_Exploring_the_Role_of_the_Heart_in_Human_Performance_An_Overview_of_Research_Conducted_by_the_HeartMath_Institute)

(Shaffer & Ginsberg, 2017). Figure 2 below offers a guide to the action of the baroreflex: as arterial pressure changes are detected at the aortic arch, these signals travel to the brain stem, and are then relayed back down to the SA and AV nodes to adjust heart rate.



**Figure 2. Baroreceptor Reflex Diagram**  
Figure from Alila Sao Mai/Shutterstock.com<sup>2</sup>

During abnormal conditions, such as during chronic stress, the SNS is continuously active without the counterbalance of activation from the PNS (Won, 2016). Low HRV may be linked to chronic stress and associated mood disorders as low HRV impairs the body's ability to manage

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<sup>2</sup>[https://www.researchgate.net/figure/Baroreflex-Credit-Alila-Sao-Mai-Shutterstockcom\\_fig5\\_276182860](https://www.researchgate.net/figure/Baroreflex-Credit-Alila-Sao-Mai-Shutterstockcom_fig5_276182860)

stressors and return to homeostasis (Kim et al., 2018). The influence of breath modulation on RSA may offer an effective means to improve HRV and stimulate PNS activity.

## **2.1 Chronic Stress Prevalence**

The most recent data from the American Psychological Association (APA) suggests that the United States is in the midst of a mental health crisis that is being exacerbated by COVID-19. Surveys from the APA indicate that 78% of all individuals have felt significant stress due to the pandemic, while 67% of individuals experienced increased levels of stress throughout the pandemic (Stress in America, 2020). The APA reported in 2020 that about one in five adults in the US believe their mental health declined in the past year. Chronic stress, or SNS overactivation, sets the stage for the development of diseases including mental illnesses such as anxiety and depression (Mariotti, 2015)(Chrousos & Gold, 1992). The CDC reports that between August 2020 and February 2021, the percentage of adults with symptoms of depression or anxiety increased by over 5%, and adults not meeting a mental health care need also increased by about 2.5% (Vahratian, 2021). Household Pulse Survey data from the CDC, NCHS, and US Census Bureau describes an even more dire situation, showing that the percentage of US adults ages 18-29 years with anxiety or depressive symptoms increased from around 11% in June 2019 to about 42.4% by December 2020 (Vahratian, 2021). Chronic stress is negatively impacting both physical and mental health. The rising rates of stress, anxiety, and depression are indicative of the mental health crisis in the US.

Individuals experiencing symptoms of mental health decline and the associated ANS imbalances are also dealing with a range of physiological issues including heightened blood pressure. When the SNS is overactive, BP can remain heightened and develop into elevated hypertension and ultimately into hypertension. While these statistics are alarming and the consequences of deteriorating health and wellbeing are becoming more obvious, many people struggle to adhere to long-term medications. As such, a greater understanding of non-pharmaceutical tools, such as breathing modification, is both warranted and necessary.

## **2.2 Chronic Stress Risks**

Individuals feeling continuously overwhelmed are at a higher risk for a wide range of serious ailments including cancer and heart disease. Interactions between the SNS and PNS are continuously occurring in the heart and vasculature to maintain cardiovascular homeostasis by responding to the various stimuli encountered in any given day. When an individual's SNS is active, catecholamines epinephrine (E) and norepinephrine (NE) are produced (Chrousos & Gold, 1992). These catecholamines modulate the release of pro-inflammatory cytokines including tumor necrosis factor (TNF) and interleukin (IL)-1 and IL-6 (Won, 2016). During typical conditions, the body works to adapt to the stressful stimulus. The PNS activates, releasing acetylcholine which is shown to significantly reduce the release of the pro-inflammatory cytokines (Borovikova et al., 2000). Blood glucose, cortisol, and E remain elevated during the adaptation phase of the stress response. However, if the stressor does not diminish and the PNS does not activate enough to bring homeostasis, then the pro-inflammatory cytokines perpetuate the continued activation of the SNS and the body exhausts its inner resources (Kim et al., 2018). In this chronically stressed, exhausted,

and inflamed state, the body becomes increasingly susceptible to disease. Continuous overactivation of the SNS may lead to ANS dysregulation and ultimately to an irregular heart rhythm (Chen et al., 2014). However, SNS overactivation is potentially manageable by improving HRV. HRV is a reliable index to measure the heart's ability to respond appropriately to stress-inducing events (Kim et al., 2018).

It is common for individuals living with chronic stress to feel unable to change their situations. This stuck feeling is compounded by their depleted resources, associated low energy, and the commonly occurring mood disorders of anxiety or depression which in turn often decrease their sleep quality in a self-reinforcing cycle. The overactive SNS creates a continued state of heightened blood pressure. Across enough time, high BP is referred to as hypertension, and along with hypertensive renal disease account for 11.1 deaths per 100,000 individuals per CDC data (CDC, 2021). Further, hypertension is a major risk factor for CVD with some estimates that half of all CVD events are attributed to it (Lawes et al., 2008)(Zhou et al., 2018). It becomes clear then how unmitigated stress puts an individual at an increased risk of illness, decreased quality of life, and earlier mortality.

## **2.3 Associations Between Slow Breathing, Cardiorespiratory Health, and Psychological Wellbeing**

Evidence is accumulating that breath modulation is a powerful tool to influence physiology and mood states. In general, studies reveal that deliberately slowing respiration rate is related to improved cardiac and pulmonary health markers and psychological wellbeing. A systematic review on slow breathing's influence on health reveals that overall slow breathing techniques

appear to increase HRV and RSA, suggesting predominance of the PNS mediated by vagal activity. Evidence was found of “increased psychophysiological flexibility linking parasympathetic activity, central nervous system activities related to emotional control and psychological well-being in healthy subjects during slow breathing techniques” (Zaccaro et al., 2018). Studies compiled in the review revealed trends between increases in the LF power of HRV resulting from techniques around 6 breaths/min and psychological outcomes such as increased relaxation, ease and comfort, along with positive energy and pleasantness, notable increases in vigor and alertness, reductions in negative affect including anger, along with decreased symptoms of anxiety and depression (Zaccaro et al., 2018). A systematic review and meta-analysis on slow breathing and BP reported that slow breathing leads to a modest reduction in BP and concluded that slow breathing “may be a reasonable first treatment for low-risk hypertensive and prehypertensive patients who are reluctant to start medication.” On average, slow breathing was found to decrease SBP by 5.62 mmHG and DBP by 2.97 mmHG (Chaddha et al., 2019). Slow breathing biofeedback periods are correlated with acute increases in LF power, total spectrum HRV, and improved sensitivity of the baroreflex (Lehrer et al., 2003)(Brown et al., 1993) (Bhimani et al., n.d.). Additionally, pranayama practices and meditation have been found to improve cardiovascular function in people regardless of differences in age, gender, and weight (Ankad et al., 2011).

An experimental study (N = 39) examined the effects of an acute five-minute slow breathing intervention, with a rate of 6 breaths/min, on HR and BP and also measured the same breathing intervention’s effect following the ingestion of a PNS-blocking drug. It was found that after the intervention, SBP and DBP were significantly reduced, and a slight reduction occurred in HR. The group taking the PNS-blocking drug recorded no significant decrease in BP or HR

(Pramanik, 2009). This study demonstrates that a slower respiration rate stimulates predominance of the PNS, which in turn reduces BP and HR. However, when the PNS is blocked, slower breathing alone does not contribute to BP and HR reductions. It is speculated that vagal mechanisms in the cardiovascular and pulmonary systems are connected and that improving one impacts the other.

## **2.4 Bhramari Pranayama's Effect on Cardiorespiratory Health and ANS Response**

In a systematic review of Bhr. P., evidence from each of the six included studies indicates PNS predominance resulting from the breathing technique. The review found reductions in HR of 2-3 beats per minute, reductions in mean BP of 5-6 mmHG, improvements in cognition based on decreased reaction time, reductions in reactivity to stress-inducing events, and reductions in overall stress levels (Kuppusamy et al., 2018). These findings should be interpreted with care as none of the systematic review studies on Bhr. P. were randomized controlled trials (RCTs); however, they serve as a foundation for future research.

In a RCT examining the effect of Bhr. P. on HRV in healthy adolescents (N = 520), it was observed that PNS-related parameters of HRV showed significant improvement (Kuppusamy et al., 2020). The intervention group (IG) practiced Bhr. P. for around 25 minutes per day, five days per week across the six-month study. The CG did not practice any pranayama. At the conclusion of the study, the average HF power of the IG increased from 40.08 to 46.87, while LF power decreased from 65.06 to 57.02; in comparison, the HF and LF power of the CG changed from

40.05 to 40.72 and 63.95 to 65.86, respectively. Time domain HRV variables saw comparable shifts from an average of 80.03 ms to 88.06 ms for SDNN in the IG and 79.05 ms to 77.82 ms in the CG, while RMSSD changed from an average of 59.91 to 62.05 in the IG and 58.78 to 58.06 in the CG. This study demonstrated the efficacy of Bhr. P. as a long-term intervention to improve HRV and PNS predominance. However, there were no data recordings during or immediately following each of the intervention sessions to determine the efficacy of Bhr. P. for shorter time periods.

A RCT (N = 70) assessed the immediate effect of an acute five-minute Bhr. P. intervention compared to a five-minute slow breath CG on BP and HRV in patients with essential hypertension. No significant differences in BP were observed between groups, but significant changes in HRV were found. Compared to the CG, participants who performed five minutes of Bhr. P. showed a significant increase in HF power and decrease in LF power during the recovery phase post-intervention (Ghati et al., 2021). This study demonstrates that compared to slow breathing alone, the hummed exhale technique of Bhr. P. produces more significant changes in HRV correlated with enhanced PNS function.

Another experimental study examined the effects of practicing Bhr. P. on 54 medical students aged 18-24 across 3 months using 15 hummed exhale breaths in the morning and evening (Jain, 2011). Before and after this 3-month intervention, participants were subjected to a cold pressor test (CPT) where one's hand is immersed in ice water for one minute to observe cardiovascular reactivity. The CPT is shown to produce SNS activation and PNS withdrawal, with typical reactions including increased BP and reduced LF and HF power (Wirch, 2006). In this study, Bhr. P. was found to counterbalance the SNS activation and associated cardiac reactivity resulting from the CPT. After three months of regular practice, the number of participants who

were hyper-reactive to the CPT dropped from 21 to 3. Blood pressure was shown to rise while the participants hands were immersed in the cold water; however, the rise in BP blunted as a result of the intervention. At the start of the study, the rise in SBP while in the CPT was 19.24 mmHG; at the end of the 3-month intervention, the SBP rise while in the CPT was 15.71 mmHG. Similarly, the rise in DBP during the CPT reduced from an average rise of 14.67 mmHG at the start to an increase of 11.62 mmHG by the study's conclusion. Bhr. P. reduced the participants' level of reactivity to the cold stressor possibly by "inducing PNS predominance and corticohypothalamomedullary inhibition" (Jain, 2011). This study provides an example of improved self-regulatory capacity resulting from Bhr. P. practice.

More recently, four different pranayama techniques, including Bhr. P. were examined for five-minute durations with a five-minute wash-out period between pranayama practices in a sample of 20 adults. The five-minute Bhr. P. practice produced cardiovascular changes from during practice to post-practice including mean decreases from about 120 mmHG to 112 mmHG for SBP and from about 79 mmHG to 72 mmHG for DBP; however, the post intervention changes were not significant compared to baseline levels. Additionally, HR decreased from about 92 beats/min to 86 beats/min post-practice while stroke volume increased from about 58 ml to 62 ml (Nivethitha et al., 2021). These results indicate that an acute five-minute Bhr. P. intervention may help improve cardiovascular health. Another acute five-minute Bhr. P. intervention examined HR and BP before and after the practice. Findings revealed a slight decrease in HR along with a significant decrease in mean BP (Pramanik et al., 2010). This study by Pramanik and colleagues concludes that Bhr. P. induced PNS predominance on the cardiovascular system.

An experimental study (N = 60) demonstrated improvements in cardiovascular biomarkers in healthy adolescents after practicing Bhr. P. The IG practiced Bhr. P. for 45 minutes at around

3-4 breaths/min, and the CG breathed at a normal pace, or around 12-16 breaths/min, for 45 minutes. The IG showed significant reductions in HR, BP, pulse pressure, mean arterial pressure, rate pressure product, and double pressure product. The data indicate a decrease in workload on the heart, signifying SNS withdrawal and PNS predominance. It is speculated that the deep breathing of Bhr. P. stimulated baroreceptor activity leading to vasodilation and decreased peripheral resistance (Kuppusamy, 2016). This study suggests that Bhr. P. practice can improve cardiovascular parameters and may be useful for reducing stress-induced cardiovascular risk.

In contrast to the previously described studies, a five-minute Bhr. P. intervention with 16 adults breathing at 6 breaths/min found evidence of PNS withdrawal during the practice (Nivethitha et al., 2017). Data from the five-minute acute intervention showed a significant reduction in HF power, while LF power and HR significantly increased. Nonsignificant increases were observed in PNS-correlated time domain metrics including the standard deviation of the interbeat intervals (IBIs) of normal sinus beats (SDNN and the root mean square of successive differences between normal heartbeats (rMSSD)). The authors conclude upon PNS withdrawal during Bhr. P. practice as they observed non-significant changes in PNS-correlated metrics and significant increases in LF Power and HR.

Longer-term Bhr. P. studies demonstrate a range of health outcomes notably modest improvements in HRV, HR, and BP along with stress reducing effects. The effects of brief interventions are less clear and require further examination to determine the strength of observations. Brief Bhr. P. interventions show modest to no effects on BP and conflicting outcomes on frequency domain measures of HRV. The current examination of Bhr. P. is comparable to the five-minute study conducted by Nivethitha and colleagues (2017) and will either support or contest those prior findings. Like Ghati and colleagues (2021), the current study

compares Bhr. P. against slow breathing to differentiate effects on HRV and BP. Unlike prior studies, the current examination will measure subjective anxiety symptoms using the Spielberger State-Trait Anxiety Inventory (STAI) to strengthen the hypothesized findings of PNS predominance of the ANS.

## **2.5 Summary**

Stress and anxiety are at or near an all-time high in modern society. Evidence indicates that persistent stress and related mood disorders are linked to a lack of psychophysiological flexibility. There are many other factors at play that are impacting mental health. This examination does not attempt to be comprehensive in the causal analysis, but rather focuses on the stress-associated imbalances in the ANS. Slow-breathing techniques, and specifically Bhr. P., are shown to increase emotional flexibility and help balance the ANS. Pranayama practices appear to help control and improve cardiovascular and pulmonary functions. However, the specific effects and strength of the evidence for individual practices, such as Bhr. P., are not yet clear.

Most studies examining pranayama practices utilize multiple techniques in their methodology, so it is important to focus on individual techniques to ascertain their potential benefits. This study examines Bhr. P. and its potential effects of reducing stress by improving HRV, decreasing BP, and reducing perceived anxiety. There remains some inconsistency regarding the specific HRV and BP outcomes resulting from the practice of Bhr. P. The current acute Bhr. P. intervention study aims to provide further clarity by assessing short-term measures of cardiovascular health and self-reported anxiety.

### **3.0 Methodology**

This study examined the effect of an acute breathing intervention on heart rate variability, blood pressure, and anxiety levels in a sample of healthy adults. To examine these research questions, the following methods and procedures were implemented.

#### **3.1 Participants**

Twenty adults were recruited to participate in this study. Upon indicating interest, potential participants were screened using an online Qualtrics survey tool. The screening process is detailed below in sections 3.2 and 3.3.

#### **3.2 Recruitment**

The participants in this study were a convenience sample recruited from students who attend the investigator's weekly yoga classes and via a recruitment flyer. The principal investigator (PI) recruited an equal number of males and females for the study. Following an initial description of the study, all prospective participants were instructed to complete a brief standardized screening questionnaire through the Qualtrics online survey tool to determine participant eligibility. While filling out the screening survey, participants provided demographic information including age and sex. The PI obtained signed informed consent from each participant after reviewing the consent document with each participant either remotely through a HIPAA-approved secure Zoom

teleconference meeting or in person. All study procedures were approved by the University of Pittsburgh Institutional Review Board.

### **3.3 Eligibility Criteria**

For study eligibility, participants needed to be between 18 and 60 years of age. Individuals were excluded from participation in the study based on the following reasons: (1) current use of medication that affects blood pressure or heart rate (i.e., diuretics, beta blockers, ACE inhibitors, angiotensin II receptor blockers, calcium channel blockers, alpha blockers, alpha-2 receptor agonists, central agonists, peripheral adrenergic inhibitors, or vasodilators); (2) self-reported smoker or alcohol addiction; (3) current symptoms of chest pain or ear infection; or (4) current pregnancy.

### **3.4 Experimental Design**

This research study used a within-subjects crossover design. In a single in-person visit that lasted approximately 60 minutes, each participant completed two experimental conditions: a five-minute Bhr. P. breathing intervention and a five-minute slow breathing control condition with a five-minute wash-out period in between conditions, which is consistent with previously published procedures (Nivethitha et al., 2021). In this repeated measure design, each participant interacted with all the variables in randomized order to counteract the possible order effects. Before and after each experimental condition, the participant's BP was measured twice, and participants completed

a questionnaire regarding anxiety symptoms. HRV was recorded at baseline and during each of the breathing methods.

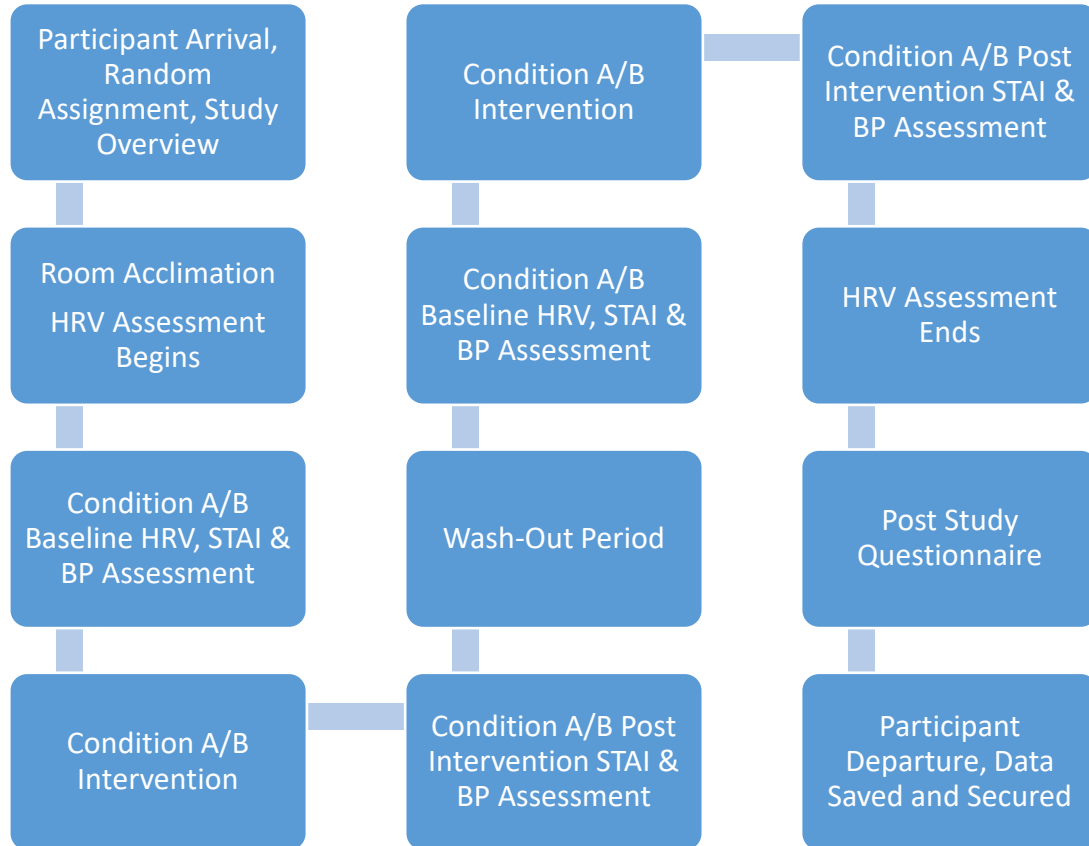
### **3.5 Intervention Components**

#### **3.5.1 Assessment Procedures**

Eligible and consenting individuals were randomly assigned to one of the two experimental conditions in a ratio of 1:1. These assignments were made using a secret envelope selection where a separate party created 20 envelopes that would determine the first breathing condition performed during the in-person session: 10 with the Bhr. P. condition listed inside, and 10 with the slow breathing condition listed inside. At the time of each in-person session, an individual other than the PI randomly selected an envelope for the PI to use in the session. Once the participant finished the first breathing condition, there was a five-minute wash-out period followed by the second breathing condition.

All testing took place in the same study room to control for room temperature, lighting, and possible distraction. To better control for diurnal influences on measurements, in-person sessions took place in the morning (0800-1200 h). In addition, participants were requested to abstain from food and caffeine for 12 hours, strenuous exercise for 24 hours, and to attain a normal night of sleep prior to testing. Upon arrival at the testing site, participants were asked to void their bladders and place their cellular devices on airplane mode or turned off and placed away from their side.

The study followed procedures as illustrated in Figure 3 below.



**Figure 3. Study Flow Diagram**

Following an illustrated guide chart, participants placed a heart rate sensor strap around their torso. The HR sensor's signal was checked by the PI to verify a reading. Next, participants were seated in a chair, which was the same for all participants. The PI measured the participant's upper left arm length and circumference to determine the appropriate blood pressure cuff size for each participant, and the cuff was secured on the participant's upper arm. Participants rested for five minutes to acclimate to the room and the wearable equipment. After the five-minute acclimation period, baseline heart rate variability measurements were recorded for five minutes. An identical procedure was implemented at the start of second breathing condition following the wash-out period.

### 3.5.2 Intervention Instructions

At the onset of the intervention, the investigator relayed the following information to the participant:

“This study will investigate the possible differences between two different types of breath interventions, five minutes of slow breathing and five minutes of hummed exhale breathing. Before and after each study condition, your level of anxiety will be assessed along with your blood pressure, which will be measured in accordance with standard protocols. Your heart rate will be monitored at time periods during the study to reveal trends in heart rate variability. During the intervention you should remain comfortably seated in an upright posture.”

Before commencing each breathing condition, the PI instructed each participant using the following scripts:

*Bhramari Pranayama*: “Please gently close your lips, relax your jaw, and place the tip of your tongue onto the roof of your mouth behind your upper front teeth. Check-in with this position frequently to ensure that your jaw is relaxed. Then, take a long, deep breath in through your nose. Tuck your chin slightly towards your throat. Exhale slowly, making a low and steady ‘hmmmm’ sound at the back of your throat. Continue breathing like this for the duration of the five-minute intervention.”

Slow Breathing: “Please gently close your lips, relax your jaw, and place the tip of your tongue onto the roof of your mouth behind your upper front teeth. Check-in with this position frequently to ensure that your jaw is relaxed. Then, take a long, deep breath in through your nose. Tuck your chin slightly towards your throat. Exhale slowly through your nose. Continue breathing like this for the duration of the five-minute intervention.”

### 3.5.3 Heart Rate Variability Measurement

Heart rate assessments were performed using a Polar H10 chest strap connected remotely to a Polar Vantage 800 watch. The Polar chest strap used in this study has been shown to be an acceptable alternative to electrocardiography (ECG) when measuring HRV metrics (Plews, 2017). Time series R-R interval data were extracted from the Vantage 800 watch and analyzed using Kubios HRV Premium software which enabled data cleaning, precise signal processing, and power estimating. Heart rate variability was measured using both frequency domain (low-frequency [LF] and high frequency [HF]) and time domain measures (standard deviation of normal-to-normal R-R intervals (SDNN) and Root Mean Square of the Successive Differences (rMSSD)). The four HRV metrics assessed are described in detail and in table 1 below. Frequency domain HRV metrics measure different frequencies, 0.15 - 0.4 Hz for HF, and 0.04 - 0.15 Hz for LF. The nature of LF is contested in the scientific community; however, it is known that LF power may be generated by both the SNS and PNS, and through baroreflex activity (Shaffer & Ginsberg, 2017)(Goldstein et al., 2011). When respiration rate is between 3 and 9 breaths/min, LF power is primarily affected by RSA (Tyagi, 2016). In a literature review on yoga and HRV, Tyagi states that “large-amplitude HR oscillations occurring in the LF range resulting from breathing at an optimal frequency may reflect resonance, also known as ‘coherence’ occurring due to entrainment between HR, BP, and the relaxation response rather than sympathetic tone.” Regarding time domain measures, the SDNN measures the standard deviation of the R spike to adjacent R spike where larger values for SDNN correlate with greater HRV. The RMSSD is a primary measure when estimating vagally mediated HRV changes. In short-term recordings of SDNN, “the primary source of variation is PNS-mediated RSA, especially with slow, paced breathing protocols” (Shaffer & Ginsberg, 2017). Shaffer and Ginsberg also note that the PNS more strongly influences RMSSD than SDNN. HRV

was recorded continuously through the study. Specific timepoints were noted in the assessment forms to extract distinct five-minute recordings for analysis. These time points included two 5-min baseline periods and two 5-min breathing conditions.

**Table 1. HRV Metric Descriptions**

<b>Time-Domain Metrics</b>	
SDNN (standard deviation of normal R-R intervals)	In brief (5-min) recordings, the main source of variation is PNS mediated RSA especially with slow breathing protocols.
RMSSD (root mean square of successive differences)	Primary measure when estimating vagally mediated HRV changes. The PNS more strongly influences RMSSD than SDNN.
<b>Frequency-Domain Metrics</b>	
HF (High Frequency)	Known as the respiratory band as it's known to reflect PNS activity and correspond to HR variations related to respiration.
LF (Low Frequency)	Produced by both the combination of the PNS and SNS and via baroreceptor activity, or primarily by the PNS, or by the baroreflex alone. The ANS branch contribution is highly dependent upon breath rate.

### **3.5.4 Blood Pressure Measurement**

Systolic and diastolic blood pressure were collected using a Dinamap digital sphygmomanometer before and after each breathing condition. The blood pressure cuff was placed on upper left limb of the participant for all but one participant; one participant needed to have their BP assessed using the right arm due to medical reasons. Following standard procedures, blood pressure was measured twice with a one-minute time period between measurements. If a difference between measurements of >10 mmHG for SBP or >6 mmHG for DBP were observed, a third measurement was recorded. Mean arterial pressure (MAP) was calculated using the following

formula:  $MAP = DBP + 1/3(SBP - DBP)$ . These methods are consistent with comparable investigations (Nivethitha et al., 2017).

### **3.5.5 Anxiety Measurement**

The 6-item Spielberger State-Trait Anxiety Inventory (STAI) was administered before and after each breathing condition to assess changes in state anxiety. The brief questionnaire produces scores comparable to those produced from the full 20-item STAI while also being more accessible and reducing time to analyze results. The STAI is shown to be a valid measurement tool to quantify anxiety in medical practices (Court, Helen. 2010). Participants provided responses including “Not at All”, “Somewhat”, “Moderately So”, and “Very Much So” to the following prompts: (1) I feel calm; (2) I feel tense; (3) I feel upset; (4) I feel relaxed; (5) I feel content; and (6) I feel worried. These 6 items represent the highest anxiety-present and anxiety-absent adjectives from the full-form STAI.

### **3.5.6 Demographic Information**

After completion of the intervention, participants completed a brief questionnaire that assessed their sex, height, weight, self-reported race and ethnicity, and their prior experience with yoga and pranayama practice. In addition, physical activity (PA) habits were assessed using the Stanford Leisure-time Activity Categorical Item (L-Cat), which is a reliable and valid tool to categorize one’s PA in a time-efficient manner (Kiernan, 2013).

### 3.6 Data Analysis

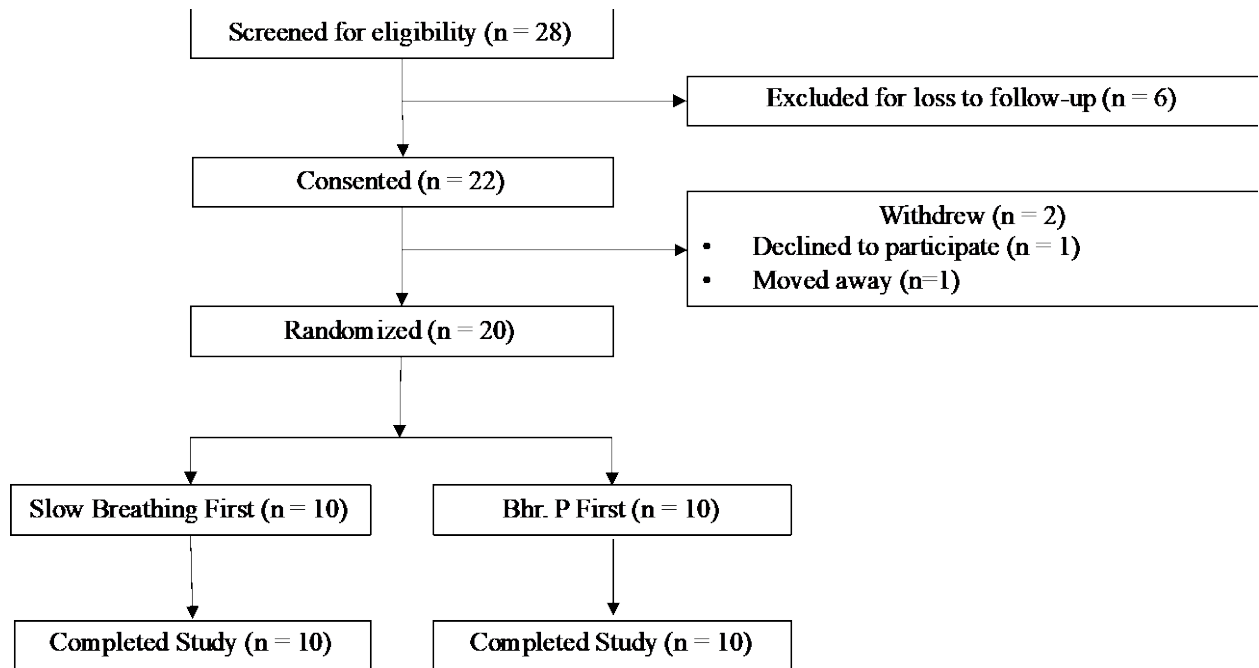
Data were analyzed using available statistical software (SPSS) with statistical significance defined as  $P < 0.05$ . Two-factor repeated measures of analysis of variance (ANOVA) were performed to examine the aims of the study. Partial eta squared effect sizes were calculated indicating small ( $\eta_p^2 = 0.01$ ), medium ( $\eta_p^2 = 0.06$ ), or large effects ( $\eta_p^2 = 0.14$ ) (Lakens, 2013). Separate analyses were conducted for each of the following outcome variables: HRV (SDNN, rMSSD, LF Power, and HF Power), BP (SBP, DBP, and MAP), and STAI assessments. The assumption of normality was tested using the Shapiro-Wilk test and the assumption of sphericity was tested using Mauchly's test. HRV variables were transformed using natural logarithm (ln; SDNN, rMSSD) or base10-logarithm (LF power, HF power) transformations prior to analysis so their ranges would be normally distributed. The SBP, DBP, MAP, and STAI ranges were not normally distributed, nor were their log transformations. As such, the PI chose to run the statistical analyses on the original units. When appropriate, post-hoc comparisons were made with the p-value adjusted using the Bonferroni procedure for multiple comparisons to determine whether breathing methods differed from each other.

## **4.0 Results**

The purpose of this experimental study was to investigate the effects of two short-term breathing exercises on autonomic function and self-reported anxiety in a sample of healthy adult participants. The results are presented below, beginning with a description of the sample and then organized by specific aims.

### **4.1 Recruitment and Enrollment**

A convenience sample was recruited from the following avenues: (1) via the principal investigator's yoga classes; (2) via a distributed recruitment flyer; and (3) via advertisement to students in the PI's undergraduate program classes. A CONSORT flow diagram in Figure 4 details the participant retention through the study. Overall, 28 individuals were screened for eligibility. None of the interested individuals who underwent screening were deemed ineligible for participation. A total of 22 individuals were consented to participate in the study. Two individuals chose to withdraw from the study after consenting. Twenty participants completed the in-person assessment. All 20 participants were randomized to perform either the slow breathing condition first ( $n = 10$ ) or Bhr. P. first ( $n = 10$ ).



**Figure 4. CONSORT flow diagram**

## **4.2 Study Participants**

Study participant information is described here and in Table 2 below. On average, participants were  $27.8 \pm 7$  years old with a body mass index of  $25.2 \pm 3.6$ . Experience with yoga practices varied widely; six participants had no prior experience, five participants had more than five years of experience, and all others had intermediate levels of experience. Participants' experience with conscious breath practices or pranayama was more limited, with eight participants having no prior experience, seven participants having between three months and two years of experience, and the remaining five participants having more than two years of experience. Participants' self-reported physical activity (PA) levels for the past month indicated that 14 of the 20 met aerobic PA guidelines (e.g., 30 min of moderate-intensity PA at least 5 d/wk) and the

remaining 6 reported insufficient levels of PA (e.g., 30 min of moderate-intensity PA on 3 d/wk).

None of the participants reported doing little to no physical activity in the past month.

**Table 2. Participant Information**

<b>Characteristics</b>	<b>Mean (SD) or N</b>
Age (years)	27.8 (7)
BMI	25.2 (3.6)
Sex	10 M, 10 F
Yoga Experience:	
No Experience	6
0 - 5 years	9
> 5 years	5
Pranayama Experience:	
No Experience	8
3 months – 2 years	7
> 2 years	5
Physical Activity Levels	
Met PA Guidelines	14
Did not meet PA Guidelines	6

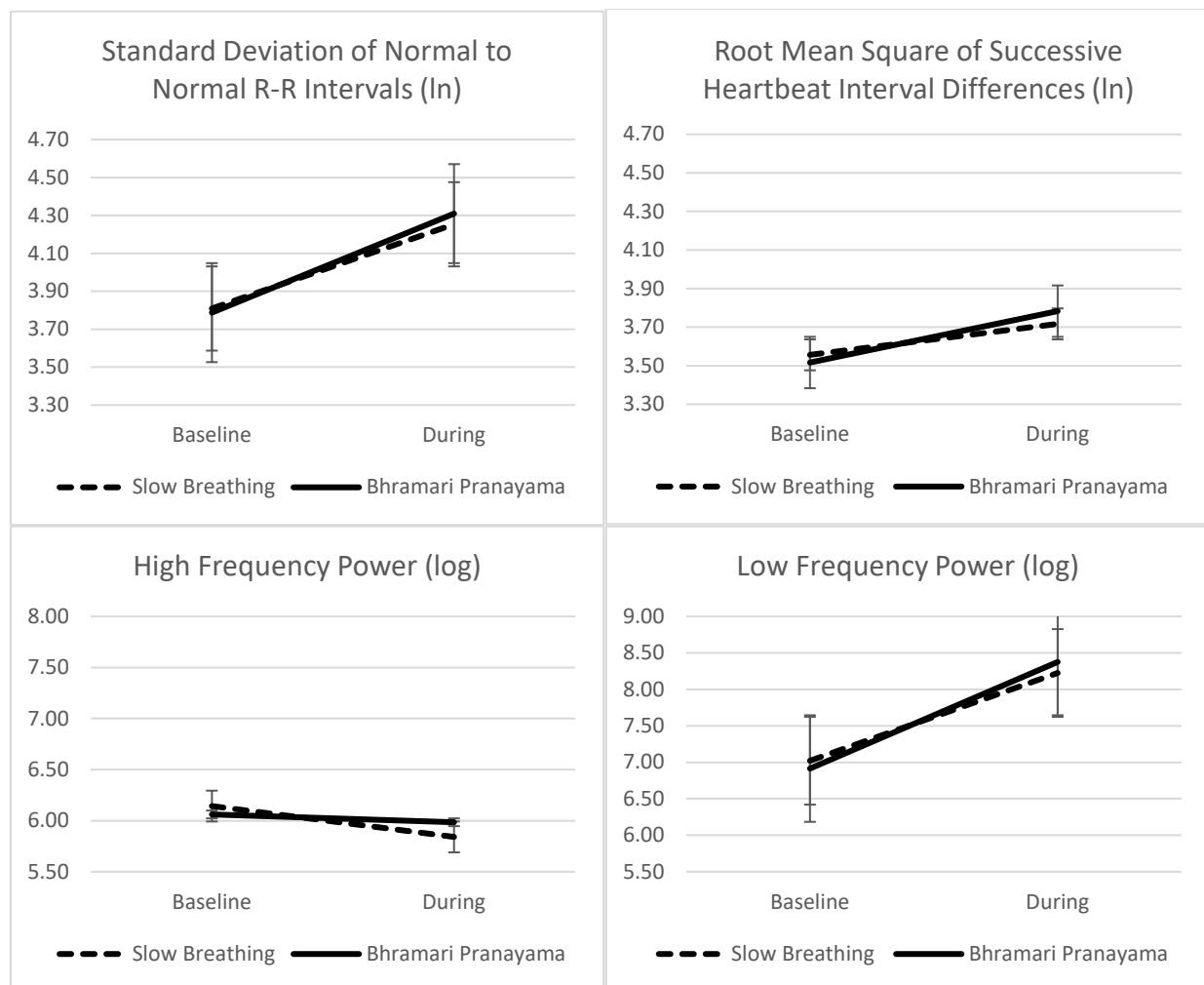
### **4.3 Specific Aim I: Heart Rate Variability**

The primary outcome of Specific Aim I was to assess changes in heart rate variability measures from baseline to a breathing condition and to determine whether these changes differed significantly between breathing conditions. Two-factor repeated measures ANOVA models showed non-significant interactions between experimental breathing conditions for all measures of HRV (each  $P \geq .155$ ). Table 3 summarizes the changes in mean HRV measures between baseline and experimental conditions as well as the interaction and main effects. Although significant interactions between experimental conditions were not present, Figure 5 details the main effects of the intervention from baseline measures to experimental conditions.

**Table 3. Changes in heart rate variability indices from baseline to during experimental conditions**

	Baseline Mean (SD)	During Mean (SD)	Main Effect (Time)		Main Effect (Breathing Condition)		Interaction (Time*Condition)	
			<i>p</i>	$\eta_p^2$	<i>p</i>	$\eta_p^2$	<i>p</i>	$\eta_p^2$
<u>SDNN(ln)</u>			<b>&lt;.001</b>	0.633	0.716	0.007	0.350	0.046
Slow Breathing	3.81 (0.50)	4.25 (0.49)						
Bhramari Pranayama	3.79 (0.44)	4.31 (0.44)						
<u>rMSSD(ln)</u>			<b>0.03</b>	0.224	0.769	0.005	0.308	0.055
Slow Breathing	3.56 (0.65)	3.72 (0.66)						
Bhramari Pranayama	3.52 (0.59)	3.78 (0.62)						
<u>HF Power(log)</u>			0.362	0.044	0.833	0.002	0.408	0.036
Slow Breathing	6.14 (1.34)	5.84 (1.35)						
Bhramari Pranayama	6.06 (1.16)	5.98 (1.50)						
<u>LF Power(log)</u>			<b>&lt;.001</b>	0.606	0.855	0.002	0.155	0.103
Slow Breathing	7.02 (1.16)	8.22 (1.12)						
Bhramari Pranayama	6.91 (1.10)	8.38 (0.91)						

Significant values are bolded. Trending values are italicized. Abbreviations:  $\eta_p^2$ : partial eta-squared effect size; *p*: p-value.



**Figure 5. Changes in HRV measures from baseline to during intervention**

For SDNN(ln), the time x condition interaction was not significant ( $P=0.350$ ,  $\eta_p^2=0.046$ ). Post-hoc analyses on the main effects revealed that SDNN(ln) significantly increased from baseline to the breathing condition with a large effect size ( $P<.001$ ,  $\eta_p^2=0.633$ ), though this change was similar between the two breathing conditions ( $P=0.716$ ,  $\eta_p^2=0.007$ ). When each breathing condition was examined separately, SDNN(ln) increased over time for breathing methods (slow breathing:  $+0.44$  [95% CI: 0.24-0.65],  $P<.001$ ; Bhramari Pranayama:  $+0.52$  [0.34-0.71],  $P<.001$ ).

For rMSSD(ln), the time x condition interaction was not significant ( $P=0.308$ ,  $\eta_p^2=0.055$ ). Post-hoc analyses revealed a significant increase in rMSSD(ln) from baseline to the breathing condition with a large effect size ( $P=0.03$ ,  $\eta_p^2=0.224$ ), with this increase being similar between

the two breathing conditions ( $P=0.769$ ,  $\eta_p^2=0.005$ ). When examining each breathing condition separately, rMSSD did not significantly change from baseline to during the slow breathing intervention ( $+0.16$   $[-0.06-0.38]$ ,  $P=0.143$ ), while rMSSD significantly increased from baseline to during the Bhr. P. breathing method ( $+0.27$   $[0.05$  to  $0.48]$ ,  $P=0.018$ ).

For HF power (log), the time x condition interaction was not significant ( $P=0.408$ ,  $\eta_p^2=0.036$ ), nor were there any significant changes over time ( $P=0.362$ ,  $\eta_p^2=0.044$ ) or between the breathing conditions ( $P=0.833$ ,  $\eta_p^2=0.002$ ).

For LF Power (log), the time x condition interaction was not significant ( $P=0.155$ ,  $\eta_p^2=0.103$ ). Post-hoc analyses revealed a significant increase in LF power (log) from baseline to the breathing condition with a large effect size ( $P<.001$ ,  $\eta_p^2=0.606$ ), with this change being similar between breathing conditions ( $P=0.855$ ,  $\eta_p^2=0.002$ ). When each breathing condition was evaluated separately, LF power increased for both breathing methods from baseline to during the intervention (slow breathing:  $+1.20$   $[0.65$  to  $1.76]$ ,  $P<.001$ ; Bhramari Pranayama:  $+1.46$   $[0.92$  to  $1.99]$ ,  $P<.001$ ).

#### **4.4 Specific Aim II: Blood Pressure**

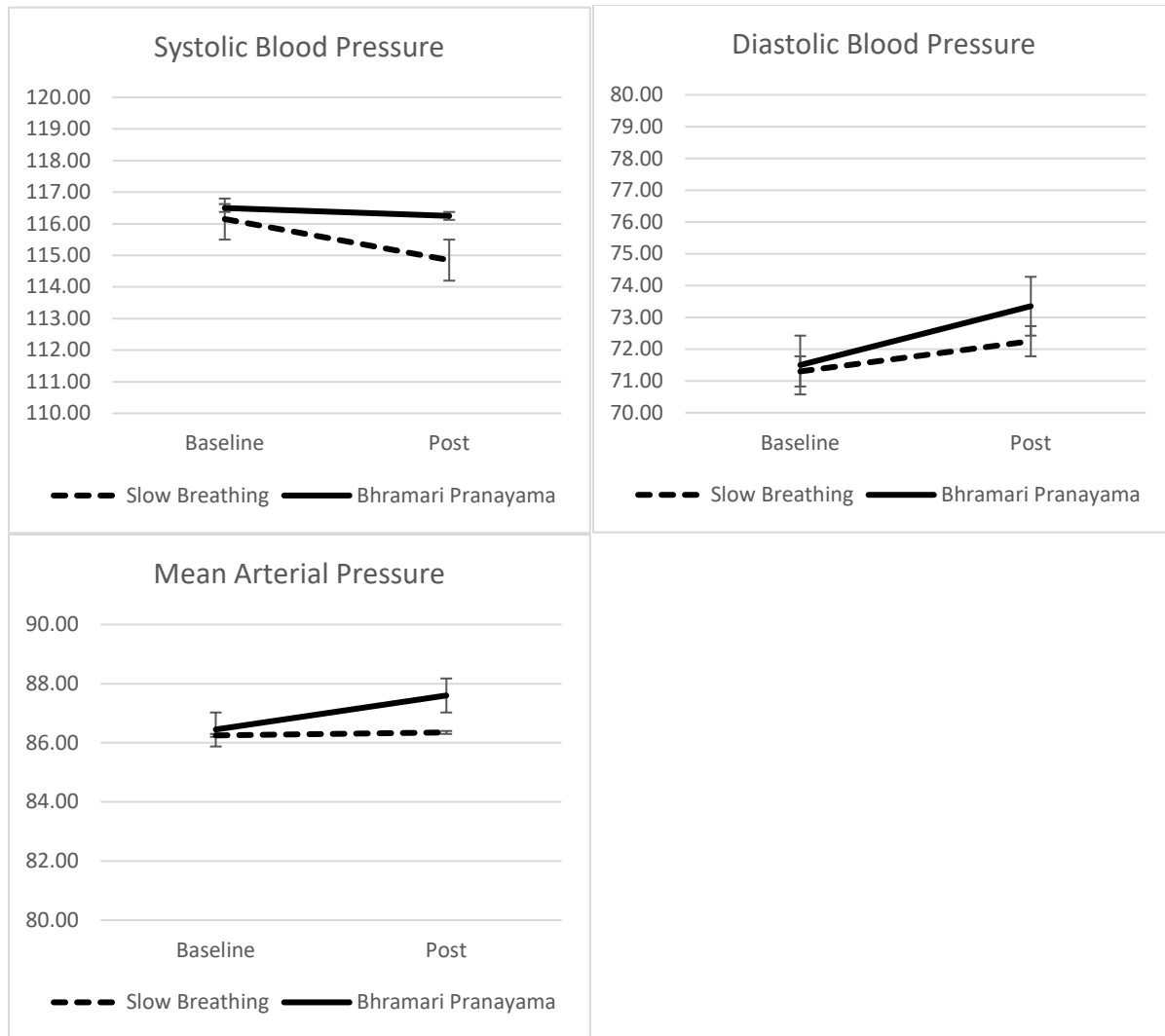
The primary outcome of Specific Aim II was to assess changes in BP measures from baseline to post-experimental condition and examine whether these changes differed between the experimental breathing conditions. Two-factor repeated measures ANOVA models showed non-significant interactions between experimental conditions for all BP measures (each  $P\geq.108$ ). Table 4 summarizes the changes in mean BP measures for each breathing condition between baseline and post-experimental conditions as well as the interaction and main effects. Although no

significant interactions between experimental conditions were present, Figure 6 displays the changes in BP from baseline to post-intervention for both breathing conditions.

**Table 4. Changes in blood pressure variables from baseline to post experimental conditions**

	Baseline Mean (SD)	Post- Experimental Mean (SD)	Main Effect (Time)		Main Effect (Breath)		Interaction (Time*Breath)	
			<i>p</i>	$\eta_p^2$	<i>p</i>	$\eta_p^2$	<i>p</i>	$\eta_p^2$
<u>Systolic BP</u>			0.209	0.082	0.517	0.022	0.411	0.036
Slow Breathing	116.15 (17.68)	114.85 (17.74)						
Bhramari	116.50 (17.77)	116.25 (19.21)						
Pranayama								
<u>Diastolic BP</u>			0.108	0.130	0.350	0.046	0.488	0.026
Slow Breathing	71.30 (12.91)	72.25 (11.56)						
Bhramari	71.50 (11.61)	73.35 (12.21)						
Pranayama								
<u>Mean Arterial Pressure</u>			0.362	0.044	0.351	0.046	0.276	0.062
Slow Breathing	86.25 (14.10)	86.35 (13.20)						
Bhramari	86.45 (12.98)	87.60 (13.87)						
Pranayama								

Significant values are bolded. Trending values are italicized. Abbreviations:  $\eta_p^2$ : partial eta-squared effect size; *p*: p-value.



**Figure 6. Changes in BP measures from baseline to post intervention**

For SBP, the time x condition interaction was not significant ( $P=0.411$ ,  $\eta_p^2=0.036$ ), nor were there any significant changes over time ( $P=0.209$ ,  $\eta_p^2=0.082$ ) or between the breathing conditions ( $P=0.517$ ,  $\eta_p^2=0.022$ ).

For DBP, the time x condition interaction was not significant ( $P=0.488$ ,  $\eta_p^2=0.026$ ), nor were there any significant changes over time ( $P=0.108$ ,  $\eta_p^2=0.13$ ) or between breathing conditions ( $P=0.35$ ,  $\eta_p^2=0.046$ ).

For MAP, the time x condition interaction was not significant ( $P=0.276$ ,  $\eta_p^2=0.062$ ), nor were there any significant changes over time ( $P=0.362$ ,  $\eta_p^2=0.044$ ) or between the breathing conditions ( $P=0.351$ ,  $\eta_p^2=0.046$ ).

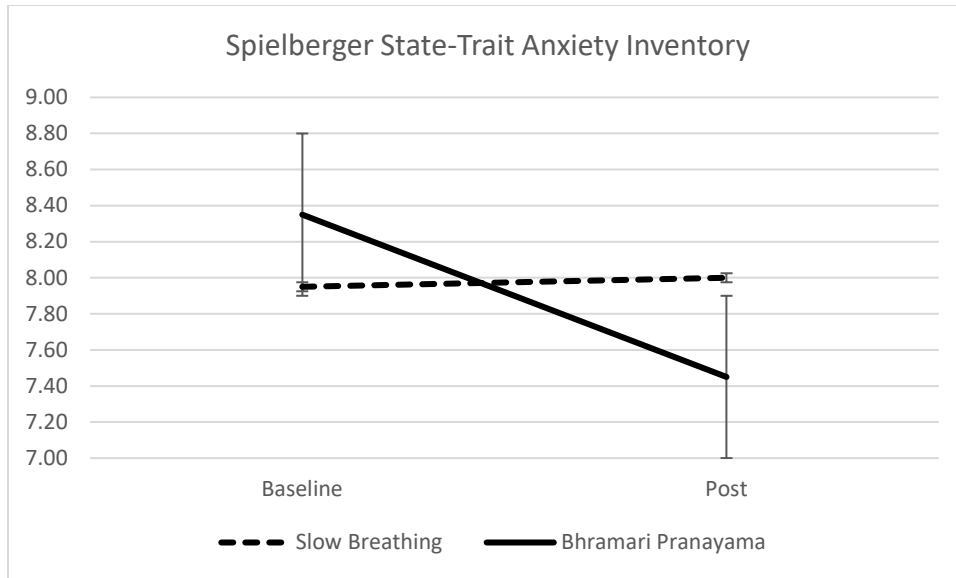
#### 4.5 Specific Aim III: Self-reported Anxiety

The primary outcome of Specific Aim III was to assess changes in self-reported anxiety measures from baseline to post-experimental condition and examine whether these changes differed between the experimental breathing conditions. A two-factor repeated measures ANOVA showed a time x condition interaction that trended towards statistical significance ( $P=0.062$ ,  $\eta_p^2=0.172$ ). Table 5 summarizes the changes in STAI scores between baseline and experimental conditions for both breathing conditions. Figure 7 displays the changes in STAI from baseline to post-intervention for both experimental conditions.

**Table 5. Changes in self-reported anxiety symptoms from baseline to post-experimental conditions**

	Baseline Mean (SD)	Experimental Mean (SD)	Main Effect (Time)		Main Effect (Breath)		Interaction (Time*Breath)	
			<i>P</i>	$\eta_p^2$	<i>P</i>	$\eta_p^2$	<i>P</i>	$\eta_p^2$
<u>STAI</u>			<i>0.084</i>	0.149	0.845	0.002	<i>0.062</i>	0.172
Slow Breathing	7.95 (1.93)	8.00 (2.53)						
Bhramari Pranayama	8.35 (2.47)	7.45 (2.11)						

Significant values are bolded. Trending values are italicized. Abbreviations:  $\eta_p^2$ : partial eta-squared effect size; *p*: p-value.



**Figure 7. Changes in self-reported anxiety measures from baseline to post intervention**

For STAI, the time x condition interaction was trending towards statistical significance with a large effect size ( $P=0.062$ ,  $\eta_p^2=0.172$ ). Post-hoc analysis revealed a change over time trending towards statistical significance with a large effect size ( $P=0.084$ ,  $\eta_p^2=0.149$ ), though this change was similar between the two breathing conditions ( $P=0.845$ ,  $\eta_p^2=0.002$ ). When each breathing condition was examined separately, STAI did not significantly change from baseline to post slow breathing intervention (+0.05 [-0.59-0.69],  $P=0.871$ ), while STAI significantly decreased from baseline to post Bhr. P. breathing intervention (-0.90 [-1.66 - -0.14],  $P=0.022$ ).

#### 4.6 Exploratory Analyses

Stratified analyses were performed to determine whether results differed based on sex and pranayama experience. No significant condition x time interactions were observed when analyses were isolated to males only ( $n=10$ ) or females only ( $n=10$ ). Two-factor repeated measures ANOVA models showed non-significant interactions between experimental conditions for all

specific aim variable measures based on sex (each  $P \geq .121$ ) Additionally, no significant condition x time interactions were observed when analyses were isolated to participants with pranayama experience greater than three months ( $n=12$ ) or no experience ( $n=8$ ). Two-factor repeated measures ANOVA models showed non-significant interactions between experimental conditions for all specific aim variable measures based on pranayama experience (each  $P \geq .10$ ).

## **5.0 Discussion**

### **5.1 Summary of Findings**

This study aimed to compare the short-term impact of two brief breathing methods on autonomic function, blood pressure, and self-reported anxiety levels. Previous research suggests that breathing practices may elicit meaningful improvements in multiple measures of health and well-being. The literature on acute bouts of pranayama practices suggests mild improvements in cardiovascular health measures including HR, BP, and HRV. The literature is limited on the short-term effects of individual breath methods or comparisons between pranayama. To help address these research gaps, we conducted an experimental study to evaluate the effects of two brief breathing protocols on measures of heart rate variability, blood pressure, and self-reported anxiety in a sample of healthy adults. Each breathing method was conducted for five minutes.

This study found no significant effects in time x condition interactions, indicating that Bhr. P. did not change any outcomes over time that differed from slow breathing; however, the self-reported state anxiety was trending towards significant. Exploratory analyses provided the following observations, including: (1) over time, from baseline measurements to experimental conditions, both breathing methods demonstrated significant changes in SDNN, rMSSD, and LF Power; (2) Bhr. P. demonstrated significant improvements to rMSSD and STAI measures over time, whereas SDNN and LF Power changed significantly over time for both breathing methods. Overall, this study suggests that performing five-minutes of either slow breathing or Bhr. P. can improve measures of heart rate variability, and Bhr. P. can reduce perceived state anxiety. In

conjunction with the findings from the current literature, both breathing methods support more optimal functioning of the autonomic nervous system.

## **5.2 Specific Aim I: Heart Rate Variability**

Specific Aim I examined the effects of a brief Bhr. P. intervention on the HRV metrics SDNN, rMSSD, HF power, and LF power and compared them to the effects of a brief slow breathing intervention. We hypothesized that Bhr. P. would improve these measures more than the slow breathing method. Our results did not support this hypothesis, as there were no significant time x condition interactions observed. Our findings contrast with the majority of the acute Bhr. P. literature. A recent study examined HF power and LF power after a five minute Bhr. P. intervention (Ghati, 2021). Unlike our current study, Ghati and colleagues found that compared to slow breathing, Bhr. P. produced significant improvements in HRV frequency domain metrics. Another investigation that examined the impact of five minutes of Bhr. P. on HRV found significant reductions in HF power and significant increases in LF power while finding no significant changes in rMSSD or SDNN (Nivethitha, 2017). The study by Nivethitha and colleagues is comparable to our current examination in terms of its intervention duration and because they observed a significant increase over time for LF power; however, unlike the study by Nivethitha and colleagues, the current examination found a significant increase over time in HRV time domain measures SDNN and rMSSD during Bhr. P. practice, though this significant increase was also observed with slow breathing suggesting that Bhr. P. and slow breathing may be effective ways to improve HRV.

This study highlights the need to assess fewer aims in future research on specific pranayama practices. This study could have produced a broader range of HRV results by focusing only on HRV allowing for reliable measurements at baseline, during, and post intervention. In summary, although we observed significant changes in SDNN and LF power in both experimental groups compared to baseline levels and found significant improvement in rMSSD in Bhr. P. over time, no significant condition x time interactions were observed. These results suggest that both Bhr. P. and slow breathing may improve HRV indices during their practice, but that Bhr. P. does not produce more favorable HRV outcomes compared to slow breathing.

### **5.3 Specific Aim II: Blood Pressure**

Specific aim II examined the effects of a brief Bhr. P. intervention on the blood pressure metrics SBP, DBP, and MAP and compared them to the effects of a brief slow breathing intervention. We hypothesized that Bhr. P. would improve these measures more than the slow breathing method. Our results did not support this hypothesis, as there were no significant condition x time interactions observed. These results are in line with findings from two comparable short-term Bhr. P. interventions that found no significant effects for BP from baseline to post-intervention (Ghati et al., 2021) (Nivethitha, 2021). However, another five-minute examination of Bhr. P. found significant decreases in diastolic blood pressure and MAP (Pramanik, 2010). The effect of brief Bhr. P. interventions on blood pressure remains unclear. The current examination found no significant effects on any measure of BP for either breath method and certainly no improvement for Bhr. P. over slow breathing. BP improvements appear to be more consistent with

longer interventions, except for the study by Pramanik and colleagues. It may be that longer intervention lengths are necessary for consistent BP effects to be observed.

#### **5.4 Specific Aim III: Self-report Anxiety**

Specific Aim III examined the effects of a brief Bhr. P. intervention on self-reported anxiety from the STAI and compared them to the effects of a brief slow breathing intervention. We hypothesized that Bhr. P. would improve these measures more than the slow breathing method. This study found the condition x time interaction was trending towards statistical significance, indicating that the reduction in STAI scores following Bhr. P. was greater than the reduction observed following slow breathing. This suggests that future studies with a larger sample may find statistically significant differences between slow breathing and Bhr. P. This study observed that over time, Bhr. P. produced a significant decrease in STAI scores.

A comparable study with a similar sample size investigating HRV and Bhr. P. makes a case for PNS withdrawal during Bhr. P. practice (Nivethitha et al., 2017). This is relevant as PNS withdrawal should not coincide with reduced anxiety; however, the current examination finds a significant decrease in STAI scores post intervention when observing Bhr. P. and a trending towards significant interaction comparing Bhr. P. to slow breathing. The literature on slow breathing alone indicates that slow breathing tends to promote PNS predominance with psychological outcomes including increased relaxation and decreased anxiety (Zaccaro et al., 2018). This study indicates that Bhr. P. may produce more potent anxiety-reducing effects than slow breathing. The mechanisms for the observed anxiety reduction are unclear as measures of PNS activity don't increase significantly more with Bhr. P. compared to slow breathing in the

present study. It is speculated that the sensation of vibration produced by Bhr. P. may induce changes in central nervous system regions correlated with reduced feelings of anxiety as a brain imaging study finding that the “neurohemodynamic correlates of ‘OM’ chanting indicate limbic deactivation” (Gangadhar, 2011). OM chanting is similar to the hummed exhale of Bhr. P. performed in the current study, though future studies should investigate the precise physiological effects underlying these practices.

The goal of Specific Aims II and III was to assess the participants’ BP and anxiety symptoms immediately before and after each of the study’s breathing conditions. However, due to the potential contaminating influences of movement on BP, the research team decided to assess BP first followed by the STAI. As a result, the STAI assessments are limited by this approach due to the potential acute changes in mood/anxiety levels resulting from following the BP assessments. Future studies focused on anxiety reduction should assess STAI immediately following the breathing condition.

In summary, we observed a condition x time interaction effect trending towards significance, providing a preliminary indication that Bhr. P. may lead to a greater decrease in state anxiety than slow breathing. This study’s findings suggest that Bhr. P. could be a useful practice for individuals to reduce their perceived level of anxiety. Further examination is warranted with a larger sample size.

### **5.5 Strengths, Limitations, and Recommendations for Future Research**

A notable strength of this study is the exploration of different breathing techniques on cardiovascular health and self-reported anxiety. Breathing exercises are gaining popularity, and

they are typically accessible for free and relatively simple to adopt; however, few studies have examined their acute impact on measures of cardiovascular health and anxiety. Additional strengths of this study include the following:

1. Participants followed standardized procedures (e.g., abstention from caffeine and strenuous exercise) to minimize any distortion of HRV measurements.
2. The laboratory design included many features that increased the experimental rigor of the study (e.g., assessments at same time of day, randomized assignment, within-subjects design, equal proportion of males and females).
3. Bhr. P. was compared against a slow-breathing condition instead of a weaker control condition (e.g., normal breathing), which allowed us to examine whether the effects of Bhr. P. could be observed above and beyond those obtained by slow breathing alone.

The findings of this study should be considered within the context of several notable limitations, including the following:

1. The study sample was small, and results may not extrapolate to a larger population.
2. The study sample had relatively low BP and anxiety levels. As a result, there was not much room for most participants to improve.
3. The level of yoga and pranayama experience varied significantly across the sample. It is unknown whether the heterogeneity in experience levels impacted the outcome variables.
4. The intervention was extremely brief, with participants engaging with each condition for only five minutes.
5. The study did not include any significant time dedicated to training or long-term practice of the breathing methods.

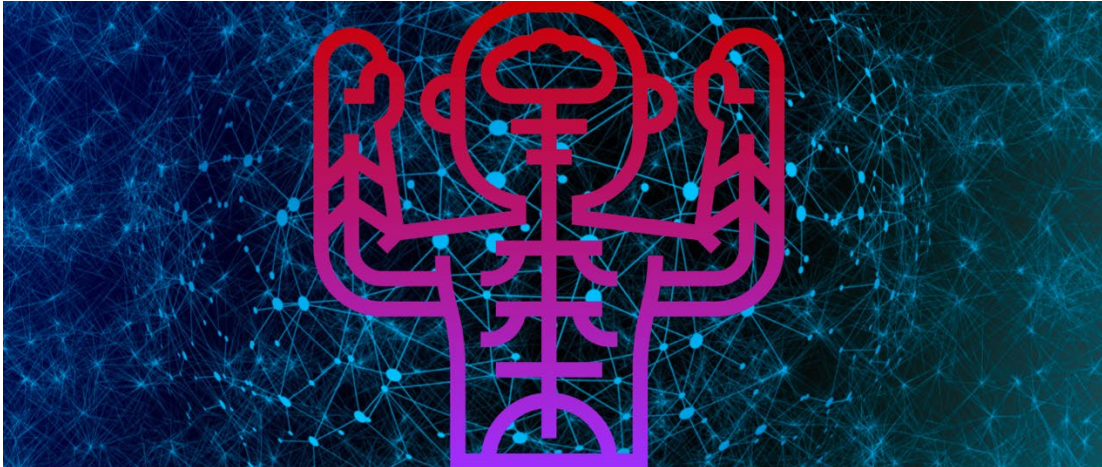
6. The study did not use an ECG to measure HRV data. Use of an ECG is known as the “gold standard” for HRV measurements.
7. The PI was the primary recruiter and study administrator and was an unblinded outcome assessor for all the participants. There are potential unconscious biases influencing the study resulting from the PI being the sole person interacting with study participants.

The PI attempted to control the respiration frequency by demonstrating and asking participants to practice each breath method prior to participants attending the in-person study and by using a written script to initially cue participants into each breath method; however, future studies should have a longer designated practice period to better standardize the implementation of breathing techniques. Previous examinations relied upon the guidance offered by the Asana Pranayama Mudra Bandha text to instruct study participants; however, beyond the text’s cues, which were also implemented in the current study, there is no standard method for ensuring practitioners adhere to the breathing technique cues (Saraswati, 2008). Future studies may find more reliable methods such as using technology to show participants the length of inhalation and exhalation or having participant breathe along with a pre-recorded video.

This study joins only a small group of investigations into the effects of a brief Bhr. P. intervention, and it is the only investigation to explore the acute impact of Bhr. P. on self-reported anxiety. Although we investigated the impact of brief breathing practices on HRV, BP, and self-reported anxiety in healthy adults, future research remains needed to test the current results and expand upon our understanding of the association between breath modulation and health outcomes. Future research on Bhr. P. should focus on population samples that have room for improvement, such as adults with elevated BP or elevated anxiety levels. Additionally, future studies should

investigate the effects of other specific pranayama practices through short-term and long-term interventions to further the evidence on health outcomes. A long-term goal is to understand how breathing practices can be used effectively as ways to enhance health, as stand-alone treatments, or in conjunction with other treatment processes.

## Appendix A – Recruitment Flyer



# PARTICIPANTS NEEDED

## FOR ANXIETY FOCUSED BREATH RESEARCH STUDY

Our nation is reporting worsening mental health across the population. Researchers at the University of Pittsburgh are investigating ancient breathing techniques and the potential effects on anxiety and heart health.

### Who do we need?



Measure  
Heart  
Response

- 18 - 60 years of age
- Not currently taking blood pressure or heart medication
- In good physical health
- Not currently pregnant, experiencing chest pain, or ear infection



Practice  
Breathing  
Technique

### Time is Compensated up to \$50

One Hour time commitment.

Location is next to Oakland and has FREE parking.

Visit the following link or QR code to sign up and determine study eligibility:

[https://pitt.co1.qualtrics.com/jfe/form/SV\\_3NSKsiLORCjcKRU](https://pitt.co1.qualtrics.com/jfe/form/SV_3NSKsiLORCjcKRU)  
or Contact John Tyler Butler via email @jtb132@pitt.edu



## Appendix B – Eligibility Screening

# Breath Anxiety Intervention

---

Start of Block: Block 1

Q2 Thank you for your interest in the Breath and Anxiety Research Study!

The study we're conducting is designed to observe potential changes in heart rate, blood pressure, and feelings of anxiety resulting from a short breathing (pranayama) practice. We are examining whether there is a difference in effect between slow breathing and hummed exhale breathing.

The first step towards participating in this research study is to determine whether you are eligible. Below are a series of questions to determine your eligibility, it will take approximately 5 minutes to complete these questions.

Completing these questions is voluntary. There is some risk of a breach of confidentiality in providing this screening information; however, that risk is greatly reduced by software that encrypts the data and by having the information stored on secured servers.

End of Block: Block 1

---

Start of Block: Default Question Block

I understand that I do not have to take part in the main study if I do the prescreening questionnaire. I am free to withdraw at any time, without having to give a reason and without detriment to me.

☐ Yes (1)

☐ No (2)

---

Q4 Please select yes if you consent to share your information with the research team. Everything you share will be kept confidential and not shared with anyone but the research team.

☐ Yes (1)

☐ No (2)

---

Q5 First Name

---

Q6 Last Name

---

---

Q7 Email

---

---

Q8 Phone Number

---

---

Q9 What is your age?

---

---

Q11 What is your sex?

☐ Male (1)

☐ Female (2)

---

Q12 Do you currently take medication that effects blood pressure or heart rate, such as diuretics, beta blockers, ACE inhibitors, angiotensin II receptor blockers, calcium channel blockers, alpha blockers, alpha-2 receptor agonists, central agonists, peripheral adrenergic inhibitors, or vasodilators?

☐ Yes (1)

☐ No (2)

---

Q13 How many alcoholic beverages do you drink each day, on average?

☐ 0 (1)

☐ 1 (2)

☐ 2 (3)

☐ 3 (4)

☐ 4 (5)

☐ 5 (6)

☐ 6+ (7)

---

Q19 Do you regularly use tobacco products such as cigarettes, electronic cigarettes, vapor cartridges, cigars, hookah, chew/snuff, or other?

☐ Yes (1)

☐ No (2)

---

Q14 Are you currently experiencing chest pain?

☐ Yes (1)

☐ No (2)

---

Q15 Are you currently experiencing an ear infection?

☐ Yes (1)

☐ No (2)

---

Q16 Are you currently pregnant?

☐ Yes (1)

☐ No (2)

---

Q17 Thank you for taking the time to answer these questions. We will review your responses to see whether you may be a good fit for this study and let you know as soon as

possible. In the meantime, please contact John Tyler Butler, our study coordinator, at [jtb132@pitt.edu](mailto:jtb132@pitt.edu) if you have any questions.

End of Block: Default Question Block

---

# Breath Anxiety Study Consent Form

---

Start of Block: Default Question Block

Q1

## Informed Consent

**Thank you for your interest in the Breathing and Anxiety Study!**

**Please review the following information about the details of this research study. Feel free to email us with any questions at [jtb132@pitt.edu](mailto:jtb132@pitt.edu). You may change your mind about participating at any time - even if you click "I consent to participate in the study" at the end of this form. It takes approximately 10-15 minutes to review this document.**

## CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

**Title:** The Breathing and Anxiety Study

**Principal Investigator:** J. Tyler Butler, B.S.

University of Pittsburgh

Department of Health and Human Development

412-992-0038

jtb132@pitt.edu

**Faculty Mentor:** Christopher E. Kline, PhD

**Thesis Committee:** Kelliann Davis, PhD

Brian Galla, PhD

**Source of Funding:** School of Education, University of Pittsburgh

**Key Information Summary**

You are being asked to participate in a research study. In this study, you will perform two distinct breathing methods for approximately five minutes each with a brief break in-between. Research studies include only people who volunteer. The study team members will explain the study to you and will answer any questions you may have. You should take time to make your decision.

Here is a summary of the research study: This study will investigate the effects resulting from two five-minute breathing interventions; The in-person meeting will take approximately one hour to complete and will involve non-invasive measurements.

Risks related to the study include those which are: Common: boredom or frustration from completing study screenings and questionnaires; Less likely: breach of confidentiality from providing personal information; Rare but serious: None identified.

## **Q2 Purpose of Research Study**

The purpose of this study is to compare and examine the changes in heart rate variability (HRV), blood pressure (BP), and your feelings of anxiety resulting from two brief breathing interventions.

### **Basic Eligibility Criteria**

To be eligible to participate, you need to be between 18 and 60 years of age, free from taking any medications that affect your blood pressure or heart rate, a non-smoker, free from chest pain or a current ear infection, consume no to mild amounts of alcohol, and not currently pregnant.

We will enroll approximately 20 individuals for this study.

### **Research Procedures to be Performed**

The total duration of study participation is approximately 90 minutes. As part of this study, you will undergo the following research procedures:

**1. Informed Consent Meeting:** Following initial pre-screening, you will schedule a time to meet with the study coordinator and have the study procedures explained to you. This meeting could occur in person or over Zoom teleconference at a time that is convenient for you. This meeting will take approximately 30 minutes.

At this meeting, you will have the study procedures described to you in detail. If you are still interested in participating in the study after all your questions are answered, you will provide written informed consent. If the meeting is in person, you will sign this document. If the meeting is over teleconference, you will be sent an online version of this consent document using a program called Qualtrics and you will indicate your consent to participate in the study by choosing the ‘I consent to participate in the study’ option in the Qualtrics consent document.

Following informed consent, you will be scheduled for an in-person study visit and given instructions to follow in preparation for participation in the study. In particular, you will be asked to follow these procedures: Please do not consume any food or caffeine in the 12 hours leading up to the visit; Please do not engage in any exercise or strenuous activity in the 24 hours leading up to the visit; Please strive to obtain a normal night of sleep on the night before your visit

Not following these guidelines can lead to inaccurate heart measurements. You will be asked whether you adhered to these procedures upon arrival at the study location. Nonadherence to these guidelines will result in you being ineligible for participation or compensation.

---

**Q3 2. In-Person Meeting (1 hour):** This visit will take place at the Oak Hill Research Center (32 Oak Hill Court, Pittsburgh, PA 15261) and will be scheduled between 8:00 am and 12:00 noon. Upon arrival, you will be asked to empty your bladder. Then you will complete two breath-focused interventions in a specific order—half will complete Condition A first, and half will complete Condition B first, before completing the other condition. The order will be assigned randomly with a procedure like flipping a coin.

In Condition A, you will first rest for approximately 5 minutes while anxiety, heart, and blood pressure measurements are completed. Then you will perform 5 minutes of slow breathing while your heart rhythm is monitored. Then, your anxiety, heart, and blood pressure will be measured after Condition A has been completed.

A brief break (approximately 5 minutes) will occur between the two conditions.

In Condition B, you will rest for approximately 5 minutes while anxiety, heart, and blood pressure measurements are completed. Then you will perform slow breathing with a hummed exhale for 5 minutes while your heart rhythm is monitored. Then, your anxiety, heart, and blood pressure will be measured after Condition B has been completed.

Your anxiety will be assessed with a questionnaire. Your blood pressure will be measured with an automated blood pressure machine using a cuff that is placed on your upper arm. Your heart rhythm will be measured with a strap worn on your chest.

After both conditions have been completed, you will be asked to complete a brief questionnaire that asks about your yoga experience, demographics, and health information.

---

#### **Q4 Study Risks and Side Effects**

Risks and side effects related to the study procedures are minimal. They include risks that are considered to be:

***Likely or common:***

<b>Research Activity</b>	<b>Risk</b>	<b>Associated Measures Taken</b>
Completing study questionnaires	Boredom, stress, or frustration	If you feel uncomfortable, you may refuse to answer a question or questions
Blood pressure assessment	Mild discomfort due to the cuff squeezing the upper arm	Assurance that discomfort is temporary and will go away once the cuff is released
Break period	Boredom, stress, or frustration	You will be given the opportunity to move around freely during this time

***Less likely:***

<b>Research Activity</b>	<b>Risk</b>	<b>Associated Measures Taken</b>
Providing personal information through questionnaires	Breach of confidentiality	All data will be coded with study numbers. Identifiable data will only be available to study staff. Study materials will be stored in a locked file cabinet in a locked office, and computer files will be password-protected and stored on a secure server
Wearing the heart monitor	Monitor may be uncomfortable	We will adjust the tightness of the strap if the monitor is causing pain or discomfort

***Rare but serious:*** None identified

**Benefits of Participating in the Study**

Participants may notice improvements to their feelings of anxiety and their overall health.

However, not all participants will receive these benefits as this is a one time visit. No direct benefit is guaranteed.

### **Costs of Participating in the Study**

Being in this research study will not cost you anything. None of the procedures described above will be billed to you or your health insurance.

---

### **Q5 Study Compensation**

You will receive \$50 if you complete this study. No payment will be provided if you do not complete both steps of participation: the informed consent meeting and the in-person intervention.

You will be paid on a reloadable debit card. Since you are being compensated for your participation in this study, your name, address, and social security number will be released to the Accounting Office. If the total reimbursement for your participation in research is greater than \$600 in a year, this will be reported to the Internal Revenue Service (IRS) as income.

Your data used in this research study may contribute to a new discovery or treatment. In some instances, these discoveries or treatments may be of commercial value and may be sold,

patented, or licensed by the investigators and the University of Pittsburgh for use in other research or the development of new products. You will not retain any property rights nor will you share in any money that the investigators, the University of Pittsburgh, or their agents may receive.

### **Additional Important Information Before You Join this Study**

***Voluntary Participation:*** Your participation in this research study is entirely voluntary. You may want to discuss this study with your family and friends and your personal physician before agreeing to participate. If there are any words you do not understand, feel free to ask us. The investigators will be available to answer your current and future questions.

Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

***If You Are Injured:*** If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC.

Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation. You do not, however, waive any legal rights by signing this form.

***Your Privacy:*** The risk of collecting your protected health information is a breach of confidentiality. This risk is minimal in this study as you will be identified only by a number, and this will only be available to the study investigators. The information collected will then only be linked to the de-identified number and will not be associated with any other identifying information. The data will be used only for research purposes. No one except for the study team will have access to this data. All electronic data will be kept behind firewalls in accordance with institutional security policies. You will not be identified by name in any publication of research results. Your research results may be shared with other investigators, but they will never be provided with information that would allow them to identify you. Per University of Pittsburgh policy, all research records must be maintained for at least 7 years following final reporting or publication of a project.

In addition to the investigators listed on this first page of this consent form and their research staff, the following may have access to identifiable information related to your participation in this research study:

The University of Pittsburgh Office of Research Protections may review your identifiable

research information for monitoring the appropriate conduct of this research study. In unusual circumstances, your identifiable information may be inspected by appropriate government agencies or may be released in response to an order from a court of law. If investigators learn that you or someone with whom you are involved is in danger or potential harm, they will need to inform the appropriate agencies, as required by Pennsylvania law.

***Use of Data:*** Once the research data are de-identified, this information may be used for future research or shared with other investigators conducting similar research. However, any data that are shared will have identifying information removed.

***Discontinuing Participation:*** After signing this form, you may end your participation at any time by contacting any of the investigators listed on the first page of this form. Your data provided prior to discontinuing will still be used by the study investigators, but you will no longer be asked to provide further data.

It is possible that you may be removed from the research study by the researchers if we find that you do not meet the study's eligibility criteria. We may also remove you from the study to protect your safety or if you are unable or unwilling to complete the research protocol. For example, if you refuse to perform the breathing intervention, you will no longer be eligible to participate in the study.

***Disclosure of Research Results:*** A summary of the results of the overall study will not be provided to you, but would be available to you at your request.

### **Questions About the Study**

If you have any questions about your rights as a research subject or wish to talk to someone other than the research team, please call the University of Pittsburgh Human Subjects Protection Advocate toll-free at 866-212-2668.

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### **Q6 VOLUNTARY CONSENT**

**The above information has been explained to me and all my current questions have been answered.** I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during this study, and that such future questions, concerns or complaints will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given.

I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator. I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and

questions; obtain information; offer input; or discuss situations that occurred during my participation.

☐ I consent to participate in the study. (4)

☐ I do not consent. I do not wish to participate in the study. (5)

---

Q7 Please provide your FULL NAME in the box below.

---

---

Q8 Please sign your name below. This will serve as your 'electronic signature' for this document.

End of Block: Default Question Block

---

## Appendix D – Assessment Forms

### Appendix D.1 Data Collection Form

#### PARTICIPANT VISIT CHECKLIST

The assessments must be completed including all the following items. Under no circumstances is this protocol to be altered unless approved by the Principal Investigator for this participant.

- |  |                |
|--|----------------|
| <input type="checkbox"/> Greet participant         | Initial: _____ |
| <input type="checkbox"/> Verify Consent            | Initial: _____ |
| <input type="checkbox"/> Prerequisite Checklist:   | Initial: _____ |
| (Food/cafeine 12hr, exercise 24hr, normal sleep)   |                |
| <input type="checkbox"/> Empty Bladder             | Initial: _____ |
| <input type="checkbox"/> Height & Weight           | Initial: _____ |
| <input type="checkbox"/> Room Acclimation          | Initial: _____ |
| <input type="checkbox"/> Randomization             | Initial: _____ |
| <input type="checkbox"/> HRV Strap Conductivity    | Initial: _____ |
| <input type="checkbox"/> HRV Strap Secure          | Initial: _____ |
| <input type="checkbox"/> Blood Pressure (twice x4) | Initial: _____ |
| <input type="checkbox"/> STAI (x4)                 | Initial: _____ |
| <input type="checkbox"/> Breath Condition A        | Initial: _____ |
| <input type="checkbox"/> Breath Condition B        | Initial: _____ |
| <input type="checkbox"/> Post Questionnaire        | Initial: _____ |
| <input type="checkbox"/> Participant Compensated   | Initial: _____ |
| <input type="checkbox"/> Study Room Clean          | Initial: _____ |

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## A. PREREQUISITE CHECKLIST

Before collecting any data, confirm the following with the participant:

- Have you consumed any food or caffeine (other than water) over the last 12 hours?YN
- Have you exercised in the past 24 hours?YN
- Did you have what you consider a 'normal' night of sleep last night?YN

**Only proceed if the participant answers No, No, and Yes to the three questions, respectively.**

## B. HEIGHT & WEIGHT

	Measurement 1	Measurement 2	Measurement 3
Height (cm)			

	Measurement 1	Measurement 2	Measurement 3
Weight (kg)			

## C. BP CUFF SIZING

- BP cuff size: PEDIATRICADULTLARGE ADULT

## D. ROOM ACCLIMATION (5 minutes)

- Exact start time for room acclimation: \_\_\_\_\_

## E. RANDOMIZATION

- Condition order:1: BHR. P.; 2: SLOW BREATHING1: SLOW BREATHING; 2: BHR. P.

## F. BASELINE #1 ASSESSMENTS

- Exact start time for HRV recording: \_\_\_\_\_
- Exact start time for 5-min baseline HRV recording: \_\_\_\_\_
- Exact end time for 5-min baseline HRV recording: \_\_\_\_\_
- Exact start time for baseline BP assessments: \_\_\_\_\_

	Measurement 1	Measurement 2	Measurement 3
SBP			
DBP			
HR			

- STAI completed? YN

**G. BREATHING CONDITION A**

- *Exact* start time for condition A: \_\_\_\_\_
- *Exact* end time for condition A: \_\_\_\_\_

**H. POST-CONDITION #1 ASSESSMENTS**

- *Exact* start time for BP assessments: \_\_\_\_\_

	Measurement 1	Measurement 2	Measurement 3
SBP			
DBP			
HR			

- STAI completed? YN

**I. WASH-OUT PERIOD**

- *Exact* start time for wash-out period: \_\_\_\_\_
- *Exact* end time for wash-out period: \_\_\_\_\_

**J. BASELINE #2 ASSESSMENTS**

- *Exact* start time for 5-min baseline HRV recording: \_\_\_\_\_
- *Exact* end time for 5-min baseline HRV recording: \_\_\_\_\_
- *Exact* start time for baseline BP assessments: \_\_\_\_\_

	Measurement 1	Measurement 2	Measurement 3
SBP			
DBP			
HR			

- STAI completed? YN

**K. BREATHING CONDITION B**

- *Exact* start time for condition B: \_\_\_\_\_
- *Exact* end time for condition B: \_\_\_\_\_

**L. POST-CONDITION #2 ASSESSMENTS**

- *Exact* start time for BP assessments: \_\_\_\_\_

	Measurement 1	Measurement 2	Measurement 3
SBP			
DBP			
HR			

- STAI completed? YN

- *Exact* end time for HRV recording: \_\_\_\_\_

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## Appendix D.2 STAI Form

A number of statements which people have used to describe themselves are given on the following pages. Read each statement and select the appropriate response to indicate how you feel right now, that is, at this very moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

		<i>Not at All</i>	<i>Somewhat</i>	<i>Moderately So</i>	<i>Very Much So</i>
1.	I feel calm	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>
2.	I feel tense	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>
3.	I feel relaxed	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>
4.	I feel upset	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>
5.	I feel content	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>
6.	I feel worried	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>

### Appendix D.3 Post Study Questions

*For each of the following questions, please circle the letter that best applies to you or fill in the blank space.*

1. What is your level of experience with yoga practice?
  - A. I have no experience with yoga
  - B. I have recently started a yoga practice (less than 3 months)
  - C. Longer than 3 months ago, but less than 2 years
  - D. 2 – 5 years
  - E. 5 – 10 years
  - F. Over 10 years
  
2. What is your experience with conscious breath practices (also called *pranayama*)?
  - A. I have no prior experience with pranayama
  - B. I have recently started a pranayama practice (less than 3 months)
  - C. Longer than 3 months ago, but less than 2 years
  - D. 2 – 5 years
  - E. 5 – 10 years
  - F. Over 10 years
  
3. Do you consider yourself to be of Hispanic or Latino ethnicity?
  - A. Yes
  - B. No
  
4. What race do you consider yourself? *Circle the letter of all that apply:*
  - A. Asian
  - B. Black or African American
  - C. White
  - D. American Indian or Alaska Native
  - E. Native Hawaiian or Other Pacific Islander

*(continued on back)*

5. During the past **month**, which statement **best** describes the kinds of physical activity you usually did? Do not include the time you spent working at a job. Please read all six statements before circling **one**.
- A. I did not do much physical activity. I mostly did things like watching television, reading, playing cards, or playing computer games. Only occasionally, **no more than once or twice a month**, did I do anything more active such as going for a walk or playing tennis.
  - B. **Once or twice a week**, I did **light activities** such as getting outdoors on the weekends for an easy walk or stroll. Or once or twice a week, I did chores around the house such as sweeping floors or vacuuming.
  - C. **About three times a week**, I did **moderate activities** such as brisk walking, swimming, or riding a bike **for about 15-20 minutes each time**. Or about once a week, I did moderately difficult chores such as raking or mowing the lawn for about 45-60 minutes. Or about once a week, I played sports such as softball, basketball, or soccer for about 45-60 minutes.
  - D. **Almost daily, that is, five or more times a week**, I did **moderate activities** such as brisk walking, swimming, or riding a bike **for 30 minutes or more each time**. Or about once a week, I did moderately difficult chores or played sports for 2 hours or more.
  - E. **About three times a week**, I did **vigorous activities** such as running or riding hard on a bike **for 30 minutes or more each time**.
  - F. **Almost daily, that is, five or more times a week**, I did **vigorous activities** such as running or riding hard on a bike **for 30 minutes or more each time**.

## Appendix D.4 Participant Payment Certificate

### PARTICIPANT PAYMENT CERTIFICATE

This is to certify that I participated as a research subject in the **Breath and Anxiety (BA)** **Research Study** on \_\_\_\_\_. I will be reimbursed for my participation as follows:

Participant Payment:

*Completion of assessment:*

☐ Assessment (\$50) \$ \_\_\_\_\_

\_\_\_\_\_  
Participant Signature

\_\_\_\_\_  
Date

-----  
**PLEASE PRINT:**

**Name:** \_\_\_\_\_

**Address:** \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**Phone Number:** \_\_\_\_\_

**Birthdate:** \_\_\_\_\_

**Social Security #:** \_\_\_\_\_

APPROVED: \_\_\_\_\_

Principal Investigator/Authorized Designee

DATE: \_\_\_\_\_

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