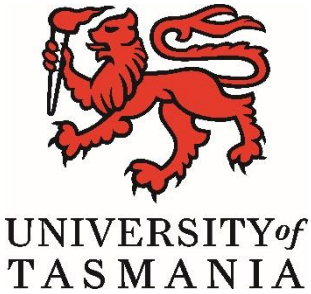

Neuromuscular, Cardiovascular, and Cortical Responses to Heart Rate Variability Biofeedback

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MSc Applied Sport and Exercise Science

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DOCTOR OF PHILOSOPHY

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Declaration of Originality

This thesis entitled "Neuromuscular, Cardiovascular, and Cortical Responses to Heart Rate Variability Biofeedback" contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, except by way of background information and duly acknowledged in the thesis. To the best of my knowledge and belief, no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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The research associated with this thesis abides by the International and Australian codes on human and animal experimentation, the guidelines by the Approval of Tasmania Health and Medical Human Research Ethics Committee (Approval number: H001658) and Swinburne University Human Research Ethics Committee (Approval number: 2017/346).

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Statement of Candidate and Co-Authorship Contribution of Jointly Published Work

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Paper Two: A Preliminary Systematic Review and Meta-Analysis on the Effect of Heart Rate Variability Biofeedback on Heart Rate Variability and Respiration of Athletes

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- The candidate completed all aspects of data collection individually. Author 3 approved the studies to be included for review.
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Paper Three: Effect of Acute Heart Rate Variability Biofeedback on H-reflex Modulation: A Pilot Study

- Located in Chapter Four.
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Paper Four: Acute Effects of Resonance Frequency Breathing on Cardiovascular Regulation

- Located in Chapter Five
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Paper Five: Effect of Acute Heart Rate Variability Biofeedback on Heart Evoked Potential Among Healthy Population

- Located in Chapter Six.
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- Author 2 and 5 helped in ethical clearance application, data gathering and analysis, and manuscript completion.
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We, the undersigned, agree with the above stated “proportion of work undertaken” for each of the above published or submitted peer-reviewed manuscripts contributing to this thesis.

Signed:

Candidate:

Author 1:

Author 2:

Author 3:

Author 4:

Author 5:

Author 6:

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This thesis comprises two reviews and three research investigations, which have been completed almost entirely by the candidate, Jeffrey Pagaduan (School of Health Sciences, College of Health and Medicine, University of Tasmania). The candidate played a leading role in the design of the studies, participant recruitment, data collection, data analysis and interpretation, and is first author on all manuscripts (chapters' two to six and appendix B). The following people and institutions also contributed to each of the studies (unless otherwise specified) as detailed:

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- Dr Sam SX Wu (Department of Health, Arts and Design, School of Health Sciences, Department of Health and Medical Sciences, Swinburne University of Technology): Advised on study design (studies one, two, three, four, and five) assisted with collection of data (studies five) and manuscript revisions (studies one, two, three, four, and five).
- Dr Yung Sheng-Chen (Department of Health and Exercise Sciences, University Taipei): assisted on data analysis (studies one, two, three), ethics application (study three), and manuscript completion (study three).
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- Dr Tatiana Kameneva (Faculty of Science, Engineering, and Technology, Swinburne University): helped with data analysis and manuscript revision in study four.

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- Leighton James (School of Health Sciences, Department of Health and Medical Sciences, Swinburne University), assisted with data collection and exercise testing (studies four), as part of honours research.

List of Publications

Chapters of this thesis have been previously published in, or are under consideration in peer-reviewed journals:

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Pagaduan JC, Wu SSX, Fell JW, Chen YS. Can heart rate variability biofeedback improve athletic performance? a systematic review. *J Hum Kinet,* 2020; 73: 103-114.

Pagaduan J, Wu SS, Kameneva T, Lambert E. Acute effects of resonance frequency breathing on cardiovascular regulation. *Physiol Rep,* 2019; 7: 14295.

Pagaduan J, Chen YS, Fell J, Wu S. A preliminary systematic review and meta-analysis on the effect of heart rate variability biofeedback on heart rate variability and respiration of athletes. *J Comp Integr Med* (in review).

Pagaduan JC, White D, Lambert E, Leighton J, Fell JW, Wu SSX. Effect of acute heart rate variability biofeedback on heartbeat evoked potential among healthy population (for submission).

Other manuscripts that have been accepted and those currently under review, prepared throughout the duration of the PhD candidature:

Pagaduan J, Chen YS. Validity of Pulse Express PRO in measurement of heart rate variability. *Ger J Exerc Sport Res,* 2021; https://doi.org/10.1007/s12662-021-00708-8

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Balite PHM, Balite PHM, Manalo MJA, **Pagaduan J.** Psychophysiological responses to competition among university swimmers. *J Hum Sport Exerc* (in press).

Raval KMR, **Pagaduan JC.** Factors that discriminate winning and losing in men's university basketball. *Monten J Sports Sci Med* (in press).

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Chen YS, Lu WA, Kuo CD, **Pagaduan JC.** A novel smart phone application Pulse Express PRO is valid and reliable tool for ultra-short-term and short-term heart rate variability. *JMIR mhealth and uhealth,* 2020; 8: e18761.

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Rhibi F, Grham A, **Pagaduan J**, Sellami M, Abderrahman A. Short-term maximal performance depend on post-activation potentiation stimuli type and recovery period. *Sport Sci Health,* 2018; 14: 235-243.

Conference Presentations

Presentations arising from studies completed throughout the candidature:

August 2018: Asian Sport Management Conference (Manila, Philippines), speaker: Low-cost technology for performance monitoring.

September 2017: Graduate Research Conference (Hobart, Tasmania), poster: Can heart rate variability biofeedback improve the health of hypertensive individuals?

Conference Proceedings

Pagaduan J, Užičanin E, Muratović M, Pojskić H. Accuracy and reliability of a free mobile HRV mobile application in measurement of heart rate variability. *J Hum Sport Exerc*, 2019; S578-S583.

Pojskić H, **Pagaduan J**, Uzicanin E, Sekulic D. The development of new sport-specific response time tests: validity, reliability, and functionality. *16th Annual Scientific Conference of Montenegrin Sports Academy "Sport, Physical Activity and Health: Contemporary Perspectives",* 2019; 16: 27.

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General Abstract

Background

Heart rate variability biofeedback (HRV BFB) is a paced breathing scheme that triggers resonance in the cardiovascular system. Whilst HRV BFB approaches have been administered in performance enhancement settings, there seems to be a scarcity in systematic literature investigating performance parameters under HRV BFB. There is also no meta-analysis that examined the effects of HRV BFB on physiological indices. In addition, there has been no study that explored neuromuscular function in HRV BFB. Lastly, there have been limited underpinnings in vagal afferent pathway with HRV BFB.

Aims

The overarching aim of this thesis was to carry out a series of pilot studies examining various physiological indices under HRV BFB. To investigate this aim, five studies were undertaken. The purpose of the first study was to conduct a systematic review on the effect of HRV BFB on gross and fine motor performance of athletes. The objective of the second study was to administer a systematic review and meta-analysis on the effects of HRV BFB on heart rate variability and respiration of athletes. The third study aimed to determine the effect of acute HRV BFB on soleus motoneuron excitability using H-reflex. The fourth study aimed to investigate the effect of HRV BFB on muscle sympathetic nervous activity (MSNA). The purpose of the fifth study was to explore the effect of HRV BFB on vagal afferent pathway utilising heart evoked potentials (HEP).

Results

Study One: The influence of HRV BFB in fine and gross motor skills in athletes demonstrated conflicting findings.

Study Two: HRV BFB reduced the respiration rate of athletes.

Study Three: HRV BFB facilitated reduction in soleus motoneuron excitability.

Study Four: An acute HRV BFB decreased MSNA compared to spontaneous breathing.

Study Five: Significant HEP differences at fronto-parietal (Fp2), frontal (F3, Fz) central (C4), parietal (P7, Pz), occipital (O1, Oz, O2), and parieto-occipital (PO1, PO2) existed between HRV BFB and CON. Specifically, HRV BFB posted higher HEP at Fp2, F3, Fz, C4, and P7 than CON. However, HRV BFB demonstrated lower HEP at Pz, O1, Oz, O2, PO1, Pz, and PO2 compared to CON.

Conclusions

The major conclusions of this thesis are:

1. The influence of HRV BFB to enhancement of fine and gross in athletic population is unclear.

2. Application of HRV BFB among athletes facilitated reduction in breathing frequency.

3. An acute HRV BFB facilitated depression in the monosynaptic reflex of the soleus muscle.

4. A ten-minute HRV BFB regulated the muscle sympathetic outflow.

5. A ten-minute HRV BFB increased cortical activity connected to cardiac regulation.

The significance of the systematic reviews and meta-analysis administered in this thesis demonstrate the potential of HRV BFB in athlete population. The novel physiological underpinnings in HRV BFB conducted in pilot studies, provide useful information towards understanding mechanisms in HRV BFB that may influence performance. Thus, this thesis contributes to the literature pertaining to HRV BFB and performance settings, and the experimental studies presented physiological insights in HRV BFB, crucial to promotion of HRV BFB.

Table of Contents

Table of Figures

Table of Tables

List of Appendices

Common Abbreviations

Selected abbreviations throughout the text

BP	blood pressure
ECG	electrocardiography
EEG	electroencephalography
HEP	heartbeat evoked potential
HRV BFB	heart rate variability biofeedback
HF	high frequency heart oscillations
HFnu	normalised high frequency
HR	heart rate
H-reflex	Hoffman reflex
HRV	heart rate variability
LF	low frequency heart oscillations
LFnu	normalised low frequency
MSNA	muscle sympathetic nervous activity
RF	resonance frequency
RMSSD	root mean square of the successive differences
RR	respiration rate
SB	spontaneous breathing
SDNN	standard deviation of normal to normal intervals
TP	total power HRV

Chapter One: Thesis Introduction and Overview

1.1 Thesis Organisation

This doctoral thesis contains a series of studies aimed at examining the effects of HRV BFB on physiological indices.

Chapter One covers a general introduction of the factors comprising the themes of this thesis, identifying the individual aims and significance of the research, in addition to the general layout of the thesis.

Chapter Two contains a systematic review on the effect of HRV BFB on performance of athletes.

Chapter Three reports a preliminary systematic review and meta-analysis based on the effect of HRV BFB on HRV and respiration of athletes.

Chapter Four provides a detailed account of the third study of this thesis, which examined the acute effect of HRV BFB on the soleus H-reflex modulation.

Chapter Five outlines the fourth study of this thesis, which investigated the muscle sympathetic nervous activity (MSNA) under spontaneous breathing, resonance frequency (RF), and one breathing frequency above RF.

Chapter Six includes the fifth and final study of this thesis designed to examine the acute effects of HRV BFB on heartbeat evoked potential (HEP).

Chapter Seven provides a discussion of the major findings of this thesis, including potential implications of the research undertaken, and provides an outline of directions for future research and an overall conclusion.

1.2 Background

Heart rate variability (HRV) refers to beat to beat fluctuations that depict autonomic regulation (Shaffer and Ginsberg, 2017; Task Force, 1996). Low HRV is associated with susceptibility to stress (Kim et al., 2018) and clinical symptoms (Koch et al., 2019; Prinsloo et al., 2014). Techniques aimed at increasing HRV have been reported to exhibit positive health outcomes (Bretherton et al., 2019; Sandercock et al., 2005; Zaccaro et al., 2018). One such technique for increasing HRV is the heart rate variability biofeedback (HRV BFB) (Gevirtz, 2013; Prinsloo et al., 2014; Wheat and Larkin, 2010).

The HRV BFB is a paced breathing technique guided by real-time physiological feedback in which typically a slowing of breathing rate impacts heart rate (HR) (Lehrer et al., 2000). The basis for this concept lies with the brain-heart interactions suggested by Claude Bernard over 150 years ago. It is suggested that because cerebral arousal and autonomic control over the cardiovascular system influence one another, modifying one will alter the other; when applied systematically, the effect may become persistent (Deschodt-Arsac et al., 2018). In the HRV BFB approach proposed by Lehrer et al. (2000), the user aims to maintain a specific breathing pace. This breathing pace (4 – 6.5 breaths/min), also known as resonant frequency (RF), activates the resonance properties in the cardiovascular system integral to autonomic regulation (Lehrer et al., 2000; Lehrer and Eddie, 2013; Vaschillo et al., 2006). Two distinct physiological characteristics are noticeable under HRV BFB: a) occurrence of maximal amplitude HR oscillation, and, b) parallel oscillations of HR and respiration (Vaschillo et al., 2002; Vaschillo et al., 2006).

1.2.1 Measurement of resonance frequency

The RF has been reported to be identified from a protocol in which paced breathing is performed at 6.0, 5.5, 5.0, 4.5 breaths/min for 2-3 minutes at each breathing frequency (Lehrer, 2000; Vaschillo et al., 2006). A respiration belt and HR sensor are attached during paced breathing to measure breathing rate and HR respectively. The breathing rate at which the peak low frequency HRV is found to occur is identified as the RF (Lehrer et al., 2000).

1.2.2 Mechanisms in heart rate variability biofeedback

Possible mechanisms through which HRV BFB is proposed to be beneficial include alterations in baroreflex, vagal afferent pathway, and respiratory sinus arrhythmia (RSA) (Lehrer, 2013; Lehrer and Gevirtz, 2014). Other potentially important physiological mechanisms that have been proposed are connected to visceral afferents/efferents, respiration, and cardiovascular parameters in HRV BFB (Fonoberova et al., 2014; Lehrer and Gevirtz, 2014).

1.2.2.1 Baroreflex

An important mechanistic path through which HRV BFB acts is the enhancement of baroreflex sensitivity (Lehrer et al., 2003; Vaschillo et al., 2004). The baroreflex is a closed-loop system and plays a crucial role in the regulation of blood pressure (BP); increases in BP decreases HR, while decreases in BP increases HR (Vaschillo et al., 2002; Vaschillo et al., 2006). BP shifts are mediated via pressure sensors known as baroreceptors that are located in the carotid artery and aorta (Lehrer, 2013). With an increased activity of baroreceptors, more action potentials are generated, these are relayed to the nucleus of the solitary tract in the medulla via the vagus nerve, which

results in stimulation of the vagal nuclei (Shaffer et al., 2014). The result is sympathetic inhibition and parasympathetic activation, maximizing the capability of baroreflex to reduce BP it is elevated. Previous research found application of acute HRV BFB exhibited increased baroreflex activation, while long-term HRV BFB facilitated increased baroreflex gain (Lehrer et al., 2003). Enhancement in baroreflex gain from long-term HRV BFB suggests intrinsic baroreflex adaptation that is independent from breathing stimulus.

1.2.2.2 Vagal afferent pathway

Improvement in the vagal afferent pathway has also been proposed as a potential mechanism of action in HRV BFB (Huang et al., 2018; Lehrer and Gevirtz, 2014; Mackinnon et al., 2013). The vagal afferent pathway, the sensory path from the heart to the brain via the vagus nerve, is known to influence the regulation of affect and mood (Grundy, 2002; Park and Blanke, 2019). The mechanism of the vagal afferent pathway under HRV BFB can be evaluated using the heartbeat evoked potential (HEP), an event-related-potential component related to the cortical processing of the heartbeat (Dirlich et al., 1998; Gray et al., 2007; Huang et al., 2018; Mackinnon et al., 2013; Schandry et al., 1986). The HEP depicts cortical signals timelocked at 200-600 ms after the R-wave of electrocardiography. Existing mainly at the frontal brain areas, the HEP topography shows a decreasing trend in from the frontal to occipital areas (Montoya et al., 1993; Schandry and Weitkunat, 1990). Generally, higher HEP exhibits better affect and self-regulation (Park and Blanke, 2019). Only two studies investigated HEP under HRV BFB (Huang et al., 2018; Mackinnon et al., 2013). Huang et al. (2018) demonstrated increased HEP with an audio distraction task after HRV BFB. In another study, Mackinnon et al. (2013) recorded lower HEP in HRV BFB compared to resting

state, negative emotion induction, and positive emotion at central brain regions among healthy individuals. More work on HEP and HRV BFB is needed to better understand the mechanism of the vagal afferent pathway in HRV BFB.

1.2.2.3 Respiratory Sinus Arrythmia

HRV BFB has also been suggested to enhance respiratory sinus arrhythmia (RSA) (Lehrer, 2003), the naturally occurring variation in HR that occurs during the respiratory cycle (Lehrer, 2014; Shaffer et al., 2014). RSA is an indicator of the vagal efferent pathway (brain to heart communication network), and is dependent on vagal outflow or acetylcholine metabolism in the vagus nerve (Shaffer and Ginsberg, 2017). During inhalation, the cardiovascular center inhibits vagal outflow and increases HR. Conversely, exhalation slows HR via restoration of vagal outflow from increased acethylcholine (Eckberg and Eckberg, 1982; Shaffer et al., 2014). The maximal HR oscillations achieved from HRV BFB increases gas exchange and enhances acetylcholine metabolism, thereby improving RSA function (Bernardi et al., 2001; Lehrer et al., 2003).

1.2.2.4 Other possible mechanisms

Improvement in the cholinergic anti-inflammatory pathway has also been recommended as a mechanism in HRV BFB (Lehrer and Gevirtz, 2014). The cholinergic anti-inflammatory pathway regulates inflammation response by suppression of pro-inflammatory markers via the afferent and efferent fibers in the viscera (Borovikova et al., 2000; Chang et al., 2003; Tracey, 2000). The enhancement in cholinergic anti-inflammatory pathway with HRV BFB is supported by research

conducted by Nolan et al. (2012) in which reduced high-sensitivity C-reactive protein were reportedly reduced among hypertensive patients after HRV BFB.

HRV BFB has also been suggested to enhance respiratory mechanics from increased tidal volume (Bernardi et al., 2001; Gevirtz, 2013; Lehrer et al., 2003) and diaphragm excursion (Russo et al., 2017; Vostatek et al., 2013).

Additionally, Fonoberova et al. (2014) suggested cardiovascular mechanisms such as increased blood flow to internal organs, improved arterial receptor gain in response to heart control period, and elevated minimum left ventricular elastance in HRV BFB.

1.2.3 Heart rate variability biofeedback and athletes

Over the past decade, HRV BFB has been used as a complementary intervention to athlete training aimed at eliciting benefits in performance. Existing studies in athlete population under HRV BFB exhibited the potential utility of HRV BFB in performance enhancement (Jiménez Morgan and Mora, 2017). Jiménez Morgan and Mora (2017) reported that enhancement of cardiac autonomic regulation with HRV BFB facilitates alteration in psychophysiological indices that in turn contribute to changes in performance.

1.2.4 Conclusion

Research to date suggests that HRV BFB is a simple and practical technique that may improve performance enhancement of athletes. However, there is a deficiency in systematic reviews in the literature investigating performance indices in athletes with HRV BFB. There is also no systematic review nor meta-analysis examining the physiological

indices among athletes under HRV BFB. Further, no study has investigated neural excitability in HRV BFB. There is no available evidence on muscle sympathetic outflow response with HRV BFB. There is also limited evidence regarding the heart to brain communication pathway under HRV BFB. Given these shortcomings, there is a need to carry out a systematic review relating to HRV BFB and performance in athletes. Conducting another systematic review and meta-analysis, identifying the effects of HRV BFB on physiological indices, may be helpful in explaining the role of HRV BFB on performance. Moreover, investigating novel neuromuscular parameters that may aid understanding the influence of HRV BFB on performance is warranted. Lastly, assessment of heart to brain communication pathway under HRV BFB may elucidate information linking vagal influence and performance.

1.3 Significance of the Research

This thesis aims to contribute to the lack of research regarding HRV BFB in athletic settings. In addition, the series of pilot studies documented in this thesis explore potential novel neuromuscular indices in HRV BFB. Further, this thesis will expand and explore recent work on the heart-to-brain communication pathway under HRV BFB. These studies aim, to some degree, to bridge the gap in understanding autonomic regulation via HRV BFB and performance.

1.4 Research Aims

The overarching aim of the series of five studies was to investigate factors that may influence the performance of athletes under HRV BFB. The first study is a systematic review, examining the effects of HRV BFB on performance. The second study is a systematic review and meta-analysis, determining the effects of HRV BFB on

physiological parameters. The third study explored the acute effect of HRV BFB on neural excitability. The fourth study investigated the acute effect of HRV BFB on muscle sympathetic outflow. A final, fifth study examined the acute effect of HRV BFB on cortical activity.

The specific aims of each study are as follows.

Study One: To conduct a systematic review on the ability of HRV BFB towards influencing gross and fine motor performance in athletes.

Study Two: To carry out a systematic review and meta-analysis on the effects of HRV BFB on HRV and respiration of athletes.

Study Three: To examine the acute effect of HRV BFB on H-reflex modulation of the soleus muscle.

Study Four: To evaluate the MSNA function under HRV BFB.

Study Five: To investigate the HEP in acute HRV BFB.

Chapter Two: Can Heart Rate Variability Biofeedback Improve Athletic Performance? A Systematic Review

An original version of this chapter has been published in the Journal of Human Kinetics as a review and appears in the literature as:

Pagaduan J, Wu SSX, Fell JW, Chen YS. Can heart rate variability biofeedback improve athletic performance? a systematic review. *J Hum Kinet,* 2020; 73: 103-114.

Rationale

There is limited published research pertaining to HRV BFB in athletic performance settings. Therefore, this review aimed to identify the effects of HRV BFB on gross and fine motor function in athlete populations.

Chapter Three: A Preliminary Systematic Review and Meta-Analysis on the Effect of Heart Rate Variability Biofeedback on Heart Rate Variability and Respiration of Athletes

An original version of this chapter has been submitted for publication with the title:

Pagaduan JC, Chen YS, Fell JW. Wu SSX. A Preliminary systematic review and meta-analysis on the effects of heart rate variability biofeedback on heart rate variability and respiration of athletes.

Rationale

Heart rate variability and respiration are common indices affected by HRV BFB. Undertaking a systematic literature review to investigate the extent to which these physiological outcomes change in athletic settings may help explain performance changes from HRV BFB practice. This is a novel review that assessed the effects of HRV BFB on heart rate variability and respiration in athletes.

3.1 Abstract

To date, there is no quantitative review examining the influence of heart rate variability biofeedback (HRV BFB) on the athlete population. Such an undertaking may provide valuable information on the autonomic and respiration responses of athletes when performing HRV BFB. Thus, purpose of this preliminary systematic review and meta-analysis on the effects of HRV BFB on HRV and respiration of athletes. Searches of Springerlink, SportDiscus, Web of Science, PROQUEST Academic Research Library, Google Scholar, and ScienceDirect were conducted for studies that met the following criteria: 1. experimental studies involving athletes that underwent randomized control trial; 2. availability of HRV BFB as a treatment compared with a control (CON)/ placebo (PLA); 3. any pre and post HRV variable and/or breathing frequency as dependent variable/s; and, 4. peer-reviewed articles written in English. Five out of 667 studies involving 143 athletes (34 females and 109 males) ages 16 to 30 years old were assessed in this review. Preliminary findings suggest the promising ability of HRV BFB to improve respiratory mechanics in athlete population. More work is needed to determine the autonomic modulatory effect of HRV BFB in athletes.

Keywords: heart rate variability, biofeedback, resonant frequency, athletes, resonance frequency breathing, autonomic nervous system

3.2 Introduction

Cardiac rhythm is controlled by the autonomic nervous system (ANS) through the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) (Bernston et al., 1997; McCraty and Shaffer, 2015; Olshansky et al., 2008; Task Force, 1996). PNS and SNS operate via the sinoatrial node (SA node) which is mainly responsible for increasing or decreasing heart rate (Shaffer et al., 2014). The interaction of PNS and SNS can be assessed using a non-invasive and reliable method called heart rate variability (HRV) (Bernston et al., 1997; Task Force, 1996; Vanderlei et al., 2010). HRV refers to fluctuations between heartbeats that represent sinus node depolarizations in the QRS complexes of the electrocardiogram (ECG). The QRS intervals, specifically the distance between R to R intervals, are computed to derive time, frequency, and non-linear domains of HRV (Nunan et al., 2010; Reyes del Paso et al., 2013; Task Force, 1996). Depressed HRV reflect sympathetic overactivation and is linked to various clinical and psychological diseases (Gang and Malik, 2003; Gevirtz, 2013; Prinsloo et al., 2014). On the other hand, the presence of high HRV is believed to represent homeostasis and resilience to stress (Gevirtz, 2013; Porges, 2003; Thayer and Sternberg, 2006).

Over the last decade, interventions aimed at increasing HRV with the goal of improving health have received attention. Among these is HRV biofeedback (HRV BFB), a non-invasive intervention utilising paced respiration assisted by visual feedback (Gevirtz, 2013; Lehrer and Eddie, 2013; Lehrer and Gevirtz, 2014; Lehrer and Vaschillo, 2008; Lehrer et al., 2000). HRV BFB was first documented in a clinical facility in Russia (Chernigovskaia et al., 1993). A typical HRV BFB set-up consists of heart rate (HR) and respiration sensors linked to a computer screen with breathing

pacer and provides real-time values of HR and respiratory rate (RR). HRV BFB use resonance frequency (RF) which presents oscillatory episodes: a) a 0-degree phase shift between HR and respiration; and, b) a 180-degree phase relationship between HR and blood pressure (BP) (Vaschillo et al., 2002; Vaschillo et al., 2004; Vaschillo et al., 2006). Additionally, researchers also discovered peak gas exchange and oxygen saturation at RF (Lehrer et al., 2003; Lehrer et al., 2004).

Autonomic responses from HRV BFB have been linked to various physiological mechanisms (Gevirtz, 2013; Lehrer and Gevirtz, 2014). Firstly, HRV BFB is believed to enhance baroreflex sensitivity (Fonoberenova et al., 2014; Lehrer and Gevirtz, 2014). The baroreflex system (BRS) plays a critical role in regulating BP that protects the body from acute blood pressure shifts (Lehrer and Gevirtz, 2014; Vaschillo et al., 2006). The BRS operates in a closed loop system wherein baroreceptors react to shifts in BP and increase or decrease HR (Vaschillo et al., 2006). Briefly, elevation in BP reduces HR, while BP depression increases HR. Conversely, increases in HR elevate BP, while decreases in HR elevates BP. The baroreceptors in the BRS are located in the heart and aortic arch that send chemical and mechanical information to the nucleus of the solitary tract (Shaffer et al. 2014). The nucleus of the solitary tract is connected to other regulatory centers in the medulla wherein SNS outflow to the heart and blood vessels are controlled. These reactions present a mechanical delay of about five seconds due to inertia and vascular plasticity (Lehrer and Eddie, 2013; Vaschillo et al., 2006). During HRV BFB, the amplitude of HR oscillations is maximized and stimulates baroreflex response (Lehrer and Eddie 2013; Vaschillo et al., 2002; Vaschillo et al., 2004; Vaschillo et al., 2006). With constant HRV BFB practice, improved baroreflex function can be achieved over time (Lehrer and Gevirtz, 2014).

Another possible mechanism in HRV BFB is the vagal afferent pathway stimulation (Gevirtz, 2013; Lehrer and Gevirtz, 2014). HRV BFB promotes the activity of subdiaphragmatic vagal afferents and enhance the vagal braking system responsible for immediate control of HR and BP. It has also been postulated that HRV BFB strengthens the parasympathetic vagal efferent pathway through accentuated antagonism, a physiological response that inhibits tonic sympathetic activation from abrupt parasympathetic stimulation under normal physiological conditions in rest and exercise (Hobson et al., 2008; Lehrer and Gevirtz, 2014; Olshansky et al., 2008). Similarly, enhancement of cholinergic anti-inflammatory pathway (CAP) may also be present in HRV BFB (Tracey 2002; Tracey, 2007). During HRV BFB, vagal activation in CAP releases acetylcholine which reduces inflammatory activity in macrophages, thereby diminishing pathogenesis (Bernik et al., 2002; Borovikova et al., 2000).

As the majority of literature reviews in HRV BFB has been conducted on healthy populations or people with chronic conditions, there remains a paucity of systematic literature on the effects of HRV BFB on autonomic and respiratory indices in athletes. These physiological responses may provide useful information towards linking HRV BFB and performance. Thus, the purpose of this study was to conduct a preliminary systematic review and meta-analysis on the effects of HRV BFB on HRV and respiration of athletes.

3.3 Methods

3.3.1 Search Strategy and Inclusion Criteria

Literature search was administered between July 1st 2017 to December 20th 2017 using the search term “heart rate variability biofeedback” AND (athletes OR athletic

population OR sport OR performance OR sport performance) in electronic databases (Springerlink, SportDiscus, Web of Science, PROQUEST Academic Research Library, Google Scholar, and ScienceDirect) adhering to the PRISMA guidelines (Jiménez Morgan and Molina Mora, 2017; Moher et al., 2009). A manual search in the reference section of relevant articles were performed to include additional studies for assessment. Studies met all the following inclusion criteria: 1. experimental studies that involved random group allocation of healthy athletes: 2. availability of HRV BFB as a treatment group compared with a control (CON)/ placebo (PLA); 3. any pre and post HRV parameter and/or breathing frequency as dependent variable/s; and, 4. peer-reviewed articles written in English.

3.3.2 Coding of Studies

Literature search and selection of studies was conducted by a single investigator (JP) with studies coded and organized in an Excel spreadsheet. Data extraction was evaluated by a second investigator (YSC). Articles included in the systematic review were encoded by author/s and year of publication, sample size information, intervention, measured HRV parameters, and results. Risk of bias in a study was also assessed by both investigators using the eight-point Consolidated Standards of Reporting Trials (CONSORT) statement (Moher et al., 2001). Each item in the CONSORT statement is answerable by 0 (absent or inadequately described) or 1 (explicitly described and present). A study with a score of 0-2 is regarded as having a high risk of bias, 3-5 with medium risk of bias, and 6-8 considered as having low risk of bias (Moher et al., 2001). Any disagreement presented in data extraction and CONSORT output was settled by a consensus between the first and second

investigator. Personal correspondence to the author/s of an included study for any clarification was also administered.

3.3.3 Meta-Analysis

A meta-analysis was carried out if at least two studies provided sufficient data to compute for effect sizes (ES). The natural logarithm of low frequency HRV (lnLF), high frequency HRV (lnHF) and total power (lnTP) were utilised as HRV markers for analysis (Shaffer and Ginsberg, 2017; Maheshwari et al., 2016; Task Force, 1996). The LF (0.04-0.15 Hz) is a marker of parasympathetic and sympathetic activity, while HF (0.15-0.40 Hz) depicts parasympathetic activity. Total power (0.04 -0.40 Hz) reflects a global marker of autonomic modulation. Additionally, normalised units of LF and HF were also used for HRV analysis (Burr, 2007; Task Force, 1996). Respiration was examined via breathing frequency. The mean difference and change in standard deviation (SD) from baseline to post-measures were computed in all the studies. Change in SD was derived based on imputed standard deviation method with correlation coefficient set at 0.40 (Becker 1998; Furukawa et al. 2006; Gu et al. 2015). The meta-analysis was conducted in a free software (Review Manager version 5.3). The standard mean difference was used to interpret ES as small = 0.20, moderate = 0.50, or large = 0.80 (Cohen, 1988; Durlak, 2009). Heterogeneity was evaluated using I^2 (Higgins et al., 2003). The I^2 represents the percentage of between-study variance due to heterogeneity versus chance based on 0% (no heterogeneity) -100% (high heterogeneity) scale. Visual inspection of a funnel plot was utilised to examine potential publication bias (Sterne and Egger, 2001). Lastly, data at resting conditions were utilised in meta-analysis.

3.4 Results

3.4.1 Literature Search

656 potential articles and 11 identified articles from reference lists were included in the database. Removal of duplicates (n = 90) led to initial screening of 570 articles on the basis of article title and abstract. After initial screening, 556 articles were eliminated by JP. Then, full articles of 14 studies were assessed for eligibility. An article was then excluded after failing to meet any of the items mentioned in the above inclusion criteria. In addition, the study of Paul and Garg (2012) was excluded as it posted the same HRV and respiration values with earlier published study (Paul et al., 2012). Four studies were eventually included in the systematic review, while three studies qualified for meta-analysis. Figure 3.1 displays the flow chart and selection process for the systematic review and meta-analysis.

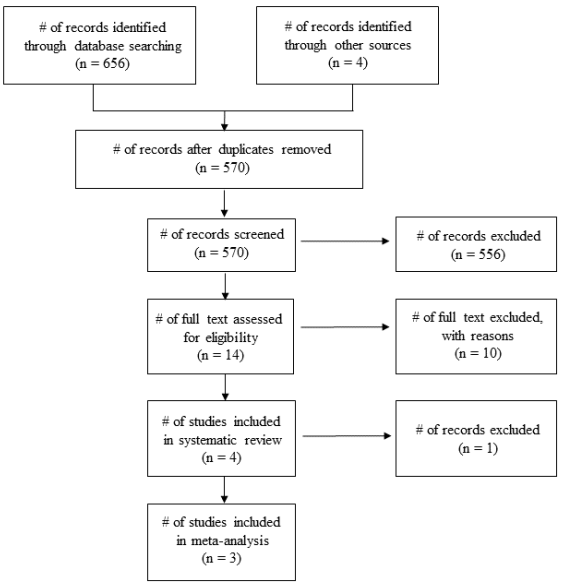

Figure 3.1. Flow diagram of search process.

Risk of bias in the study is displayed in Table 3.1. Two studies scored 5 points (Paul et al., 2012; Rusciano et al., 2017) while two studies scored 3 points (Choudhary et al., 2016; Dziembowska et al. 2016).

3.4.2 Experimental Protocols

Participants in the five studies involved 115 athletes (25 females and 90 males) which comprised 28 male and 13 female basketball athletes, 50 male football athletes, 12 male and 12 female track and field athletes with ages ranging from 16 to 30 years old.

All five studies included comparison of HRV BFB and CON (Choudhary et al., 2016; Dziembowska et al., 2016; Paul et al., 2012; Rollo et al., 2017; Rusciano et al., 2017), while only two studies compared HRV BFB and PLA (Paul et al., 2012; Rollo et al., 2017). CON involved regular sport training in all studies. Paul et al. (2012) implemented ten consecutive days of HRV BFB with each session lasting for 20 minutes. Additionally, motivational videos were used in PLA. Dziembowska et al., (2016) administered HRV BFB for ten 20-minute sessions within three weeks. Choudhary et al. (2016) conducted ten formal sessions of HRV BFB alongside with two 20-minute daily HRV BFB practice at convenience for ten weeks.

Different physiological parameters were identified from the studies above. Five studies included LF as a parameter for comparison (Dziembowska et al., 2016; Paul et al., 2012; Rollo et al., 2017; Rusciano et al., 2017). Three studies utilised high frequency (HF) and total HRV for assessment (Dziembowska et al., 2016; Paul et al., 2012; Rollo et al., 2017). One study compared LF/HF output (Choudhary et al., 2016). Respiration rate (RR) was differentiated in three studies (Paul et al., 2012; Rollo et al., 2017; Rusciano et al., 2017). The characteristics of studies are presented in Table 3.2.

3.4.3 HRV BFB vs CON

lnLF

There was no significant difference in lnLF between HRV BFB and CON, ES = 0.12 [-0.39, 0.62], $Z = 0.65$, $p < 0.05$ (Fig 3.2).

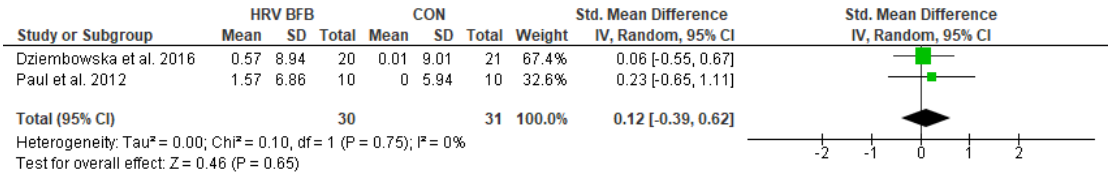

Figure 3.2. Forest Plot of lnLF in HRV BFB vs CON.

LFnu

The LFnu in HRV BFB was significantly higher compared to CON, ES = 0.46 [0.02, 0.91], $Z = 2.05$, $p < 0.05$ (Fig 3.3).

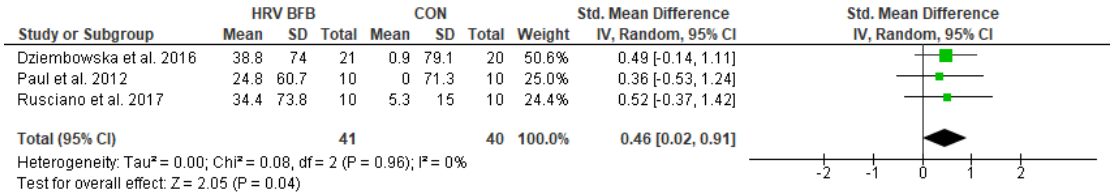

Figure 3.3. Forest Plot of LFnu in HRV BFB vs CON.

lnHF

The lnHF was not significantly different in HRV BFB and CON, ES = -0.04 [-0.55, 0.46], $Z = 0.05$, $p > 0.05$ (Fig 3.4).

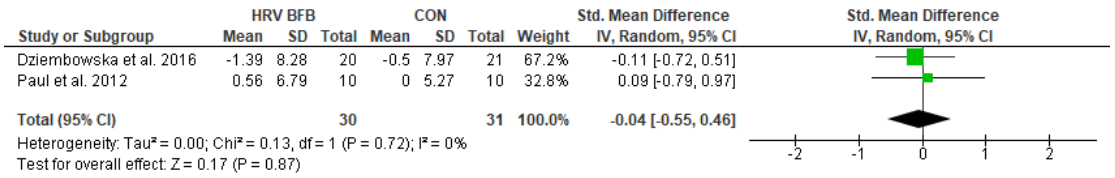

Figure 3.4. Forest Plot of lnHF in HRV BFB vs CON.

Table 3.1 CONSORT scores of HRV BFB studies included in systematic review.

	Were the groups comparable at baseline on key characteristics?	Did the study include a true control group (randomised participants – not a comparison group)	Was the randomisation procedure adequately described and carried out?	Did the study report a power calculation and was the study adequately powered to detect intervention effects?	Were the assessors blinded to treatment allocation at baseline and posttest?	Did at least 80% of participants complete follow up assessments?	Did the study analyses account for potential differences at baseline?	Did the study report effect sizes?	Total
Choudhary et al. 2016	1	0	0	0	0	1	1	0	3
Dziembowska et al. 2016	0	0	1	0	0	1	1	0	3
Paul et al. 2012	1	0	1	0	1	1	1	0	5
Rusciano et al. 2017	1	0	1	0	0	1	1	1	5

Table 3.2. HRV BFB and physiology of athletes.

Authors	Participants	Intervention	Outcome
Choudhary et al. 2016	18-25 yr old male (n = 12) and female (n = 12) university, state, and national level long distance runners age: 22.54 ± 1.72 yrs	HRV BFB: 10-week HRV BFB; once a week formal HRV BFB; 2 x 20-min/day home practice; regular sport training CON: regular sport training	LF/HF in HRV BFB: post < pre LF/HF in CON: post ↔ pre
Dziembowska et al. 2016	16-22 yr old male basketball and football players with at lest 3-year experience	HRV BFB (n = 20): Ten 20-minute HRV BFB in 3 weeks CON (n = 21): regular sport training	HRV BFB: post LF, total HRV > pre LF, total HRV; post HF > pre HF CON: post LF, HF, total HRV ↔ pre LF, HF, total HRV
Paul et al. 2012	18 to 28 yr old male (n = 16) and female (n = 14) university, state, and national basketball athletes age: 21.70 ± 2.71 yrs	HRV BFB (males: n = 8; females: n = 2): 10 consecutive days of 20-min HRV BFB; regular sport training PLA (males: n = 2; females: n = 8) motivational video clips for 10 days at 10 min/day; regular sport training CON (males: n = 7; females: n =3): regular sport training only	LF: HRV BFB > PLA; HRV BFB > CON HF: HRV BFB > PLA; HRV BFB > CON Total HRV: HRV BFB > PLA; HRV BFB > CON RR: HRV BFB < PLA; HRV BFB < CON
Rusciano et al. 2017	20 male professional football players age: 30.4 ± 4.1 yrs; height: 181.7 ± 55.9 cm; weight 79.0 ± 6.3 kg	HRV BFB: Fifteen 30-minute biofeedback feedback sessions (2x/week); 4th - 9th session: HRV BFB + SCL + EMG + hand temperature 10th - 15th session: HRV BFB + math tasks + hyperventilation + videos of matches won/lost regular sport training CON: regular sport training	LF: HRV BFB > CON RR: HRV BFB < CON

HRV BFB - heart rate variability biofeedback; PLA - placebo; CON - control; LF - low frequency; HF - high frequency; RR - respiration rate.

HFnu

Meta-analysis of HFnu between HRV BFB and CON exhibited lower HFnu in HRV BFB than CON, ES = -0.78 [-1.31, -0.25], $Z = 2.88$, $p < 0.01$ (Fig 3.5).

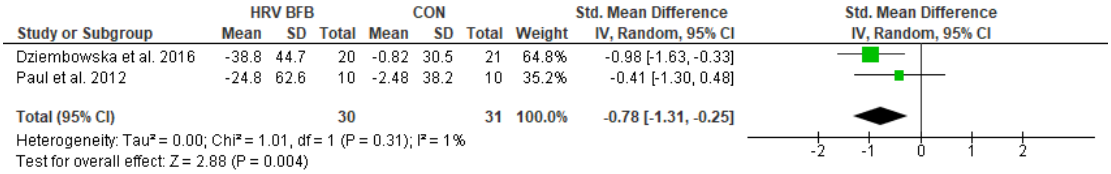

Figure 3.5. Forest Plot of HFnu in HRV BFB vs CON.

lnTP

There was no significant difference in lnTP in HRV BFB and CON, ES = -0.36 [-1.20, 0.48] $Z = 0.84$, $p > 0.05$ (Fig 3.6).

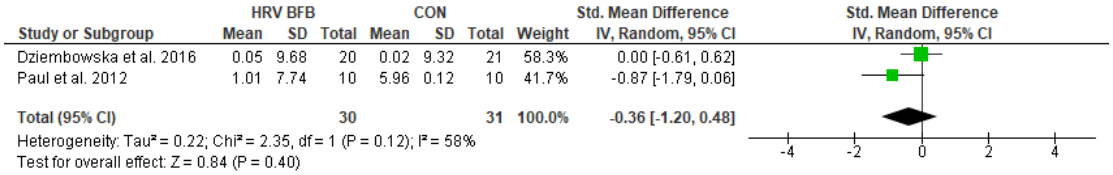

Figure 3.6. Forest Plot of lnTP in HRV BFB vs CON.

RR

Meta-analysis of RR between HRV BFB and CON posted significant reductions in RR of HRV BFB than CON, ES = -4.30 [-5.53, -3.08], $Z = 6.90$, $p < 0.01$ (Fig 3.7).

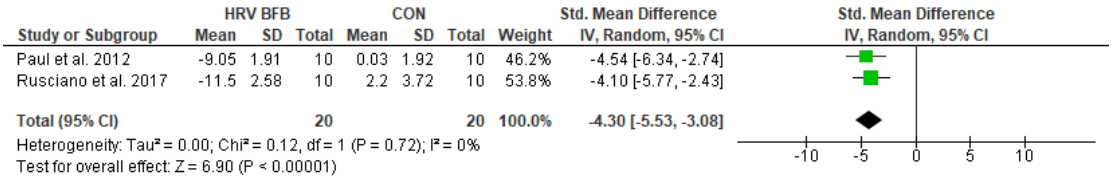

Figure 3.7. Forest Plot of RR in HRV BFB vs CON.

The funnel plots of HRV and respiration measures in the meta-analyses are demonstrated in Figure 3.8. The HRV values and respiration in HRV BFB and CON are displayed in Table 3.3.

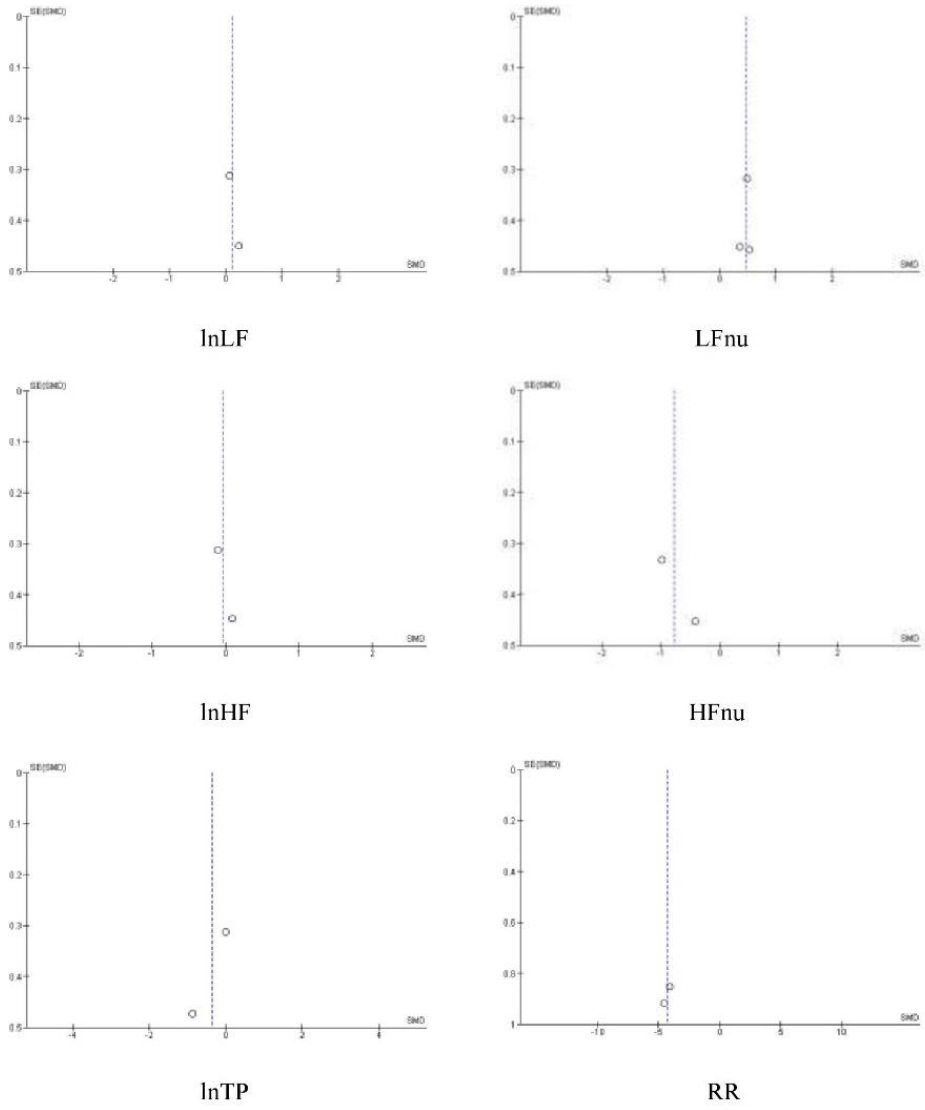

Figure 3.8. Funnel Plots of HRV Indices and RR in HRV BFB vs CON.

Table 3.3. Heart rate variability and respiration in HRV BFB and CON.

	HRV BFB			CON		
	n	Pre	Post		Pre	Post
HRV						
lnLF (ms^2)				n		
Dziembowska et al. 2016	20	8.47 ± 8.08	9.04 ± 8.25	21	8.26 ± 8.27	8.27 ± 8.18
Paul et al. 2012	10	5.54 ± 5.39	7.11 ± 6.92	10	5.50 ± 5.43	5.50 ± 5.42
LFnu				n		
Dziembowska et al. 2016	20	47.8 ± 51.3	86.6 ± 77.6	21	62.6 ± 72.4	63.5 ± 72.0
Paul et al. 2012	10	39.7 ± 32.1	64.6 ± 65.9	10	65.2 ± 65.1	65.2 ± 65.0
Rusciano et al. 2017	10	52.2 ± 11.3	60.1 ± 11.9	10	49.0 ± 15.7	54.3 ± 10.4
lnHF (ms^2)						
Dziembowska et al. 2016	20	8.56 ± 8.03	7.17 ± 7.00	21	7.74 ± 7.31	7.71 ± 7.24
Paul et al. 2012	10	5.96 ± 6.14	6.51 ± 6.26	10	4.88 ± 4.80	4.88 ± 4.80
HFnu						
Dziembowska et al. 2016	20	52.2 ± 48.7	13.4 ± 22.4	21	36.4 ± 27.6	36.5 ± 28.0
Paul et al. 2012	10	60.3 ± 67.9	35.4 ± 34.1	10	37.4 ± 34.9	34.4 ± 35.0
lnTP (ms^2)						
Dziembowska et al. 2016	20	9.38 ± 8.99	9.43 ± 8.68	21	8.90 ± 8.50	8.92 ± 8.52
Paul et al. 2012	10	6.70 ± 6.71	7.71 ± 7.39	10	6.12 ± 5.96	6.12 ± 5.96
RR (breaths/min)						
Paul et al. 2012	10	15.3 ±2.00	6.25 ± 0.25	10	14.6 ± 1.77	14.6 ± 1.73
Rusciano et al. 2017	10	17.1 ± 2.80	5.60 ± 0.90	10	16.7 ± 3.00	18.9 ± 3.70

lnLF-log-transformed low frequency HRV; LFnu – normalised low frequency; lnHF – log-transformed high frequency HRV; HFnu – normalised high frequency; lnTP – log- transformed total power HRV; RR – respiration rate.

3.5 Discussion

The purpose of this study was to conduct a systematic review and meta-analysis on the effect of HRV BFB on physiological indices among athletes. Meta-analyses revealed the following outcomes comparing HRV BFB and CON: 1. HRV BFB reduced breathing rate compared to CON (ES = -4.34; large); 2. Greater LFnu in HRV BFB than CON (ES = 0.46; moderate); 3. relatively, HRV BFB posted lower HFnu than CON (ES = -0.78; moderate).

In this review, HRV BFB demonstrated reduction in breathing frequency compared to CON. This is supported by increased LFnu seen in HRV BFB. HRV BFB practice facilitates respiratory homeostasis by decreasing chemoreceptor activation (Bernardi et al., 1998; Bernardi et al., 2001; Lehrer et al., 2004). This in turn increases arterial oxygen saturation, and reduces breathing frequency (Bernardi et al., 1998; Lehrer et al. 2003; Lehrer et al., 2004). The lower breathing frequency attained with HRV BFB may be crucial to reduction of psychophysiological stressors of athletes, thereby improving performance (Paul and Garg, 2012). Thus, HRV BFB can serve as a promising intervention to improving the respiration mechanics of athletes in a resting condition.

Another finding in this review is the non-enhancement in baroreflex function with HRV BFB. At resting conditions, LF represents baroreceptor activity from PNS, SNS, and blood pressure regulation from PNS (Goldstein et al., 2011; McCraty and Shaffer, 2015; Nunan et al., 2010; Reyes del Paso et al., 2013; Shaffer et al., 2014; Task Force, 1996). HRV BFB activates resonance in the cardiovascular system and creates oscillatory vagal outflow coinciding with the baroreflex function (Ahmed et al., 1982;

Lehrer et al., 2000; Lehrer et al., 2003; Lehrer and Eddie, 2013; McCraty and Shaffer, 2015). This resonance produces large increases in HR amplitudes and 'exercises' the baroreflex (Lehrer et al., 2000; Lehrer et al., 2003; Vaschillo et al., 2006). Findings revealed non-differences in lnLF, lnHF and lnTP between HRV BFB and CON. Therefore, the results of these HRV indices under HRV BFB are linked to no enhancement in baroreflex function. Possible factors contributing to non-significant results in autonomic markers supporting baroreflex function are ambiguous. More studies are needed to elucidate information on the mechanism of baroreflex under HRV BFB in athlete population.

This review also revealed an absence of effect in modulating the cardiac vagal tone after HRV BFB among athletes. The cardiac vagal tone characterises the contribution of PNS in cardiac regulation (Laborde, 2017). The lnHF was utilised as an index of vagal modulation in this review (Task Force, 1996; Maheswari et al., 2016). Results revealed non-significant lnHF in HRV BFB and CON. The limited availability of published research constrained the researchers to conduct additional analysis that can identify possible variables that led to non-improvement in lnHF. Further investigation utilising HRV BFB in athlete population should be carried out to demonstrate vagal influence of HRV BFB in athletes.

Adaptations in respiratory sinus arrhythmia (RSA) with HRV BFB in athlete population is unclear (Eckberg and Eckberg, 1982; Prinsloo et al., 2014; Shaffer et al., 2014; Shaffer and Ginsberg, 2017; Task Force, 1996). RSA is a cyclical change in HR synchronized with respiration, resulting to vagal discharge in the medulla. Specifically, RSA accelerates and slows down HR during inhalation and exhalation respectively.

Inhalation inhibits the vagal outflow from the cardiovascular center and speeds up HR. Conversely, exhalation facilitates vagal outflow by acetylcholine release. HRV BFB is believed to increase RSA (Julien, 2006; Laborde et al., 2017; Lehrer et al., 2003; Lehrer and Eddie, 2013; Russo et al., 2017). Although the change in LFnu and decreased breathing frequency may suggest RSA shift from HF to LF, additional HRV and HR indices (maximal HR and minimum HR) in future HRV BFB studies can allow the robust interpretation of RSA in HRV BFB.

From a methodological perspective, statistical inferences from this preliminary review are not worthwhile due to small sample sizes. The small number of studies limits the statistical power to reasonably interpret heterogeneity and publication bias (Jackson and Turner, 2017). Additionally, subgroup analyses for potential covariates (e.g. age, level of ability, HRV BFB duration, gender) crucial for understanding autonomic function with HRV BFB were not determined. Also, utilising a common performance marker (e.g. cardiovascular endurance) and relate it to HRV adaptations with HRV BFB was not achieved. In regard to HRV markers in meta-analyses, normalised LF and HF do not reflect unique physiological occurrences within the ANS (Burr, 2007). As such, LFnu and HFnu were only used to depict breathing dominance within HRV frequency band. Other time-domain HRV parameters that may be helpful for determining autonomic phenomena with HRV BFB in athletes were not available (Buchheit, 2014; Saboul et al., 2013). Despite these limitations, this study has a noteworthy strength in that it is the first meta-analysis on the topic of HRV BFB in athletes.

3.6 Conclusion

Application of HRV BFB suggests enhancement of respiratory mechanics in athlete population. More studies are needed to identify the effect of HRV BFB on autonomic modulation among athletes in the resting condition.

Chapter 4: Effect of Acute Heart Rate Variability Biofeedback on H-reflex Modulation: A Pilot Study

An original version of this chapter has been published in the Journal of Human Kinetics as an original investigation and appears in the literature as:

Pagaduan J, Wu SSX, Fell JW, Chen YS. Effect of acute heart rate variability biofeedback on H-reflex modulation: a pilot study. *J Human Kinet,* 2021; 76: 83-88.

Rationale

Changes in performance occurring with HRV BFB (Study 1) are usually linked to autonomic regulation via HRV and RR (Study 2). Examining neuromuscular pathways may also provide crucial information that can help justify alteration in performance in HRV BFB. This study was to the first to identify the effect of HRV BFB on a neural excitability utilising H-reflex.

4.1 Abstract

Heart rate variability biofeedback (HRV BFB) is a paced breathing scheme that stimulates resonance in the cardiovascular system. This study aimed to investigate the effect of a single-session HRV BFB on Hoffman reflex (H-reflex) of the soleus muscle. Twelve healthy males (height: 173.7 ± 7.18 cm; weight: 72.7 ± 17.7 kg; age: 24.0 ± 5.02 yrs) completed a randomized-crossover intervention involving a 10-minute HRV BFB and normal breathing (CON) separated by 48 hours. Results revealed significantly lower 1a afferent activation after HRV BFB. Similarly, the HRV BFB also demonstrated a lower proportion of activated motor neurons from 1a afferents. In conclusion, an acute HRV BFB influenced the reduction in motoneuron excitability in the resting condition.

Keywords: biofeedback, resonance frequency breathing, H-reflex, neural excitation

4.2 Introduction

The Hoffman reflex (H-reflex) is a tool for assessing the monosynaptic reflex in the spinal cord, which is modulated by input of 1a afferents to the alpha motoneural pool (MN) (Chen et al., 2012; Gajewski and Mazur-Różycka, 2016; Knikou, 2008; Palmieri et al., 2004; Pierrot-Deseilligny and Mazevet, 2000). Percutaneos electrical stimulation to the mixed peripheral nerve is applied to evoke H-reflex, targetting the monosynaptic reflex arc and efferent motor response (Zehr, 2002). This process begins with gradually increasing low-intensity stimulus that leads to primary depolarization of 1a afferents in the muscle spindle (Palmieri et al., 2004). Stimulation of 1a afferents result in action potentials being directed to the spinal cord. Adequate activation of 1a afferents depolarize the presynaptic terminal, releasing neurotransmitters in the synaptic cleft of 1a alpha MN synapse. Then, post synaptic potentials in the MNs occur. When the excitory post synaptic potentials are capable of MN depolarization, acethycholine is released at the neuromuscular junction. This leads to muscle contraction, eliciting noticeable H-reflex tracing on electromyography (EMG) recording. Researchers suggest that regulation of H-reflex happens in the primary motor area of the central nervous system (Rollnik et al., 2000; Tanaka, 2015; Tanaka et al., 2012). The H-reflex is a useful technique in clinical, exercise, and sport settings (Gajos et al., 2014; Palmieri et al., 2004; Zehr, 2002).

Heart rate variability biofeedback (HRV BFB) is a non-invasive, visually-guided paced breathing intervention believed to improve psychophysiological indices (Reiner, 2008; Gevirtz, 2013; Lehrer and Gevirtz, 2014). HRV BFB involves breathing at a pace (0.075 – 0.12 Hz or 4.5 – 7.2 breaths/min) known as resonance frequency (RF), that generates rhythmical changes in the baroreflex and activates the resonant

properties in the cardiovascular system (Lehrer and Eddie, 2013; Lehrer and Gevirtz, 2013; Lehrer et al., 2000; Vaschillo et al., 2006). The baroreflex plays a crucial role in the regulation of blood pressure that operates in a negative feedback loop, such that increasing blood pressure reduces heat rate and vice versa. Key features in HRV BFB include occurrence of maximal heart rate oscillations and simultaneous oscillations of both heart rate and respiration. Regular HRV BFB practice enhances baroreceptor activity, vagal afferents and efferents that facilitate beneficial health changes (Gevirtz, 2013; Gruzelier et al., 2014; Lehrer and Eddie, 2014; McCraty and Shaffer, 2015; Paul and Garg, 2012; Russo et al, 2017).

The application of HRV BFB may influence motor function through the central autonomic network (Beissner et al., 2013; Benarroch, 1993). However, no study has investigated neural adaptation within HRV BFB context. Examining the magnitude of neural excitability from HRV BFB may provide a worthwhile consideration of HRV BFB in neuromuscular settings. Thus, the purpose of this study was to determine motoneural excitability from acute HRV BFB.

4.3 Methods

4.3.1 Participants

Twelve healthy males (height: 173.7 ± 7.18 cm; weight: 72.7 ± 17.7 kg; age: 24.0 ± 5.02 yrs) with no reported lower body injury two months prior to experimentation were recruited for this pilot study. Participants were asked to avoid any strenuous physical activity and caffeine containing food/beverage 24 hours prior to experimentation. The last meal intake by participants was at least two hours before testing. A written informed consent was collected before any further experimentation. Ethical clearance

for this study was approved by the University of Tasmania Human Research Ethics Committee (H0016508), in agreement with the University of Taipei. The protocol of this study was in adherence with the Declaration of Helsinki for human experimentation.

4.3.2 Procedures

The participants attended three experimentation sessions, separated by 48 hours, at the Exercise Performance Laboratory of University of Taipei. Anthropometric measurements (height and weight), identification of individual RF were administered in the first session. Participants executed a randomized breathing at RF (HRV BFB) or normal breathing (CON) in the second session. A crossover HRV BFB or CON was performed in the third session. HRV BFB was carried out using a commercial HRV BFB equipment (HRV Starter System, Thought Technology, Canada). In CON, participants underwent normal breathing for ten minutes with gaze focused at the computer screen. Figure 1 displays the experimental protocol for this study. Pre and post H-reflex indices in HRV BFB and CON were subjected for analysis.

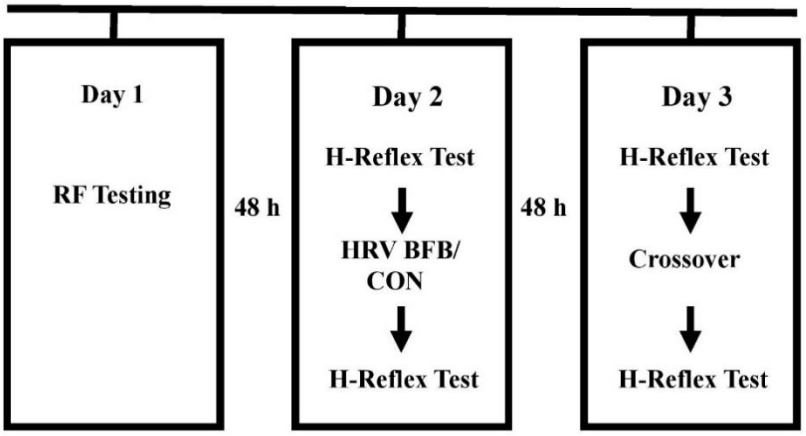

Figure 4.1. Experimental protocol.

4.3.3 Assessment

4.3.3.1 Resonance Frequency

A commercial biofeedback equipment (HRV Starter System, Thought Technology, Canada) measuring respiration and HRV was used to identify RF. The RF testing involved two-minute breathing at various frequencies (6.5, 6.0, 5.5, 5.0, 4.5 times per min). Prior to RF testing, participants underwent a five-minute familiarization of diaphragmatic breathing with nasal inhalation and pursed lip exhalation. RF was established as the breathing pace that demonstrated the highest amplitude in low frequency HRV (Lehrer et al., 2000; Task Force, 1996).

4.3.3.2 H-Reflex

H-reflex was measured from the SOL electromyography (EMG) of the dominant leg at resting condition. A surface electrode (TSD150A, Biopac Systems, USA) was placed 2/3 from the medial condyle of the femur to the medial malleolus and central position in the medial-lateral direction of the border of SOL. In addition, a reference electrode was attached on the dominant hand of the participants. Further, an adhesive electrode (10 ´ 10 mm, FA 25, Gem-Stick, Australia) was placed on the right popliteal fossa as cathode and another (50 ´ 50 mm, Life Care, Taiwan) adhesive electrode was fixed over the right patella as the anode. Participants were asked to sit with the dominant knee extended at approximately 180 degrees, while the non-dominant knee flexed at 90-120 degrees. Hip flexion was 90-120 degrees. Recording of EMG signals were acquired using a commercial data acquisition system (MP150, Biopac Systems, USA) filtered with a band-pass range of 10-500 Hz and amplified with a gain of 1,000 times. The common mode rejection ratio for the EMG amplifier was 100dB. Sampling rate was set to 2.5 kHz. A single electrical impulse was applied to the right posterior

tibial nerve via an electrical stimulator (DSH7, Digitimer, Herfordshire, UK) with 1000μs pulse duration to identify SOL H-reflex response. To determine maximal levels of H-wave and M-wave, stimulation intensity was increased with 10 mA increments until maximum M-wave (Mmax) was identified. The stimulation intensity for exhibiting maximum H-wave (Hmax) was determined by using 2 mA increment when the H-reflex threshold was identified. The stimulation intensity was set at 10% - 20% Mmax to elicit an H-reflex response in the ascending portion of the recruitment curve (Chen and Zhou, 2011). Each stimulation intensity was applied four times with 10 s inter-stimulation interval. A commercial data acquisition system (Acqknowledge 4.2.1 software, Biopac Inc., USA) with custom-written program was used to synchronize the electrical stimulation trigger and EMG recording. During H-reflex testing, participants were encouraged to breathe normally. A sample of H wave and M wave recruitment curves from one representative participant is displayed on Figure 4.2.

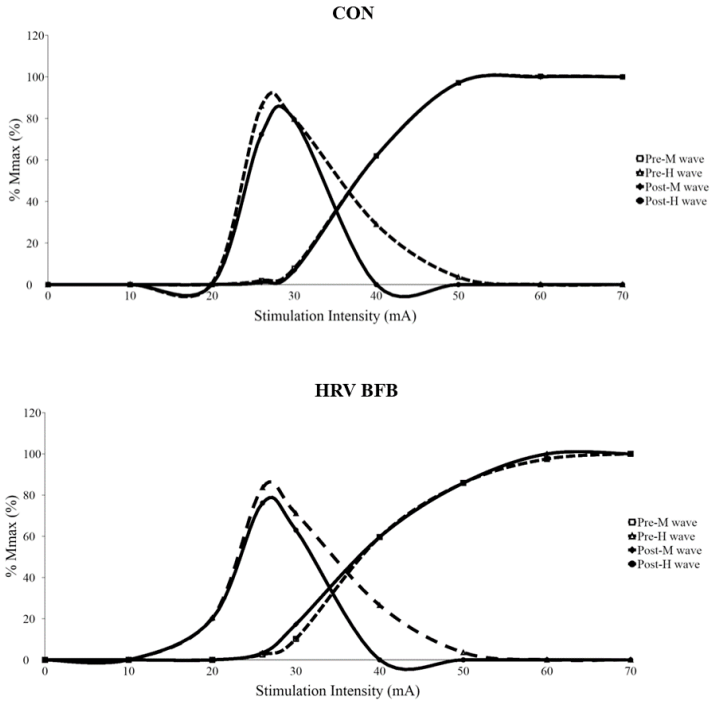

Figure 4.2. Sample of H wave and M wave curves.

The following H-reflex indices were measured offline: a) M wave at maximal H-reflex (M wave at Hmax); b) Hmax; c) Mmax; and, d) Hmax/Mmax ratio (maximal Hmax response to normalised Mmax). Hmax estimates motoneuron excitability activated by Ia afferents, elicited from electrical stimulations (Gajewski and Mazur-Różycka, 2016; Knikou, 2008). Mmax represents recruitment of motor axons and provides an estimate of the size of motoneural pool response (Chen et al., 2012; Chen et al., 2015; Palmieri et al., 2004; Pierrot-Deseilligny and Mazevet, 2000). Hmax/Mmax ratio is the proportion of the entire MN pool recruited by Ia afferents (Kipp et al., 2011; Palmieri et al., 2004). M wave at Hmax refers to the proportion of motor fibers activated by electrical stimulations (Chen et al., 2015; Knikou, 2008). Additionally, the M wave at Hmax was utilised as a confirmatory marker to determine the consistency of relative stimulation intensity at the tibial nerve (Zehr, 2002).

4.4 Statistical Analysis

Data are expressed as mean ± standard deviation. The Wilcoxon-Signed Rank Test was carried out to determine significant difference in pre and post indices in HRV BFB and CON. The effect size was computed from matched-pairs rank-biserial correlation (*r*) using the simple difference formula (Kerby, 2014). The *r* is interpreted as small (0.10), medium (0.30), large (0.50), very large (0.70) (Cohen, 1988; Rosenthal, 1996). The statistical analyses were administered using a commercial statistical package (SPSS version 22, IBM Corp, USA) with alpha set at 0.05 level.

4.5 Results

The RF of participants ranged from 4.4 to 7.4 breaths per minute (0.09 ± 0.02 Hz). Non-significant difference in of M wave at H max confirmed the same relative stimulation intensity at tibial nerve in HRV BFB and CON.

4.5.1 H-Reflex

HRV BFB

Table 4.1 depicts H-reflex indices from HRV BFB and CON. There was a significant difference in pre-Hmax and post-Hmax at Z = -2.40, p = 0.04, r = -0.67. No difference was observed in pre-Mmax and post-Mmax at Z = -0.89, p = 0.37, r = -0.30. A significant difference existed in pre-Hmax/Mmax ratio and post-Hmax/Mmax ratio at Z = -2.35, p = 0.02, r = -0.84.

CON

CON exhibited non-significant differences in pre and post Hmax (Z = -0.16, p = 0.88, r = -0.05), Mmax (Z = 0.00, p = 1.00, r = 0.00), and Hmax/Mmax ratio (Z = -0.04, p = 0.67, r = -0.14). Table 4.1 displays the pre and post H-reflex indices in HRV BFB and CON.

4.6 Discussion

This study examined the effect of an acute HRV BFB on the SOL motoneural pathway using H-reflex. The findings revealed a significant reduction in Hmax (r = - 0.67, large) and Hmax/Mmax ratio (r = - 0.87, very large) after HRV BFB.

Indeed, HRV BFB facilitated changes in SOL neural transmission in this study. This can be observed by decreased resting Hmax/Mmax after HRV BFB. The alteration in Hmax/Mmax ratio is attributed to maximal H-reflex amplitude rather than maximal M-wave amplitude, supported by reduced Hmax and non-difference in Mmax (Palmieri et al., 2004). The decreased Hmax/Mmax ratio reflects increased presynaptic inhibition from afferent depolarization (Kipp et al., 2011; Pierrot-Deseilligny and Mazevet, 2000; Zehr, 2002). It seems that the acute HRV BFB reduced the neural excitability between Ia afferents and alpha MNs. However, the exact mechanisms lowering 1a afferent activation after HRV BFB are unclear. A recent study by Bagheri et al. (2018) reported a negative relationship of resting Hmax/Mmax with a vertical jump and balance task. This suggests the importance of resting neural excitability in neuromuscular tasks. More work is needed to establish the association of Hmax/Mmax attenuation from HRV BFB in voluntary muscle contraction and proprioception.

Various limitations of the current study are acknowledged. First, generalization of results should be avoided as the neural outcomes from acute HRV BFB may only be applicable to the sample population involved. Also, assessment of H-reflex was administered in the resting condition. Inclusion of voluntary muscle contraction task whilst investigating H-reflex after HRV BFB may exhibit alteration of H-reflex from abolition of post-activation depression, clearer distinction of neural characteristics at lower stimulation intensities, and identification active motoneuron pool (Burke et al., 1989). Lastly, concurrent measurement of HRV with H-reflex after HRV BFB can determine trends in vagal reactivity from neural excitation.

4.7 Conclusion

The findings of this study serve as an impetus for future research within the context of HRV BFB and neuromuscular function. In conclusion, an acute HRV BFB facilitated depression of soleus motoneural excitability in the resting condition.

Table 4.1. H-reflex indices in HRV BFB and CON.

Parameter		HRV BFB				CON			
		Pre	Post	p-value	*r*	Pre	Post	p-value	*r*
M wave at Hmax	(mV)	0.38 ± 0.23	0.39 ± 0.25	0.50	0.22	0.59 ± 0.61	0.51 ± 0.44	0.37	-0.29
Hmax	(mV)	1.84 ± 0.74	1.67 ± 0.90	0.04	-0.67	1.83 ± 0.77	1.83 ± 0.80	0.88	-0.05
Mmax	(mV)	3.32 ± 0.91	3.26 ± 1.00	0.37	-0.30	3.36 ± 1.08	3.37 ± 1.18	1.00	0.00
Hmax/Mmax ratio		0.55 ± 0.15	0.50± 0.18	0.02	-0.84	0.54 ± 0.15	0.54 ± 0.15	0.67	-0.14

** M wave at Hmax – M wave at Hmax; Hmax – maximal H-reflex response; Mmax – maximal M- wave response; Hmax/Mmax ratio – proportion of the motoneural pool recruited by 1a afferents.*

Chapter Five: Acute Effects of Resonance Frequency Breathing on Cardiovascular Regulation

An original version of this chapter has been published in Physiological Reports as an original investigation and appears in the literature as:

Pagaduan J, Wu S SX, Kameneva T, Lambert E. Acute effects of resonance frequency breathing on cardiovascular regulation. *Physiol Rep.,* 2019; 7: e14295.

Rationale

Alterations in performance with HRV BFB (Study 1) may also be associated with resting muscle sympathetic outflow. Additionally, the neural excitability from HRV BFB (Study 2) may be affected by muscle sympathetic outflow. This study was undertaken to examine muscle sympathetic nervous activity in HRV BFB.

5.1 Abstract

Acute slow breathing may have beneficial effects on cardiovascular regulation by affecting hemodynamics and the autonomic nervous system. Whether breathing at the resonance frequency (RF), a breathing rate that maximizes heart rate oscillations, induces differential effects to that of slow breathing is unknown. We compared the acute effects of breathing at either RF and RF + 1 breaths per minute on muscle sympathetic nervous activity (MSNA) and baroreflex function. Ten healthy men underwent MSNA, blood pressure (BP), and heart rate (HR) recordings while breathing for 10 min at their spontaneous breathing (SB) rate followed by 10 min at both RF and RF + 1 randomly assigned and separated by a 10-min recovery. Breathing at either RF or RF + 1 induced similar change in HR and HR variability, with increased low frequency and decreased high frequency oscillations ($p < .001$ for both). Both respiration rates decreased MSNA (−5.6 and −7.3 bursts per min for RF and RF + 1 $p < .05$), with the sympathetic bursts occurring more often during mid-inspiration to early expiration (+57% and + 80%) and longer periods of silence between bursts were seen ($p < .05$ for RF + 1). Systolic BP was decreased only during RF (−4.6 mmHg, $p < .05$) but the decrease did not differ to that seen during RF + 1 (−3.1 mmHg). The sympathetic baroreflex function remained unchanged at either breathing rates. The slope of the cardiac baroreflex function was unaltered but the cardiac baroreflex efficiency was improved during both RF and RF + 1. Acute breathing at either RF or RF + 1 has similar hemodynamic and sympatho-inhibitory effects in healthy men.

Keywords: resonance frequency, sympathetic nervous activity, baroreflex, blood pressure

5.2 Introduction

Slow breathing has long been recognized to exert beneficial effects in a range of disorders including those related to the cardiovascular system and those associated with anxiety. Breathing at a low pace (5–6 breaths per min) has been shown to acutely decrease blood pressure (BP) in patients with post-traumatic stress disorder (Fonkoue et al., 2018), and in the longer-term decrease BP in patients with hypertension (Hering et al., 2013). In patients with heart failure, slow breathing either performed acutely or chronically reduced the cardiovascular reactivity to mental stress and improved various aspects of health-related quality of life (Lachowska et al., 2020). Recently, an intervention has emerged consisting of breathing at a specific and individualized (slow) breathing rate, termed resonance frequency (RF), where oscillations in heart rate (HR) and breathing synchronize. This intervention typically involves the use of a device which guides an individual to breathe at their RF via visual or auditory feedback. Breathing at RF maximizes HR oscillations by creating a 0-degree phase shift between HR and respiration, while BP response from HR exhibit a 180-degree phase shift occurring at approximately 5-s delay as a result of mechanical response (Vaschillo et al, 2006). Similar to slow breathing at a set pace, RF breathing has emerged as a promising tool to enhance performance, reduce stress and anxiety (Jester et al., 2019; Lehrer and Gevirtz, 2014) and positively influence clinical symptoms in a number of disorders including depression (Lin et al., 2019), asthma (Taghizadeh et al. 2019), and prehypertension (Lin et al., 2012).

The primary proposed mechanistic pathway for slow breathing or breathing at RF has been an improved vagal tone, associated with improved cardiac baroreflex function. Powerful within-breath respiratory modulation of sympathetic vasoconstrictor activity

has been well documented in humans (Macefield and Wallin, 1995; St Croix et al., 1999). During spontaneous breathing, muscle sympathetic nerve activity (MSNA) is inhibited during mid-inspiration to mid-expiration, with MSNA activation occurring during late expiration (Eckberg et al., 1985; Limberg et al., 2013). Interventions aiming at altering the breathing frequency may affect the within-breath modulation of MSNA and hence modulate steady state sympathetic tone. Acute slow breathing for 10–15 min was found to decrease MSNA in patients with hypertension (Hering et al., 2013; Oneda et al., 2010), post-traumatic stress disorder (Fonkoue et al., 2018) and those with heart failure (Harada et al., 2014), but failed to induce any change in healthy subjects when the breathing exercise was of a three-minute duration (Limberg et al., 2013).

Breathing at RF is thought to maximize heart rate variability (HRV) and influence autonomic nervous system (Lehrer and Gevirtz, 2014; Lehrer et al., 2000; Lehrer et al., 2003). However, it is not known whether breathing at a precise respiration rate at an individualized RF may result in different changes in cardiovascular regulation compared to breathing at a set slow pace. The aim of the present study was therefore to compare the acute effects of breathing at an individual's RF relative to breathing at 1 breath above (RF + 1) on HR, HRV, BP, MSNA, within-breath modulation of MSNA, and vascular and sympathetic baroreflex functions.

5.3 Methods

5.3.1 Participants

Ten healthy non-smoking males (age: 28.7 ± 4.8 years; height: 170 ± 7 cm; weight: 68.4 ± 8.0 kg) with no history of clinical disease volunteered in this study. Participants

were required not to consume any food or any caffeine containing beverage for at least two hours before experimentation. The protocol of this study conformed to the Declaration of Helsinki for human experimentation. All subjects provided written informed consent prior to participation. The experimental protocol was approved by the Swinburne University Human Ethics Committee.

5.3.2 Procedures

This study involved two experimentation sessions separated by 24-hrs. Determination of participant's RF was administered in the first session while breathing schemes were facilitated in the second session (HRV Starter System, Thought Technology). The RF was determined according to the protocol described by Lehrer and colleagues (2000) from 2-min paced breathing at various frequencies (6.5, 6.0, 5.5, 5.0, 4.5) while monitoring HR and respiration. At the end of ten min, the breathing frequency that displayed the highest occurrence of low frequency in HRV (LF; 0.05–0.15 Hz) was selected as the RF. Prior to RF testing, participants underwent a biofeedback-assisted paced breathing familiarization involving nasal inhalation and pursed-lip exhalation.

On the second session, participants were placed in a semi-supine position and instrumented for BP, HR, respiration, and MSNA recordings. After they had rested for at least 10–15 min, all parameters were continuously recorded for the ten-minute baseline period where they were required to breathe at their spontaneous frequency. Following baseline recording, participants were asked to breathe at either their RF rate or RF + 1 for ten min. Participants were allowed to rest for 10–15 min and told to breathe at their spontaneous rate until BP and MSNA values had returned to baseline before undergoing another ten-min period of breathing at either their RF rate or RF +

1. Each participant therefore completed baseline first, followed by RF and RF + 1 in a random manner. During spontaneous breathing (SB), participants were asked to fix gaze at a blank computer screen. Paced breathing at RF or RF + 1 was guided by a visual pacer, with heart and respiratory oscillations displayed on screen.

5.3.3 Assessment

5.3.3.1 Muscle Sympathetic Nerve Activity, Respiration, Heart Rate, Blood Pressure

Recording of multiunit postganglionic MSNA was made with participants resting in a semi-supine position as described previously (Lambert and Schlaich, 2004). A tungsten microelectrode (FHC) was inserted directly into the right peroneal nerve just below the fibular head. A subcutaneous reference electrode was positioned 2–3 cm away from the recording site. The nerve signal was amplified (350,000), filtered (bandpass 700–2,000 Hz), and integrated. During MSNA recording, BP was measured continuously using the Finometer system (Finapress Medical System BV), HR was determined using a lead III echocardiogram and respiration was assessed using a piezoelectric belt. BP, electrocardiogram, respiration, and MSNA were digitized with a sampling frequency of 1,000 Hz (PowerLab Recording System, model ML 785/8SP; ADI Instruments).

5.3.3.2 MSNA Analysis

Sympathetic bursts were visually identified and the number of bursts was averaged over the ten-min period during SB, RF, and RF + 1. The MSNA was expressed as burst frequency (burst/min) and burst incidence (bursts/100 heartbeats). For analysis with simultaneous signals (BP, respiration), the MSNA signals were advanced 1.5 s to

account for peripheral sympathetic nerve conduction delays (Fagius and Wallin, 1980). HR and BP were averaged over the ten-min period during SB, RF, and RF + 1.

5.3.3.2.1 Dynamic Patterns of MSNA Spiking

The inter-burst interval was used to measure dynamic patterns of MSNA activity. The inter-burst interval was used as a measure of burst-suppression, when a cluster of bursts is followed by a silence period. The difference in inter-burst intervals during each condition was calculated for individual participants and then averaged for the whole cohort.

5.3.3.2.2 Instantaneous Phase of the Respiration Signal

To calculate the phase of the respiration signal, a Hilbert transform was applied to the signal. The Hilbert transform is a linear operator which converts a function of real variables into a complex plane (King, 2009). The instantaneous phase of the respiration is calculated as an arctangent of a ratio of real and imaginary parts of the signal, measured between $-\pi$ and π, where π is the end of inspiration and early expiration and $-\pi$ is the end of expiration and early inspiration. A histogram of MSNA burst occurrence was plotted as a function of an instantaneous phase of the respiration signal. The MSNA bursts histograms were normalised to the maximum value for each breathing period and compared among SB, RF, and RF + 1 breathing schemes.

5.3.3.3 Spontaneous Arterial Baroreflex Control of MSNA

Over each period (SB, RF, RF + 1), diastolic BPs associated with individual heartbeats were grouped in intervals of 2 mmHg and, for each interval, the percentage of diastoles associated with a sympathetic burst was plotted against the mean of the pressure

interval (Lambert et al., 2002). The sensitivity of the sympathetic baroreflex gain was defined as the slope of the regression line and was expressed as bursts/100 heartbeats/mmHg.

5.3.3.4 Spontaneous Cardiac Baroreflex Sensitivity

Baroreflex sensitivity was assessed using the sequence method (Parati et al., 2004). This procedure identifies the 'spontaneous' sequences of three or more consecutive beats in which systolic BP progressively rose and cardiac interval progressively lengthened (type 1 sequences), or systolic BP progressively fell and cardiac interval progressively shortened (type 2 sequences), with a lag of one beat. For each sequence, the linear correlation coefficient between cardiac interval and systolic BP was computed and the sequence validated when $r > 0.85$. The slope between cardiac interval and systolic BP was calculated for each validated sequence and expressed as msec/mmHg. The baroreflex efficacy index (BEI) (Di Rienzo et al., 2001) was assessed as the total number of cardiac intervals/systolic BP sequences divided by the total number of systolic BP ramps.

5.3.3.5 Heart Rate Variability

HRV was assessed from the resting electrocardiogram (ECG) obtained during the MSNA recording and was determined using commercially available software (HRV Module for Chart 5 Pro; ADI Instruments, Bella Vista, Australia). Parameters derived were standard deviation of normal-to-normal intervals (SDNN) and standard deviation of heart rate (*SD* rate) in the time domain analysis. LF (0.04–0.15 Hz) and high frequency (HF: 0.15–0.4 Hz) in the frequency domain analysis expressed as percentage and normalised units. Additionally, LF/HF ratio was also included in HRV analysis.

Statistical Analysis

In order to compare the variables under the three conditions (SB, RF, and RF + 1) a repeated measures ANOVA followed by pairwise multiple comparison procedure (Bonferroni *t*-test) was used when the data were normally distributed. Otherwise, the Friedman's test for repeated measures was used and Wilcoxon signed rank test for paired samples was administered for post hoc analysis. Statistical analyses were carried out using a commercial statistical package (SPSS ver 25, IBM) with alpha set at 0.050 level. Data are presented as mean ± standard deviation or mean ± interquartile range.

5.5 Results

Figure 5.1 contains hemodynamic recordings from one subject showing the effects of breathing at the SB, RF, and RF + 1. Fluctuations in the ECG and BP signals are obvious at both RF and RF + 1. In this participant, MSNA was more pronounced during early inspiration, and subsequently inhibited from mid-inspiration to mid-expiration. This observation was more obvious at RF and RF + 1, compared with SB.

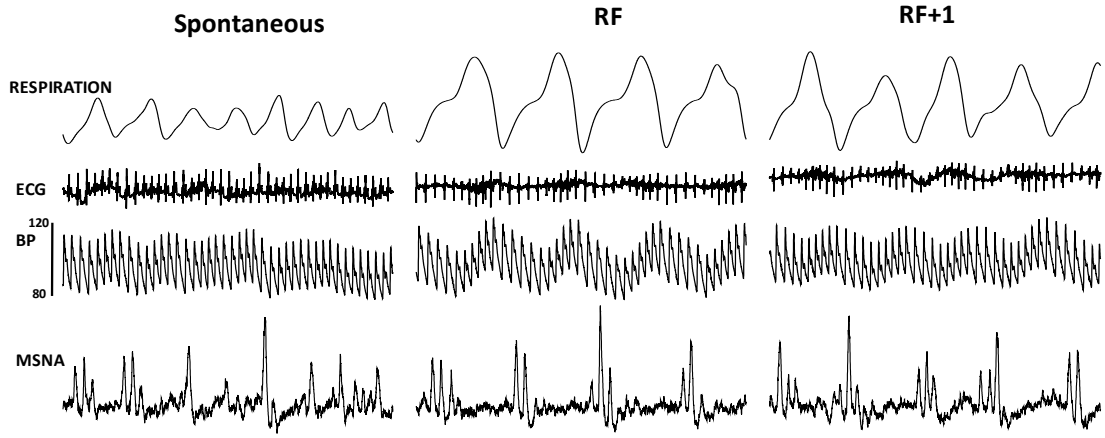

Figure 5.1. Original traces of respiration, ECG, blood pressure (BP), and muscle sympathetic nerve activity (MSNA) in one participant while breathing at their spontaneous breathing rate, at the resonance frequency (RF), and at the RF + 1. In this participant, MSNA is more pronounced during early inspiration and inhibited from mid-inspiration to mid-expiration with the effect being more obvious at RF and RF + 1.

5.5.1 Respiration, Blood Pressure, Heart Rate

As expected, the respiration rates during both RF and RF + 1 were significantly lower compared to that during SB ($p < .01$ for both) with RF + 1 being also significantly higher compared to RF ($p < .01$) (Table 1). Systolic BP was significantly lower during RF compared with SB ($p < .05$) but no difference was noted during RF + 1 (Table 5.1). No significant difference in either diastolic BP or HR were observed between breathing conditions.

Table 5.1. Respiration, blood pressure, and heart rate across breathing.

	SB	RF	RF+1
Haemodynamics			
Respiration rate, breaths/min	14.2±3.13	5.44±0.88**	6.43±0.88▲•
Systolic blood pressure, mmHg	123.6±12.13	119.0±14.66*	120.5±14.07
Diastolic blood pressure, mmHg	80.8±11.8	78.7±12.3	80.1±11.5
Heart rate, bpm	71.2±6.47	72.6±7.73	72.5±7.15

**p< 0.050, RF versus SB, **p< 0.010, RF versus SB, ▲p< 0.010, RF+1 vs SB, • p< 0.010, RF versus RF+1.*

5.5.2 Muscle Sympathetic Nervous Activity

5.5.2.1 Burst Frequency and Incidence

A significant difference in MSNA burst frequency and incidence was observed during both breathing schemes: Burst frequency during RF was significantly lower than SB ($p < .05$) (Table 5.2). Similarly, RF + 1 exhibited lower MSNA burst frequency than SB ($p < .01$). Likewise, lower burst incidence was seen during RF compared to SB ($p < .05$) and during RF + 1 compared to SB ($p < .01$). Difference in burst incidence and burst frequency between RF and RF + 1 was non-significant.

5.5.2.2 Pattern of Sympathetic Activity

Figure 5.2 shows an example of MSNA data with corresponding respiration and the calculated instantaneous phase of the signal, normalised histogram of burst occurrence as a function of instantaneous phase of the respiration signal and normalised inter-burst interval histogram.

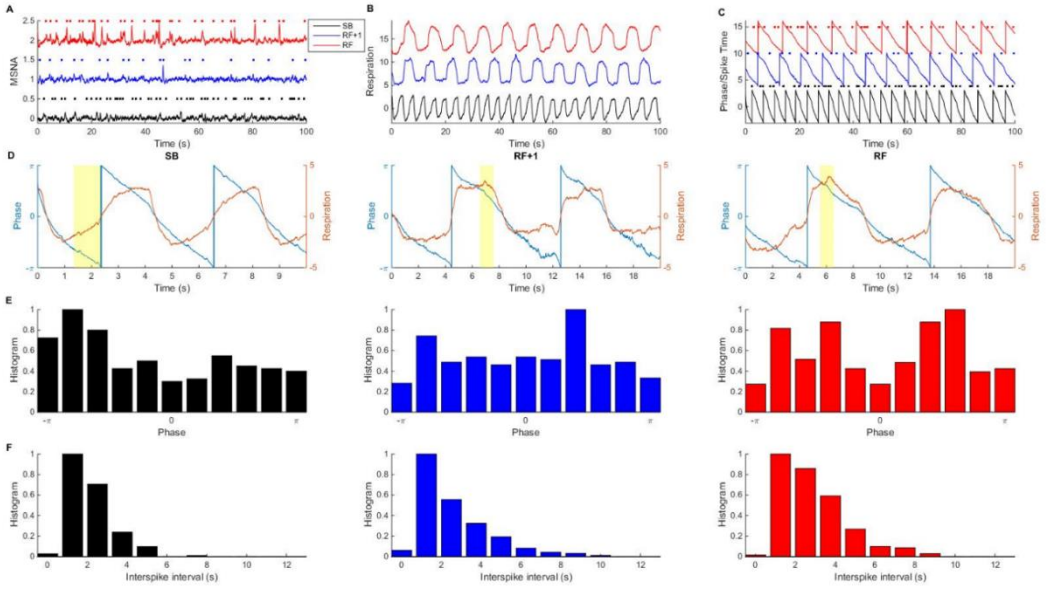

Figure 5.2. Examples of recorded and analysed data from a participant. (a–d) illustrate short extracts of data for an individual participant. (e and f) illustrate data for the whole period of recording for the same participant. All three conditions are shown in subplots (a–b), shifted along vertical axis for clarity, and color-coded: Black—spontaneous breathing (SB), Red—resonance frequency (RF), Blue—at (RF + 1). (a) An extract of muscle sympathetic nerve activity (MSNA) data (solid lines) and detected bursts (stars). (b) Respiration signal for three conditions. (c) Detected phase of respiration signal from b (solid lines) and MSNA bursts from a (stars), aligned in time. (d) An extract of respiration data (left y-axis) and detected phase of the signal (right y-axis). Yellow rectangles: the phase of the signal when maximum likelihood of spiking occurs. (e) Normalised burst time histogram as a function of the instantaneous phase of respiration. (f) Normalised histogram of inter-burst intervals. (d–f) Data for baseline SB (left plot), RF + 1 (middle), and RF (right).

5.5.2.3 Burst Histogram as a Function of Respiration Phase

The average, median, maximum, and differences in MSNA burst occurrence for the phase intervals $-\pi$ to 0 and 0 to π are presented in Table 5.2 and the average across all phases is illustrated in Figure 5.3. For the phase intervals $-\pi$ to 0, no significant difference existed between breathing frequencies. For the phase interval 0 to π, there was a difference in the area under the curve of burst occurrence histogram from 0 to π

with both RF and RF + 1 displaying significantly greater area under the curve of burst occurrence histogram compared to SB ($p < .05$ and $p < .01$, respectively).

5.5.2.4 Inter-burst Intervals

Inter-burst intervals were divided in duration of <4 ms and ≥4 ms. The mean and maximum inter-burst intervals for each duration are presented in Table 5.2 and Figure 5.3b illustrates inter-burst interval data averaged between all participants. There was no difference between breathing schemes in inter-burst intervals for the <4 ms. However, there was a significant difference in inter-burst intervals at ≥4 ms with RF + 1 displaying higher inter-burst intervals compared to SB ($p < .05$). The area under the curve of inter-burst intervals ≥4 ms was larger during RF + 1 compared to SB ($p < .05$). Similar trends were noticed during RF but these did not reach statistical significance.

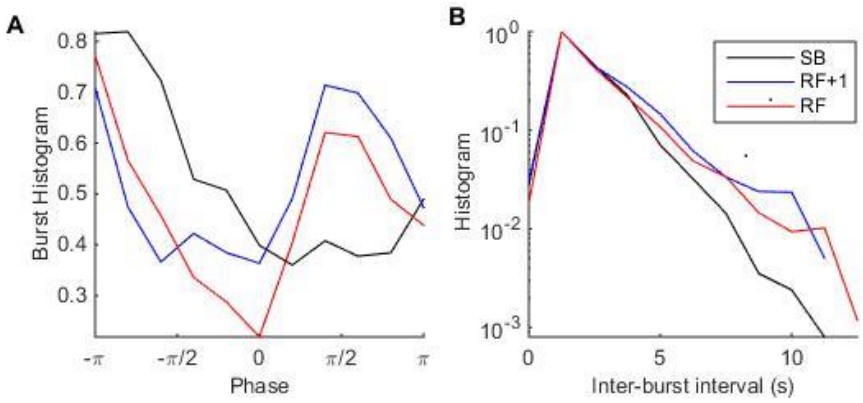

Figure 5.3. (a) Burst occurrence as a function of respiration phase averaged between all participants and normalised to a maximum value. There is a significant increase in burst occurrence on the 0 to π interval during breathing at resonance frequency (RF) and at the RF + 1. At this interval, the maximum likelihood of burst occurs at π phase for a baseline condition (spontaneous breathing, SB), while for the RF and at the RF + 1 the maximum occurrence of burst is seen at −π/2. On average, there is a 68% increase in burst occurrence at the π/2 phase during RF and at the RF + 1 compared to the SB. (b) inter-bursts interval data averaged between all participants. It can be seen that inter-burst intervals become longer during RF and at the RF + 1 breathing patterns, that is, there are more silence periods.

5.5.3 Baroreflex Function

The slopes of both sympathetic and cardiac baroreflex functions were unchanged during breathing exercises. The BEI of the cardiac baroreflex function was significantly increased during RF and RF + 1 ($p < .05$ for both).

Table 5.2. Muscle sympathetic nerve activity (MSNA) and baroreflex function during spontaneous breathing (SB), breathing at the resonance frequency (RF) and at the RF+1..

	SB	RF	RF+1
Muscle Sympathetic Nerve Activity			
Burst frequency (Bursts per minute)	33.5±9.67	27.9±8.14*	26.1±6.95▲▲
Burst incidence (Bursts per 100 heartbeats)	47.5±15.0	38.9±12.0*	36.5±11.2▲▲
Burst occurrence as a function of respiratory phase [-π,0]			
Normalised average burst occurrence	0.63 ± 0.09	0.44 ± 0.19	0.45± 0.22
Normalised median burst occurrence	0.65	0.43	0.46
Normalised maximum burst occurrence (corresponding phase)	0.82 (-π)	0.77(-π)	0.71(-π)
Difference in burst occurrence compared to SB at -π/2	-	-37%	-36%
Area under the curve of burst occurrence histogram	0.32 ± 0.07	0.36 ± 0.13	0.28± 0.08
Burst occurrence as a function of respiratory phase [0,π]			
Normalised average burst occurrence	0.40 ± 0.08	0.47 ± 0.15	0.56 ± 0.18
Normalised median burst occurrence	0.41	0.46	0.58
Normalised maximum burst occurrence (corresponding phase)	0.49(π)	0.62(π/2)	0.72(π/2)
Difference in burst occurrence compared to SB at π/2	-	+57%	+80%
Area under the curve of burst histogram	0.15 ± 0.05	0.28 ± 0.14*	0.33± 0.13▲▲
Inter bursts intervals corresponding to <4 ms			
Average	0.41 ± 0.09	0.41 ± 0.12	0.44 ± 0.13
Median	0.38	0.40	0.47
Maximum (corresponding interval)	1 (1.25 ms)	1 (1.25 ms)	1 (1.25 ms)
Difference compared to SB	-	+0.02	+0.13
Area under the curve of inter burst interval	1.12 ± 0.08	1.11 ± 0.12	1.07 ± 0.11
Inter bursts intervals corresponding to ≥4 ms			
Average inter bursts interval	0.02 ± 0.02	0.03 ± 0.03	0.05 ± 0.04▲
Median inter bursts interval	0.01	0.02	0.04
Maximum (corresponding interval)	0.09 (5 ms)	0.27 (5 ms)	0.19 (5 ms)
Difference compared to SB	-	+0.13	+0.26
Area under the curve of inter burst interval histogram	0.12 ± 0.09	0.16 ± 0.11	0.19± 0.09▲
Sympathetic baroreflex function slope, bursts/100 heartbeats/mmHg	-3.77 ± 2.71	-4.23 ± 2.35	-4.55 ± 2.96
Cardiac baroreflex function slope, msec/mmHg	14.6±3.83	13.8±4.51	14.1±5.51
Cardiac baroreflex function efficacy index	39.14 ± 13.97	56.23 ± 23.98*	60.37 ± 20.44▲

**p< 0.050, RF vs SB, ▲p< 0.050, RF+1 vs SB, ▲▲p< 0.010, RF+1 vs SB*

5.5.4 Heart Rate Variability

All changes in HRV for time domain and frequency domain analysis are presented in Table 5.3. In the time domain *SD* rate was higher during RF and RF + 1 ($p < .05$ and $p < .01$). In the frequency domain, RF and RF + 1 displayed greater LF power and lower HF power compared to SB but no significant difference was found between RF and RF + 1.

Table 5.3. Heart rate variability parameters during spontaneous breathing (SB), breathing at the resonance frequency (RF) and at the RF+1.

	SB	RF	RF+1
Time domain analysis			
SD Rate	5.10±1.45	7.30±2.41*	7.20±2.30▲▲
SDNN ms	48.0 ±25.4	46.2±26.9	52.3±28.2
Frequency domain analysis			
LF Power (%)	28.0±12.6	69.6±13.2**	64.6±19.1▲▲
LF Power (nu)	46.8±20.8	88.3±6.11**	82.1±12.4▲▲
HF Power (%)	32.2±16.3	9.30±6.03**	12.8±8.90▲
HF Power (nu)	51.2±19.5	11.5±5.76**	17.2±11.8▲
LF/HF	1.60±2.22	10.9±8.47**	8.90±7.55▲

**p< 0.050, RF vs SB, ▲p< 0.050, RF+1 vs SB, ▲▲p< 0.010, RF+1 vs SB.*

5.6 Discussion

This study examined the effects of short-term breathing at RF on cardiovascular regulation compared to breathing at 1 breath/min above the RF. We addressed this issue by assessing hemodynamics including direct sympathetic nerve recording and assessment of cardiac and arterial baroreflex function. Major findings are: (a) Both RF and RF + 1 induced similar changes in MSNA including a decrease in the incidence and frequency of bursts, with the bursting pattern associated with longer periods of burst silencing and a shift of burst occurrence towards mid-inspiration to early expiration, (b) RF and RF + 1 were both associated with improved cardiac baroreflex efficacy but did not affect the sympathetic baroreflex function (c) RF induced a significant reduction in systolic BP.

This is the first study to examine the modulatory effects of respiration at RF and RF + 1 on sympathetic activity investigating both the global sympathetic tone (burst incidence and frequency) and the dynamic pattern of sympathetic firing including inter-burst intervals and burst occurrence as a function of the respiration phase. A significant reduction in sympathetic burst frequency and incidence were seen during either RF or RF + 1 compared with SB. In the healthy state, MSNA represents global sympathetic outflow to the skeletal muscle linked to BP regulation with strong feedback from the arterial baroreceptors (Wallin, 2006) and respiratory modulation (Habler et al, 1994). The reduction in MSNA observed during slow breathing at either RF or RF + 1 was similar to that demonstrated in previous studies where slow breathing was of 10–15 min duration (Fonkoue et al., 2018; Harada et al., 2014; Hering et al., 2013; Oneda et al., 2010). Two prior studies in healthy subjects indicated no effect of slow breathing on MSNA but the duration of the breathing exercise was much shorter being 3 (Limberg et al., 2013) or 4 min (Raupach et al., 2008). Our data indicate that MSNA is reduced during slow breathing in healthy subjects with a longer breathing task. Lower burst frequency and burst incidence with RF or slow breathing in general may be due to activation of pulmonary mechanoreceptors in response to the increased tidal volume that accompanies slow breathing. This is supported by the findings of Lehrer and colleagues (2003) who demonstrated greater tidal volume among healthy male participants under RF breathing compared with SB. The increased tidal volume under slow breathing might have activated lung stretch receptors and reduced chemoreflex response thus suppressing the activation of MSNA (Bernardi et al., 2001; Seals et al., 1990). The modulatory influence of breathing on MSNA has previously been described with approximately 70% of the activity occurring during low lung volumes (initial half of inspiration and latter half of expiration) and MSNA decreasing

progressively and markedly from onset to late inspiration (Seals et al., 1993). The analysis of the pattern of sympathetic activity using the respiration phase analysis and the inter-burst intervals is novel and allows to demonstrate that RF and RF + 1 have significant effect on the pattern of sympathetic activity. In accordance with that described by Seals et al. (1993), we documented that during normal spontaneous breathing, MSNA is more likely to occur during the $-\pi$ to $-\pi/2$ phase indicating higher activity during early inspiration and late expiration. MSNA is more inhibited during the 0 to π phase, hence during the late inspiration to early expiration. The decrease in burst incidence and frequency observed during either RF or RF + 1 is associated with a change in the pattern of bursting with the bursts being less frequent during the respiration phase interval $-\pi/2$ to 0 (expiration to onset of inspiration) and more frequent around $\pi/2$ phase (late inspiration). In addition, the inter-burst interval analysis revealed longer periods of burst silencing during RF and RF + 1. Hence, the sympatho-inhibition observed during either RF or RF + 1 seems to occur as a result of changes in the bursting pattern of MSNA imposed by the ability of the respiration to modulate the timing of bursts. Such changes are potentially critical because the respiratory-modulated bursting of sympathetic activity has been shown to modulate vascular resistance (Briant et al., 2015).

Slow breathing exercises have been reported to decrease BP albeit to a modest extent (Fonkoue et al., 2018; Grossman et al, 2001; Hering et al., 2013; Rosenthal et al., 2001). In line with these previous studies, we noticed a small but significant decrease in systolic BP during the RF breathing with no difference in systolic BP between RF and RF + 1 indicating that breathing at RF as opposed to RF + 1 did not trigger a better systolic BP response. Breathing at RF has been proposed to modulate autonomic

cardiovascular regulation, affecting cardiopulmonary reflexes, arterial baroreflexes, sympathetic vascular tone, and peripheral resistance, which in turn may result in systemic vasodilatation and decreased BP (Lehrer and Gevirtz, 2014).

Enhanced baroreflex sensitivity has been suggested to occur during slow breathing (Bernardi et al., 2001; Raupach et al., 2008) or breathing at RF (Lehrer et al., 2000), possibly as a result of reduced chemoreflex sensitivity, which in turn may lead to decreased sympathetic tone. Our study indicates that the slope of the cardiac baroreflex function (assessed as a combination of up and down sequences) remains unchanged during RF or RF + 1 but the efficacy of the baroreflex as assessed by BEI was improved during both RF + 1 and RF. BEI has been suggested to be a good representation of the baroreflex function in healthy subjects as it quantifies the number of times the baroreflex is effective in driving the sinus node (Di Rienzo et al., 2001). Tzeng et al. (2009) demonstrated that slow breathing in healthy subjects did not affect the arterial baroreflex when measured using the gold standard modified Oxford method, and suggested that results indicating improvement in baroreflex function during slow breathing may have occurred as commonly used baroreflex assessment techniques may not be accurate in this setting. The sympathetic baroreflex function was also explored as a possible contributor to the changes in MSNA as Fonkue and colleagues (2018) observed that slow breathing improved the sympathetic baroreflex function. However, this was not observed in our study as neither RF nor RF + 1 improved the slope of the sympathetic baroreflex function. Overall, the effects of slow breathing schemes in this study on baroreflex function are still unclear as results vary depending on the method used (Tzeng et al., 2009).

Within the context of this study, we found that breathing at RF or RF + 1 induced significant hemodynamic and autonomic changes but we were unable to detect any differences between the two breathing schemes. This raises the question as to whether precise measurement of the RF is essential for the reported beneficial clinical effects of individualized RF or a standardized paced breathing at 5–7 breaths per min is all that is required. Lin et al. (2012) investigated the effects or either paced breathing at RF compared to slow breathing (6 breaths/min) in individuals with prehypertension and found that both schemes of breathing resulted in a decrease in BP over a period of 5 weeks and that the decrease in BP was more marked in those breathing at RF except for the first session where the BP fall was identical with either RF or RF + 1. They also found that RF induced stronger changes in HRV indices and in the baroreflex function compared with slow breathing which may explain better hemodynamic changes. In this study, we noticed that there was neglible difference in HRV and the respiratory modulation of MSNA between acute RF and RF + 1. These findings indicate occurrence of sympathoinhibition regardless of RF or RF + 1. In a recent study comparing RF and RF + 1 on BP levels, Steffen et al. (2017) noticed that both RF and RF + 1 decreased systolic BP to the same extent but subjects allocated to the RF exercise exhibited lower systolic BP in response to a stress test compared to subjects allocated to RF + 1. This was accompanied by better mood, suggesting a preferential effect of RF compared to RF + 1 on the stress response. Hering et al. (2013) showed that slow breathing at six breaths per min induced significant decrease in BP in the long term but not acutely in hypertensive subjects. Interestingly, they found, that long-term paced breathing selectively attenuated pressor and tachycardic responses to mental stress but the corresponding MSNA responses remained unaltered; however, in their study the resonance frequency was not imposed. Hence, while slow breathing in

general is undoubtedly a strategy to improve BP and stress responses, whether breathing at the specific RF is more efficient in the longer term is uncertain. More studies are certainly needed to fully examine long-term effect of breathing at RF on hemodynamic markers.

Limitations in the current study should be acknowledged. This study was conducted in a small group of healthy young males. Hemodynamic and MSNA results in this cohort might not be applicable in other populations. Slow breathing may be more beneficial in individuals with elevated sympathetic nervous activity and decreased baroreflex function such as those with hypertension, heart failure, chronic obstructive pulmonary diseases, or in individuals with anxiety disorders where sympathetic and vagal function may be altered. Also, this study was conducted in a single session and respiratory rate was assessed using a standard respiratory belt hence parameters such as tidal volume was not available. Future studies should employ long-term applications of RF versus RF + 1 to identify dose–response relationship on hemodynamic and autonomic nervous system.

5.7 Conclusion

Breathing exercises remain an attractive non-invasive strategy to modulate autonomic nervous system function. While breathing at the specific RF is suggested to maximize the effect, we found that breathing at RF or 1 breath above RF induced the same acute changes on the sympathetic nervous system and BP, with both breathing paradigms inducing similar changes in the pattern of sympathetic firing.

Chapter 6: Effect of Acute Heart Rate Variability Biofeedback on Heartbeat Evoked Potential Among Healthy Population

An original version of this chapter has been prepared for review titled:

Pagaduan J, White D, Lambert E, Leighton J, Fell J, Wu SSX. Effect of acute heart rate variability biofeedback on heart evoked potential among healthy population.

Rationale

The afferent information may have an effect on muscle sympathetic outflow from HRV BFB (Study 4). Moreover, physiological and performance changes (Studies 1, 2, and 3) with HRV BFB may be initiated by alterations in the vagal afferent pathway. However, there is limited published research examining the vagal afferent pathway under HRV BFB. Thus, this study investigated the cortical processing of cardiac signals via HEP in HRV BFB.

Abstract

Heart rate variability biofeedback (HRV BFB) is a paced breathing technique that activates resonance properties in the cardiovascular system (Lehrer & Eddie, 2013). There is little evidence of the influence of HRV BFB on the cortical processing of cardiovascular activity. This study aimed to examine the acute effect of HRV BFB on the vagal afferent pathway using the heartbeat evoked potential (HEP). Thirty males and fourteen healthy females were semi-randomised and counterbalanced according to sex, into either HRV BFB or a control group (CON) breathing at their spontaneous breathing rate for ten minutes. The HEP between 200-300 ms time-locked to the ECG R-peak as contrasted between the two conditions. Results revealed significant differences at fronto-parietal (Fp2), frontal (F3, Fz) central (C4), parietal (P7, Pz), occipital (O1, Oz, O2) parieto-occipital (PO1, PO2) between HRV BFB and CON. HRV BFB displayed significantly higher HEP at Fp2, F3, Fz, C4, and P7 than CON. On the other hand, HRV BFB showed significantly lower HEP at P7, Pz, O1, Oz, O2, PO1, Pz, and PO2 compared to CON. These findings suggest HRV BFB can result in acute alteration in afferent pathways beneficial to cardiovascular regulation.

Keywords: event related potential, biofeedback, resonance frequency breathing

6.2 Introduction

Afferent neural information plays a crucial role in maintaining the homeostasis of brain centers responsible for autonomic cardiovascular control (Craig, 2002; McCraty et al., 2009; Shaffer et al., 2014). One method of assessing the afferent pathway is through the heartbeat evoked potential (HEP). The HEP represents the cortical activity in the brain occurring 200-600 ms after each R peak of the electrocardiograph (Schandr et al., 1986; Schandry and Weikunat, 1990; Verberne and Owens, 1998). HEP is believed to exist mainly at the somatosensory cortex and frontal/pre-frontal areas (Aziz et al., 1995; Gray et al., 2007; Leopold and Schandry, 2001; Pollatos and Schandry, 2004). HEP at frontal areas have been reported to reflect cardiovascular regulation from cardio-afferent signals in the insular cortex (Aziz et al., 1995; Pollatos and Schandry, 2004). Others reported HEP at central areas (Montoya et al., 1993; Petzschner et al., 2019), while other primarily observed the HEP at parietal areas (Babo-Rebelo et al., 2016; Dirlich et al., 1998). The variability in HEP topography can be attributed to neural and methodological factors in measurement of HEP (Park and Blanke, 2019). Two physiological mechanisms have been proposed for HEP occurrence: the phase-reset and additive evoked potential model (Park et al., 2018; Sauseng et al., 2007). The phase-reset model posits that the generation of HEPs result from resetting of ongoing oscillations in each single trial. On the other hand, the additive evoked potential model implies that stimulus-evoked responses in each trial are added to produce HEPs. A recent finding by Paul et al. (2018) suggests that the phase-resetting mechanism is the underlying mechanism in HEP rather than the additive evoked potential mechanism after evidence of stimulus–induced phase concentration without enhancement in spectral power, and non-relationship of intertrial coherence and spectral power across recording sites. HEP amplitude is reduced under pathological conditions such as heart

disease (Gray et al., 2007), anxiety (Pang et al., 2019), depression (Terhaar et al., 2013), and sleep disorder (Immanuel et al., 2014). Thus, interventions that increase HEP may in turn play a role in improving associated health outcomes (Huang et al., 2018; Mackinnon et al., 2013; Schandry and Weitkunat, 1990).

Heart rate variability biofeedback (HRV BFB) is a non-invasive, paced breathing intervention believed to enhance HEP (Gevirtz, 2013; Lehrer et al., 2000; Lehrer and Gevirtz, 2014; Mackinnon et al., 2013; Huang et al., 2018). During HRV BFB, an individual follows a visual/auditory pacer while real time information on heart rate and respiration are displayed on a screen. HRV BFB uses a breathing pace that resonates within the naturally occurring oscillations in the cardiovascular system (Lehrer and Eddie, 2013; Vaschillo et al., 2006). The mechanistic properties under HRV BFB stimulates an increase to the efficacy of the baroreflex receptors that provide afferent neural information to the brain (Lehrer et al., 2003; Pagaduan et al., 2019; Vaschillo et al., 2002; Vaschillo et al., 2006). Specifically, the baroreceptors located in the carotid sinus and/or aortic arch increase sensitivity and send efficient sensory information to the nucleus solitary tract (NST), which is connected to the medulla via the vagus nerve (McCraty and Shaffer, 2015; Shaffer and Venner, 2013; Lehrer et al., 2003). Thus, HRV BFB may positively influence vagal afferent pathway as measured by HEP.

Limited research investigated the impact of HRV BFB on HEP using healthy populations (Huang et al., 2018; Mackinnon et al., 2013). For instance, Huang et al. (2018) compared the HEP in HRV BFB and electromyography BFB (EMG BFB) with progressive muscle relaxation among healthy individuals for 4-weeks. HEP was analysed at a site wherein minimal interference occurred. Five percent of the

participants were subjected to HEP analysis at Cz. Fifty-eight percent of the participants were assessed for HEP at F3, while HEP at Fz was examined from 13% of the participants. Twenty-four percent of participants were investigated for HEP at Pz. It was discovered that HEP in the HRV BFB group was higher during a 10-minute baseline audio distraction than EMG BFB group after four weeks. On the other hand, HEP in the ten-minute HRV BFB and EMG BFB across sessions were not significantly different. In another study, Mackinnon et al. (2013) examined HEP at central brain areas (C3, Cz, C4) under normal breathing, positive emotion, negative emotion, and HRV BFB at 0.1 Hz among healthy participants in a single session. The study revealed that HRV BFB increased HEP at Cz compared to normal breathing. On the other hand, no difference in HEP at C3 and C4 were seen between the HRV BFB and normal breathing. In addition, HRV BFB induced greater HEP at Cz and C4 compared with the positive emotion. All three sites demonstrated higher HEP in HRV BFB than negative emotion. Nevertheless, HEP were only evaluated using limited areas of the brain in both studies. In addition, the existing studies failed to disclose artefact correction techniques in analysing HEP, which may contribute to bias in interpretation.

Understanding HEP topography in HRV BFB can aid in the prospective application of HRV BFB for health and performance enhancement. However, there is a scarcity of published research addressing HEP with HRV BFB. Thus, the purpose of this study was to examine the effect of a single session HEP utilising 32-channel EEG.

6.3 Methods

6.3.1 Participants

Thirty males and fourteen females volunteered to participate in this study. During the time of experimentation, the participants were classified as apparently healthy. They were semi-randomly assigned based on the order of recruitment, and equally distributed according to sex into either HRV BFB (males, n = 15: 29.3 ± 6.15 yrs; females, n = 7: 30.6 ± 8.20 yrs) or CON (males, n = 15: 30.5 ± 7.70 yrs; females, n = 7: 28.0 ± 5.28 yrs) group. Avoidance of any food intake two hours before experimentation was requested. Non-ingestion of caffeine containing food/beverage on testing day, was also instructed. A written informed consent was obtained from the participants before further participation. This study was approved by the Swinburne University Human Research Ethics Committee (Ethics No: 2017/346).

6.3.2 Procedures

This study occurred across two sessions separated by 24 hours. In the first session, participants visited a quiet room in the university between 0900-1800 hrs. The HRV BFB group performed resonance frequency (RF) testing. In contrast, the CON performed five-minute spontaneous breathing whilst attached with biofeedback sensors. In the second session, participants completed the experiment in an electrically shielded and dimly lit room. The participants in the HRV BFB group underwent 10-minute paced breathing at individual RF. In addition, HRV BFB group executed abdominal breathing technique with nasal inhalation and pursed lip exhalation. Also, HRV BFB group was encouraged to focus on the pacer. CON group breathed normally with their gaze fixed at a black computer screen. Both groups were asked to facilitate an ‘empty mind’ whilst completing a breathing task. The ten-minute

electroencephalography (EEG), electrocardiography (ECG), and electrooculogram (EOG) were recorded in HRV BFB and CON.

6.3.3 Measures

6.3.3.1 Resonance Frequency

Five ´ two-minute paced breathing frequencies at decreasing order (6.6, 6.0, 5.5, 5.0, 4.5) were completed by the participants in HRV BFB group. RF is defined as the breathing pace that displays the highest power exhibited at low frequency (LF) in HRV (Task Force, 1996). A five-minute 0.1 Hz paced breathing familiarization with the biofeedback equipment (HRV Starter System, Thought Technology, Canada) was facilitated before RF testing.

6.3.3.2 Electroencephalography

EEG, ECG, and EOG were acquired using a 40-channel NuAmps amplifier and Curry 7 software (Compumedics Neuroscan, USA). Data was digitised at a sampling rate of 1000 Hz. Thirty-two Ag/AgCl EEG electrodes arranged in accordance with the extended International 10-20 System) (Jasper, 1958) were fitted to the scalp of the participants via an electrode cap (Quickcaps, Compumedics Neuroscan, USA). The ground electrode was positioned anterior to electrode Fz (AFz) and bilateral mastoid electrodes served as the on-line reference. Impedances were kept below 5 kΩ.

6.3.3.3 Electrocardiography

A single electrode connected to EEG cap was placed over the left radial artery to record ten-minute ECG at 1000 Hz sampling rate.

6.3.3.4 Electroculogram

Eye movement was monitored concurrently with EEG recordings by attachment of a single electrode above the left eye.

An eight-minute stabilization period was facilitated after electrode attachment, during which participants were asked to breathe normally, focus their gaze at the computer screen, and enable thought clearance. The ten-minute HRV BFB/CON epochs were saved as DAT files.

6.3.3.5 R-peak Detection

The ten-minute recordings were imported to EEGLAB version 14.1.2b, and subjected to R-peak detection using HEPLAB (Delorme and Makeig; Perakakis and Ciria, 2018). The success of R-peak detection was confirmed via visual inspection, and correction was facilitated by deleting any R-peak detection error or manual identification of R-peak. Then the EEG file is saved as a Matlab file (.set).

Pre-processing of raw EEG data was administered in Automagic (Pedroni et al., 2019). Non-scalp reference was selected as the reference channel. The default PREP pipeline was used for bad channel detection (Bigdely-Shamlo et al., 2015; Mullen 2012). Artefact correction configurations involved electrooculogram regression and Multiple Artifact Rejection Algorithm with a temporary high pass filter of 1 Hz (Parra et al., 2005; Winkler et al., 2014). Filter settings included 50 Hz line power, 1 Hz high pass, and 40 Hz low pass. The default settings for bad channel interpolation via spherical interpolation method, and quality assessment thresholds were utilised.

All the pre-processed data resulted to acceptable quality rating and exported to BIDS file format.

6.3.3.6 Heartbeat Evoked Potential

Epochs from -200 ms to 600 ms after each R-peak were extracted using Brainstorm software (Tadel et al., 2011). Baseline correction was applied to each epoch by subtracting the mean of the period -200 to -50 ms from R-peak. The average HEP was obtained for each participant across the 10-minute recording and underwent analysis.

A comparison of the ECG signal from 200-300 ms in HRV BFB and CON was carried out to confirm HEP as a result of cortical activity rather than cardiac artefact. The 10-minute epoch were average referenced, then filtered to 1 Hz high pass and 40 Hz low pass. Baseline correction of -200 to -50 ms to 800 ms recording. ECG signals were exported to an excel spreadsheet for further analysis.

6.3.3.7 Heart Rate Variability

Ten-minute R-peak occurrences displayed on Brainstorm were exported to an excel spreadsheet, the time interval between two R-peaks was computed, then saved for further processing. The excel file was imported to a free HRV software (Kubios ver 3.1.0., Finland) to derive HRV indices (Tarvainen et al., 2014). Medium artefact correction and smoothness priors were applied for HRV analysis (Tarvainen et al., 2002). The standard deviation of the normal-to-normal (NN) interbeat intervals (SDNN) and the square root of the mean square differences of successive normal heartbeats (RMSSD) were the time-based HRV measures that were subjected for analysis (Task Force, 1996; Shaffer and Ginsberg, 2017). SDNN is a global measure

of HRV, while RMSSD is linked to parasympathetic activity. Further, low frequency (LF; 0.04 – 0.15 Hz), high frequency (HF; 0.15 – 0.4 Hz), and total power (TP) spectral HRV measures were also examined (Montano et al., 2009; Task Force, 1996). The LF is believed to reflect baroreceptor activity from PNS, SNS, and blood pressure regulation from PNS at resting conditions (McCraty and Shaffer, 2015; Nunan et al., 2010; Reyes del Paso et al., 2013; Shaffer et al., 2014; Task Force, 1996). On the other hand, HF depicts parasympathetic activity and respiratory sinus arrhythmia (RSA). TP is a global index of HRV which is the sum of very low frequency (≤ 0.04 Hz), LF, and HF (Task Force, 1996). Time and frequency HRV values were transformed to natural logarithm reduce bias. In addition, normalised units of LF (LFnu) and HF (HFnu) were used to identify breathing frequency range (Burr, 2007; Task Force, 1996). Lastly, RSA was determined from heart rate difference (HR-diff) by subtracting maximum HR and minimum HR (Shaffer and Ginsberg, 2017).

6.4 Statistical Analysis

Data are expressed as mean ± standard deviation. HEP amplitude at each point in the window from 200-300ms following the R-peak was contrasted using independent groups t-test with cluster-based permutation (n = 1000) in Fieldtrip toolbox under Brainstorm (Maris and Oostenveld, 2007; Oostenveld, et al., 2011). This method was performed to estimate the null distribution of test statistics by generating surrogate data and analysing it with the original data in order to control for multiple comparisons across electrodes and time points. Additionally, independent t-test was utilised to compare HRV indices and ECG signals between groups at 0.05 alpha level. Effect size was calculated using Cohen's *d*, interpreted as trivial (0.00–0.20), small (0.20–0.60), moderate (0.60–1.20), large (1.20–2.00) and very large (>2.00) (Cohen, 1988).

6.5 Results

6.5.1 Electrocardiography

Figure 6.1 Displays the ECG signals in HRV BFB and CON. There was no significant difference in ECG signals between HRV BFB and CON, $t(42) = 0.21$, $p = 0.84$, $d = 0.06$.

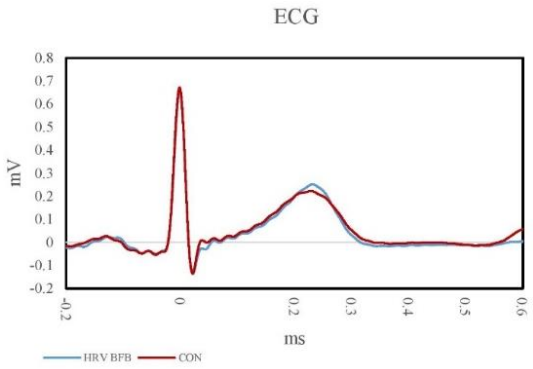

Figure 6.1. ECG of HRV BFB and CON.

6.5.2 Heart Evoked Potential

Testing the difference of HEP between HRV BFB and CON in the latency range from 200-300 ms post-R peak, the cluster-based permutation test revealed a significant difference between HRV BFB and CON ($p < 0.05$). In this latency range, the difference was most pronounced over Fp2, F3, Fz, C4, P7, Pz, O1, Oz, O2, PO1, Pz, and PO2. Figure 6.2 displays HEP from -200 – 600 ms. The HRV BFB showed higher HEP in Fp2, F3, Fz, and C4 under HRV BFB compared to CON. Contrastingly, HRV BFB displayed lower HEP in P7, Pz, O1, Oz, O2, PO1, Pz, PO2 compared to CON.

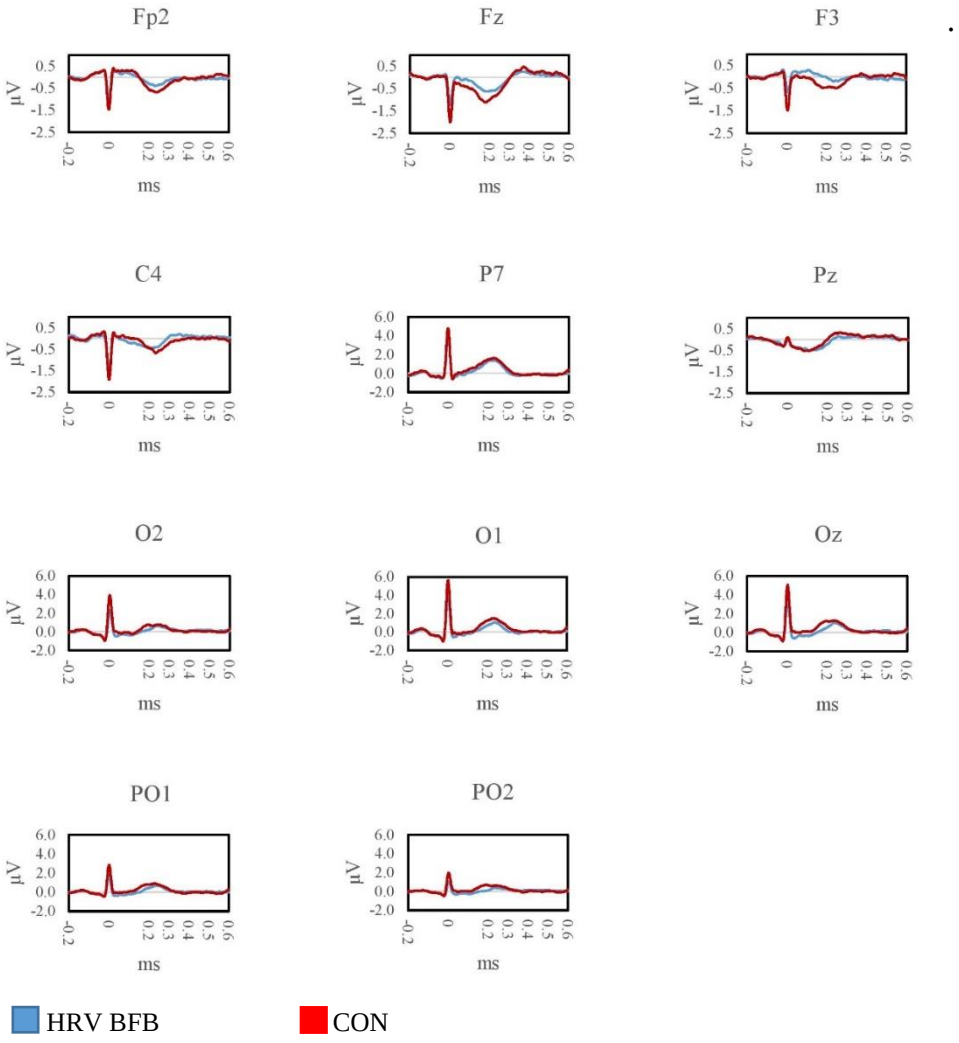

Figure 6.2. HEP in HRV BFB and CON.

6.5.3 Heart Rate Variability

The ten-minute HRV BFB showed greater lnSDNN, $t(42) = 4.71$, $p = 0.00$, $d = 1.42$. lnLF was significantly higher in HRV BFB than CON, $t(42) = 6.34$, $p = 0.00$, $d = 2.07$. HRV BFB exhibited significantly higher lnHF than CON, $t(42) = 5.52$, $p = 0.00$, $d = 1.68$. LFnu was significantly greater in HRV BFB than CON, $t(42) = 7.93$, $p = 0.00$, $d = 2.39$. Relatively, HFnu was significantly lower in HRV BFB compared to CON $t(42) = 7.92$, $p = 0.00$, $d = 2.39$.

Table 6.1. HRV indices in HRV BFB and CON.

		HRV BFB	CON
lnSDNN	(ms)	4.21 ± 0.30	3.50 ± 0.64**
lnRMSSD	(ms)	3.58 ± 0.29	3.28 ± 0.69
HR-difference	(beats/min)	31.0 ± 8.29	26.1 ± 10.4
lnLF	(ms^2)	8.36 ± 0.62	6.23 ± 1.45**
lnHF	(ms^2)	2.12 ± 0.07	1.80 ± 0.26**
lnTP	(ms^2)	8.43 ± 0.61	6.93 ± 1.32**
LFnu	(%)	94.9 ± 3.63	58.0 ± 21.6**
HFnu	(%)	5.02 ± 3.62	41.9 ± 21.6**

***significant at the 0.01 level.*

6.6 Discussion

The purpose of this study was to examine heart evoked potential under HRV BFB among apparently healthy adults. The HEP represents cortical processing of signals from the heart to the brain via the afferent pathway (Pollatos et al., 2005; Aziz et al., 1995). Novel findings demonstrated higher HEP in Fp2, F3, Fz, and C4 under HRV BFB compared to CON. On the other hand HEP in P7, Pz, O1, Oz, O2, PO1, Pz, PO2 under HRV BFB was lower than CON.

In this study, an acute HRV BFB demonstrated efficient afferent pathway. Results revealed greater HEP at Fp2, F3, Fz, and C4 in HRV BFB than CON. These findings are in partial agreement with previous studies that exhibited higher HEP under HRV BFB (Mackinnon et al., 2013; Huang et al. 2018). The aforementioned sites cover the insular cortex, which regulates cardiovascular activity (Aziz et al., 1995; Pollatos and Schandry, 2004). Higher HEP at this area suggest transmission of information by visceral afferents rather than via somatosensory pathways (Montoya et al., 1993; Schandry and Montoya, 1996). Thus, HRV BFB can be a useful intervention to

facilitate enhancement of heart to brain communication pathway (Aziz et al., 1995; Mackinnon et al., 2013).

The improvement in afferent pathway with HRV BFB is partially supported by higher lnLF, LFnu, lnSDNN, and lnTP compared to CON. The increased lnLF and LFnu verify slow breathing within the LF band. When this is accompanied by increased SDNN and TP, baroreflex enhancement may be possible (Lehrer et al., 2003; Fonoberova et al., 2014). Lehrer et al. (2003) suggested that baroreflex gain in acute HRV BFB is related with respiratory frequency and tidal volume. Inclusion of baroreflex markers with HEP under HRV BFB in future studies can help establish the baroreflex function in vagal afferent enhancement in HRV BFB.

Other novel findings in this study included lower HEP at P7, Pz, O1, Oz, O2, PO1, Pz, PO2 in HRV BFB than CON. The decreasing trend in HEP topography from frontal to posterior regions is in agreement with previous studies, supporting visceral afferent pathway in processing of heartbeats (Montoya et al., 1993; Schandry and Weitkunat, 1990).

Within this study, assessment of efferent pathway via RSA under 10-minute HRV BFB was also conducted using the HR-difference method (Giardino et al. 2003; Shaffer and Ginsberg, 2017; Yilmaz et al., 2018). A non-significant change in RSA function between CON and HRV BFB was found. RSA reflects the synchrony of heart rate and respiration, wherein HR rises with inspiration and vice versa (Eckberg, 1982; Hayano et al., 1996; Yasuma and Hayano, 2004; Task Force, 1996). Lower respiration rate in HRV BFB can generate increased RSA independent from changes in vagal firing

(Shaffer and Ginsberg, 2017). In this study, non-significant difference in RSA existed between HRV BFB and CON. This is supported by non-difference in lnRMSSD between groups (Shaffer and Ginsberg, 2017). More studies are necessary to demonstrate the vagal efferent influence of HRV BFB.

Whilst the results of this study provide some evidence supporting the beneficial effects of HRV BFB, limitations are acknowledged. First, HEP was evaluated in limited conditions. Inclusion of HEP assessment with higher intrinsic level of arousal after HRV BFB may provide valuable information on HEP response under induced affect (Huang et al., 2018; Luft and Bhattacharya, 2015). Moreover, HRV BFB was administered for a single session. Utilising longitudinal application of HRV BFB may facilitate distinct HEP changes from consistent HRV BFB practice. A practical suggestion from this study is to apply the results to populations dealing with depression and/or anxiety, who may benefit from the positive influence of HRV BFB towards HEP enhancement.

6.7 Conclusion

In conclusion, evidence of alterations in HEP at indicated areas in the brain during acute HRV BFB emphasise the cardiovascular regulatory influence of HRV BFB.

Chapter Seven: Thesis summary, limitations, future directions, and conclusions

7.1 Thesis Summary

The overarching aim of this thesis was to investigate motoneural excitation, muscle sympathetic nervous activity, and vagal afferents that may influence the alteration of performance in athletes under HRV BFB. To date, there exists a limited availability of published research in systematic reviews investigating the effect of HRV BFB on performance. There is no available meta-analysis examining the effects of HRV BFB on physiological indices in athletic population. Additionally, there are no studies that have investigated neuromuscular responses under HRV BFB. Further, initial work on HEP and HRV BFB lacked information on techniques to control for cardio-artefact, crucial for interpreting vagal afferent pathway. These gaps in literature prompted the design of the five studies which unpin this PhD thesis.

The investigation of HRV BFB in athletic settings, through a systematic review of related literature, may clarify current understanding of HRV BFB in athlete setting. Hence, study one (Chapter Two) is a systematic review that examined the effects of HRV BFB on fine and gross motor ability of athletes. In this review, HRV BFB was compared with CON/PLA. Findings revealed different effects between HRV BFB and CON in fine and gross motor ability of athletes. Interestingly, HRV BFB depicted improvement in short-duration gross motor ability compared to PLA. Another finding showed no difference in fine motor skill function between HRV BFB and PLA. The confounding results in fine and gross motor function with HRV BFB practice among athletes may be attributed to the low number of studies included in the review. Thus, further research examining the dose-response relationship of HRV BFB and performance are needed.

The alteration of performance among athletes in HRV BFB has been attributed to autonomic regulation (Jiménez Morgan and Mora, 2017). It may also be possible that reduced cognitive overload with resting RR may play a crucial role in determining performance (Grassmann et al., 2016). Whilst the HRV and RR are the common physiological markers in HRV BFB studies, no systematic review nor meta-analysis has been conducted invovling these indices. Therefore, study two (Chapter Three) is a preliminary systematic review and meta-analysis that investigated the effects of HRV BFB on HRV and RR in athletes. In this study, HRV BFB was compared to CON. Results revealed that HRV BFB demonstrated greater reduction in breathing frequency than CON. Neither the baroreflex function nor RSA was affected by HRV BFB. These findings imply that reduced breathing frequency, rather than changes in autonomic function may influence alteration of performance among athletes in HRV BFB. Further investigation is warranted to establish the link autonomic regulation and performance in an athletic population.

Whilst alteration in performance from HRV BFB (Study One) may be influenced by HRV and RR (Study Two), examining neuromuscular pathways in HRV BFB settings provides a valuable insight that may link autonomic regulation via HRV BFB on fine and gross motor function. Autonomic modulation and muscle activity are regulated by the central command (Boulton et al., 2014; Taylor et al., 2019; Williamson et al., 2005). Additionally, changes in autonomic activity could affect motor unit recruitment (Seki et al., 2008). In the athletic environment, measurement of motoneuron excitability using H-reflex method has been utilised to assess neuromuscular response to various interventions (Palmieri et al., 2004). Determining the resting motoneuron excitability from HRV BFB may be crucial in for understanding any subsequent

changes in neuromuscular performance. Study three (Chapter Four) is a novel research that investigated the effect of a single-session, ten-minute HRV BFB on soleus H-reflex modulation. In this study, participants underwent an acute HRV BFB whilst evaluated for pre and post measures of H-reflex. Results revealed a reduction in neural excitability of the soleus muscle after HRV BFB in the resting condition. The resting H-reflex can be useful to explain the alteration in performance with HRV BFB, with alpha motoneurons activated before movement initiation (Porter and Hore, 1969; Porter and Muir, 1971; Fetz and Cheney, 1980). The resting H-reflex achieved from HRV BFB may provide favourable specific H-reflex facilitation prior to a movement, improving ability for stimulus identification and response selection (Eichenberger and Rüegg, 1984). The specific facilitation of the H-reflex, which originates in the motor cortex, is caused by presynaptic inhibition at 1a terminals or interneuronal activation interspersed in the polysynaptic components of the H-reflex. It may be possible that improvement in reaction time with HRV BFB from earlier studies in athletes (Paul et al., 2012; Rusciano et al., 2017) may have resulted from low resting neural excitability, leading to advantageous specific H-reflex facilitation. In addition, low resting neural excitability has been related to low anxiety and higher jump performance (Bagheri et al., 2018). Paul and Garg (2012) demonstrated reduced anxiety alongside with increased efficiency in dribbling, passing, and shooting after HRV BFB among basketball athletes. The enhancement in resting neural excitability with HRV BFB may also play a critical role to improving the gross motor performance in athletes (Bagheri et al., 2018; Paul and Garg, 2012). Future studies are needed to determine the exact role of H-reflex modulation via HRV BFB in voluntary neuromuscular contraction and performance.

The motoneuron excitability from HRV BFB (Study Three) may be modulated by muscle sympathetic outflow via muscle sympathetic nervous activity (Kamibayashi et al., 2009). The MSNA is a neuromuscular marker that reflects baroreflex function (Benarroch, 2008). Examining MSNA under HRV BFB may provide novel information on muscle sympathetic outflow response with RF. Therefore, study four (Chapter Five) identified the MSNA under ten-minute RF, RF + 1 and SB in healthy males. Results revealed lower MSNA burst and incidence in both RF and RF + 1 compared with SB. Moreover, burst occurrence is increased by an average of 68% at $\pi/2$ in RF and RF + 1 with both depicting more silence periods. The novel findings in MSNA under RF highlight the beneficial effects of breathing at RF in reducing MSNA. The reduced MSNA with HRV BFB may be an important factor in increasing the capacity for vasodilation and maximal vascular conductance in continuous muscular activity in athletes (Joyner and Casey, 2015; Fairfax et al., 2013; Snell et al., 1987). In relation to this, lowering MSNA with HRV BFB may contribute to regulation of resting blood pressure among athletes, assisting beneficial adaptations in maximal oxygen consumption and ventilatory anaerobic threshold (Choudhary et al., 2016; Mazic et al., 2014). More investigations involving resting MSNA after HRV BFB, and MSNA during voluntary muscle contraction are needed to determine the function of autonomic regulation via MSNA on performance.

The enhancement in MSNA (Study Four) and reduction in RR (Study Two) with HRV BFB may arise from efficient afferent information, as depicted by HEP (Gray et al., 2007; Incognito et al., 2019). Additionally, the effects of HRV BFB on neural excitability (Study Three) and performance outcomes (Study One) may be triggered by HEP response via the central command (Boulton et al., 2016; Williamson, 2005).

The HEP has been associated with somatosensory detection (Al et al., 2020), visual perception (Park and Thayer, 2014), self-consciousness (Park et al., 2018), and various mental processes critical for decision-making (de Lange et al., 2018; Friston, 2009; Park and Blanke, 2019). There is limited literature examining the vagal afferent response under HRV BFB. Additional studies may provide crucial evidence on HEP with HRV BFB practice. Therefore, study five (Chapter Six) examined the HEP under HRV BFB. Specifically, HEP was compared in HRV BFB (ten-minute; single-session) and CON (spontaneous breathing). Issues pertaining to cardio-artefact were addressed using robust techniques to deriving HEP. Results revealed significant differences occurring at fronto-parietal (Fp2), frontal (F3, Fz) central (C4), parietal (P7, Pz), occipital (O1, Oz, O2), and parieto-occipital (PO1, PO2) between HRV BFB and CON. Specifically, HRV BFB showed higher HEP at Fp2, F3, Fz, C4, and P7 than CON. Higher HEP exhibited by HRV BFB at these areas depict efficient transmission of afferent signals (Montoya et al., 1993; Schandry and Montoya, 1996). Other results showed lower HEP at P7, Pz, O1, Oz, O2, PO1, Pz, and PO2 in HRV BFB compared to CON. The presence of lower HEP topography with HRV BFB compared to CON suggests processing of heartbeats via the visceral afferent pathway (Montoya et al., 1993; Schandry and Weitkunat, 1990). The findings in this study emphasise HRV BFB as a non-invasive scheme for vagal afferent enhancement. The improvement in vagal afferents enhance regulatory brain networks that may be crucial to performance (Mather and Thayer, 2018; Silvani et al., 2015). However, further research is needed to establish the relationship of HEP and performance outcomes within HRV BFB context.

The three studies (Studies Three, Four, and Five) carried out in this thesis led to proposing additional physiological pathways under HRV BFB (Figure 7.1) (Gevirtz, 2013; Lehrer and Gevirtz, 2014; Lehrer et al., 2003; Fonoberova et al., 2014; Pagaduan et al., 2021; Pagaduan et al., 2019; Palmieri et al., 2004; Prinsloo et al., 2014; Russo et al., 2017) The HRV BFB increases the activity of vagal afferents (Study 5), which are autonomic fibers innervating the brainstem at the nucleus tractus solitarius in the medulla (Prinsloo et al., 2014; Shaffer et al., 2014; Zoccal et al., 2014). This leads to the stimulation of the cardio-inhibitory area, increasing vagal outflow, reducing heart rate, and increasing HRV (Lehrer et al., 2003; Fonoberova et al., 2014; Pagaduan et al., 2019; Prinsloo et al., 2014). HRV BFB also reduces respiration rate (Lehrer et al., 2003; Lehrer et al., 2004), decreasing intra-thoracic pressure (Russo et al., 2017). The reduction in intra-thoracic pressure with HRV BFB may increase stroke volume and cardiac output (Fonoberova et al., 2014; Lehrer and Gevirtz, 2014; Russo et al., 2017), reducing blood pressure (Lehrer et al., 2003; Pagaduan et al., 2019). Improvement in vagal afferents may also result to inhibitory sympathetic outflow from the vasomotor center in the medulla to vasculature. This is represented by reduction in muscle sympathetic outflow (Study 5) (Pagaduan et al., 2019). The adaptive responses modulated in the medulla with HRV BFB may affect the neuromuscular pathways via the central command (Smith et al., 2017; Skrelov et. al., 2019; Valenza et al., 2019; Williamson, 2015). Specifically, HRV BFB may influence reduction in resting motoneural excitability (Study 3) (Pagaduan et al., 2021; Palmieri et al., 2004). Aside from improvement in vagal afferents, the aforementioned physiological changes with HRV BFB may also be triggered by enhancement of baroreflex sensitivity (Lehrer et al., 2003; Pagaduan et al., 2019; Lehrer and Gevirtz, 2014). Thus, the findings in this thesis provide additional information on physiological pathways affected by HRV BFB.

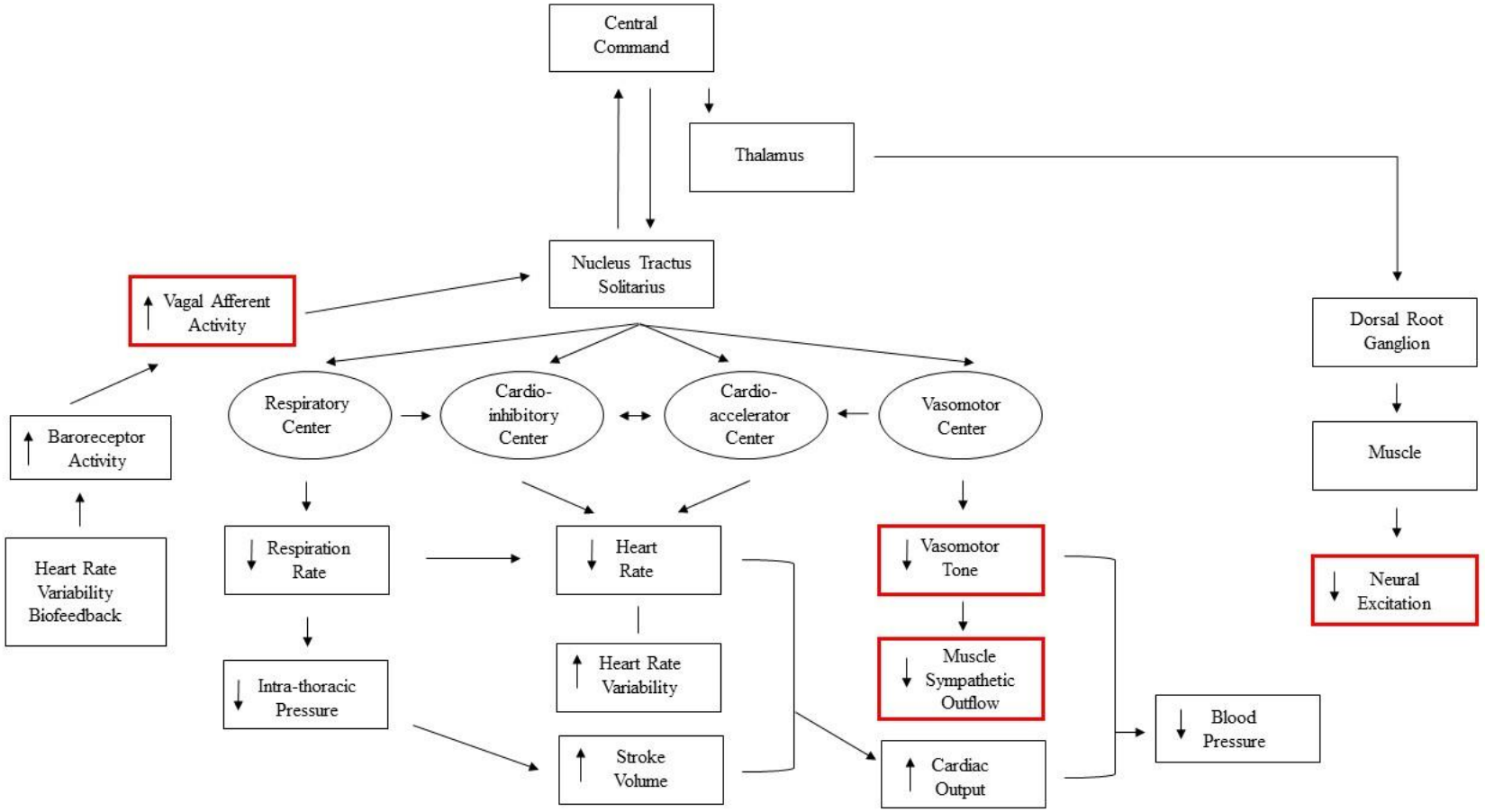

Figure 7.1. Physiological pathways in HRV BFB. The highlighted boxes indicate the pathways arising from this thesis. Study 3 demonstrated reduced neural excitation after HRV BFB. Study 4 showed decreased muscle sympathetic outflow with HRV BFB. Study 5 exhibited increased vagal afferent activity reflecting cardiovascular regulation under HRV BFB.

To extend the results of this thesis in applied settings, an example is provided. An athlete might participate in HRV BFB practice (ten-minute session/day for six consecutive days a week at convenience) during the pre-season, lasting six weeks, using a portable HRV BFB tool. Prior to HRV BFB, a pretest of physiological (H-reflex, HEP, HRV, RR, MSNA) and performance measures (leg power, aerobic capacity, reaction time) would be administered. Testing of RF, utilised in HRV BFB, would also be conducted. The physiological and performance tests, excluding RF, would be replicated after six weeks to establish change in physiological and performance outcomes with HRV BFB. However, the measurement of H-reflex, HEP, and MSNA requires technical expertise, and testing equipment for these measurements is commonly only available in research institutions. In a practical sense, choosing H-reflex or HEP or MSNA, together with RR and HRV is the ideal scenario. Also, daily monitoring of three-minute HRV (Chen et al., 2020; Mueller et al., 2020) and subjective wellness (McFarland and Bird, 2014) within an hour after waking up, would be executed to determine the trends in autonomic regulation and wellness respectively. One of four outcomes is expected after a six-week HRV BFB (Table 7.1). After six weeks, enhancement in performance could be facilitated by autonomic regulation via HRV BFB if increased HEP and HRV, whilst decreases in RR, resting motoneuron excitability, and MSNA would be observed (Outcome A). This would be supported by increased wellness and weekly trends in HRV. Alternatively, if performance remains the same whilst exhibiting similar physiological and subjective results previously mentioned (Outcome B), autonomic regulation might be achieved, but not of a sufficient level to enhance performance. No improvement in performance with HRV BFB may be due to lack of physical/tactical/technical training stimulus. When performance is diminished or remains the same after HRV BFB practice (Outcomes C

and D), complemented by decreased HRV and HEP, whilst increased RR, HEP, and MSNA, autonomic dysfunction may have occurred. This can be confirmed by reduction in wellness scores. Most likely, the autonomic dysfunction would be a result of fatigue from physical/tactical/technical training.

Table 7.1. Potential physiological, performance, and wellness adaptations to HRV BFB training.

	A	B	C	D
Heart rate variability	↑	↑	↓	↓
Respiration rate	↓	↓	↑	↑
Heartbeat evoked potential	↑	↑	↓	↓
Motoneuron excitability	↓	↓	↑	↑
Muscle sympathetic nervous activity	↓	↓	↑	↑
Wellness	↑	↑	↓	↓
Performance	↑	↔	↓	↔

*↑ - increase; ↓ - decrease; ↔ - no change; *Outcome A: performance enhancement with adaptive physiological and wellness responses; Outcome B: no improvement in performance with adaptive physiological and wellness changes; Outcome C: decreased performance with maladaptive physiological and wellness responses; Outcome D: no improvement in performance with maladaptive physiological and wellness changes.*

7.2 Limitations

There are a number of limitations in this thesis. Firstly, the experimental studies were conducted in acute conditions. Longer-duration HRV BFB (> 4 weeks) may enhance the value of HRV BFB in performance settings. Chronic HRV BFB facilitates intrinsic baroreflex, wherein autonomic regulation is no longer dependent on breathing rate (Lehrer et al., 2003). Such mechanisms may be crucial for detecting adaptive neuromuscular response. Secondly, the vigorous procedures employed in H-reflex and MSNA testing, alongside other challenges encountered (e.g. laboratory availability, participant recruitment) resulted in a small sample study population using healthy individuals in all three studies (Studies 3, 4, and 5). Increasing the sample size using H-reflex and MSNA parameters, in addition to including well-trained athletes in future HRV BFB studies will increase the power of the study design, and may provide greater certainty of findings connecting HRV BFB and performance. Lastly, studies 4 and 5

employed measures related to autonomic regulation. Additional markers for voluntary muscle contraction and performance in the future will add importance to the applicability of HRV BFB.

7.3 Future Studies Arising from Thesis Results

Future research to gain greater insight into the translation of these findings will be best served by including the aforementioned physiological measures whilst HRV BFB in performance settings. Adding these measures in athletic populations may aid in the confidence of using HRV BFB as part of training regimen. Alternatively, assessment of these physiological indices in clinical populations may help promote HRV BFB as a complementary intervention for health improvement. Further, concurrent physiological monitoring and completion of task demands can help establish trends in vagal reactivity with HRV BFB (Laborde et al., 2018). Lastly, analysing phase oscillations of physiological indices under HRV BFB may identify the impact of oscillatory properties on physiological mechanisms (Lehrer et al., 2020).

7.4 Concluding Comments Pertaining to Practical Application

This thesis investigated neuromuscular, cardiovascular, and cortical responses that may link HRV BFB and performance. In addition, the findings in this work contribute to existing central and peripheral mechanisms in HRV BFB, providing further evidence for considering HRV BFB as a complementary scheme in performance settings. The results support the ten-minute, single-session HRV BFB as a non-invasive scheme for promoting short-term autonomic regulation.

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Appendix A, i: Participant Information Sheet (Study Three)

PARTICIPANT INFORMATION SHEET

A Pilot Study into the Acute Effect of Heart Rate Variability Biofeedback (HRV BFB) on H-Reflex

1. Invitation

You are invited to participate in a pilot study examining the acute effect of heart rate variability biofeedback (HRV BFB) on autonomic activity, reflex mechanism, and muscle contraction.

This study is being conducted by:

- Dr. James Fell, School of Health Sciences, University of Tasmania
- Dr. Sam Wu, School of Health Sciences, Swinburne University
- Jeffrey Pagaduan, School of Health Sciences, University of Tasmania;
- Dr. Yung Shung Chen, Department of Health and Exercise Sciences, University of Taipei

2. What is the purpose of this study?

This pilot study aims to examine the acute effect of HRV BFB on heart rate variability (HRV), H-reflex, and maximal voluntary contraction (MVC) of plantar flexors.

Why have I been invited to participate?

You are eligible to take part in this study because you are an individual meeting the following inclusion criteria:

i) Male aged between 18 to 55 years old

ii) Physically active or preferably competing at a high level of sport

iii) No reported lower body injury 2 months prior to participation

iv) No known clinical disease (such as heart disease requiring a pacemaker)

v) Not currently taking any prescribed medication, and no history of smoking/substance abuse

3. What is required of the participant?

You are requested to visit the exercise science laboratory for three 40-60 minute sessions each session one day apart. In the first session, your height and weight will be measured. Subsequently, you will undergo a seated 10-minute paced breathing test with various frequencies (6.5 – 4.5 breaths per minute), each 2 minutes in duration to determine your prescribed breathing frequency. This test is administered with surface sensors attached to your middle finger and abdominal region to monitor heart rate and breathing rate. Thereafter, you will perform three seated 3-second maximal voluntary contractions of your lower calf muscle, with a rest interval of 1 minute in between trials. A surface electrode will be placed on the lower calf muscle of your right leg to examine muscle activity.

On day 2, your baseline HRV and H-reflex will be determined after arrival at the testing facility. Prior to that, surface electrodes will be placed on specific areas (lower calf muscle, front, and back part of your knee cap) of your right leg. Then, heart rate and respiration sensors from Day 1 will also be attached. Thereafter, HRV testing will ensue with you seated, relaxed, and eyes closed for 5 minutes. This will be followed by H-reflex where you will experience incremental electrical stimulation behind your knee cap until a maximum muscular reflex is elicited by your calf muscle. H-reflex testing will be succeeded by a randomly assigned activity of either 10-minute HRV BFB or 10-minute normal breathing. HRV BFB involves breathing at your pre-determined specific breathing frequency with inhalation and exhalation guided by visual feedback. The control condition involves normal breathing. Subsequently, 1-minute closed eyes HRV, H-reflex, and MVC tests will be repeated. Day 3 will include all activities performed on day 2, with the exception that you will undergo the activity (HRV BFB or normal breathing) that you did not perform during day 2.

4. Are there any possible benefits from participation in this study?

This pilot study will provide information on your current heart rate variability, H-reflex, and maximal voluntary contraction of plantar flexors. The findings will extend our knowledge of the mechanisms of HRV BFB training.

5. Are there any possible risks from participation in this study?

This study involves temporary low risk for injury. The risk is no more than that of a normal physical activity such as a game of tennis and that any injuries are likely to be at most minor and resolved in one to two days. H-reflex testing due to electrical stimulation could potentially cause slight discomfort, mild electric-shock like sensation, or in rare occasions, low-degree electric-shock burn injury (e.g. similar to moderate sunburn). To minimise this, stimulation will be done progressively. You may also feel slight discomfort during maximal voluntary contraction testing and there is a small risk of a muscle strain. At all times during testing sessions, a certified

first aider will be present. In any occasion of injury, the first aid trained person will manage the injury at no expense to the participant.

What if I change my mind during or after the study?

You are free to withdraw at any time during the study, and can do so without providing an explanation. However, if you choose to withdraw after the study, it will not be possible to remove your data, as it will be stored anonymously.

6. What will happen to the information when this study is over?

Data collected during the experiment will be placed in computer files as it is recorded throughout the data collection process, where it will be used for data analysis following completion of data collection.

Data will be stored on a password protected hard drive, where the password is only known to immediate researchers, and will be located in a password protected spreadsheet. Hard copies of the data will be stored in a locked filing cabinet which only the experimental researchers will have access to.

Data will be stored in this manner for a total of 5 years from publication, and at this point the data will be permanently erased from the hard drive of the researcher with the data.

7. How will the results of the study be published?

All data in this study will be anonymous. Data from this study will be discussed and may be published. If you wish to be notified on the results of this study, please feel free to contact us.

8. What if I have questions about this study?

If you have any queries, concerns or issues with this study, please feel free to contact us:

- Jeffrey Pagaduan: jeffrey.pagaduan@utas.edu.au
- Yung Shung Chen, PhD: yschen@utaipei.edu.tw
- James Fell, PhD: james.fell@utas.edu.au
- Sam Wu, PhD: sswu@swin.edu.au

This study has been approved by the Tasmanian Health and Medical Human Research Ethics Committee. If you have concerns of complaints about the conduct of this study you should contact the Executive Officer of the HREC (Tasmania)

Network on (03) 6226 6254 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote H0016508.

Thank you for your time

A Pilot Study into the Acute Effect of Heart Rate Variability Biofeedback (HRV BFB) on H-Reflex (H0016508)

Appendix A, ii: Consent Form (Study Three)

FACULTY OF HEALTH

CONSENT FORM

A Pilot Study into the Acute Effect of Heart Rate Variability Biofeedback (HRV BFB) on H-Reflex

1. I agree to take part in this research study.
2. I have read and understood the Information Sheet for this study.
3. The nature and possible effects of the study have been explained to me.
4. I understand that the study involves:

    - Attachment of heart rate and respiratory sensors for heart rate variability (HRV) and HRV BFB
    - Resonant frequency (RF) testing
    - 10-minute intervention (HRV BFB) and control (quiet seating) sessions
    - Spinal reflex (H-reflex) testing
    - Maximal voluntary contraction testing of plantar flexors

5. I understand that participation involves the low-risks (possible discomfort/pain/injury during H-reflex/MVC testing) associated with undertaking the tests.
6. I understand that I can withdraw at any test (RF/HRV/H-reflex/MVC) or intervention/control (HRV BFB/normal breathing). However, if I choose to withdraw after the study, it will not be possible for my data to excluded in the analysis.
7. I understand that all research data will be securely stored on a password-secured database for five years from the publication of the study results, and will then be destroyed unless I give permission for my data to be stored in an archive.

    I agree to have my study data archived.

    Yes ☐ No ☐
8. Any questions that I have asked have been answered to my satisfaction.
9. I understand that the researchers will maintain confidentiality and that any information I supply to the researchers will be used only for the purposes of the research.
10. I understand that the results of the study will be published so that I cannot be identified as a participant.

A Pilot Study into the Acute Effect of Heart Rate Variability Biofeedback (HRV BFB) on H-Reflex (H0016508)

11. I understand that my participation is voluntary and that I may withdraw at any time without any effect.
12. I understand that I will not be able to withdraw my data after completing the study, as it will be stored anonymously.

Participant's name:

_____

Participant's signature:

_____

Date: _____

Statement by Investigator

☐ I have explained the project and the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation.

If the Investigator has not had an opportunity to talk to participants prior to them participating, the following must be ticked.

☐ The participant has received the Information Sheet where my details have been provided so participants have had the opportunity to contact me prior to consenting to participate in this project.

Investigator's name:

Jeffrey Pagaduan

Investigator's signature:

Date

A Pilot Study into the Acute Effect of Heart Rate Variability Biofeedback (HRV BFB) on H-Reflex (H0016508)

Appendix B, i: Consent Information Sheet (Studies Four and Five)

Consent Information Statement

Project Title

Heart Rate Variability Biofeedback and Muscle Sympathetic Nervous Activity in Apparently Healthy Adults: A Pilot Study

Investigators and Other Project Personnel

Dr Sam Wu – Chief investigator A
Dr Elisabeth Lambert
Dr Gavin Lambert
Dr David White
Dr Clare MacMahon
Jeffrey Pagaduan, PhD (candidate)
Dr James Fell
Leighton James - Student Investigator

Introduction to Project and Invitation to Participate

You are invited to participate in a pilot study examining the acute and long term effects of a paced breathing strategy called heart rate variability biofeedback (HRV BFB) on activities of your heart, brain, lower body muscle nerve, as well as cognitive ability, subjective mood and stress. In HRV BFB, you will be asked to breathe following a visually guided pattern on a mobile application. You are eligible if you are between 18-50 years old, have no known illnesses, no reported lower body injury 2 months prior to participation, no known clinical disease (such as heart disease requiring a pacemaker), not currently taking any prescribed medication, and no history of smoking/substance abuse.

What this project is about and why it is being undertaken

This study will help identify the short and long-term effects of HRV BFB, a paced breathing intervention aided by visual feedback, on psychophysiological indices, cognition, and subjective mood and stress.

Project and researcher interests

This project will aid us in better understanding the mechanisms operating under HRV BFB. Student researchers may be involved during data collection to help satisfy their course requirements.

What participation will involve

We will ask that you attend four 2 hour sessions at the Clinical Research Laboratory of the Iverson Health Innovation Research Institute at Swinburne University of Technology. The first two and last two sessions are separated by 6 weeks. The same tasks will be completed in the first two and last two sessions. These are detailed below:

1st day

- Measurement of height and weight
- Questionnaires for stress and mood
- Computer based reaction time task
- 10 minute paced breathing test

24 h monitoring using wearable devices between 1st and 2nd day

2nd day (same time of day as 1st day)

- Heart rate sensors, respiratory sensor, brain activity electrodes, lower body muscle nerve electrode will be attached onto you (nerve electrode is a micro electrode; you may feel slight discomfort or nothing at all)
- Normal breathing for 10 minutes
- Paced or normal breathing for 10 minutes (paced breathing will be guided a visual pacer with heart rate and respiration displayed on a laptop monitor).

- Normal breathing for 10 minutes.

Sessions three and session four will commence after 6 weeks following the same procedures from session 1 and 2, excluding the paced breathing test.

For the 6 weeks, you will be assigned to a paced or normal breathing group. Within this period, you are not allowed to undergo any form of psychological intervention/counselling or any exercise that involves paced breathing (e.g. yoga, pilates). In the paced breathing group, you will be asked to perform paced breathing for 10 minutes per day for 6 days a week (preferably at any time of day at a location of your convenience). The first paced breathing session will start on a Monday after the second testing session. A monitoring sheet will be handed out for you to log the time you administered the paced breathing.

Participant rights and interests – Risks & Benefits/Contingencies/Back-up Support
The study may provide you with an information of your current health status based on heart signals, brain activity, muscle nerve activity, and subjective mood and stress. This study involves a low risk of injury. Although unlikely, slight discomfort (lightheadedness) may be experienced during paced breathing but this is temporary and will cease right after the test. You will be seated or lying supine during tests. Also, the investigators will familiarise you with the proper breathing technique to avoid any incidence of discomfort. For muscle nerve activity monitoring, you might experience a temporary ant-bite pain with the attachment of a probe. An expert tester will measure this parameter that will ensure the least pain (if any) as possible. At all times during testing there will be researchers on site who have current first aid training. Your responses and performance will be kept strictly confidential and also not associated with any information that can identify you.

As you will be lying supine (facing up) or seated, the risk of traumatic injuries will be minimal. In the event of any injury, appropriate first aid will be applied. If you wish to seek further medical advice, we advise you contact Swinburne University Health Service:

Phone: +61 3 9214 8483
Level 4, George Swinburne Building: 4/34 Wakefield Street, Hawthorn

Monday	8:30am–7pm
Tuesday	8:30am–7pm
Wednesday	8:30am–5pm
Thursday	8:30am–7pm
Friday	8:30am–5pm

Participant rights and interests
Your participation is voluntary. You are free to withdraw at any time, and can do so without providing an explanation. However, if you choose to withdrawal after the study, it will not be possible to remove your data, as it will be stored anonymously. If you are a Swinburne student, your decision to participate or not in the project will have no bearing on your results or treatment. You will receive AUD100 in full upon completion of the study as a reimbursement for your time. Your consent will be sought with a signed consent form. We ask that you inform us if medication is taken at any time during the course of the study. Only sign after you have understood what is required of you, and have had all questions satisfactorily answered.

Any information obtained in connection with this project and that can identify you will remain confidential. Your data will not have your name associated with it. Forms with identifying information will be stored separately from other study information in a locked filing cabinet in the researcher's office or in a password protected electronic file. A unique code will be used on all forms and data collected from you, and not with your name or any other identifying information. These data will be stored on a password protected computer. Only the investigators will have access to the data. Sharing of data with investigators outside of Swinburne will occur only in a coded, anonymous way and no identifying or personal information will be shared.

None of the information provided will be made public in any form that would reveal a participant's identity to an outside party, thus all participants will remain anonymous. The results of this project will be

discussed at scientific conferences in a pooled format, thus your identity and personal information will not be disclosed, and information will be provided in such a way that you cannot be identified. This data may be used for future research.

All data will be de-identified and stored on a password protected secure Swinburne server for a period of 5 years after publication, after which stage it will be destroyed. In accordance with the Freedom of Information Act 1982 (Vic), you have the right to access and to request correction of information held about you by Swinburne University.

Research output
Participants' names will not be used in any reports and/or scientific journals. The intention is that this research will be presented at research conferences and in peer-reviewed publications. Personal identity will not be revealed in any publication. If you wish to be notified on the results of this study, please feel free to contact us.

Further information about the project – who to contact
If you would like further information about the project, please do not hesitate to contact:

Dr Sam Wu
Swinburne University of Technology
SPW221, Hawthorn
Victoria, 3122
Contact Number: 92145566
Email: sswu@swin.edu.au

Concerns/complaints about the project – who to contact:

This project has been approved by or on behalf of Swinburne's Human Research Ethics Committee (SUHREC) in line with the *National Statement on Ethical Conduct in Human Research*. If you have any concerns or complaints about the conduct of this project, you can contact:

Research Ethics Officer, Swinburne Research (H68),
Swinburne University of Technology, P O Box 218, HAWTHORN VIC 3122 Australia.
Tel (03) 9214 3845 or +61 3 9214 3845 or resethics@swin.edu.au

Appendix B, ii: Consent Form (Study Four and Five)

Consent Form

Swinburne University of Technology

Project Title: Heart Rate Variability Biofeedback and Muscle Sympathetic Nervous Activity in Apparently Healthy Adults: A Pilot Study

Principal Investigator(s):
Dr Sam Wu
Dr Elisabeth Lambert
Dr Gavin Lambert
Dr David White
Dr Clare MacMahon
Jeffrey Pagaduan (PhD candidate)
Dr James Fell
Leighton James – Student Investigator

1. I consent to participate in the project named above that will last for up to 8 weeks. I have been provided a copy of the project consent information statement to which this consent form relates and any questions I have asked have been answered to my satisfaction.

2. ***In relation to this project, please circle your response to the following:***

▪ I agree to take part in this research study (8 weeks)	**Yes**	**No**
▪ I agree to visit the Clinical Research Laboratory of the Iverson Health Innovation Research Institute at Swinburne University of Technology for four testing sessions at 2 hours per session. The first two and last two sessions are separated by 6 weeks	**Yes**	**No**
▪ I agree to complete questionnaires asking me about my subjective mood and stress (first and third testing session)	**Yes**	**No**
▪ I agree to wear physiological monitors for 24 hours for two days (after first and third testing sessions)	**Yes**	**No**
▪ I agree to be measured for height and weight (first and third testing sessions)	**Yes**	**No**
▪ I agree to be examined for reaction time (first and third testing sessions)	**Yes**	**No**
▪ **I agree to undergo a 6-week paced or normal breathing intervention (paced involves an assigned breathing rate for 10 minutes/day for 6 times a week for 6 weeks at same time of day and location of convenience)**	**Yes**	**No**
▪ I agree to wear heart rate and respiratory sensors succeeded by a 10-minute paced breathing test (first testing session)	**Yes**	**No**
▪ I agree to wear heart, brain, and lower body muscle activity sensors for 30 minutes. The first and last 10 minutes will be monitored under normal breathing conditions. The second 10 minutes will be an assigned paced breathing or normal breathing (second and fourth testing sessions)	**Yes**	**No**
▪ I agree not to participate in any form of psychological intervention/counselling or any exercise with paced breathing (e.g yoga, pilates)	**Yes**	**No**
▪ I agree to report my paced breathing task when requested	**Yes**	**No**
▪ I understand that participation involves risks associated with this study.		

These may include lightheadedness during paced breathing and/or ant-bite pain during muscle sympathetic nerve testing. **Yes** **No**

3. I acknowledge that:
   (a) my participation is voluntary and that I am free to withdraw from the project at any time without explanation;
   (b) the Swinburne project is for the purpose of research and not for profit;
   (c) any identifiable information about me which is gathered in the course of and as the result of my participating in this project will be (i) collected and retained for the purpose of this project and (ii) accessed and analysed by the researcher(s) for the purpose of conducting this project;
   (d) I understand the length of time researcher/s will have access to this information;
   (e) my anonymity is preserved and I will not be identified in publications or otherwise without my express written consent.

By signing this document I agree to participate in this project.

Name of Participant: .....

Signature & Date: .....

Appendix C, i: Manuscript Publication (Study Two)

Journal of Human Kinetics volume 73/2020, 103-114 DOI: 10.2478/hukin-2020-0004 103
Section II - Exercise Physiology & Sports Medicine

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Can Heart Rate Variability Biofeedback Improve Athletic Performance? A Systematic Review

by
Jeffrey Cayaban Pagaduan[1], Yung-Sheng Chen[2], James William Fell[1], Sam Shi Xuan Wu[3]

This systematic review was conducted to evaluate the effect of heart rate variability biofeedback (HRV BFB) on performance of athletes. Six electronic databases (Springerlink, SportDiscus, Web of Science, PROQUEST Academic Research Library, Google Scholar, and ScienceDirect) and article references were searched. Eligibility criteria were: 1. experimental studies involving athletes randomly allocated among groups (randomized control trial); 2. availability of HRV BFB as a treatment compared to a control condition (CON) that involves regular sport/dance training, a placebo (PLA) or other methods of BFB; 3. performance-related variables such as a dependent index; and, 4. peer-reviewed articles written in English. Out of 660 articles, six studies were included in the systematic review which involved 187 athletes (females: n = 89; males n = 98). Six studies compared HRV BFB with a CON, three studies compared HRV BFB with a PLA, and two studies differentiated HRV BFB with other methods of BFB. Findings support HRV BFB as a potential intervention to improve fine and gross motor function in athletes.

Key words: *heart rate variability, biofeedback, athletes, performance, resonant frequency breathing.*

Introduction

Biofeedback (BFB) as a performance enhancement strategy in athletes has been receiving notable attention among sports practitioners (Brown and Fletcher, 2016; Keilani et al., 2016; Pusenjak et al., 2015). BFB provides real-time understandable physiological information to an individual that enhances psychophysiological and affective indices (Galloway and Lane, 2005; Wilson et al., 2014). Among various approaches to BFB there is a breathing strategy known as heart rate variability biofeedback (HRV BFB) (Lehrer et al., 2000). HRV BFB is executed by paced breathing at a specific frequency, known as resonance frequency (RF), that elicits maximal heart rate oscillations. RF usually ranges from 4 to 6.5 breaths/min (Lehrer et al., 2000). In addition, RF exhibits a 0-degree phase shift between the heart rate and respiration as well as a 180-degree phase shift between the heart rate and blood pressure (Vaschillo et al., 2006). The physiological phenomena with HRV BFB are believed to improve autonomic function from baroreflex gain and vagal activation (Gevirtz, 2013; Lehrer et al., 2000; Lehrer and Gevirtz, 2014; Prinsloo et al., 2014; Vaschillo et al., 2006).

In an athletic setting, the first documented HRV BFB intervention was administered on wrestlers who exhibited reduced muscle relaxation disorders and an improved rate of relaxation (Vaschillo et al., 1998). A recent review conducted by Jiménez Morgan and Molina-Mora (2017) synthesized the effect of HRV BFB on athletic performance and found that 86% (n = 6) of studies reviewed performance enhancement via improvement in psychophysiological variables.

[1] - *College of Health and Medicine, School of Health Sciences, University of Tasmania, Launceston, Tasmania, Australia.*
[2] - *Department of Exercise and Health Sciences, University of Taipei, Taipei, Taiwan.*
[3] - *Faculty of Health, Arts, and Design, School of Health Sciences, Department of Health and Medical Sciences, Swinburne University, Melbourne, Australia.*

Authors submitted their contribution to the article to the editorial board.
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One notable strength of the previous study was the employment of systematic procedures. On the other hand, the review included different quantitative study designs (two case reports, one quasi-experimental study, four experimental studies) which may have contributed to bias in findings. Thus, the purpose of this study was to conduct a systematic review of studies only employing randomized trials on the effect of HRV BFB on psychophysiology and exercise performance of athletes.

Methods

Search Strategy

The search was conducted between July 1st and August 31st 2017 using the search term "heart rate variability biofeedback" and (athletes or athletic population or sport or performance or sport performance) in electronic databases: Springerlink, SportDiscus, Web of Science, PROQUEST Academic Research Library, Google Scholar, and ScienceDirect (Jiménez Morgan and Molina-Mora, 2017). In addition, a manual reference search was administered on the records found. PRISMA guidelines were used for reporting.

Eligibility Criteria

To be included in the systematic review, the studies had to meet the following criteria: 1. experimental studies involving athletes randomly allocated among groups (randomized control trial); 2. availability of HRV BFB as a treatment compared with a control condition (CON)/ a placebo (PLA)/other BFB; 3. any performance-related variable as a dependent index; and, 4. peer-reviewed articles written in English.

Study Selection

Literature search and selection of studies were completed by a single investigator (JP). All studies were coded and organized in an Excel spreadsheet. The second investigator (YSC) evaluated data extraction. For each article included in the systematic review, the following data were encoded: author/s and year of publication, sample size information, intervention, measured performance variable/s, and results. These studies were also assessed for 'risk of bias' using an eight-point scale from Consolidated Standards of Reporting Trials (CONSORT) statement wherein each item is answerable by 0 (absently or inadequately described) or 1 (explicitly described and present). A study with a score of 0-2 is regarded as having a high risk of bias, 3-5 with medium risk of bias, and 6-8 considered as having low risk of bias (CONSORT, 2001). A consensus was reached between JP and YSC for any disagreement presented in data extraction and CONSORT output (Table 1).

Statistical Analysis

Independent T-tests using pre-post mean differences and standard deviation of variables from HRV BFB and groups' comparison were administered. Then, corresponding Cohen's d as effect size (ES) with 95% confidence limits were calculated (Cohen, 1988; Lee, 2016; Morris, 2008). Missing pre-post mean differences and SD in studies were computed based on previous methods (Gu et al., 2015; Zu et al., 2013). ES was interpreted as small (d = 0.20), medium (d = 0.50), or large (d = 0.80) (Cohen, 1988). Statistical power calculation from post hoc was also conducted using G*Power ver 3.1 (Faul et al., 2007). Indices with alpha = 0.00 were set at alpha = 0.01.

Results

Figure 1 shows the flowchart and selection process of the studies. The database search indicated 656 potential articles with an additional 4 identified articles from reference lists. After removal of duplicates (n = 90), 570 articles underwent initial screening based on the article title/abstract. This process led to excluding 557 articles after failing to meet all the items in the inclusion criteria. Further assessment for eligibility of 13 full articles led to removal of seven studies leaving six articles included in the analysis.

Participants

The six studies included a total of 187 (females: n = 89; males n = 98) athletes including: 60 (females: n = 27; males: n = 33) university, state, and national basketball athletes; 24 (females: n = 12; males: n = 12) university, state, and national standard long distance runners; 20 male professional soccer players; and, 84 (females: n = 51; males: n = 33) university student dancers.

Experimental protocols

Of the six studies included, all studies compared HRV BFB and a control condition (CON) wherein a CON involved regular dance/sport training. Three studies also compared

HRV BFB and a placebo (PLA) with two studies using motivational video (Paul and Garg, 2012; Paul et al., 2012) and one using choreology (Gruzelier et al., 2014). Two studies compared HRV BFB with the alternative BFB intervention neurofeedback (NFB) which utilized electronenchaphalogram (EEG) signals (Gruzelier et al., 2014; Raymond et al., 2005).

Interventions

The studies applied a variety of HRV BFB protocols. Two studies utilized 10 consecutive days of HRV BFB administered at 20-min/day (Paul and Garg, 2012; Paul et al., 2012). Other studies followed protocols for HRV BFB suggested by Lehrer et al. (2000) using 10 formal sessions with two 20-min daily sessions at convenience (Choudhary, et al., 2016; Gruzelier et al., 2014; Raymond et al., 2005). Two studies following a protocol set by Lehrer et al. (2000) lasted for 10 weeks, while the study of Raymond et al. (2005) was carried out for 4 weeks.

Rusciano et al. (2017) administered HRV BFB for fifteen 30-min sessions (twice a week). The first three sessions followed the protocol set by Lehrer et al. (2000). Then sessions 4 – 9 combined HRV BFB with other biofeedback schemes including a skin conductance level (SCL), electromyography (EMG) of masseter and a posterior cervical region and hand temperature. The remaining sessions (10^{th} – 15^{th}) integrated HRV BFB with math tasks, hyperventilation, and winning and losing video games.

Instruments

Different HRV BFB equipment was utilized for HRV BFB. Two studies utilized freeze-framer from Boulder Creek, California, US (Gruzelier et al., 2015; Raymond et al., 2005). Three studies used Biograph Pro Comp Infinity 5.0 from Thought Technology Ltd., Canada (Choudhary et al., 2016; Paul and Garg, 2012; Paul et al., 2012). Rusciano et al. (2017) used HRV BFB with Nexus 10 Mark II hardware and Biotrace1 commercial software from Mind Media, Herten, Netherlands.

Performance Evaluation

Studies included in the review assessed various performance related variables. Changes in performance (%) and effect sizes with 95% confidence limits of HRV BFB and groups' comparison, as well as study power are displayed in Table 2.

Choudhary et al. (2016) determined 5-km performance of track athletes. The study of Paul et al. (2012) assessed performance using movement and choice reaction time (RT) along with a 3-min shooting score. Paul and Garg (2012) measured weaving in and around cones whilst dribbling for 30 s, 30-s passing with specified targets on the wall, and 3-min shooting at marked perimeters. Rusciano et al. (2017) established visual tracking RT of professional soccer players with and without a target. Accuracy under congruent and incongruent stimuli using a Stroop task was also determined. Further, injury prevention was identified from attendance records (days present and absent or differential training days – the number of days on which athletes followed personalized training due to recent injuries). In the study of Gruzelier et al. (2014), four dance experts rated dance performance for artistry and technique. Raymond et al. (2005) utilised two qualified dance assessors that evaluated dance performance considering a technicality, musicality, timing, partnering skill, performing flair, and overall execution from a customized scale with scoring of one to five. In addition, rating scores were divided by the number of practice sessions for each dancer to derive "improvement per practice session". Then, scores were averaged to get the group score from "practice-corrected average difference". Characteristics of each study are presented in Table 3.

Performance Outcomes

HRV BFB vs. CON

For gross motor skill executed in relatively short duration comparing HRV BFB and a a CON, Paul and Garg (2012) demonstrated non-significant difference in dribbling between the HRV BFB and the CON group at $p = 0.06$, ES[95% CL] = 0.89[0.60, 1.18]. On the other hand, the HRV BFB group presented significant enhancement in passing and shooting at $p < 0.001$, 2.14[1.79, 2.49] and $p < 0.001$, 2.00[1.66, 2.34], respectively. In a similar study, Paul et al. (2012) found significant improvement in 3-min shooting after HRV BFB compared to a CON at $p = 0.01$, 1.38[1.07, 2.36]. Raymond et al. (2005) observed non-significant difference in performance scores of dancers at $p = 0.40$, 0.55[-0.06, 1.77]. No significant difference in practice-corrected difference scores at $p = 0.31$, 0.66[0.04, 1.89] was also found.

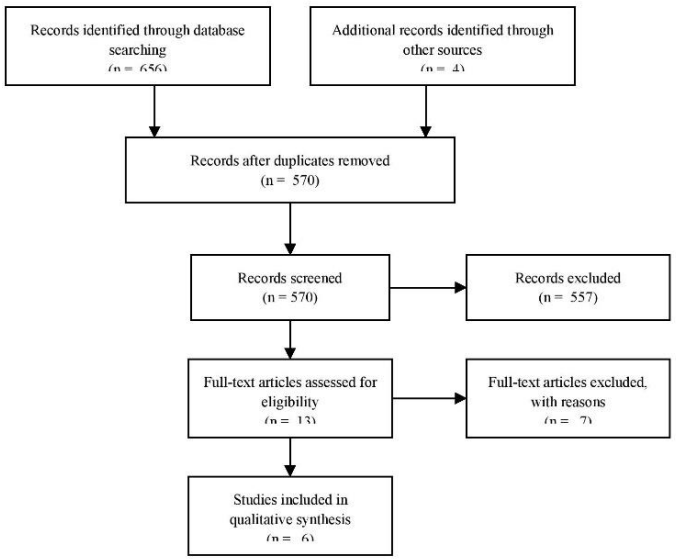

Figure 1

Flow diagram of the search process

Table 1

CONSORT scores of articles included for systematic review

References	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Total
Choudhary et al. (2016)	1	0	0	0	0	1	1	0	3
Gruzelier et al. (2014)	0	0	1	0	0	1	1	0	3
Paul and Garg (2012)	1	0	1	0	1	1	1	0	5
Paul et al. (2012)	1	0	1	0	1	1	1	0	5
Raymond et al. (2005)	1	0	1	0	0	1	1	0	4
Rusciano et al. (2017)	1	0	1	0	0	1	1	1	5

Item 1 *– Were the groups comparable at baseline on key characteristics?* ***Item 2*** *– Did the study include a true control group (randomised participants – not a comparison group)?* ***Item 3*** *- Was the randomisation procedure adequately described and carried out?* ***Item 4*** *– Did the study report a power calculation and was the study adequately powered to detect intervention effects?* ***Item 5*** *– Were the assessors blinded to treatment allocation at baseline and posttest?* ***Item 6*** *– Did at least 80% of the participants complete follow-up assessments?* ***Item 7*** *– Did the study analyses account for potential differences at baseline?* ***Item 8*** *– Did the study compute effect sizes?*

Table 2

Effect Size with 95% Confidence Interval of Studies in HRV BFB vs Comparison Group

Study	Parameter	% Change		p-value	ES	95% Confidence Interval		Power
HRV BFB vs CON		HRV BFB	CON			LL	UL	
Choudhary et al. (2016)	5-km running	-13.0	-0.71	0.16	-0.60	-0.84	0.22	0.51
Paul and Garg (2012)	dribbling	25.1	6.54	0.06	0.89	0.60	1.18	0.50
	passing	76.6	13.2	0.00	2.14	1.79	2.49	0.96
	shooting	104	9.43	0.00	2.00	1.66	2.34	0.93
Paul et al. (2012)	shooting	98.1	27.3	0.01	1.38	1.07	2.36	0.59
	movement time	-17.1	0.00	0.05	-0.95	-1.24	-0.02	0.52
	choice RT	-21.3	0.00	0.01	-1.23	-1.53	-0.27	0.47
Raymond et al. (2005)	dance performance	15.1	8.46	0.40	0.55	-0.06	1.77	0.56
	practice-corrected difference	NA	NA	0.31	0.66	0.04	1.89	0.53
Rusciano et al. (2017)	target absent RT	-29.4	-1.58	0.00	-1.72	-2.05	-0.70	0.82
	target present RT	-22.6	-11.0	0.31	-0.47	-0.72	0.42	0.53
	congruent task accuracy	10.5	0.57	0.00	2.64	2.26	3.84	1.00
	incongruent task accuracy	15.6	0.95	0.00	2.94	2.54	4.20	1.00
	days present	NA	NA	0.00	1.51	1.20	2.50	0.69
	days absent	NA	NA	0.00	-1.52	-1.83	-0.52	0.69
	differential training	NA	NA	0.18	-0.62	-0.90	0.28	0.51
HRV BFB vs PLA		HRV BFB	PLA					
Paul and Garg (2012)	dribbling	25.1	3.06	0.04	0.99	0.70	1.92	0.51
	passing	76.6	23.9	0.01	1.38	1.07	2.36	0.59
	shooting	104	28.8	0.00	1.63	1.31	2.64	0.77
Paul et al. (2012)	shooting	98.1	30.8	0.00	1.39	1.08	2.37	0.60
	movement time	-17.1	0.00	0.42	-0.37	-0.64	0.52	0.56
	choice RT	-21.3	0.00	0.00	-1.51	-1.82	-0.51	0.69
HRV BFB vs NFB		HRV BFB	NFB					
Raymond et al. (2005)	dance performance	15.1	12.2	0.86	0.12	-0.51	1.39	0.86
	practice-corrected difference	NA	NA	0.94	0.05	-0.58	1.31	0.94

ES - effect size; LL - lower limit; UL - upper limit; NA - not applicable

Table 3a

HRV BFB and Athlete Performance

References	Participants			Training Modality	Conclusion/s
	N/sex/age	Training	Discipline		
Choudhary et al. (2016)	n = 24 12M; 12F 22.5 ± 1.72 yrs	regular sport training for HRV BFB/ CON	university, state, national level long distance runners	HRV BFB: once a week formal HRV BFB training; 2 x 20 min/day home practice	5-km time: HRV BFB ↔ CON
Gruzelier et al. (2014)	n = 64 22M; 42F NR	regular dance training for HRV BFB/ CON/ NFB	university dancers	HRV BFB: 10 HRV BFB sessions at 20 min/session	Artistry: HRV BFB ↔ CON HRV BFB ↔ PLA HRV BFB ↔ NFB
				NFB: 10 alpha/theta training at 20 min/session	Technique: HRV BFB ↔ CON HRV BFB ↔ PLA HRV BFB ↔ NFB
Paul and Garg (2012)	n = 30 17M; 13F 21.1 ± 2.82 yrs	regular sport training for HRV BFB/ PLA/CON	university, state and national basketball athletes	HRV BFB: 10 consecutive HRV BFB at 20 min/session PLA: motivational video clips for 10 days at 10 min/day	Dribbling: HRV BFB ↔ CON HRV BFB >PLA Passing: HRV BFB > CON HRV BFB > PLA Shooting: HRV BFB > CON HRV BFB > PLA

***HRV BFB** - heart rate variability biofeedback; **PLA** - placebo; **CON** - control; **NFB** - neurofeedback; **NR** - not reported; **SCL** - skin conductance level; **EMG** - electromyography; **RT** - reaction time*

Table 3b

HRV BFB and Athlete Performance

References	Participants			Training Modality	Conclusion/s
	N/sex/age	Training	Discipline		
Paul et al. (2012)	n = 30 16M; 14F 21.7 ± 2.71 yrs	regular sport training for HRV BFB/ PLA/CON	university, state and national basketball athletes	HRV BFB: 10 consecutive HRV BFB at 20 min/session PLA: motivational video clips for 10 days at 10 min/day	Choice RT: HRV BFB < CON HRV BFB < PLA Movement RT: HRV BFB ↔ CON HRV BFB ↔ PLA Shooting: HRV BFB > CON HRV BFB > PLA
Raymond et al. (2005)	n = 18 9M; 9F 21.6 yrs	regular dance practice for HRV BFB/ NFB./CON	university dancers	HRV BFB: 10 formal HRV BFB training at 20 min/session in 4 weeks NFB = 10 alpha/theta training sessions at 20 min/session	Performance HRV BFB ↔ CON HRV BFB ↔ NFB Practice-Corrected Difference Score HRV BFB ↔ CON HRV BFB ↔ NFB
Rusciano et al. (2017)	n = 20 20M 30.4 ± 4.10 yrs	regular sport training for HRV BFB/ CON	professional football players	HRV BFB: Fifteen 30-min biofeedback sessions at twice/week 4th-9th session: HRV BFB + SCL + EMG + hand temperature 10th -15th session: HRV BFB + math tasks + videos of matches won/lost	Target-Absent Visual Task RT: HRV BFB < CON Target-Present Visual-Task RT: HRV BFB ↔ CON Congruent Task: Accuracy: HRV BFB > CON Incongruent Task Accuracy: HRV BFB > CON Date Present: HRV BFB > CON Days Absent: HRV BFB < CON Differential Training Days: HRV BFB ↔ CON

Gruzelier et al. (2014) found that university dancers exhibited non-significant outcomes in artistry and technique between HRV BFB and CON groups.

In exercise of longer duration, Choudhary et al. (2016) reported no significant improvement in gross motor skill movement via 5-km performance between the HRV BFB group and a CON at $p = 0.16$, ES[95% CL] = -0.60[-0.84, 0.22].

In fine motor ability, Paul et al. (2012) found no significant reduction in movement time seen after HRV BFB and a CON at $p = 0.05$, -0.95[-1.24, -0.02]. On the other hand, there was significantly shorter choice reaction time found after HRV BFB compared to a CON at $p = 0.01$, -1.23[-1.53, -0.27]. Rusciano et al. (2017) detected significant improvement in visual tracking scores with a target absent stimulus among soccer athletes after HRV BFB compared to a CON at $p = 0.00$, -1.72[-2.05, -0.70]. However, visual tracking with a target present stimulus did not significantly improve after HRV BFB compared to a CON at $p = 0.31$; ES = -0.47[-0.75, 0.42]. Furthermore, soccer players after HRV BFB significantly increased accuracy under congruent stimuli at $p = 0.00$, 2.64[2.26, 3.84] compared to a CON. Similarly, the HRV BFB group showed significantly higher accuracy following incongruent stimuli compared to a CON at $p = 0.00$, 2.94[2.54, 4.20].

Rusciano et al. (2017) found significantly reduced absence from sport training sessions compared with a CON at $p = 0.00$, 1.51[1.20, 2.50]. The number of absences from training in the entire sport season that followed treatment from injury was also significantly lower in the HRV BFB group than a CON at $p = 0.00$, -1.52[-1.83, -0.52]. Differential training between the HRV BFB group and a CON was not significantly different, $p = 0.18$, -0.62[-0.90, 0.28].

HRV BFB vs. PLA

Paul and Garg (2012) discovered a significantly higher dribbling score in the HRV BFB group than a PLA at $p = 0.04$, 0.99[0.70, 1.92]. Passing also significantly improved in the HRV BFB group compared with a PLA at $p = 0.01$, 1.38[1.07, 2.36]. The HRV BFB group significantly increased shooting performance compared to a PLA, $p = 0.00$, 1.63[1.31, 2.64]. In the study of Paul et al. (2012), athletes after HRV BFB significantly increased shooting scores at $p = 0.01$, 1.39[1.08, 2.37]. In dance, artistry and technique were similar in both the HRV BFB and the PLA group (Gruzelier et al., 2014).

For fine motor task ability examining HRV BFB and a PLA, non-significant improvement in movement time was identified between the HRV BFB group and a PLA at $p = 0.42$, -0.37[-0.64, 0.52]. However, the HRV BFB group presented significantly lower choice reaction time than the PLA at $p = 0.00$, -1.51[-1.82, -0.51].

HRV BFB vs. other BFB intervention

Raymond et al. (2005) presented similar both performance and practice-corrected performance after HRV BFB and NFB at $p = 0.86$, 0.12[-0.51, 1.31] and $p = 0.94$, 0.05[-0.58, 1.31], respectively. Gruzelier et al. (2014) recorded non-significant difference in artistry and technique between HRV BFB and NFB.

Discussion

The purpose of this systematic review was to examine the effect of HRV BFB on performance indices of athletes. When compared to a control condition (CON) that involved regular training only, HRV BFB displayed contrasting effects on the gross motor skills during short duration performance. In addition, the effect of HRV BFB on gross motor function during exercise of longer duration is unclear. Confounding results in fine motor skills after HRV BFB were also identified in comparison with a CON.

In this study, short duration (< 10 minutes) gross motor tasks after HRV BFB delivered conflicting results. For example, Paul and Garg (2012) showed improved shooting and passing performance in basketball players after HRV BFB compared to a CON. However, non-significant difference in dribbling was seen after HRV BFB and a CON. In a similar study, Paul et al. (2012) recorded increased shooting following HRV BFB compared to a CON. Raymond et al. (2015) presented no significant difference in dance performance and practice-corrected difference in HRV BFB and a CON. The conflicting results in short duration gross motor ability can be attributed to underpowered trials (Table 3). Future research in HRV BFB and gross motor abilities should employ adequate sample size to facilitate enough power to detect meaningful difference (Biau et al., 2008).

The influence of HRV BFB on longer duration (> 10 min) gross motor performance in comparison with a CON is unclear due to limited literature. In the study of Choudhary et al. (2016), 5-km performance of athletes in the HRV BFB and the CON group was not significantly different. However, a notable trend in running enhancement of 13.0% (ES = -0.60) was demonstrated by athletes from the HRV BFB group. On the other hand, the CON group displayed a 0.71% change in performance. Better performance exhibited by athletes following HRV BFB suggests the positive influence of HRV BFB on longer duration gross motor ability. HRV BFB may have reduced physiological stress of athletes from increased blood flow to internal organs, elevated minimum left ventricular elastance, baroreflex sensitivity gain, and improved pulmonary function (Fonoberova et al., 2014; Lehrer et al., 2003; McEwen and Seewan, 2003). More studies are needed to establish the effect of HRV BFB on gross motor ability.

In another light, the fine motor ability of athletes after HRV BFB and a CON displayed conflicting results. Paul et al. (2012) observed improved choice reaction time after HRV BFB. Conversely, no significant enhancement in movement reaction time existed between the HRV BFB and the CON group. Rusciano et al. (2017) found enhancement in reaction time with a target absent stimulus after HRV BFB. However, there was no significant change in the target present stimulus in both groups. Non-significant findings in fine motor ability between HRV BFB and CON can also be due to insufficient sample size leading to low statistical power. In the study of Paul et al. (2012), employment of unequal sample size of males and females between groups may have influenced the non-significant results (Rusticus and Lovato, 2014). Males and females utilize different processing strategies in reaction time wherein males demonstrate faster reaction time than females (Adam et al., 1999; Dane and Ezurumluoglu, 2003). This can be supported by the greater vagal activity observed in females due to the presence of more oestrogen than in males (Dart et al., 2002; Du et al., 2006; Koenig and Thayer, 2016). Oestrogen improves the activity of choline uptake and synthesis of acetylcholine, thereby increasing vagal function (Dart et al., 2002). In addition to unequal distribution of males and females between groups, inclusion of athletes from various competitive levels in the study by Paul et al. (2012) may have increased the variability in fine motor skill execution (Carillo et al., 2011; Mückschel et al., 2014). Indeed, previous studies have shown better reaction times in elite athletes compared to non-elite athletes (Loureiro Jr and Freitas, 2012; van de Water et al., 2017; Verbugh et al., 2016). Addressing the aforementioned shortcomings when examining fine motor skill with HRV BFB training in future studies should be warranted.

Although inconsistent results were observed in HRV BFB and a CON, there is a favourable trend in performance enhancement towards HRV BFB in overall motor function. Possible physiological mechanisms of HRV BFB can be explained by the neurovisceral integration model (Porges, 2009; Thayer and Lane, 2009). HRV BFB increases activation of the vagal nerve (Gevirtz, 2013; Lehrer and Gevirtz, 2014). The vagal nerve is connected to the anterior cingulate cortex, the brain region that plays a crucial role for multi-component behaviour (Duncan, 2010; Mayer, 2011; Mückschel et al., 2014). HRV BFB may have facilitated the production of neurotransmitters responsible for improving fine and gross motor function (Beste et al., 2016; Hassert et al., 2004; Juster et al., 2010; Sellaro et al., 2015; Steenbergen et al., 2015; Yildiz et al., 2014). However, the exact mechanism affecting performance from improvement in vagal function is unknown. Future studies should be sufficiently powered, and include biochemical markers to elucidate the mechanistic properties of HRV BFB.

An interesting finding in this review is the increased attendance of athletes in training with HRV BFB. Rusciano et al. (2017) recorded an ~86% presence in training and ~4% absent rate out of 240 training sessions in soccer players in HRV BFB training. For athletes from a CON group, the rates of attendance and absence were ~73% and ~14%, respectively. Although differential training was not significantly different between groups, athletes following HRV BFB presented lower differential training of ~9% compared to a CON (~13%). The increased attendance, reduced absences, and a lower trend in differential training among athletes under HRV BFB may be related to improved physiological adaptations leading to resilience to stressors (Ivarsson and Johnson, 2010;

Porges, 2007, 2009; Thayer and Lane, 2009). Thus, HRV BFB can also be a promising intervention in increasing athlete's attendance in training by reduction of the risk of injuries.

Aside from comparing HRV BFB and a CON, the researchers also found improvement in short-duration gross motor performance with HRV BFB compared to a PLA. Paul and Garg (2012) displayed increased dribbling, shooting, and passing after HRV BFB compared to a PLA. Similarly, Paul and Garg (2012) showed improved shooting after HRV BFB compared to a PLA. Implications from these results point to HRV BFB as a superior alternative compared with a PLA in improving gross motor function of short duration.

The influence of HRV BFB on fine motor skill differentiated with a PLA is vague due to the scarcity of literature. Paul et al. (2012) recorded non-significant difference in movement time between HRV BFB and a PLA. On the other hand, HRV BFB presented enhanced choice reaction time in HRV BFB compared to a CON. Therefore, additional HRV BFB studies including a PLA are needed.

It is necessary to acknowledge that the outcomes of this study are limited to the type of athletes included in the analyses. Generalization of results should be avoided. In addition, this review evaluated performance indices to provide practitioners with a simple and direct link on HRV BFB and performance.

Conclusion

Findings of this study indicate the potential ergogenic ability of HRV BFB in improving fine and gross motor skills of athletes.

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ORIGINAL RESEARCH

Physiological Reports

Acute effects of resonance frequency breathing on cardiovascular regulation

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Abstract

Acute slow breathing may have beneficial effects on cardiovascular regulation by affecting hemodynamics and the autonomic nervous system. Whether breathing at the resonance frequency (RF), a breathing rate that maximizes heart rate oscillations, induces differential effects to that of slow breathing is unknown. We compared the acute effects of breathing at either RF and RF + 1 breaths per minute on muscle sympathetic nervous activity (MSNA) and baroreflex function. Ten healthy men underwent MSNA, blood pressure (BP), and heart rate (HR) recordings while breathing for 10 min at their spontaneous breathing (SB) rate followed by 10 min at both RF and RF + 1 randomly assigned and separated by a 10-min recovery. Breathing at either RF or RF + 1 induced similar changes in HR and HR variability, with increased low frequency and decreased high frequency oscillations ($p < .001$ for both). Both respiration rates decreased MSNA (−5.6 and −7.3 bursts per min for RF and RF + 1 $p < .05$), with the sympathetic bursts occurring more often during mid-inspiration to early expiration (+57% and + 80%) and longer periods of silence between bursts were seen ($p < .05$ for RF + 1). Systolic BP was decreased only during RF (−4.6 mmHg, $p < .05$) but the decrease did not differ to that seen during RF + 1 (−3.1 mmHg). The sympathetic baroreflex function remained unchanged at either breathing rates. The slope of the cardiac baroreflex function was unaltered but the cardiac baroreflex efficiency was improved during both RF and RF + 1. Acute breathing at either RF or RF + 1 has similar hemodynamic and sympatho-inhibitory effects in healthy men.

KEYWORDS

baroreflex, blood pressure, resonance frequency, sympathetic nervous activity

1 | INTRODUCTION

Slow breathing has long been recognized to exert beneficial effects in a range of disorders including those related to the cardiovascular system and those associated with anxiety. Breathing at a low pace (5–6 breaths per min) has been shown to acutely decrease blood pressure (BP) in patients with post-traumatic stress disorder (Fonkoue et al., 2018), and in the longer term decrease BP in patients with hypertension (Hering et al., 2013). In patients with heart failure, slow breathing either performed acutely or chronically reduced the cardiovascular reactivity to mental stress and improved various aspects of health-related quality of life (Lachowska, Bellwon, Morys, Gruchala, &

Hering 2019). Recently, an intervention has emerged consisting of breathing at a specific and individualized (slow) breathing rate, termed resonance frequency (RF), where oscillations in heart rate (HR) and breathing synchronize. This intervention typically involves the use of a device which guides an individual to breathe at their RF via visual or auditory feedback. Breathing at RF maximizes HR oscillations by creating a 0 degree phase shift between HR and respiration, while BP response from HR exhibit a 180 degree phase shift occurring at approximately 5-s delay as a result of mechanical response (Vaschillo, Vaschillo, & Lehrer, 2006). Similar to slow breathing at a set pace, RF breathing has emerged as a promising tool to enhance performance, reduce stress and anxiety (Jester, Rozek, & McKelley, 2019; Lehrer & Gevirtz, 2014) and positively influence clinical symptoms in a number of disorders including depression (Lin et al., 2019), asthma (Taghizadeh, Eslaminejad, & Raoufy, 2019), and prehypertension (Lin et al., 2012).

The primary proposed mechanistic path for slow breathing or breathing at RF has been an improved vagal tone, associated with improved cardiac baroreflex function. Powerful within-breath respiratory modulation of sympathetic vasoconstrictor activity has been well documented in humans (Macefield & Wallin, 1995; St Croix, Satoh, Morgan, Skatrud, & Dempsey, 1999). During spontaneous breathing, muscle sympathetic nerve activity (MSNA) is inhibited during mid-inspiration to mid-expiration, with MSNA activation occurring during late expiration (Eckberg, Nerhed, & Wallin, 1985; Limberg, Morgan, Schrage, & Dempsey, 2013). Interventions aiming at altering the breathing frequency may affect the within-breath modulation of MSNA and hence modulate steady state sympathetic tone. Acute slow breathing for 10–15 min was found to decrease MSNA in patients with hypertension (Hering et al., 2013; Oneda, Ortega, Gusmao, Araujo, & Mion, 2010), post-traumatic stress disorder (Fonkoue et al., 2018) and those with heart failure (Harada et al., 2014), but failed to induce any change in healthy subjects when the breathing exercise was of 3 min duration (Limberg et al., 2013).

Breathing at RF is thought to maximize heart rate variability (HRV) and influence autonomic nervous system (Lehrer & Gevirtz, 2014; Lehrer, Vaschillo, & Vaschillo, 2000; Lehrer et al., 2003); however, it is not known whether breathing at a precise respiration rate at an individualized RF may result in different changes in cardiovascular regulation compared to breathing at a set slow pace. The aim of the present study was therefore to compare the acute effects of breathing at an individual's RF relative to breathing at 1 breath above (RF + 1) on HR, HRV, BP, MSNA, within-breath modulation of MSNA, and vascular and sympathetic baroreflex functions.

2 | METHODS

2.1 | Participants

Ten healthy non-smoking males (age: 28.7 ± 4.8 years; height: 170 ± 7 cm; weight: 68.4 ± 8.0 kg) with no history of clinical disease volunteered in this study. Participants were required not to consume any food or any caffeine containing beverage for at least 2 hr before experimentation. The protocol of this study conformed to the Declaration of Helsinki for human experimentation. All subjects provided written informed consent prior to participation. The experimental protocol was approved by the Swinburne University Human Ethics Committee.

2.2 | Procedures

This study involved two experimentation sessions separated by 24-hrs. Determination of participant's RF was administered in the first session while breathing schemes were facilitated in the second session (HRV Starter System, Thought Technology). The RF was determined according to the protocol described by Lehrer and colleagues (2000) from incremental 2-min paced breathing at various frequencies (6.5, 6.0, 5.5, 5.0, 4.5) while monitoring HR and respiration. At the end of 10 min, the breathing frequency that displayed the highest occurrence of low frequency in HRV (LF; 0.05–0.15 Hz) was selected as the RF. Prior to RF testing, participants underwent a biofeedback-assisted paced breathing familiarization involving nasal inhalation and pursed-lip exhalation.

On the second session, participants were placed in a semi-supine position and instrumented for BP, HR, respiration, and MSNA recordings. After they had rested for at least 10–15 min, all parameters were continuously recorded for the ten minute baseline period where they were required to breathe at their spontaneous frequency. Following baseline recording, participants were asked to breathe at either their RF rate or RF + 1 for 10 min. Participants were allowed to rest for 10–15 min and told to breathe at their spontaneous rate until BP and MSNA values had returned to baseline before undergoing another 10-min period of breathing at either their RF rate or RF + 1. Each participant therefore completed baseline first, followed by RF and RF + 1 in a random manner. During spontaneous breathing (SB), participants were asked to fix gaze at a blank computer screen. Paced breathing at RF or RF + 1 was guided by a visual pacer, with heart and respiratory oscillations displayed on screen.

2.3 | Muscle sympathetic nerve activity, respiration, heart rate, and blood pressure

Recording of multiunit postganglionic MSNA was made with participants resting in a semi-supine position as described

previously (Lambert & Schlaich, 2004). A tungsten microelectrode (FHC) was inserted directly into the right peroneal nerve just below the fibular head. A subcutaneous reference electrode was positioned 2–3 cm away from the recording site. The nerve signal was amplified (350,000), filtered (bandpass 700–2,000 Hz), and integrated. During MSNA recording, BP was measured continuously using the Finometer system (Finapress Medical System BV), HR was determine using a lead III echocardiogram and respiration was assessed using a piezoelectric belt. BP, electrocardiogram, respiration, and MSNA were digitized with a sampling frequency of 1,000 Hz (PowerLab recording system, model ML 785/8SP; ADI Instruments).

2.4 | MSNA analysis

Sympathetic bursts were visually identified and the number of bursts was averaged over the 10-min period during SB, RF, and RF + 1. The MSNA was expressed as burst frequency (burst/min) and burst incidence (bursts/100 heartbeats). For analysis with simultaneous signals (BP, respiration), the MSNA signals were advanced 1.5 s to account for peripheral sympathetic nerve conduction delays (Fagius & Wallin, 1980). HR and BP were averaged over the 10-min period during SB, RF, and RF + 1.

2.4.1 | Dynamic patterns of MSNA spiking

The inter-burst interval was used to measure dynamic patterns of MSNA activity. The inter-burst interval was used as a measure of burst-suppression, when a cluster of bursts is followed by a silence period. The difference in inter-burst intervals during each conditions was calculated for individual participants and then averaged for the whole cohort.

2.4.2 | Instantaneous phase of the respiration signal

To calculate the phase of the respiration signal, a Hilbert transform was applied to the signal. The Hilbert transform is a linear operator which converts a function of real variables into a complex plane (King, 2009). The instantaneous phase of the respiration is calculated as an arctangent of a ratio of real and imaginary parts of the signal, measured between $-\pi$ and π, where π is the end of inspiration and early expiration and $-\pi$ is the end of expiration and early inspiration. A histogram of MSNA burst occurrence was plotted as a function of an instantaneous phase of the respiration signal. The MSNA bursts histograms were normalized to the maximum value for each breathing period and compared among SB, RF, and RF + 1 breathing schemes.

2.5 | Spontaneous arterial baroreflex control of MSNA

Over each period (SB, RF, RF + 1), diastolic BPs associated with individual heartbeats were grouped in intervals of 2 mmHg and, for each interval, the percentage of diastoles associated with a sympathetic burst was plotted against the mean of the pressure interval (Lambert et al., 2002). The sensitivity of the sympathetic baroreflex gain was defined as the slope of the regression line and was expressed as bursts/100 heartbeats/mmHg.

2.6 | Spontaneous cardiac baroreflex sensitivity

Baroreflex sensitivity was assessed using the sequence method (Parati et al., 2004). This procedure identifies the 'spontaneous' sequences of three or more consecutive beats in which systolic BP progressively rose and cardiac interval progressively lengthened (type 1 sequences), or systolic BP progressively fell and cardiac interval progressively shortened (type 2 sequences), with a lag of one beat. For each sequence, the linear correlation coefficient between cardiac interval and systolic BP was computed and the sequence validated when $r > 0.85$. The slope between cardiac interval and systolic BP was calculated for each validated sequence and expressed as msec/mmHg. The baroreflex efficacy index (BEI) (Di Rienzo et al., 2001) was assessed as the total number of cardiac intervals/systolic BP sequences divided by the total number of systolic BP ramps.

2.7 | Heart rate variability

HRV was assessed from the resting electrocardiogram (ECG) obtained during the MSNA recording and was determined using commercially available software (HRV Module for Chart 5 Pro; ADI Instruments, Bella Vista, Australia). Parameters derived were standard deviation of normal to normal intervals (SDNN) and standard deviation of heart rate (*SD* rate) in the time domain analysis. LF (0.04–0.15 Hz) and high frequency (HF: 0.15–0.4 Hz) in the frequency domain analysis expressed as percentage and normalized units. Additionally, LF/HF ratio was also included in HRV analysis.

2.8 | Data analysis

In order to compare the variables under the three conditions (SB, RF, and RF + 1) a repeated measures ANOVA followed by pairwise multiple comparison procedure (Bonferroni *t*-test) was used when the data were normally distributed. Otherwise, the Friedman's test for repeated measures was used and Wilcoxon signed rank test for

paired samples was administered for post hoc analysis. Statistical analyses were carried out using a commercial statistical package (SPSS ver 25, IBM) with alpha set at 0.050 level. Data are presented as mean ± standard deviation or mean ± interquartile range.

3 | RESULTS

Figure 1 contains hemodynamic recordings from one subject showing the effects of breathing at the SB, RF, and RF + 1. Fluctuations in the ECG and BP signals are obvious at both RF and RF + 1. In this participant, MSNA was more pronounced during early inspiration, and subsequently inhibited from mid-inspiration to mid-expiration. This observation was more obvious at RF and RF + 1, compared with SB.

3.1 | Respiration, blood pressure, and heart rate

As expected, the respiration rates during both RF and RF + 1 were significantly lower compared to that during SB ($p < .01$ for both) with RF + 1 being also significantly higher compared to RF ($p < .01$) (Table 1). Systolic BP was significantly lower during RF compared with SB ($p < .05$) but no difference was noted during RF + 1 (Table 1). No significant difference in either diastolic BP or HR were observed between breathing conditions.

3.2 | Muscle sympathetic nervous activity

3.2.1 | Burst frequency and incidence

A significant difference in MSNA burst frequency and incidence was observed during both breathing schemes: Burst frequency during RF was significantly lower than SB ($p < .05$) (Table 2). Similarly, RF + 1 exhibited lower MSNA burst frequency than SB ($p < .01$). Likewise, lower burst incidence was seen during RF compared to SB ($p < .05$) and during RF + 1 compared to SB ($p < .01$). Difference in burst incidence and burst frequency between RF and RF + 1 was nonsignificant.

3.2.2 | Pattern of sympathetic activity

Figure 2 shows an example of MSNA data with corresponding respiration and the calculated instantaneous phase of the signal, normalized histogram of burst occurrence as a function of instantaneous phase of the respiration signal and normalized inter-burst interval histogram.

3.2.3 | Burst histogram as a function of respiration phase

The average, median, maximum, and differences in MSNA burst occurrence for the phase intervals $-\pi$ to 0 and 0 to π are presented in Table 2 and the average across all phases is illustrated in Figure 3a. For the phase intervals $-\pi$ to 0, no significant difference existed between breathing frequencies. For the phase interval 0 to π, there was a difference in the area under the curve of burst occurrence histogram from 0 to π with both RF and RF + 1 displaying significantly greater area under the curve of burst occurrence histogram compared to SB ($p < .05$ and $p < .01$, respectively).

3.2.4 | Inter-burst intervals

Inter-burst intervals were divided in duration of <4 ms and ≥4 ms. The mean and maximum inter-burst intervals for each duration are presented in Table 2 and Figure 3b illustrates inter-burst interval data averaged between all participants. There was no difference between breathing schemes in inter-burst intervals for the <4 ms. However, there was a significant difference in inter-burst intervals at ≥4 ms with RF + 1 displaying a higher inter-burst intervals compared to SB ($p < .05$). The area under the curve of inter-burst intervals ≥4 ms was larger during RF + 1 compared to SB ($p < .05$). Similar trends were noticed during RF but these did not reach significance.

3.3 | Baroreflex function

The slopes of both sympathetic and cardiac baroreflex functions were unchanged during breathing exercises. The BEI of the cardiac baroreflex function was significantly increased during RF and RF + 1 ($p < .05$ for both).

3.4 | Heart rate variability

All changes in HRV for time domain and frequency domain analysis are presented in Table 3. In the time domain *SD* rate was higher during RF and RF + 1 ($p < .05$ and $p < .01$). In the frequency domain, RF and RF + 1 displayed greater LF Power and lower HF power compared to SB but no significant difference were found between RF and RF + 1.

4 | DISCUSSION

This study examined the effects of short term breathing at RF on cardiovascular regulation compared to breathing at

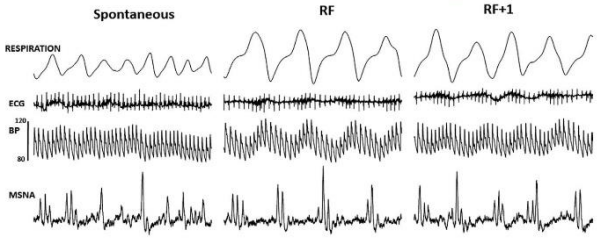

FIGURE 1 Original traces of respiration, ECG, blood pressure (BP), and muscle sympathetic nerve activity (MSNA) in one participant while breathing at their spontaneous breathing rate, at the resonance frequency (RF), and at the RF + 1. In this participant, MSNA is more pronounced during early inspiration and inhibited from mid-inspiration to mid-expiration with the effect being more obvious at RF and RF + 1

TABLE 1 Hemodynamic parameters during spontaneous breathing (SB), breathing at the resonance frequency (RF) and at the RF + 1

	SB	RF	RF + 1
Hemodynamics			
Respiration rate, breaths/min	14.2 ± 3.13	5.44 ± 0.88**	6.43 ± 0.88▲●
Systolic blood pressure, mmHg	123.6 ± 12.13	119.0 ± 14.66*	120.5 ± 14.07
Diastolic blood pressure, mmHg	80.8 ± 11.8	78.7 ± 12.3	80.1 ± 11.5
Heart rate, bpm	71.2 ± 6.47	72.6 ± 7.73	72.5 ± 7.15

Note: Data are presented as mean ± *SD*.

*$p < .050$, RF versus SB, **$p < .010$, RF versus SB, ▲$p < .010$, RF + 1 versus SB, ●$p < .010$, RF versus RF + 1

1 breath/min above the RF. We addressed this issue by assessing hemodynamics including direct sympathetic nerve recording and assessment of cardiac and arterial baroreflex function. Major findings are: (a) Both RF and RF + 1 induced similar changes in MSNA including a decrease in the incidence and frequency of bursts, with the bursting pattern associated with longer periods of burst silencing and a shift of burst occurrence towards mid-inspiration to early expiration, (b) RF and RF + 1 were both associated with improved cardiac baroreflex efficacy but did not affect the sympathetic baroreflex function (c) RF induced a significant reduction in systolic BP.

This is the first study to examine the modulatory effects of respiration at RF and RF + 1 on sympathetic activity investigating both the global sympathetic tone (burst incidence and frequency) and the dynamic pattern of sympathetic firing including inter-burst intervals and burst occurrence as a function of the respiration phase. A significant reduction in sympathetic burst frequency and incidence were seen during either RF or RF + 1 compared with SB. In the healthy state, MSNA represents global sympathetic outflow to the skeletal muscle linked to BP regulation with strong feedback from the arterial baroreceptors (Wallin, 2006) and respiratory modulation (Habler, Janig, & Michaelis, 1994). The reduction in MSNA observed during slow breathing at either RF or RF + 1 was similar to that demonstrated in previous studies where slow breathing was of 10–15 min duration (Fonkoue et al., 2018; Harada et al., 2014; Hering et al., 2013; Oneda et al., 2010). Two prior studies in healthy subjects indicated no effect of slow breathing on MSNA but the duration of the breathing exercise was much shorter being 3 (Limberg et al., 2013) or 4 min (Raupach et al., 2008). Our data indicate that MSNA is reduced during slow breathing in healthy subjects with a longer breathing task. Lower burst frequency and burst incidence with RF or slow breathing in general may be due to activation of pulmonary mechanoreceptors in response to the increased tidal volume that accompanies slow breathing. This is supported by the findings of Lehrer & colleagues (2003) who demonstrated greater tidal volume among healthy male participants under RF breathing compared with SB. The increased tidal volume under slow breathing might have activated lung stretch receptors and reduced chemoreflex response thus suppressing the activation of MSNA (Bernardi, Gabutti, Porta, & Spicuzza, 2001; Seals, Suwarno, & Dempsey, 1990). The modulatory influence of breathing on MSNA has previously been described with approximately 70% of the activity occurring during low lung volumes (initial half of inspiration and latter half of expiration) and MSNA decreasing progressively and markedly from onset to late inspiration (Seals

TABLE 2 Muscle sympathetic nerve activity (MSNA) and baroreflex function during spontaneous breathing (SB), breathing at the resonance frequency (RF), and at the RF + 1

	SB	RF	RF + 1
Muscle sympathetic nerve activity			
Burst frequency (Bursts per minute)	33.5 ± 9.67	27.9 ± 8.14*	26.1 ± 6.95▲▲
Burst incidence (Bursts per 100 heartbeats)	47.5 ± 15.0	38.9 ± 12.0*	36.5 ± 11.2▲▲
Burst occurrence as a function of respiratory phase [-π,0]			
Normalized average burst occurrence	0.63 ± 0.09	0.44 ± 0.19	0.45 ± 0.22
Normalized median burst occurrence	0.65 (0.06)	0.43 (0.23)	0.46 (0.23)
Normalized maximum burst occurrence (corresponding phase)	0.82 (−π)	0.77(−π)	0.71(−π)
Difference in burst occurrence compared to SB at −π/2	–	−37%	−36%
Area under the curve of burst occurrence histogram	0.32 ± 0.07	0.36 ± 0.13	0.28 ± 0.08
Burst occurrence as a function of respiratory phase [0,π]			
Normalized average burst occurrence	0.40 ± 0.08	0.47 ± 0.15	0.56 ± 0.18
Normalized median burst occurrence	0.41(0.10)	0.46 (0.17)	0.58 (0.30)
Normalized maximum burst occurrence (corresponding phase)	0.49(π)	0.62(π/2)	0.72(π/2)
Difference in burst occurrence compared to SB at π/2	–	+57%	+80%
Area under the curve of burst histogram	0.15 ± 0.05	0.28 ± 0.14*	0.33 ± 0.13▲▲
Inter bursts intervals corresponding to < 4 ms			
Average	0.41 ± 0.09	0.41 ± 0.12	0.44 ± 0.13
Median	0.38 (0.12)	0.40 (0.12)	0.47 (0.18)
Maximum (corresponding interval)	1 (1.25 ms)	1 (1.25 ms)	1 (1.25 ms)
Difference compared to SB	–	+0.02	+0.13
Area under the curve of inter burst interval	1.12 ± 0.08	1.11 ± 0.12	1.07 ± 0.11
Inter bursts intervals corresponding to ≥ 4 ms			
Average	0.02 ± 0.02	0.03 ± 0.03	0.05 ± 0.04▲
Median	0.01(0.02)	0.02 (0.04)	0.04 (0.04)
Maximum (corresponding interval)	0.09 (5 ms)	0.27 (5 ms)	0.19 (5 ms)
Difference compared to SB	–	+0.13	+0.26
Area under the curve of inter burst interval histogram	0.12 ± 0.09	0.16 ± 0.11	0.19 ± 0.09▲
Sympathetic baroreflex function slope, bursts/100 heartbeats/mmHg	−2.92 (2.55)	−4.07 (3.27)	−4.04 (4.19)
Cardiac baroreflex function slope, msec/mmHg	14.6 ± 3.83	13.8 ± 4.51	14.1 ± 5.51
Cardiac baroreflex function efficacy index	47.3 (30.5)	52.9 (33.1)*	61.3 (20.1)▲

Note: Data are presented as mean ± *SD*. *$p < .050$, RF versus SB, ▲$p < .050$, RF + 1 versus SB, ▲▲$p < .010$, RF + 1 versus SB. Data are presented as mean ± *SD* or median (interquartile range).

et al., 1993). The analysis of the pattern of sympathetic activity using the respiration phase analysis and the inter-burst intervals is novel and allows to demonstrate that RF and RF + 1 have significant effect on the pattern of sympathetic activity. In accordance to that described by Seals et al. (1993) we documented that during normal spontaneous breathing, MSNA is more likely to occur during the −π to −π/2 phase indicating higher activity during early inspiration and late expiration. MSNA is more inhibited during the 0 to π phase, hence during the late inspiration to early expiration. The decrease in burst incidence and frequency observed during either RF or RF + 1 is associated with a change in the pattern of bursting with the bursts being less frequent during the respiration phase interval −π/2 to 0 (expiration to onset of inspiration) and more frequent around π/2 phase (late inspiration). In addition, the inter-burst interval analysis revealed longer periods of burst silencing during RF and RF + 1. Hence, the sympatho-inhibition observed during either RF or RF + 1 seems to occur as a result of changes in the bursting pattern of MSNA imposed by the ability of the respiration to modulate the timing of bursts. Such changes are

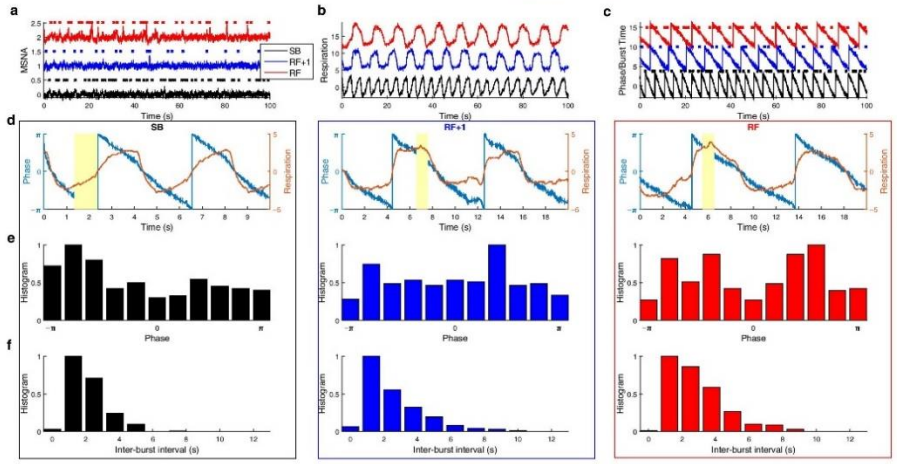

FIGURE 2 Examples of recorded and analyzed data from a participant. (a–d) illustrate short extracts of data for an individual participant. (e and f) illustrate data for the whole period of recording for the same participant. All three conditions are shown in subplots (a–b), shifted along vertical axis for clarity, and color-coded: Black—spontaneous breathing (SB), Red—resonance frequency (RF), Blue—at (RF + 1). (a) An extract of muscle sympathetic nerve activity (MSNA) data (solid lines) and detected bursts (stars). (b) Respiration signal for three conditions. (c) Detected phase of respiration signal from b (solid lines) and MSNA bursts from a (stars), aligned in time. (d) An extract of respiration data (left y-axis) and detected phase of the signal (right y-axis). Yellow rectangles: the phase of the signal when maximum likelihood of spiking occurs. (e) Normalized burst time histogram as a function of the instantaneous phase of respiration. (f) Normalized histogram of inter-burst intervals. (d–f) Data for baseline SB (left plot), RF + 1 (middle), and RF (right)

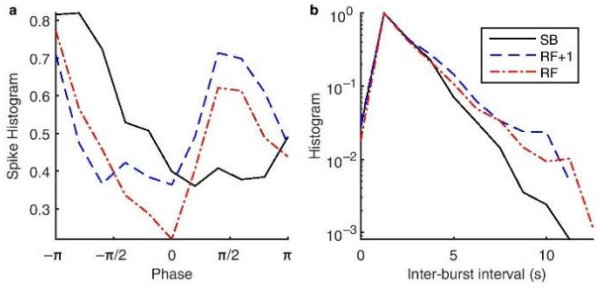

FIGURE 3 (a) Burst occurrence as a function of respiration phase averaged between all participants and normalized to a maximum value. There is a significant increase in burst occurrence on the 0 to π interval during breathing at resonance frequency (RF) and at the RF + 1. At this interval, the maximum likelihood of burst occurs at π phase for a baseline condition (spontaneous breathing, SB), while for the RF and at the RF + 1 the maximum occurrence of burst is seen at −π/2. On average, there is a 68% increase in burst occurrence at the π/2 phase during RF and at the RF + 1 compared to the SB. (b) inter-bursts interval data averaged between all participants. It can be seen that inter-burst intervals become longer during RF and at the RF + 1 breathing patterns, that is, there are more silence periods

potentially critical because the respiratory-modulated bursting of sympathetic activity has been shown to modulate vascular resistance (Briant, O'Callaghan, Champneys, & Paton, 2015).

Slow breathing exercises have been reported to decrease BP albeit to a modest extent (Fonkoue et al., 2018; Grossman, Grossman, Schein, Zimlichman, & Gavish, 2001; Hering et

TABLE 3 Heart rate variability parameters during spontaneous breathing (SB), breathing at the resonance frequency (RF), and at the RF + 1

	SB	RF	RF + 1
Time domain analysis			
SD rate	5.10 ± 1.45	7.30 ± 2.41*	7.20 ± 2.30▲▲
SDNN ms	48.0 ± 25.4	46.2 ± 26.9	52.3 ± 28.2
Frequency domain analysis			
LF power (%)	28.0 ± 12.6	69.6 ± 13.2**	64.6 ± 19.1▲▲
LF power (nu)	46.8 ± 20.8	88.3 ± 6.11**	82.1 ± 12.4▲▲
HF power (%)	32.2 ± 16.3	9.30 ± 6.03**	12.8 ± 8.90▲
HF power (nu)	51.2 ± 19.5	11.5 ± 5.76**	17.2 ± 11.8▲
LF/HF	1.60 ± 2.22	10.9 ± 8.47**	8.90 ± 7.55▲

Note: Data are presented as mean ± *SD*. *$p < .050$, RF versus SB, ▲$p < .050$, RF + 1 versus SB, ▲▲$p < .010$, RF + 1 versus SB.

Abbreviations: HF, High frequency; LF, Low frequency; *SD*, Standard deviation; SDNN, standard deviation of normal to normal intervals.

al., 2013; Rosenthal, Alter, Peleg, & Gavish, 2001). In line with these previous studies, we noticed a small but significant decrease in systolic BP during the RF breathing with no difference in systolic BP between RF and RF + 1 indicating that breathing at RF as opposed to RF + 1 did not trigger a better systolic BP response. Breathing at RF has been proposed to modulate autonomic cardiovascular regulation, affecting cardiopulmonary reflexes, arterial baroreflexes, sympathetic vascular tone, and peripheral resistance, which in turn may result in systemic vasodilatation and decreased BP (Lehrer & Gevirtz, 2014).

Enhanced baroreflex sensitivity has been suggested to occur during slow breathing (Bernardi et al., 2001; Raupach et al., 2008) or breathing at RF (Lehrer et al., 2000), possibly as a result of reduced chemoreflex sensitivity, which in turn may lead to decreased sympathetic tone. Our study indicates that the slope of the cardiac baroreflex function (assessed as a combination of up and down sequences) remains unchanged during RF or RF + 1 but the efficacy of the baroreflex as assessed by BEI was improved during both RF + 1 and RF. BEI has been suggested to be a good representation of the baroreflex function in healthy subjects as it quantifies the number of times the baroreflex is effective in driving the sinus node (Di Rienzo et al., 2001). Tzeng et al. (2009) demonstrated that slow breathing in healthy subjects did not affect the arterial baroreflex when measured using the gold standard modified Oxford method and suggested that results indicating improvement in baroreflex function during slow breathing may have occurred as commonly used baroreflex assessment techniques may not be accurate in this setting. The sympathetic baroreflex function was also explored as a possible contributor to the changes in MSNA as Fonkue and colleagues observed that slow breathing improved the sympathetic baroreflex function (Fonkoue et al., 2018). However, this was not observed in our study as neither RF nor RF + 1 improved the slope of the sympathetic baroreflex function. Overall, the effects of slow breathing schemes in this study on baroreflex function are still unclear as results vary depending on the method used (Tzeng et al., 2009).

Within the context of this study, we found that breathing at RF or RF + 1 induced significant hemodynamic and autonomic changes but we were unable to detect any differences between the two breathing schemes. This raises the question as to whether precise measurement of the RF is essential for the reported beneficial clinical effects of individualized RF or a standardized paced breathing at 5–7 breaths per min is all that is required. Lin et al. (Lin et al., 2012) investigated the effects or either paced breathing at RF compared to slow breathing (6 breaths/min) in individuals with prehypertension and found that both schemes of breathing resulted in a decrease in BP over a period of 5 weeks and that the decrease in BP was more marked in those breathing at RF except for the first session where the BP fall was identical with either RF or RF + 1. They also found that RF induced stronger changes in HRV indices and in the baroreflex function compared with slow breathing which may explain better hemodynamic changes. In this study, we noticed that there was neglible difference in HRV and the respiratory modulation of MSNA between acute RF and RF + 1. These findings indicate occurence of sympathoinhibition regardless of RF or RF + 1. In a recent study comparing RF and RF + 1 on BP levels, Steffen et al. (Steffen, Austin, DeBarros, & Brown, 2017) noticed that both RF and RF + 1 decreased systolic BP to the same extent but subjects allocated to the RF exercise exhibited lower systolic BP in response to a stress test compared to subjects allocated to RF + 1. This was accompanied by better mood suggesting a preferential effect of RF compared to RF + 1 on the stress response. Hering et al. (Hering et al., 2013) showed that slow breathing at 6 breaths per min induced significant decrease in BP in the long term but not acutely in hypertensive subjects. Interestingly, they found, that long-term paced breathing selectively attenuated pressor

and tachycardic responses to mental stress but the corresponding MSNA responses remained unaltered; however, in their study the resonance frequency was not imposed. Hence, while slow breathing in general is undoubtedly a strategy to improve BP and stress responses, whether breathing at the specific RF is more efficient in the longer term is uncertain. More studies are certainly needed to fully examine long-term effect of breathing at RF on hemodynamic markers.

Limitations in the current study should be acknowledged. This study was conducted in a small group of healthy young males. Hemodynamic and MSNA results in this cohort might not be applicable in other populations. Slow breathing may be more beneficial in individuals with elevated sympathetic nervous activity and decreased baroreflex function such as those with hypertension, heart failure, chronic obstructive pulmonary diseases, or in individuals with anxiety disorders where sympathetic and vagal function may be altered. Also, this study was conducted in a single session and respiratory rate was assessed using a standard respiratory belt hence parameters such as tidal volume were not available. Future studies should employ long-term applications of RF versus RF + 1 to identify dose–response relationship on hemodynamic and autonomic nervous system.

5 | CONCLUSION

Breathing exercises remain an attractive non-invasive strategy to modulate autonomic nervous system function. While breathing at the specific RF is suggested to maximize the effect, we found that breathing at RF or 1 breath above RF induced the same acute changes on the sympathetic nervous system and BP, with both breathing paradigms inducing similar changes in the pattern of sympathetic firing.

CONFLICT OF INTEREST

None declared.

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Motor Control

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Effect of Acute Heart Rate Variability Biofeedback on H-reflex Modulation: A Pilot Study

by
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Heart rate variability biofeedback (HRV BFB) is paced breathing scheme that stimulates resonance in the cardiovascular system. This study aimed to investigate the effect of a single-session HRV BFB on Hoffman reflex (H-reflex) of the soleus muscle. Twelve healthy males (height: 173.7 ± 7.18 cm; weight: 72.7 ± 17.7 kg; age: 24.0 ± 5.02 yrs) completed a randomized-crossover intervention involving a 10-minute HRV BFB and normal breathing (CON) separated by 48 hours. Results revealed significantly lower 1a afferent activation after HRV BFB. Similarly, the HRV BFB also demonstrated lower proportion of activated motor neurons from 1a afferents. In conclusion, an acute HRV BFB influenced the reduction in motoneuron excitability at resting condition.

Key words: *biofeedback, resonance frequency breathing, H-reflex, neural excitation.*

Introduction

The Hoffman reflex (H-reflex) is a tool for assessment of monosynaptic reflex in the spinal cord, modulated by input of 1a afferents to the alpha motoneural pool (MN) (Chen et al., 2012; Gajewski and Mazur-Różycka, 2016; Knikou, 2008; Palmieri et al., 2004; Pierrot-Deseilligny and Mazevet, 2000). Percutaneos electrical stimulation to the mixed peripheral nerve is applied to evoke H-reflex, targetting the monosynaptic reflex arc and efferent motor response (Zehr, 2002). This process begins with gradually increasing low-intensity stimulus that leads to primary depolarization of 1a afferents in the muscle spindle (Palmieri et al., 2004). Stimulation of 1a afferents result to action potentials directed to the spinal cord. Adequate activation of 1a afferents depolarize the presynaptic terminal, releasing neurotransmitters in the synaptic cleft of 1a alpha MN synapse. Then, post synaptic potentials in the MNs occur. When the excitory post synaptic potentials are capable of MN depolarization, acethycholine is released at the neuromuscular junction. This leads to muscle contraction, eliciting noticeable H-reflex tracing on electromyography (EMG) recording. Researchers suggest that regulation of H-reflex happens in the primary motor area of the central nervous system (Rollnik et al., 2000; Tanaka et al., 2015; Tanaka et al., 2012). The H-reflex is a useful technique in clinical, exercise, and sport settings (Gajos et al., 2014; Palmieri et al., 2004; Zehr, 2002).

Heart rate variability biofeedback (HRV BFB) is a non-invasive, visually-guided paced breathing intervention believed to influence autonomic modulation (Lehrer et al., 2000; Mackinnon et al., 2013; Huang et al., 2018). HRV BFB involves breathing at a pace (0.075 – 0.12 Hz or 4.5 – 7.2 breaths/min) known as resonance frequency (RF), that generates rhythmical changes in the baroreflex and activates the resonant properties in the cardiovascular system (Lehrer and Eddie, 2013; Lehrer and Gevirtz, 2013; Lehrer et al., 2000; Vaschillo et al., 2006). The baroreflex

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plays a crucial role in the regulation of blood pressure that operates in a negative feedback loop, such that increasing blood pressure reduces heat rate and vice versa. Key features in HRV BFB include occurrence of maximal heart rate oscillations and simultaneous oscillations of both heart rate and respiration. Regular HRV BFB practice enhances baroreceptor activity, vagal afferents and efferents that facilitate beneficial health changes (Gevirtz, 2013; Lehrer et al., 2003; McCraty and Shaffer, 2015; Prinsloo et al., 2014).

The application of HRV BFB may influence motor function through the central autonomic network (Beissner et al., 2013; Bennaroch, 1993). However, no study has investigated neural adaptation within HRV BFB context. Examining the magnitude of neural excitability from HRV BFB may provide noteworthy consideration of HRV BFB in neuromuscular settings. Thus, the purpose of this study was to determine motoneural excitability from acute HRV BFB.

Methods

Participants

Twelve healthy males (height: 173.7 ± 7.18 cm; weight: 72.7 ± 17.7 kg; age: 24.0 ± 5.02 yrs) with no reported lower body injury 2 months prior to experimentation were recruited for this pilot study. Participants were asked to avoid any strenuous physical activity and caffeine containing food/beverage 24 hours prior to experimentation. The last meal intake by participants was at least 2 hours before testing. A written informed consent was collected before any further experimentation. Ethical clearance for this study was approved by the University of Tasmania Human Research Ethics Committee (H0016508). The protocol of this study was in adherence with the Declaration of Helsinki for human experimentation.

Procedures

The participants attended three experimentation sessions, separated by 48 hours, at the Exercise Performance Laboratory of University of Taipei. Anthropometric measurements (height and weight), identification of individual RF were administered in the first session. Participants executed a randomized breathing at RF (HRV BFB) or normal breathing (CON) in the second session. A crossover HRV BFB or CON was performed in the third session. HRV BFB was carried out using a commercial HRV BFB equipment (HRV Starter System, Thought Technology, Canada). In CON, participants underwent normal breathing for 10 minutes with gaze focused at the computer screen. Figure 1 displays the experimental protocol for this study. Pre and post H-reflex indices in HRV BFB and CON were subjected for analysis.

Measures

Resonance Frequency

A commercial biofeedback equipment (HRV Starter System, Thought Technology, Canada) measuring respiration and HRV was used to identify RF. The RF testing involved two-minute breathing at various frequencies (6.5, 6.0, 5.5, 5.0, 4.5 times per min). Prior to RF testing, participants underwent a 5-minute familiarization of diaphragmatic breathing with nasal inhalation and pursed lip exhalation. RF was established as the breathing pace that demonstrated the highest amplitude in low frequency HRV (Lehrer et al., 2000; Task Force, 1996).

H-reflex

H-reflex was measured from the soleus (SOL) EMG of the dominant leg at resting condition. A surface electrode (TSD150A, Biopac Systems, USA) was placed 2/3 from the medial condyles of the femur to the medial malleolus and central position in the medial-lateral direction of the border of SOL. In addition, a reference electrode was attached on the dominant hand of the participants. Further, an adhesive electrode (10 × 10 mm, FA 25, Gem-Stick, Australia) was placed on the popliteal fossa as cathode and another (50 × 50 mm, Life Care, Taiwan) adhesive electrode was fixed over the patella as the anode. Participants were asked to sit with dominant knee extended at approximately 180 degrees, while non-dominant knee flexed at 90-120 degrees. Hip flexion was 90-120 degrees. Recording of EMG signals were acquired using a commercial data acquisition system (MP150, Biopac Systems, USA) filtered with a band-pass range of 10-500 Hz and amplified with a gain of 1,000 times. The common mode rejection ratio for the EMG amplifier was 100 dB. Sampling rate was set to 2.5 kHz. A single electrical impulse was applied to the posterior tibial nerve via an electrical stimulator (DSH7, Digitimer, Herfordshire, UK) with 1000 μs pulse duration to identify SOL H-reflex response. To

determine maximal peak-to-peak amplitude of H-wave and M-wave, stimulation intensity was increased with 10 mA increments until maximum M-wave (Mmax) was identified. The stimulation intensity for exhibiting maximum H-wave (Hmax) was determined by using incremental 2 mA until the H-reflex threshold is identified (see Figure 1). The stimulation intensity was set at 10% - 20% Mmax to elicit an H-reflex response in the ascending portion of the recruitment curve (Chen and Zhou, 2011). Each stimulation intensity was applied four times with 10 s inter-stimulation interval. A commercial data acquisition system (Acqknowledge 4.2.1 software, Biopac Inc., USA) with custom-written program was used to synchronize the electrical stimulation trigger and EMG recording. During H-reflex testing, participants were encouraged to breathe normally. A sample of H wave and M wave recruitment curves from one representative participant is displayed on Figure 2.

The following H-reflex indices were measured offline: a) M wave at maximal H-reflex (M wave at Hmax); b) Hmax; c) Mmax; and, d) Hmax/Mmax ratio (maximal Hmax response to normalized Mmax). Hmax estimates motoneuron excitability activated by Ia afferents, elicited from electrical stimulations (Gajewski and Mazur-Różycka, 2016; Knikou, 2008). Mmax represents recruitment of motor axons and provides an estimate of the size of motoneural pool response (Chen et al., 2012; Chen et al., 2015; Palmieri et al., 2004; Pierrot-Deseilligny and Mazevet, 2000). Hmax/Mmax ratio is the proportion of the entire MN pool recruited by Ia afferents (Kipp et al., 2011; Palmieri et al., 2004). M wave at Hmax refers to the proportion of motor fibers activated by electrical stimulations (Chen et al., 2015; Knikou, 2008). Additionally, the M wave at Hmax was utilised as a confirmatory marker to determine the consistency of relative stimulation intensity at the tibial nerve (Zehr, 2002).

Statistical Analysis

Data are expressed as mean ± standard deviation. The Wilcoxon-Signed Rank Test was carried out to determine significant difference in pre and post indices in HRV BFB and CON. Effect size was computed from matched-pairs rank-biserial correlation (*r*) using the simple difference formula (Kerby, 2014). The *r* is interpreted as small (0.10), medium (0.30), large (0.50), verylarge (0.70) (Cohen, 1988; Rosenthal, 1996). Statistical analysis was administered using a commercial statistical package (SPSS version 22, IBM Corp, USA) with alpha set at 0.05 level.

Results

The RF of participants ranged from 4.4 to 7.4 breaths per minute (0.09 ± 0.02 Hz). Non-significant difference in of M wave at H max confirmed the same relative stimulation intensity at tibial nerve in HRV BFB (Z = -0.67, p = 0.50) and CON (Z = -0.90, p = 0.37). Table 1 displays the pre and post H-reflex indices in HRV BFB and CON.

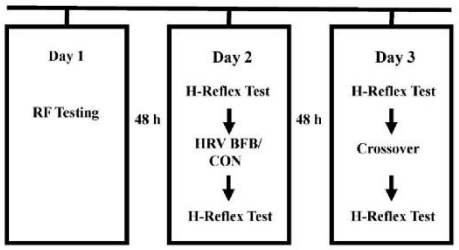

Figure 1

Experimental protocol

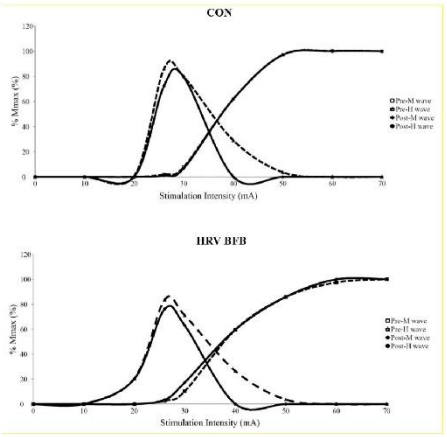

Figure 2

Sample of H wave and M wave recruitment curves

Table 1

H-reflex indices in HRV BFB and CON

Parameter	HRV BFB				CON			
	Pre	Post	p-value	*r*	Pre	Post	p-value	*r*
M wave at Hmax (mV)	0.38 ± 0.23	0.39 ± 0.25	0.50	0.22	0.59 ± 0.61	0.51 ± 0.44	0.37	-0.29
Hmax (mV)	1.84 ± 0.74	1.67 ± 0.90	0.04	-0.67	1.83 ± 0.77	1.83 ± 0.80	0.88	-0.05
Mmax (mV)	3.32 ± 0.91	3.26 ± 1.00	0.37	-0.30	3.36 ± 1.08	3.37 ± 1.18	1.00	0.00
Hmax/Mmax ratio	0.55 ± 0.15	0.50 ± 0.18	0.02	-0.84	0.54 ± 0.15	0.54 ± 0.15	0.67	-0.14

** M wave at Hmax – M wave at Hmax; Hmax – maximal H-reflex response; Mmax – maximal M-wave response; Hmax/Mmax ratio – proportion of the motoneural pool recruited by 1a afferents.*

There was a significant difference in pre-Hmax and post-Hmax at Z = -2.40, p = 0.04, r = -0.67. No difference was observed in pre-Mmax and post-Mmax at Z = -0.89, p = 0.37, r = -0.30. A significant difference existed in pre-Hmax/Mmax ratio and post-Hmax/Mmax ratio at Z = -2.35, p = 0.02, r = -0.84. The M wave at Hmax/Mmax ratio was not significantly different in pre and post-test conditions at Z = -0.72, p = 0.47, r = 0.25.

CON

CON exhibited non-significant pre and post differences in Hmax (Z = -0.16, p = 0.88, r = -0.05), Mmax (Z = 0.00, p = 1.00, r = 0.00), Hmax/Mmax ratio (Z = -0.04, p = 0.67, r = -0.14), and M wave at Hmax/Mmax ratio (Z = -0.59, p = 0.56, r = -0.19).

Discussion

This novel study examined the effect of an acute HRV BFB on the SOL motoneural pathway using H-reflex. Findings revealed significant reduction in Hmax (r = - 0.67, large) and Hmax/Mmax ratio (r = - 0.87, very large) after HRV BFB.

Indeed, HRV BFB facilitated change in SOL neural transmission in this study. This can be observed by decreased resting Hmax/Mmax after HRV BFB. The alteration in Hmax/Mmax ratio is attributed tom maximal H-reflex amplitude rather than maximal M-wave amplitude, supported by reduced Hmax and non-difference in Mmax (Palmieri et al., 2004). The decreased Hmax/Mmax ratio reflects increased presynaptic inhibition from afferent depolarization (Kipp et al., 2011; Pierrot-Deseilligny and Mazevet, 2000; Zehr, 2002). It seems that the acute HRV BFB reduced the neural excitability between Ia afferents and alpha MNs. However, the exact mechanisms lowering 1a afferent activation after HRV BFB are unclear. A recent study by Bagheri et al. (2018) posted a negative relationship of resting Hmax/Mmax with vertical jump and balance task. This suggests the importance of resting neural excitability in neuromuscular tasks. More work is needed to establish the association of Hmax/Mmax attenuation from HRV BFB in voluntary muscle contraction and proprioception.

Limitations of the current study are acknowledged. First, generalization of results should be avoided as the neural outcomes from acute HRV BFB are only applicable to the sample population involved. Also, assessment of H-reflex was administered at resting condition. Inclusion of voluntary muscle contraction task whilst investigating H-reflex after HRV BFB may exhibit alteration of H-reflex from abolition of post-activation depression, clearer distinction of neural characteristics at lower stimulation intensities, and identification active motoneuron pool (Burke et al., 1989). Lastly, concurrent measurement of HRV with H-reflex after HRV BFB can determine trends in vagal reactivity from neural excitation.

The findings of this study serve as an impetus for future research within the context of HRV BFB and neuromuscular function. In conclusion, an acute HRV BFB facilitated depression of soleus motoneural excitability in the resting condition.

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