Cardiorespiratory Optimized Guided-Breathing for Post-Stress Recovery in a Group Setting

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Abstract

Stress is the feeling of emotional strain in response to a perceived threat that disturbs the homeostasis and affects our health and well-being. Short-term stress has some beneficial effects such as improving alertness and performance and boosting memory, but prolonged stress responses can have deleterious effects on human health, including tissue damage and disease. Thus regulating stress levels is important for dealing with difficult situations to mitigate negative impacts. Prevailing approaches to treating stress have some limitations and drawbacks. Slow breathing/Resonant frequency breathing or HRV biofeedback and Music Therapy are some of the widely used methods for dealing with stress and anxiety. These methods are thought to stimulate the vagus nerve that promotes autonomic balance and hence reduce symptoms of stress. The current study investigated the effects of relaxing music and slow breathing/resonance frequency breathing on heart rate variability and respiration as well as on subjective measures of perceived stress. Although relaxing techniques are often administered in group classes, research studies in groups are rare. To our knowledge, this is the first study to investigate the effects of music listening and slow breathing in reducing stress evoked by watching a stressful movie in a group setting. The study sought to evaluate the effectiveness of the aforementioned interventions in reducing stress, measured by psychophysiological and self-report measures. Thirty-two participants were recruited and randomly assigned to two groups (Music, Breathing). We hypothesized that after watching the stressful movie, the Breathing group would show greater physiological and self-report changes marking greater stress reduction compared to the Music group. Results indicated that slow Breathing affected perceived stress as well as HRV, whereas Music affected perceived stress, but had no significant effect on HRV. Also, results indicated that Slow Breathing and not Music reduced the complexity of heart and respiration signals. Moreover, the study found that respiration and heart rhythm synchronized maximally during slow breathing. The results suggest that the interventions studied in this research can be used as an effective stress reduction tool in a group setting.

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Contents

List of Figures

List of Tables

Declaration of Authorship

I, Debanjan Borthakur, declare that this thesis titled, "Cardiorespiratory optimized guided-breathing for post-stress recovery in a group setting" and the work presented in it are my own.

Chapter 1

Introduction

1.1 Introduction

Stress has a considerable effect on health. Regulating stress levels is important for practitioners in high-stress occupations like first responders and also for dealing with stressful situations in an everyday context. have a substantial negative impact on cardiovascular disease, anxiety, depression, chronic pain, and addiction (Arpaia and Andersen 2019). Stress is also connected to the occurrence of hypertension (Spruill 2010), metabolic syndrome (Kyrou and Tsigos 2006) and obesity (Brunner et al. 2007). also impact our memory, cognition and immune system. Studies have shown that cause functional and structural changes in the hippocampus (McEwen 1999) and can lead to atrophy and neurogenesis disorders (Lupien and Lepage 2001). Stress can affect cognitive abilities in general (Scholey et al. 2014). Stress can also affect the immune system (Khansari et al. 1990). It has also been found that acute or chronic stress has a deleterious effect on the function of the cardiovascular system (Rozanski et al. 1999; Herd 1991). Short-term stress has some beneficial effects from preserving homeostasis of cells to engaging responses in dangerous situations that aid in survival (Yaribeygi et al. 2017) but chronic stress has harmful effects as outlined above. Therefore, stress reducing techniques can have a positive effect on our health and well-being. In this thesis we investigate effects of slow guided breathing and listening to relaxing music on physiological responses and self-report measures of stress.

Studies have found that regulating mental stress reduces a number of risks including cardiac diseases (Steptoe and Kivimäki 2012). It is not surprising that effective stress management systems are under continuous research and development. Music therapy and Resonance breathing have been found effective in treating stress-related symptoms and promoting relaxation (Jerath et al. 2006; Ventura et al. 2012). Studies have shown anxiety and respiratory rate reduction in voluntary breathe-holding and guided breathing techniques (Meuret et al. 2018; Fuchs et al. 2018). Besides breathing, other non-invasive techniques for stress reduction include cognitive behavioral therapy, mindfulness-based stress reduction (Grossman et al. 2004), yoga (Chong et al. 2011), meditation (Peterson and Pbert 1992) and biofeedback based on heart rate variability (HRV) (Hassett et al. 2007; Moss 2004; Lehrer et al. 2003).

Music is another technique that targets the brain's motivation and reward pathways to induce psychophysiological changes (Salamon et al. 2003; Esch et al. 2004). Reduction in blood pressure, respiratory rate and psychological distress were observed in Cardiac patients who listened to 30 minutes of symphonic music with nature sounds (Cadigan et al. 2001). Self-selected relaxing music can also reduce anxiety in surgery patients (Cadigan et al. 2001). Studies have found that music was effective in reducing cortisol levels (Suda et al. 2008). For example, one study observed a lower increase in cortisol levels following a stressor when compared to a non-music control condition (Khalfa et al. 2003). In the present research, we are interested in the comparison of the effectiveness of these two Intervention (Breathing, Music) as measured by reported stress, and physiological indicators of stress (Heart Rate Variability, Respiration Rate, and Galvanic skin Responses).

In the following sections, we will discuss how stress affects Autonomic Nervous System and how effective interventions can be used to mitigate the effects of stress.

1.2 Stress and Autonomic Nervous System

The part of the nervous system associated with involuntary regulation of the bodily functions is called the Autonomic Nervous System. The Autonomic Nervous System (ANS) consists of sympathetic nerves and parasympathetic nerves. The two divisions are complementary, with, for example, activity of sympathetic nervous system (SNS) increasing heart rate and activity of the parasympathetic nervous system (PNS) decreasing heart rate.

ANS can be substantially impacted by Stress. A cascade of stress hormones can lead to physiological changes in the body in response to stressors such as a work deadline or a natural disaster. The heart rate can go up and sweating can happen. This process evolved as a survival mechanism and is called the fight or flight response. Quoting from Nesse and Young (2000) "..Most stresses in modern life arise not from physical dangers or deficiencies, but from our tendency to commit ourselves to personal goals that are too many and too high. When our efforts to accomplish these goals are thwarted or when we cannot pursue all the goals at once and must give something up, the stress reaction is expressed. In short, much stress arises, ultimately, not from a mismatch between our abilities and the environment's demands, but from a mismatch between what we desire and what we can have." Prolonged exposure to stressors takes a toll on the body and can contribute to anxiety and depression. There are two phases of the stress response. The first one is the rapid activation of the Sympathetic Adreno-Medullar (SAM) axis and the other one is the Hypothalamus-Pituitary-Adrenal (HPA) axis (Godoy et al. 2018). In the first phase of the stress response, SAM provides a rapid physiological adaptation that results in short-lasting responses that include increased alertness, vigilance and appraisal of the situation (Kloet et al. 2005). The slower HPA axis can result in an amplified and long-lasting response. The two axes (SAM, HPA) are shown in Figure 1.1

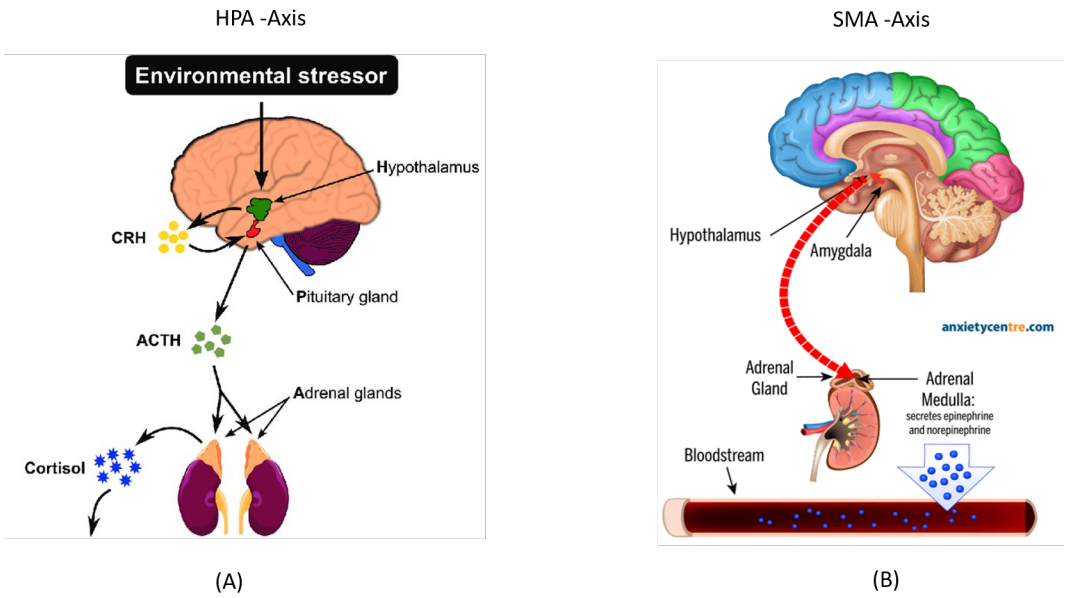

FIGURE 1.1: HPA and SMA axes. A shows the Hypothalamic- pituitary-adrenal (HPA) axis. When the brain experiences a stressor, the hypothalamus secretes corticotrophin- releasing hormone (CRH). CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) in the pituitary gland. The cortex of the adrenal glands then produce glucocorticoids (cortisol) in response to ACTH, which will generate a stress response. B shows the SMA axis. The hypothalamus activates the adrenal medulla, which results in the production of the hormone adrenaline which prepares the body for a fight or flight response. It leads to the arousal of the sympathetic nervous system and reduced activity in the parasympathetic nervous system. The figures are adapted from *anxietycentre.com*

The sympathetic nervous system is activated by the hypothalamus by sending signals through the autonomic nerves to the adrenal glands. In response, these glands pump the hormone epinephrine into the bloodstream (Godoy et al. 2018). On the other hand, If the brain continues to perceive something as potentially threatening, the hypothalamus releases corticotropin-releasing hormone (CRH), which travels to the pituitary gland, which triggers the release of adrenocorticotropic hormone (ACTH). ACTH travels to the adrenal glands, and this leads to the release of cortisol (Godoy et al. 2018). When the stressor disappears, cortisol levels fall. The parasympathetic nervous system then takes over and suppresses the stress response.

The activation of sympathetic nervous system is reflected in several psychophysiological changes, including increased heart rate, blood pressure, pupil dilation, sensory perception, and blood sugar. An effective intervention can bring about an optimal balance between sympathetic and parasympathetic nervous systems and thereby help reduce the stress response.

1.3 Stress and Heart Rate

Heart rate is modulated by the Autonomic Nervous System in two ways, 1) by vagal/parasympathetic modulation, which slows the heart and 2) by sympathetic modulation, which increases heart rate. The tenth cranial or vagus nerve is responsible for the vagal/parasympathetic modulation of the heart (Breit et al. 2018). The vagus nerves innervate the sinoatrial node, atrioventricular conducting pathways and the atrial myocardium (Hainsworth 1998). The heart rate slows down with the slowing of the rate of spontaneous depolarization of the pacemaker cells. The sympathetic innervation to the heart originates in the cells of the intermediolateral column of the spinal cord. All regions of the heart are innervated, including pacemaker and conducting tissue, and the atrial and ventricular myocardium (Hynynen 2011), with the increased heart rate preparing the body for the fight or flight response. It is noteworthy that sympathetic responses are much slower than parasympathetic responses (Figure 1.2).

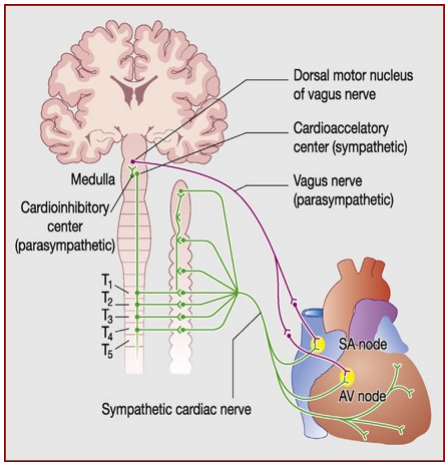

FIGURE 1.2: Autonomic Innervation of Heart. Anatomy of the sympathetic and parasympathetic innervation of the heart where AV stands for atrioventricular and SA for sinoatrial. The Figure is adapted from (Scridon et al. 2018)

HR can be calculated from Electrocardiogram (ECG) or Photoplethysmogram (PPG) recordings. Most published studies have used electrocardiagram (ECG) to record cardiac activity. ECG has drawbacks as several leads need to be connected to the participant, thus limiting mobility, convenience and flexibility. Pulse plethysmography (PPG) is an optical measurement method that can be used to replace ECG. Previous literature confirms that PPG provides accurate interpulse intervals and HRV measures can be accurately derived in healthy participants under ideal condition (Lu et al. 2009). Hence, the limitations associated with ECG can be overcome by the use of state of the art PPG sensors, which are inexpensive and more suitable for ecological settings. Our previous pilot studies have also shown that PPG can actually be used in lieu of ECG in ideal conditions. Moreover, we have compared publicly available simultaneous PPG and ECG

signals and found that there was no visible differences in the HRV calculated from the aforesaid signals.

1.4 Stress and Heart Rate Variability

HRV refers to the variation in the time interval between heartbeats and is measured as the beat-to-beat interval in millisecond (ms). HRV is related to emotional arousal and is known to be reduced in individuals with anxiety or stress disorders. HRV, is affected by both parasympathetic input or withdrawal (Berntson et al. 1997). Low HRV is associated with adverse chronic psychological stress (Prinsloo et al. 2011). Studies have reported a reduction in the high-frequency component of HRV, which is considered a proxy for vagal activation, in stressor compared to the control sessions (Hjortskov et al. 2004).

HRV-biofeedback can help increase HRV and is known to have a positive effect on anxiety and anxiety disorders for a variety of user groups (Kennedy and Parker 2019; Stern 2012; Firth-Clark et al. 2019). Optimal heart rate variability is associated with optimal responses to environmental input and lower stress levels (Shaffer and Ginsberg 2017). It is also observed that breathing rate increases with higher stress levels (Ristiniemi et al. 2014) and galvanic skin responses (reflecting sweating) increase with higher stress levels due to ionic filling of the skin's sweat glands in response to sympathetic nervous activation (Healey and Picard 2005; Lunn and Harper 2010) .

1.5 Stress and HRV Guided Breathing

HRV breathing biofeedback systems guide users to an appropriate breathing rate which reduces heart rate and maximizes HRV, causing an increased parasympathetic activation which is accompanied by the subjective experience of stress relief and eventual reduction in stress levels. Some examples of biofeedback systems are emWave (Ratanasiripong et al. 2015), Wild Divine (Cutshall et al. 2011), StressEraser (Lee and Finkelstein 2015) etc.

Researchers have created various HRV biofeedback real-time applications such as adaptive bio-feedback games, guided breathing apps, and biofeedback based on sonic , haptic, or visual interfaces. In Borthakur et al. (2019) authors have proposed sonification of HRV features. Both open and closed loop designs have been proposed in the literature. Open-loop systems attempt to guide breathing without feeding physiological state data back to the user, while closed loop systems feed physiological state data back to the user. Visual systems use some kind of visual feedback such as an opening and closing circle (Plans et al. 2019). Auditory systems may present music syncing the phasic relationship between sound and respiration via the target phase defined on the MIDI score (Sato and Moriya 2019). Closed-loop systems typically present user's physiological state (such as HRV) as measured using a stress related physiological marker, potentially in addition to a visual or auditory signal that guides the user's breathe. A common

approach is to visualize transformed inter-(heart)beat-interval (IBI) as an respiratory sinus arrythmia (RSA) wave. This allows users to see the real-time fluctuations of their pulse rate (Yu et al. 2016). Common are adaptive biofeedback games with breathing frequency as an input signal (Parnandi et al. 2013), or haptic interfaces designed with inflatable airbags (Yu 2016). All forms of guided breathing should have a substantial effect on Heart Rate Variability. The *breathe with touch* a tactile interface provides breathing guidance through shape changing airbags. The airbag inflates and deflates at a specific rhythm that simulates the targeted respiratory pattern. A comparison of auditory and visual feedback showed that auditory feedback was as effective as visual feedback (Yu et al. 2015). Veterans with combat-related PTSD exhibit significantly depressed HRV, which is a sign of ANS dysregulation, compared to non-PTSD. Veterans receiving HRV biofeedback have shown a reduction in PTSD symptoms post-treatment (Tan et al. 2011). Respiratory sinus arrhytmia (RSA) is heart rate variability in synchrony with respiration, whereby the R-R interval (the distance between two consecutive R peaks in ECG or Peak-to-Peak in PPG) on an ECG is shortened during inspiration and prolonged during expiration (Yasuma and Hayano 2004). RSA can be useful in measuring emotional arousal. Increased skin conductance and decreased RSA have been associated with arousal independent of valence. RR interval was related to affective valence and not arousal (Frazier et al. 2004). Group level studies that have examined HRV-guided breathing biofeedback are rare. Wallmark et al. (2013), investigated the affects of a Buddhist meditation intervention on empathy, perceived stress, mindfulness, and self-compassion. The meditators (n=20) were divided into two smaller groups of 10 participants each, and two meditation sessions were conducted in successive order. They found an increase in altruistic orientation in the intervention group, decrease in perceived stress, and increases in self-compassion and mindfulness. Changes in HRV have also been seen in Heartfulness Meditation (Léonard et al. 2019).

The coupling between heart rate and respiration has also been studied. Significantly increased coherence between heart rate and breathing during meditation compared to baseline was observed previously (Peng et al. 2004).

Different effects of HRV can be observed for high and low frequency components of the heart signal. Taking a psychological test was found to lead to a significant reduction in the high frequency component of HRV and a significant increase in the low frequency component (Delaney and Brodie 2000). There was also a significant increase in the low frequency to high frequency ratio. These physiological effects were related to self-evaluation of physical tension and emotional state measured on visual analog scales (VAS).

In sum, the efficacy of HRV breathing biofeedback has been established in the literature and such systems have been used effectively for reducing stress in a variety of situations. In the present study, we used a design process that follows principles of somasthetics appreciation in order to make use of the embodied nature of the breathing entrainment process. Aesthetically appealing embodied sounds are less likely to distract

or annoy users over long exposure. The breathing stimuli used in the present study was designed by Muvik Labs *https://muviklabs.io/*

1.6 Stress and Music

Several studies have investigated the effect of music and music therapy in reducing stress. For example, one study examined passively listening to music before, during and after opthalmic surgery (Fernell 2002). They found that patients in the Music group had lower HR and BP levels compared to patients in the no Music group in the preoperative period, during surgery, and also after surgery. Another used music as a cognitive behavioural intervention for anxiety and pain in elderly cataract patients awaiting surgery (Reilly 2000). Robb et al. (1995) investigated the effects of music with a combination of progressive muscle relaxation, imagery and passive listening to music in pediatric patients. Some clinical and laboratory-based studies have revealed that listening to music may reduce stress by decrease sympathetic activity (Bartlett 1996; Standley 1992). Han et al. (2010) claimed that music reduces stress by entraining respiration and blood pressure. On the other hand, Koelsch et al. (2011) argued that the psychological effects of music are channelled through various neurological pathways that include the mesolimbic dopaminergic system and the central nucleus of the amygdala before they exert influence on hormones, cells and blood pressure and thus reduce stress.

Music effects HRV as well as synchronization of cardiac and respiration rhythms. Authors in Vickhoff et al. (2013) showed that coherence analysis of HR and respiration mirrors music structure. Music structure determines respiration rates and in those rates respiration/HRV entrainment is seen. In their work the coherence was high during the mantra (at 0.1 and the 0.2 Hz harmonic) as well as during the hymn (at 0.05, 0.1, and 0.2Hz).

Previous studies have compared musically driven resonance breathing with selected relaxing music (Fuchs et al. 2018), In this research, the music therapist received direct physiological feedback of patients' respiratory frequency curves in order to musically guide the participants to breathe at a low frequency (close to 6 bpm). Given that it is not always possible to have a music therapist present, our present study focuses on somasthetically designed breathing sounds created by Muvik labs to guide participants to breathe at 0.1 Hz.

1.7 Measures of Heart Rate Variability

HRV reflects autonomic modulation of heart. Heart Rate (HR) and heart rhythm are controlled by the ANS (European Society of Cardiology et al. 1996). The balance between SNS and PNS control the Heart Rate. Release of acetylcholine from the vagus nerve is responsible for the parasympathetic influence. Similarly, the sympathetic influence on HR is mediated by the release of epinephrine and norepinephrine. An increase of SNS activity or reduction in PNS activity results in acceleration of the heart

rhythm, whereas decreased SNS or increased PNS activity results in deceleration of heart (Acharya et al. 2006). Under resting conditions vagal/parasympathetic activity dominates over sympathetic activity, and the interplay between the two systems results in the variations in the heart period (HRV). Thus, heart rate variability provides information about the functioning of nervous control on the HR and the heart's ability to respond to moment to moment challenges (Acharya et al. 2006). A number of measures of heart rate variability have been devised. Early studies found that increases in stress were associated with decreases in the RR interval (Sloan et al. 1994). Authors also found that psychological stress was significantly associated with an increase in the low frequency to high frequency (LF/HF) ratio, suggesting increased sympathetic activity during stress. Here LF is defined as 0.04–0.15 Hz and HF as 0.15–0.40 Hz. However, when the breathing rate is slowed to .1, the RSA will transfer from the HF band to the LF band, increasing the LF/HF ratio with involvement of the parasympathetic system (Russo et al. 2017)

Time-domain indices of HRV quantify the amount of variability in interbeat intervals (IBI), also called NN (normal to normal) intervals, which are the time periods between successive heartbeats. Some of the time-domain indices are shown in Table: 1.1 (Shaffer and Ginsberg 2017).

Table 1.1: HRV Time-Domain Measures.

Measures	**Definition**
SDNN	The standard deviation of NN intervals.
RMSSD	The square root of the mean of the squares of the successive differences between adjacent NNs.
SDSD	The standard deviation of the successive differences between adjacent NNs.
NN50	The number of pairs of successive NNs that differ by more than 50 ms.
pNN50	The proportion of NN50 divided by total number of NNs.
NN20	The number of pairs of successive NNs that differ by more than 20 ms.
pNN20	The proportion of NN20 divided by total number of NNs.

The standard deviation of the IBI (RR) of normal sinus beats (SDNN) is measured in milliseconds. "Normal" means that abnormal beats, like ectopic beats, have been removed. To measure SDNN a 5 min recording is typically made (European Society of Cardiology et al. 1996) , although reliable ultra-short term HRV (10, 30, 40, 50 sec) have also been documented previously (Salahuddin et al. 2007; Castaldo et al. 2016) , Both SNS and PNS activity contribute to SDNN (Umetani et al. 1998), In short-term resting recordings, the primary source of the variation in SDNN is parasympathetically-mediated RSA (naturally occurring variation in HR that occurs during the breathing cycle), most specifically with slow, paced breathing protocols (Shaffer et al. 2014), The low Frequency band (LF) makes the most contribution to SDNN. SDNN is also considered an index of physiological resilience against stress. Kang et al. (2004) found that SDNN was significantly lower in a high strain group than in a low strain group. A higher SDNN

indicates higher parasympathetic dominance and a reduced level of stress, whereas lower SDNN indicates lower parasympathetic dominance and a higher level of stress. HR Max-HR Min also reflects RSA. RSA is directly proportional to HRV (Thompson et al. 2015). Both SNS and PNS activity contribute to SDNN and SDNN is highly correlated with both LF band power and total power (Umetani et al. 1998). As well, greater power than the HF band might contribute to SDNN. In the present thesis, we examined SDNN and HR, but see Shaffer and Ginsberg (2017) for a discussion of other time domain measures.

Frequency-domain measurements estimate the distribution of absolute or relative power into four frequency bands, ultra-low-frequency (ULF), very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF) (European Society of Cardiology et al. 1996). Some frequency domain indices are shown in Table: 1.2 : The ULF and VLF

Table 1.2: HRV Frequency-Domain Measures.

Measures	**Definition**
ULF power	Absolute power of the ultra-low-frequency band (below 0.003 Hz).
VLF power	Absolute power of the very-low-frequency band (0.0033–0.04 Hz)
LF power	Absolute power of the low-frequency band (0.04–0.15 Hz).
HF power	Absolute power of the high-frequency band (0.15–0.4 Hz).
LF/HF	Ratio of LF to HF power.

are often ignored due to the lack of long enough data recordings to accurately resolve these frequencies (Ramshur 2010). Here we discuss LF, HF and LF/HF ratio, which were analysed in this work. The amount of power contained within a frequency band is obtained by integrating the Power Spectral Density (PSD) between the band frequency limits.

The LF band (0.04–0.15 Hz) is also called the baroreceptor range because it mainly reflects baroreceptor activity during resting conditions (McCraty and Shaffer 2015). LF power is affected by the SNS, but it is primarily affected by the PNS (Shaffer and Ginsberg 2017) and by BP regulation via baroreceptors (Berntson et al. 2007; Goldstein et al. 2011; Shaffer et al. 2014). When we breath at a very slow rate such as 0.1 Hz, the RSA shifts to the LF band, and under these conditions, the LF predominantly reflects parasympathetic activity through vagal activity (Ahmed et al. 1982; Tiller et al. 1996). Thus, LF HRV increase should be interpreted as being sympathetically driven during a stressful situation with normal or accelerated breathing, but it reflects almost entirely parasympathetic activity during slow breathing (Shaffer et al. 2014; Lehrer 2007). Pharmacological blockade studies also confirm this. In Kromenacker et al. (2018) authors blocked the paraysmpathtic tone by Glycopyrrolate which is a synthetic anticholinergic agent that inhibits the muscarinic actions of acetylcholine on autonomic nerve endings. They found, in the parasympathetic blockade condition, the peak power is suppressed in slow breathing condition which is not the case with sympathetic blockade. This would mean that LF power during slow breathing is parasympathetic (vagally mediated).

In normal breathing the HF band reflects primarily parasympathetic activity. It is also called the respiratory band as it corresponds to the HR variations related to the respiration. It is also noteworthy that HF power is considered as the index of vagal modulation of HR but it does not represent vagal tone although LnHF power can be used to estimate vagal tone or RSA (Egizio et al. 2011). It's because the HF oscillations coincide with the typical respiration frequency in normal breathing condition (10 and 24 breath cycles per minute). At 0.1 Hz or 6 bpm RSA also resonates with the LF baroreflex that integrates breathing frequency and Mayer waves (Julien 2006). The baroreflex is the body's homeostatic mechanisms that help to maintain blood pressure at a constant level. This creates a resonance and maximises RSA as well as inflates the power in LF band. Both HRV (RSA) and baroreflex sensitivity are maximised when respiration is slowed to 6 breaths per min through resonance, although this resonant frequency does vary between individuals (Bernardi 2001; Radaelli et al. 2004; Badra et al. 2001).

LF/HF ratio reflects the ratio between SNS and PNS activity (Shaffer et al. 2014). Sympathovagal balance in LF/HF reflects the weight of sympathetic versus parasympathetic autonomic control, A higher LF/HF HRV ratio reflects sympathetic dominance and a lower ratio reflects parasympathetic dominance (Malliani et al. 1991; Akselrod et al. 1981) but in resonance frequency breathing a high LF/HF ratio can be interpreted as higher levels of baroreflex and vagal nerve activity (Steffen et al. 2017). It is noteworthy that interpretation of LF/HF changes during slow breathing as discussed above. So, interpretation of LF as well as LF/HF should be specific to measurement conditions. For instance, similar to slow breathing, when LF is calculated while sitting upright during resting conditions, the primary contributors to LF are PNS activity and baroreflex activity and not SNS activity (Kember et al. 2001; Eckberg 1983).

Non-linear HRV measures help us quantify the unpredictability of a time series (Stein and Reddy 2005). HRV in general displays the characteristics of a nonlinear signal and non-linear interaction between the PNS and SNS may contribute to heart beat complexity in healthy participants (Levy 1971). The heart beat signal also shows 1/f-like scaling which also points to the Non Linear nature of the series (Ivanov et al. 1999). Some Non-Linear measures are shown in Table 1.3.

A Poincaré plot (return map) is a scatter plot of RR interval against the prior interval. This plot can be used to differentiate between a pathological state and a healthy state. A healthy participant typically displays a 'comet' shaped plot. This shape does not vary with respiration rate (Guzik et al. 2007). On the other hand, patients with heart failure display atypical 'torpedo' or 'complex' patterns (Woo et al. 1992).

Similar to approximate entropy, the sample entropy measures the regularity and complexity of a time series (here the RR and the Respiration Time Series). It was designed to provide a less biased and more reliable measure of signal regularity and complexity (Lippman et al. 1994). A higher value of Sample Entropy means a low predictability of fluctuations in successive RR intervals. Similarly, a lower value of Sample Entropy means that the signal is more predictable and less complex.

Table 1.3: HRV Non Linear Measures.

Measures	**Definition**
S	Area of the ellipse which represents total HRV
SD1	Poincaré plot standard deviation perpendicular the line of identity
SD2	Poincaré plot standard deviation along the line of identity
SD1/SD2	Ratio of SD1-to-SD2
ApEn	Approximate entropy, that measures the regularity and complexity of a time series.
SampEn	Sample entropy, measures the regularity and complexity of a time series
DFA $\alpha 1$	Detrended fluctuation analysis, which describes short-term fluctuations.
DFA $\alpha 2$	Detrended fluctuation analysis, which describes long-term fluctuations.
D2	Correlation dimension, which estimates the minimum number of variables required to construct a model of system dynamics

Detrended fluctuation analysis extracts the correlations between successive RR intervals over different time scales. This DFA analysis results in slope $\alpha 1$, that describes short term fluctuations, and slope $\alpha 2$, describes long-term fluctuations. The short-term correlations reflect the baroreceptor reflex, and long-term correlations reflect the regulatory mechanisms that limit fluctuation of the beat cycle. Weippert et al. 2015, found that in slow breathing $\alpha 1$ was increased and $\alpha 2$ was decreased. Descriptions of other non-linear measures are beyond the scope of the present thesis and can be found in the work of Shaffer and Ginsberg 2017.

Slow breathing exercises decrease nonlinear behaviour of heart rate dynamics. A decreased complex behaviour of HRV through symbolic analysis, Entropies and DFA during slow breathing has been reported previously (Porto et al. 2018). Weippert et al. (2015) also observed elevation in Detrended Fluctuation Analysis (DFA) and entropy was lowered during slow breathing. While some authors assume complexity and regularity measures being fundamentally different and uncorrelated from traditional HRV indices (Schmidt and Morfill 1995; MÄKIKALLIO et al. 1996), some other authors found some correlations between traditional HRV metrics and non-linear measures (Bigger Jr et al. 1996; Perkiomaki et al. 2002)

1.8 HRV/Breathing Biofeedback

HRV biofeedback has been found to be promising for a variety of disorders. Lehrer et al. (2000)'s work with cardiorespiratory intervention has been labeled HRVB or respiratory sinus arrhythmia (RSA) biofeedback, or resonance frequency feedback (RFF). The procedure consists of feeding back beat by beat heart rate data during slow breathing maneuvers such that the participant tries to maximize RSA by looking at the changes in heart rate with a final goal of reaching a sine-wave-like curve of peaks and valleys of the cardiac rhythm. The participant can use feedback or a breath pacing procedure to produce the sinusoidal maximized RSA. A higher RSA amplitude in HRV Breathing

might be a key point to consider while discussing the efficacy of this method. Respiratory sinus arrhythmia, which is controlled by the vagus nerve can reflect aspects of the ANS. Lehrer and Gevirtz (2014) argue that greater vagus nerve traffic will therefore produce greater amplitudes of RSA, and many scientists equate RSA (or HF HRV) with "cardiac vagal tone," or parasympathetic influence on the heart (Berntson et al. 1997). We considered 6 bpm (0.1 Hz) guided breathing for this study based on prior literature, which indicates the highest coherence between heart and respiratory rhythms at this rate. A higher amplitude of sinusoidal rhythm of RR time series is shown in Figure 1.3 B. At this breathing frequency the heart rate oscillation and breathing align exactly in

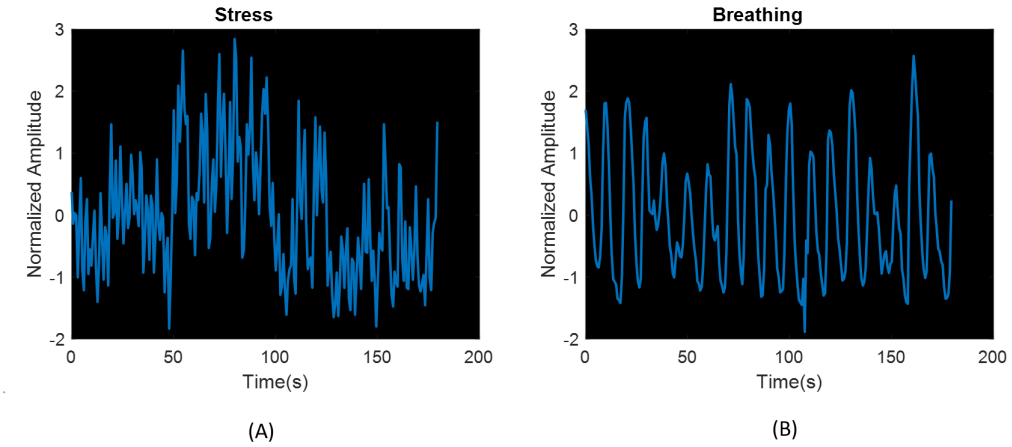

FIGURE 1.3: RR interval for Stress and Breathing condition. Plot A shows the RR interval in the Stress condition for participant 15 in the Breathing group. Plot B shows the RR interval in the Intervention condition for participant 15 in the Breathing group.

phase. Another reason why slow breathing might be helpful is that it optimizes alveolar ventilation and reduce dead space. Six breaths per min breathing (0.1 Hz) was found to be optimal for improving alveolar ventilation and reducing dead space in healthy as well as chronic heart failure patients in terms of increased arterial oxygen saturation and ease and sustainability in terms of respiratory effort (Bernardi et al. 1998). Slow breathing at 6 breaths per min has also been found to increase venous return (Dick et al. 2014). The baroreflex which is a reflex mediated by blood pressure sensors in the aorta and carotid artery that modulate blood pressure fluctuations, has a role to play in HRV Breathing. Stress receptors in the aorta and carotid artery detect changes in blood pressure and modulates vagal activity at the sinoatrial node producing changes in the heart rate oscillations (Eckberg and Sleight 1992; Vaschillo et al. 2002). When HRV biofeedback is practiced twice daily at home over about a 3 month period, an increase in resting baroreflex gain was observed by Lehrer and Gevirtz (2014). We already mentioned that at 0.1 Hz RSA also resonates with the LF baroreflex that integrates frequency and Mayer waves and both HRV (RSA) and baroreflex sensitivity are maximised when respiration is slowed to 6 breaths per min through resonance. This is the reason why the power

in the LF band increases as well as the amplitude of RSA. Vagus nerve stimulation (it involves delivering electrical impulses to the vagus nerve) for severe depression and seizure disorders have been documented (Sackeim et al. 2001; Nahas et al. 2005). It is speculated that stimulation especially of the sub diaphragmatic pathways through slow deep breathing techniques might be stimulating the same vagal efferent pathways and thus having an effect on depressive/anxiety symptoms (Porges 2011; Brown et al. 2013). From this discussion we can say that increased baroreflex leading to improved homeostasis (Lehrer and Gevirtz 2014) and also the stimulation of the vagal efferent system might play roles as possible mechanisms for the effectiveness of HRV Biofeedback. We will revisit these ideas in discussion chapter of this thesis.

1.9 Stress and Galvanic Skin Response (GSR)

The GSR also known as EDA, measures the skin conductance and varies with the state of sweat glands in the skin which is controlled by the sympathetic nervous system (Martini et al. 2015). The sweat gland activity is directly proportional to the sympathetic branch of the nervous system thus skin conductance can be considered as a measure of emotional and sympathetic responses (Carlson 2012).

Skin conductance has been previously used to detect stress (Zhai et al. 2005). Emotion classification has also been an area where GSR finds it's application such as emotion evocation by watching videos (Wu et al. 2010). To assess the internal emotional state of the participant along with HR the GSR can be used (Christoforou et al. 2015), and is a convenient way of indexing changes in sympathetic arousal associated with emotion and also cognition and attention (Critchley 2002). Authors in (Brouwer and Hogervorst 2014) used skin conductance (GSR) as a measure of physiological effects of mental stress, which was higher during the stressful condition. Two major components of GSR:

1. Tonic component: Skin conductance level (SCL) or Tonic level is a slowly changing part of the GSR signal which can be computed as the mean value of skin conductance over a specific window.

2. Phasic component: Phasic component is a fast changing part of the GSR signal also called skin conductance response (SCR), that result from sympathetic neuronal activity and occurs in relation to a single stimulus.

In the Figure 1.4 we have shown the GSR components. Several open source tooboxes exist for GSR/EDA signal analysis. EdaExplorer (Taylor et al. 2015) is one of them that is able to find peaks in GSR and also label epochs in the data. cvxEDA (Greco et al. 2015) is another toolbox that is widely used for GSR analysis that uses a convex optimization procedure to decompose the GSR into tonic component, phasic component and a noise term. LEDALAB is also a widely used toolbox for GSR data analysis (Benedek and Kaernbach 2010). Previous studies have investigated the effects of Music, Visual stimuli and deep breathing on GSR. One study found that aversive visual stimulation increases skin conductance level(SCL) and also skin conductance response frequency. The SCL was increased during the aversive stimuli and decreased during

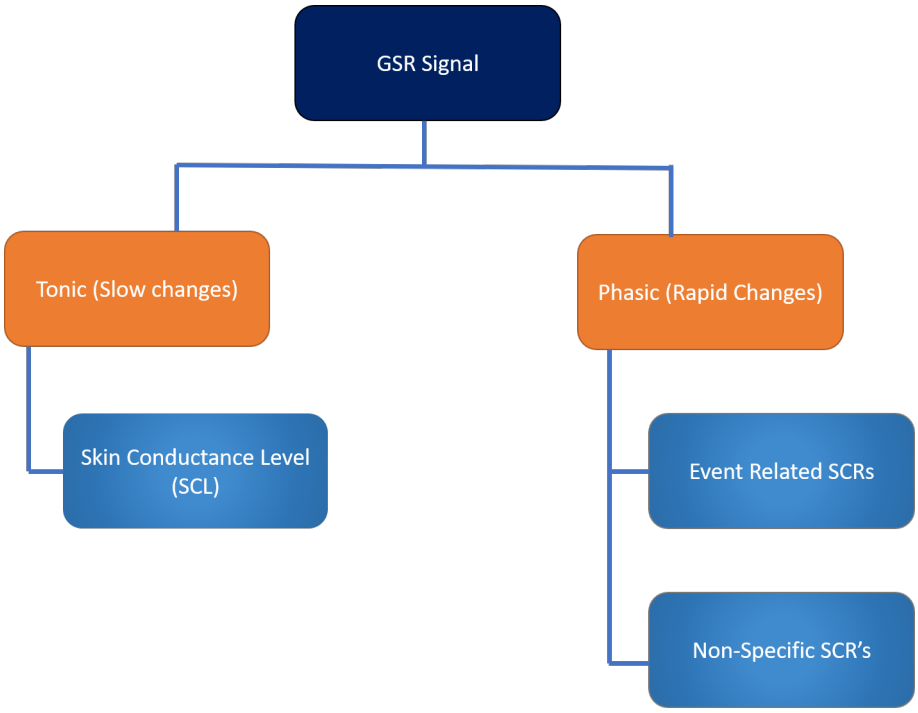

FIGURE 1.4: GSR components. The Figure shows the flow of GSR components.

music listening (Sokhadze 2007). In GN et al. (n.d.) authors recorded GSR before and after practising deep breathing exercise daily for three months. They found that GSR was significantly increased ($p < 0.001$) after practicing deep breathing. Authors conclusion was that practicing deep breathing daily as indicated in their method, is suggesting lowered sympathetic activity and increased parasympathetic activity. Tonic measures of SC (SCL) have also been used in assessment of personality, aggressive and antisocial behaviour (Crider 2008; Norris et al. 2007; Gatzke-Kopp et al. 2002).

1.10 Motivation and Relevance

This research work aims to investigate how the interventions of slow breathing and relaxing music compare in reducing stress induced by watching a stressful movie, as measured by HR, HRV, Breathing, GSR and self-reported stress levels. It is of interest to compare how these two interventions affect physiological responses and self-reported stress levels during the interventions and whether any benefits carry over once the interventions end. In addition, the interventions were performed in group settings as, if shown to be effective in this context, this would open many opportunities to apply the interventions in clinical and other settings. Furthermore, the exploration of nonlinear HRV measures has rarely been done in a study of this type and will contribute to our knowledge of how slow breathing and relaxing music in a group setting affect these physiological parameters.

1.11 Hypotheses and Research Questions

We investigated the effects of undergoing stress followed by one of two intervention conditions (Breathing, Music) on HR, HRV, GSR, Respiration Rate, and self-report stress levels of the participants (Taelman et al. 2009; Han et al. 2010). The central research question was: can slow breathing or relaxing music be used for stress reduction? We tested the following hypothesis:

1. Participants in the Music group will show significantly different psychophysiological response (HRV, GSR, Respiration etc) than participants in the Breathing group only in the Intervention condition.
2. Participants in the Music group will show significantly different Visual Analogue Scale (VAS) ratings than participants In the Breathing group only in the Intervention condition.
3. The stressful movie would induce physiological and self-report changes consistent with stress, and both the slow breathing and the music interventions would induce physiological and self-report changes consistent with stress reduction.
4. The slow breathing conditions would show greater physiological and self-report changes consistent with greater stress reduction compared to the music condition.
5. The positive effects of the interventions would carry over to the immediately following baseline measurement in both the Breathing and Music conditions.

We also tested some secondary hypothesis:

1. Participants in the Breathing group would show a more regular, predictable and less complex cardiac rhythm in the Intervention condition compared to the Music group. Also, cardiac rhythm complexity would increase during the Stressful movie in both groups.
2. The synchronization between Cardiac and Respiratory Rhythm would increase to a greater extent in the Breathing group in the Intervention condition compared to Music group, characterized by a high correlation and coherence between RR time series and Respiration.

To summarize, this thesis intends to investigate the efficacy of the slow breathing and music interventions as tools to reduce psychological stress in group settings by altering autonomic modulation.

Chapter 2

Experimental Design

We investigated two interventions (Slow Breathing and Relaxing Music) on the effects of undergoing stress, measured by Heart Rate Variability (HRV), Galvanic Skin Response (GSR), Respiration Rate and self-report measures of stress level of the participants. These are some of the most commonly used physiological measures of stress (Taelman et al. 2009; Han et al. 2010). Participants were tested either in a group setting or individually to evaluate the effects of group experience, although we were not able to finish testing or analyze those tested individually.

2.0.1 Participants

32 healthy adults between the ages of 18 and 37 (Mean= 20, SD= 3.7) participated in the slow Breathing group and 24 adults between the ages of 18 and 44 (Mean= 20, SD= 7) participated in the Music group. All participants were recruited from the Psychology student database (SONA) at McMaster University and were given course credit or cash compensation for participation. Participants were asked to avoid drinking caffeine two hours prior to the study. Participants were randomly sorted and assigned into two groups: either the Music group or the Breathing group, and within each of these conditions, to either group testing (8 participants/group) or Individual testing. Testing of individual participants was not completed due to the shutdown of the lab during the COVID-19 pandemic, so this thesis only analyzes those tested in the group testing conditions. In the group testing setting two groups of 8 participants were tested in each of the Music and Breathing groups for a total of 32 participants.

2.0.2 Ethics

The Experimental procedure was approved by the McMaster University Research Ethics Board. All participants gave their informed consent by signing a consent form. Participants either received SONA credits or $10 cash.

2.0.3 Stimuli

The auditory stimuli consisted of a synthesized human breath sound that simulated the inhale and exhale breathing sound as described below. Visual instructions were

presented on a large video display wall situated about 6m in front of the participants, who were seated in a semicircle (see Figure 2.1 and detailed description in section 2.0.5). The experimental protocol is shown in section 2.2.

The breath sound stimulus was provided by Muvik labs. It was created using a white noise substractive synthesizer, with filter coefficients modelled from a female breath recording spectrum at the beginning and end of inhale/exhale. The breath recording was analyzed and used to create a filter bank applied to the white noise with changing coefficients. The steps included were:

- Female subject recorded inhale and exhale
- Spectrum produced formant peaks at certain frequencies.
- Formant peaks and amplitude used to create band-passe filter bank.

Previous research has found that researcher-selected music stimuli has shown greater effects on stress reduction than music stimuli of subject's choice (Pelletier 2004). We have selected Standardized music stimuli for the Relaxing Music Experimental condition. In our study, we used a music stimulus ("Peaceful Journey" by composer and sound therapist Jonathan Goldman) which had already been used as relaxing music in previous study (Fuchs et al. 2018). So we assumed that this stimulus had stress-attenuating capacity that should be independent of individual preferences.

2.0.4 Physiological measures and Self-Reports

We collected Pulseplethysmography (PPG) data, Galvanic Skin Response, Respiration as physiological measures. We also collected behavioural responses in the form of questionnaires. Detailed descriptions of the data collection are in section 2.0.6.

2.0.5 Experimental Procedure

Participants were assigned randomly to either the Breathing group or the Music group, assigned according to the slot they signed up for. When participants arrived, they were seated in the main experiment room in a random seat in the arrangement shown in the Figure 2.1 and asked to fill out a consent form as well as a demographic information form that contained questions on age, sex, musical and dance background, language skills, education, employment status, annual household income, the region of residence, handedness, and any potential hearing problems. Participants could choose to not answer a question if it made them feel uncomfortable. After the consent and demographic forms, they were asked to complete the ISMA Stress questionnaire, the Perceived Stress Scale, the STAI-T questionnaire, the TIPI personality questionnaire, and the VAS for stress. Before the main experiment began, each participant was taken individually into a separate room, which was separate from the one in which the experiment was being conducted. Each participant was asked to sit in the Live Lab facing the screen (see setup in Figure 2.1) and fitted with three physiological sensors: a respiration belt with a SleepSense Double Loop Piezo Crystal Respiratory Effort Sensor, a G.Tec galvanic

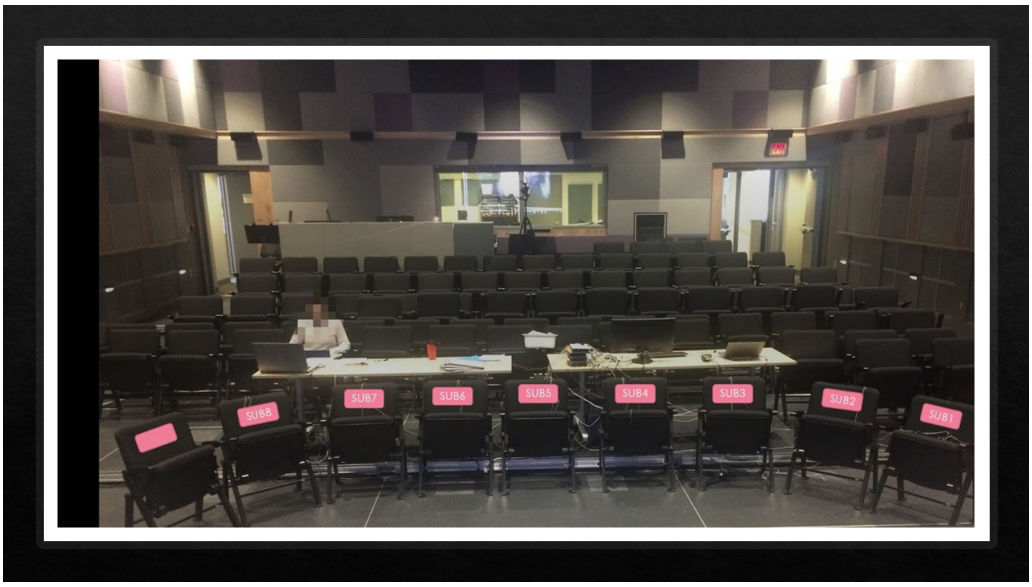

FIGURE 2.1: Experimental Setting: In the group testing condition, participants sat in a semicircle to watch the video. The physiological sensors were connected to computers that captured the data on the desk behind them.

skin response (GSR) sensor, and a photoplethysmogram (PPG) sensor (pulsesensor) available from *https://pulsesensor.com*. The respiration belt was wrapped around the participant's chest and was used to track the participant's respiration rate. GSR and PPG sensors were placed on specific locations on the non-dominant hand of the participants. The GSR sensor was placed on the proximal phalanges of the second and third fingers of the participants' hand, with their palm facing up. This GSR sensor measured participants' skin conductance. The PPG sensor sensed the blood volume pulse (BVP) which measures cardiovascular dynamics by detecting changes in the arterial translucency. Data collected from PPG sensor was converted into a digital format via custom Arduino software, with four of the PPG sensors connected to each Arduino. We have used a 9600 baud rate, which means the serial port is capable of transferring a maximum of 9600 bits per second. The sensor was placed on the participants' tip of their index finger of non-dominant hand and the BVP was used to extract nonlinear and linear metrices of their heart rate variability (HRV). All participants were asked not to talk and to minimize movements of their fingers that were attached to the sensors during the procedure. A video-screen then guided participants through the conditions of the experiment. The statistical software IBM SPSS Statistics V25 and R were used to do the statistical analysis of the HRV, Respiration, and GSR data collected from each participant. Custom built scripts in Matlab were used to analyze the data along with toolboxes (HRVAS) based on Matlab scripts (Ramshur 2010).

Once participants were hooked up with the sensors (PPG, GSR, Respiration belt),

both groups completed the four conditions of the main experiment: 1. Baseline1 condition (five minutes) 2. Stress condition (five minutes) 3. Intervention condition, either slow guided breathing or relaxing music condition (five minutes). 4. Baseline2 condition (five minutes) (see Figure 2.1). Thus, conditions 1, 2 and 4 were identical for the Breathing and Music groups. There was a 30-second gap after each Condition during which participants rated their stress using a VAS scale from 1 (no stress) to 4 (very stressed). In the Baseline1 condition (1), participants were instructed to breathe normally without any guided assistance. In the stressor condition (2), participants were shown a stressful movie scene from the movie 'Vertical Limit'. During the Breathing Intervention condition (3), participants were guided to breathe at a rate of 0.1 Hz (6 breath per minute) using the auditory cue. During the Relaxing Music Intervention, participants listened to calming music, "A Peaceful Journey" by Jonathan Goldman (Fuchs et al. 2018). During the Baseline2 condition (4), participants were again asked to breathe normally without any guided assistance.

Participants in the Breathing group were given the following specific instructions: "During the experiment, you will see videos and instructions on the screen and you just need to follow these instructions. When you see 'Baseline' on the screen, please breath normally and relax. When you see 'Breath Slowly' on the screen, please breathe slowly following the rhythm of a breathing sound. Breathe easily and comfortably. Do not try too hard, just follow the rhythm of the sound'. Again, when the 'Baseline' appears on the screen, just relax and breathe normally. When 'Stress Rating' appears on the screen, use your hand that is not attached to sensors to mark your stress level at this moment on the Stress Rating scale on the page on your clipboard.".

Participants in the Music group were given the following specific instructions: "During the experiment, you will see videos and instructions on the screen and you just need to follow these instructions. When you see 'Baseline' on the screen, please breath normally and relax. When you see 'Relaxing music' on the screen, please listen to the music, relax and breathe normally. Again when the 'Baseline' appears on the screen, just relax and breathe normally. When 'Stress Rating' appears on the screen, please use your hand that is not attached to sensors to mark your stress level at this moment on the Stress Rating scale on the page on your clipboard."

Once the main experiment was complete, participants were unhooked from the equipment. The participants then completed their demographic form (if it was not previously completed). Participants were provided with a debriefing form summarizing the experiment, the hypotheses, and the independent and dependent variables.

2.0.6 Data Collection

Questionnaires

Before the experiment began, participants completed several questionnaires. The ISMA Stress questionnaire (ISMA 2011) gives a general overview of a participant's Baseline stress level. The Perceived Stress Scale (Cohen et al. 1994) measures the perception

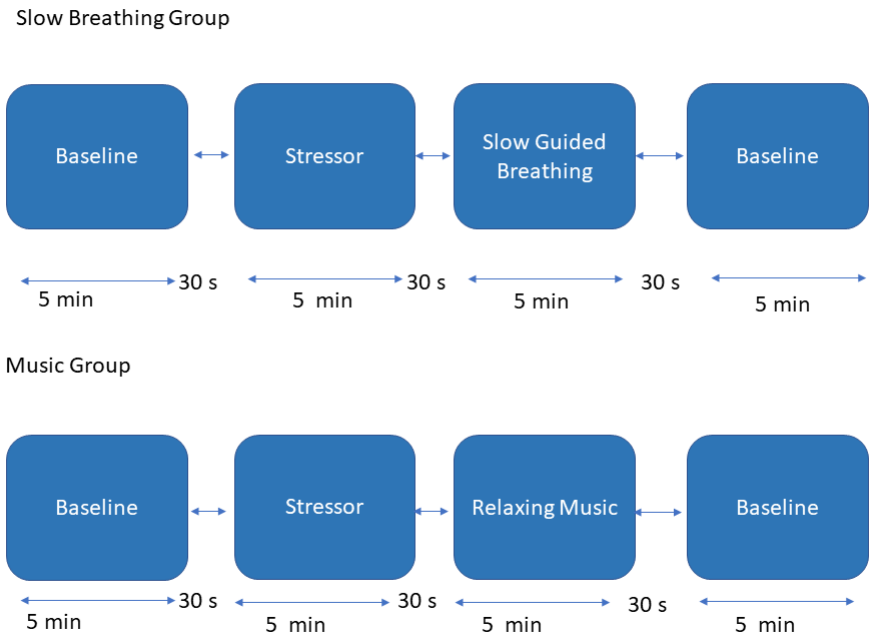

FIGURE 2.2: Experimental Protocol. The Figure shows the experimental protocol. The top blocks shows the Experimental conditions for the Breathing group. The Experimental conditions include Baseline1, Stressor, Slow Guided Breathing and Baseline2. Similarly bottom block shows the Experimental conditions for the Music group. The Experimental Conditions include Baseline1, Stressor, Relaxing Music and Baseline2

of stress using a questionnaire with 10 different stressful situations. Each situation has a scale associated with it, that ranges from 0–4, with 0 being 'almost never', 1 being 'never', 2 being 'sometimes', 3 being 'fairly often', and 4 being 'almost always'. The STAI-T (Spielberger et al. 1983) has 20 descriptions of situations people were previously in. Participants indicate to what degree each description matches themselves using a scale of 1–4, with 1 being 'almost never', 2 being 'sometimes', 3 being 'often', and 4 being 'almost always'.

The TIPI (Gosling et al. 2003) is a measure of the big five personality traits, derived from ratings (from 1–7, with 1 being 'disagree strongly' and 7 being 'agree strongly') of 10 questions. The five personality traits are extroversion agreeableness, conscientiousness, emotional stability, and openness.

The Visual Analogue Scale (VAS) has 4 identical questions asking a participant how stressed they feel at the current moment using a scale from 0–10, with 0 being 'No' and 10 being 'Severe'. Each of the four questions was associated with each of the four different points during the video in which 'Stress Rating' appeared on the screen.

TABLE 2.1: Participant Data at Baseline $Mean \pm SD$ of participant characteristics provided for both Breathing and music group.

Categories	**Breathing (16)**	**Music (16)**
Age	$18.25 \pm .45$	21.12 ± 6.80
Sex (M, F, Unknown)	(0, 12, 4)	(2, 13, 1)
ISMA Score	15.87 ± 3.64	13.81 ± 5.20
Perceived Stress Scale Score	22.59 ± 6.71	20.93 ± 7.49
STAI Score	53.18 ± 10.02	50.06 ± 11.39
TIPI Extroversion Score	4.03 ± 1.60	4.09 ± 1.38
TIPI Agreeableness Score	$4.75 \pm .93$	$4.78 \pm .96$
TIPI Conscientiousness Score	4.93 ± 1.22	5.12 ± 1.00
TIPI Emotional Stability Score	3.84 ± 1.60	4.34 ± 1.85
TIPI Openness Score	4.62 ± 1.13	5.68 ± 1.09

Photoplethysmogram (PPG)

Heart beats cause variations in blood volume or blood flow in the body which can be detected and registered by plethysmograph. We used a pulse or photoelectric plethysmography to detect the heart beats. A PPG sensor consist of a light source and a detector to detect a cardio-vascular pulse waves that propagate through the body. The signal reflects movement of blood in the vessels (Evans and Geddes 1988). An invisible infrared light is sent into the tissue and the amount of the back scattered light varies with the variation of the blood volume (Alnaeb et al. 2007). The intensity of back scattered light and blood volume are thus related. The benefits of PPG over the more traditional ECG measure is that it is low-cost and simple to use. PPG is widely used in healthcare where there is a demand for non-invasive, accurate and simple-to-use diagnostic techniques. Previous studies have compared the HRV signals extracted from PPG and ECG signals. They found that in monitoring healthy individuals, the PPG signal offers excellent potential to replace ECG recordings (Bolanos et al. 2006). The advent of embedded devices such as Raspberry Pi and the Arduino platforms facilitated more diverse data collection and thus popularized PPG as compared to traditional ECG, which is considered too invasive or sometimes too disruptive for experiments.

We used The Pulse Sensor Amped which is an Arduino based heart-rate sensor for the recording of PPG signals from participants during the experiment. The sensor and Arduino are shown in Figure 2.3.

The Pulse sensor specifications are as follows:

- Diameter = 0.625" (16mm)
- Overall thickness = 0.125" (3mm)
- Working Voltage = 3V to 5V
- Working Current = 4mA at 5V

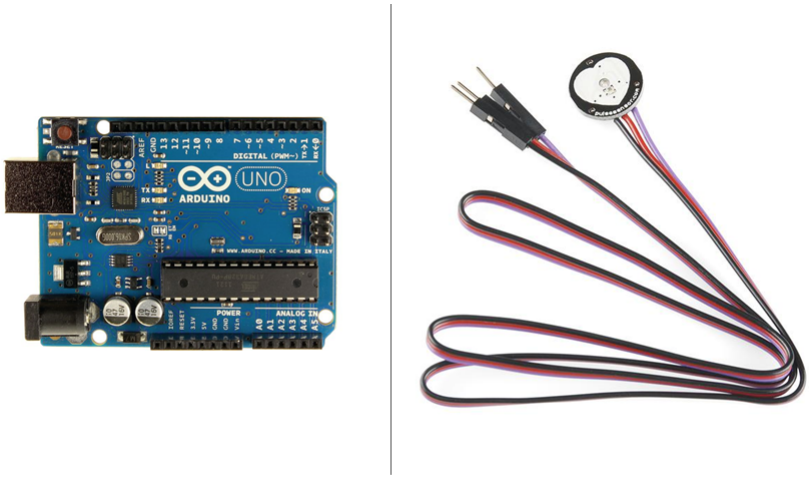

FIGURE 2.3: Arduino and pulse sensor

Steps to connect Pulse Sensor to Arduino:

- Connect + to +5
- Connect – to GND
- Connect S to A0.

To save the serial data as csv, we used PLX-DAQ. PLX-DAQ is a Parallax microcontroller data acquisition add-on tool for Microsoft Excel (Silva et al. 2014). The steps followed were:

1. Click on the connect button as shown in the Figure 2.4. It will setup a connection for serial data transfer.
2. The port number need to be selected based on the Arduino configuration.
3. Check step 2 before step 1.
4. The data will be stored in the CSV as soon as the connect button is pressed.
5. The data can be saved by clicking on the csv as save as.
6. After step 5, clear the data and repeat the process.

This add on for Microsoft Excel helped us to store excel sheets for recorded PPG amplitudes. This application requires Microsoft Excel in the PC in order to start recording serial data from Arduino.

Pre-Processing in PPG signal

PPG signal quality depends on several factors such as the location and the properties of the subject's skin at measurement, which includes the subjects' skin structure and blood

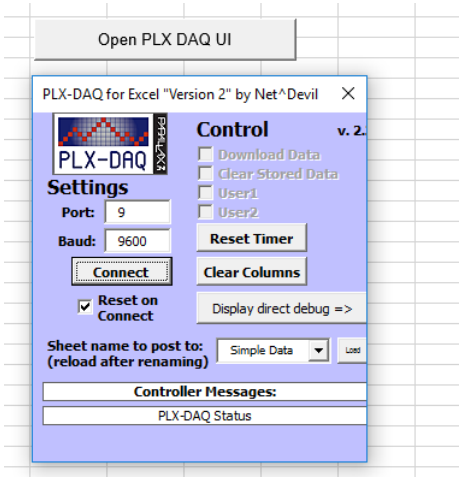

FIGURE 2.4: PLX-DAQ framework

oxygen saturation, blood flow rate, and skin temperature (Elgendi 2012). Poor contact to the fingertip photo sensor and excessive movement of the participant can cause artifacts. The signals were sampled at 62 Hz for first two groups and at 31 Hz for the other two groups. We lowered the sampling rate due to issues with signal acquisition with PLX-DAQ (Silva et al. 2014). Previous studies suggest from analyses of variability in the time and frequency domain that PPG may be potentially as reliable as ECG, provided that fs $\geq$ 25 Hz sampling frequency is used (Choi and Shin 2017). We instructed the participants to limit the movement of the hand where the sensor was attached. The peak detection and RR signal acquisition was performed using python implementation of the algorithm HeartPy (Gent et al. 2019). The RR intervals thus acquired were used for the calculation of the Time, Frequency and Non-linear HRV matrices. The raw signals were Bandpass filtered with a butterworth second order filter in the range of .05-2 Hz. The resultant RR intervals in ms were used to calculate the time and frequency as well as non-linear HRV matrices using the Matlab implementation of the toolbox (Ramshur 2010).

Both electrocardiogram (ECG) and the Photoplethysmogram (PPG) are widely used for detection of heart beats. However, algorithms that work with ECG like Pan-Tompkins algorithm (Pan and Tompkins 1985), might not be suitable for PPG. The basic difference between ECG and PPG is that ECG measures the electrical activity of the heart using electrodes attached to the body whereas, the PPG uses a small optical sensor with a light source to measure the discoloration of the skin as blood perfuses through it after each heartbeat. Thus, measuring of fast electrical activation and slower pressure waves requires very different specialized signal processing. We used the HeartPy algorithm as it works well for PPG (Gent et al. 2019).

We will now discuss the HeartPy algorithm and associated preprocessing methods. If a PPG peak was clipped, indicating that the signal was too large for the sensor limits, a clipping function was used to interpolate the 'missing' signal peak using a cubic spline,

which takes into account 100 ms of data on both ends of the clipped portion of the signal; then the reconstructed R-peak is overlaid on the original signal and used for further analysis. Our analysed data did not have any peaks that were clipped.

The Heartpy toolbox employs an adaptive peak detection threshold which is followed by several steps of outlier detection and rejection. A moving average is calculated using a window of 0.75 s on both sides of each data point and the first and last 0.75 s of the signal are not analyzed. Gent et al. (2019) describe Regions of interest (ROI) as areas marked between two points of intersection where the signal amplitude is larger than the moving average and thus a peak is detected and R-peaks are marked at the maximum of each ROI. A more detailed explanation of peak detection can be found in (Gent et al. 2019). The toolbox also rejects peaks based on threshold value for the RR-intervals (the intervals between successive heartbeats). The mean of the RR-intervals in the segments are considered for threshold computation. Gent et al. (2019) describes, the threshold as RR (mean) +/- (30% of RR(mean) (+ or - for upper and lower threshold, respectively). If the RR-interval exceeds one of the thresholds, it is ignored. We thus get a set of RR intervals, also often called Inter Beat Intervals (IBI). In the next step, we used a Matlab based toolbox for analysing the RR intervals (Ramshur 2010). HRVAS was chosen because this toolbox has been extensively used and cited (Zenonos et al. 2016; Munla et al. 2015) and in (Bernardi et al. 2017; Bhagat et al. 2017) etc. More detailed description of heart rate variability analysis from RR signals thus acquired will be discussed in the HRV measurements and analysis section.

Galvanic Skin Response

A G.Tec galvanic skin response (GSR) sensor was attached to the palmar surface of the proximal phalanges of the second and third fingers of each participant to measure skin conductance during different experimental conditions. The signals thus acquired were z transformed with respect to the Baseline condition and decomposed into tonic and phasic components. Tonic components include slow drifts of the baseline skin conductance level and spontaneous fluctuations in skin conductance (Boucsein 2012). The phasic component is also known as skin conductance response (SCR) and reflects short time responses to the stimulus. In our analysis, we only considered the Tonic skin conductance (SC) because the tonic level gives us the average skin conductance over each of the four conditions of the experiment. We used the toolbox cvxEDA (Greco et al. 2015) for decomposing the SC signal to the two different components. The analysis is done in MATLAB.

We downsampled the raw GSR from 256 Hz to 16 Hz. Downsampling GSR does not pose any significant risk of losing important aspects of the tonic signal whereas filtering smooths the GSR curve and removes the tonic component of the signal. Previous studies such as Brouwer and Hogervorst (2014) did not decompose the signal and just used raw GSR.

It is recommended to transform the data before using cvxEDA. A convex-optimization-based EDA model was applied to each participant's GSR after downsampling and Z-score

transformation. Z-score transformation is performed in order to standardize the dataset and increase the velocity of the optimization procedure (Greco et al. 2016). We used the default parameters in cvxEDA. We assumed fast time constant of the Bateman function (tau1)= 0.7, slow time constant of the Bateman function (tau0)= 2.0, penalization for the sparse SMNA driver (alpha)=.0008, penalization for the tonic spline coefficients (gamma)=.01 and a sparse Quadratic programming (QP) solver 'quadprog'. The convex optimization approach does not need any preprocessing step (Greco et al. 2016). The output returns the following objects:

1. Phasic component.
2. Sparse SMNA driver of phasic component.
3. Tonic component.
4. Coefficients of tonic spline.
5. Offset and slope of the linear drift term.
6. Model residuals.
7. Value of objective function.

We only considered the tonic component that was used for quantifying the skin conductance during different conditions of the experiments.

The details of the mathematical modelling approach can be found in (Greco et al. 2015). We will mention the assumptions related to the model. Greco et al. (2015) modelled the EDA (GSR) generation process based on the following assumptions:

1. SCRs are preceded by temporally discrete episodes of bursts from the sudomotor nerves controlling the sweat glands (Macefield and Wallin 1996; Nishiyama et al. 2001)
2. The relationship between the number of sweat glands recruited and the amplitude of a firing burst is linear (Nishiyama et al. 2001). The system is considered as linear time-invariant.
3. The sweat diffusion process has a subject-specific impulse response function (IRF) which is relatively stable for all skin conductance responses from the same subject (Benedek and Kaernbach 2010).
4. This phasic activity is superimposed over a slowly varying tonic activity with spectrum below 0.05 Hz.

In this way, the tonic component is extracted from the SCR signal. In the next step we considered the last one minute of each Experimental condition (i.e., Baseline1, Stress, Intervention, Baseline2). The reasoning behind considering the last one minute is that the stressful movie that was displayed during the stressor condition showed the stressful part during the last 2 minutes of the total duration. We then took median of the last one minute of tonic activity for all the conditions.

Respiration

A respiration belt with a SleepSense Double Loop Piezo Crystal Respiratory Effort Sensor is used to collect the respiratory signal from all the subjects in the group. Signals were resampled from 256 Hz to 16 Hz. A Butterworth second order bandpass filter in the range [.07 - 5] hz was applied to the raw respiratory signals. Then, individuals peaks were detected using Matlab's *findpeak* function. Peak counts are used to calculate the breathing rate per minute. We have seen that in the stressful movie condition the respiration rate was higher than Baseline1 and it dropped in the Intervention condition for both the groups. Our results are in agreement with previous research (Han et al. 2010).

HRV Measurements and Analysis

As discussed in the previous sections, the peak detection and RR signal acquisition is performed using python implementation of the algorithm HeartPy, (Gent et al. 2019). For short term HRV measurements, 5-minute segments are considered appropriate (Camm et al. 1996), although ultra-short term HRV (10, 30, 40, or 50 sec) have also been documented previously (Salahuddin et al. 2007; Castaldo et al. 2016). We extracted 3-minute segments from the continuous HRV data for Baseline1, Stressor, Intervention and Baseline2 period. A few subjects (n = 8 in the Breathing group and n = 8 in the Music group) had some portion of missing data in the Baseline2 due to technical issues. As a result we considered 90 seconds of the Baseline2 for those participants. We analyzed the standard deviation of NN intervals SDNN, low frequency (LF) power and ratio of LF/HF power. We also looked at various nonlinear measures of HRV. The low frequency band of power spectrum, ranging between 0.04 and 0.15 Hz, is thought to represent both sympathetic and parasympathetic nervous system activity. The high frequency spectrum, ranging between 0.15 and 0.4 Hz, is thought to estimate cardiac vagal tone which also represents RSA. In the case of low breathing rates used in this study in the Guided Breathing intervention, RSA falls within the LF band. Therefore, slow relaxed breathing should increase the LF power, which would indicate increased vagal outflow (Quintana and Heathers 2014).

In this section we discuss time, frequency and nonlinear HRV measures. Time-based metrics measures the variance in the temporal domain. Frequency-based metrics analyze power within certain frequency bands of the RR signal. Nonlinear-based metrices evaluate complexity and self-similarity. We also investigated time-frequency metrics that examine the signal in both the time and frequency domains simultaneously. We will first define RR and NN and IBI. In standard nomenclature "NN" (normal-to- normal) is used in place of IBI or RR to indicate IBIs containing no ectopic intervals. We will use IBI, RR, and NN interchangeably to represent IBI. In RR interval R is a point that corresponds to the peak of the QRS complex of the ECG wave and RR is the interval between successive R's. IBI is a time series signal. The IBI time series of an ECG

segment for N beats is given by:

$$IBI(n) = R(n+1) - R(n) : 1 \leq n \leq N-1 \tag{2.1}$$

where R(n) is the time location of the nth beat.

Several pre-processing steps are used with IBI time series data. Most important pre-processing is the removal of ectopic beat and IBI resampling. Ectopic beats are heartbeats which are not caused by a normal sinus node pace, rather they are caused by an electrical potential originating in some other areas (Luna and Fisch 1995). Ectopic beats can cause errors in the HRV analysis (Thuraisingham 2006). Ectopic detection is the first step. The percentage filter locates intervals that change by more than a user defined percentage (often 20%) from the previous interval (Aubert et al. 1999). This method implemented in the toolbox HRVAS locates any sudden or abrupt IBI changes. A standard deviation filter can also be used to detect ectopic intervals, by marking outliers as intervals that lie beyond the overall mean IBI by a user defined value of standard deviations (Aubert et al. 1999). We performed the ectopic interval detection using the percent filter (20%) and standard deviation filter (3 SD). Detrending was accomplished using the wavelet packet detrending. For ectopic correction, we used spline interpolation. We have chosen these specific parameters following the work of (Ramshur 2010) .

Spectrum estimates from irregularly sampled time series signals can introduce additional harmonics into the power spectrum (Niskanen et al. 2004). So, we have to resample the IBI time series before power spectrum estimation. Other studies e.g., (Cao et al. 2020) also found that interpolation and resampling of unevenly sampled RR interval signals improves the discrimination of chronic heart failure patients from healthy controls. In our analysis, we have chosen an IBI interpolation rate of 2 Hz. In Time domain measures of HRV, standard deviation of the NN interval series (SDNN) was calculated. The standard deviation of each IBI segment is first calculated and then the mean value of the SDNN is considered. The mean value of RR intervals (RR) is denoted by RR_m. The standard deviation of RR intervals (SDNN) is defined as:

$$SDNN = \sqrt{\frac{1}{N-1}\sum_{j=1}^{N}(RR_j - RR_m)^2} \tag{2.2}$$

RR_j denotes the value of j'th RR interval and N is the total number of successive intervals. The SDNN reflects both short-term and long-term variation within the RR interval series.

Power spectrum density (PSD) can be used to quantify the power within the RR time series. The PSD estimates can inform us about the amount of power in different frequency bands. In our analysis, we concentrated on the Low Frequency and High Frequency bands. The LF band (0.04–0.15 Hz) is affected by breathing from 3 to 9 bpm (slow breathing rate). Whereas, the HF, which is also called the respiratory band

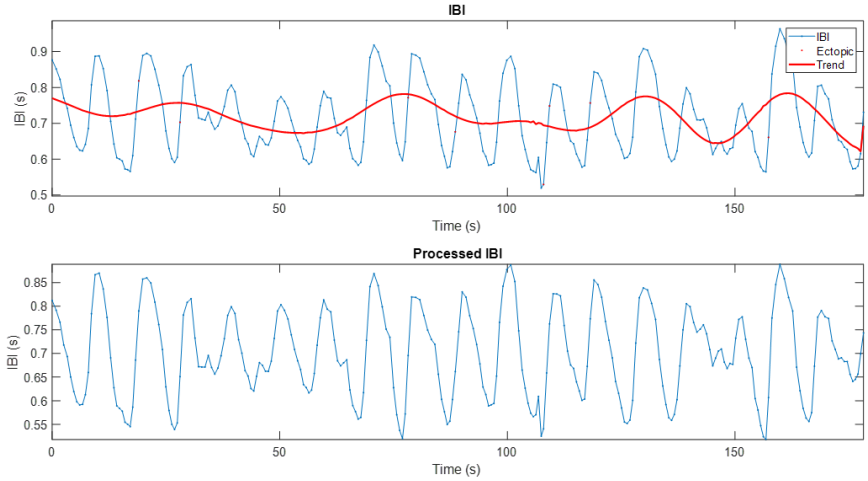

FIGURE 2.5: The IBI signal before and after detrending and ectopic interval removal. The IBI time series is shown for subject 15 in the Intervention condition in the Breathing group. Ectopic beats are shown in red dots if any.

(0.15–0.40 Hz) is influenced by breathing from 9 to 24 bpm (European Society of Cardiology et al. 1996). The ratio of LF to HF power (LF/HF ratio) can also be considered as the ratio between sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) activity under controlled conditions.

Estimating the PSD can be performed using many methods, but methods based on Fast-Fourier Transform (FFT) and autoregressive (AR) modelling are popularly used for spectral analysis of HRV (Clifford et al. 2006). Some commonly used FFT based methods are developed by (Bartlett 1996; Blackman and Tukey 1958; Welch 1967). FFT is a non-parametric method whereas the AR power spectrum methods do make assumptions and are called parametric. Ramshur (2010) HRVAS toolbox allows analysis using both parametric and non parametric methods. Welch's method has some advantages over FFT such as being robust to non-stationarity, are being less sensitive to noise, although with a reduced spectral precision. The PSD values discussed in this thesis are calculated using welch method (Welch 1967).

For time frequency analysis, the Ramshur (2010) toolbox has the provision for the windowed Burg periodogram and the windowed Lomb-Scargle periodogram (Carvalho et al. 2003; Thong et al. 2004). The windowed Lomb-Scargle periodogram is computed by windowing the entire data series and then breaking it into segments of equal lengths. For the time frequency analysis we used the Lomb-Scargle periodogram method with a window of 30 sec and a 15 sec overlap.

Nonlinear measures are used to characterize HRV. Several nonlinear measures have

been used to quantify the behaviour of heart rhythms, such as Poincaré plot (Brennan et al. 2001), Approximate and Sample Entropy (Richman and Moorman 2000) , Detrended Fluctuation Analysis (Penzel et al. 2003; Peng et al. 1995) as well as Correlation (Trulla et al. 1996; Webber Jr and Zbilut 1994) and Recurrence plots (Henry et al. 2001; Guzzetti et al. 1996). The physiological interpretation of the nonlinear results are difficult. Studies have shown that reduction of IBI (RR) signal complexity (Thuraisingham 2006; Papaioannou et al. 2006) may be a feature of cardiac pathology. On the other hand, for respiration signals, Caldirola et al. (2004) found that greater respiratory entropy could be a factor in vulnerability to panic attacks. Similarly, other studies have found that 0.1 Hz breathing is the most dynamic state which is characterized by a specific complexity pattern and is potentially beneficial for cardiopulmonary rehabilitation and conditioning (Matić et al. 2020).

One commonly used nonlinear method is Poincaré plot. The Poincaré plot is the plot of RR intervals versus the previous RR interval. In a Poincaré plot SD1 represents the Standard Deviation (SD) of the instantaneous beat to beat variability or short term variability and SD2 represents the SD of the continuous or long term variability (Kamen and Tonkin 1995; Brennan et al. 2002). To parameterize, the shape an ellipse is fitted to the plot. SD1 is the width and SD2 is the length of the ellipse. SD1 describes short term variability and is mainly caused by RSA. SD1 is also related to the time domain measure SDSD (Brennan et al. 2001).

Sample entropy (SampEn) is another non linear measure that has been used for assessing the complexity of physiological time series signals, and also for diagnosing diseased states (Richman and Moorman 2000). Larger values of SampEn represent higher complexity. If we have a time-series data set of length $N = \{y_1, y_2, y_3, .., y_N\}$ with a constant time interval τ. We define a template vector of length m, such that $Y_m(i) = \{y_i, y_{i+1}, y_{i+2}, ., y_{i+m-1}\}$ and the distance function $d[Y_m(i), Y_m(j)]$ (i≠j) is to be the Chebyshev distance. Euclidean distance can also be used. The sample entropy can be defined as:

$$SampEn = -\log \frac{A}{B} \tag{2.3}$$

Where, A = number of template vector pairs having $d[Y_{m+1}(i), Y_{m+1}(j)] < r$
B = number of template vector pairs having $d[Y_m(i), Y_m(j)] < r$ So it can be considered as the negative logarithm of the conditional probability of randomly selecting two m-length sequences (embedding dimension) from a signal, that have a distance less than r (tolerance) between them given that they also have a distance less than r if their lengths are increased to m+1. SampEn was designed to reduce the bias of approximate entropy and is in agreement with the theory for data with known probabilistic content (Lake et al. 2002). The embedding dimension (m) is set to 2 and tolerance (r) is set to 0.2 times the standard deviation of the data for our analysis. These values are commonly used for clinical HRV data (Pincus 1991; Yentes et al. 2013)

Another non-linear measure we used is the Detrended fluctuation analysis (DFA)(Peng

et al. 1994) which tries to quantify the fractal like or self-similar properties of non-stationary time series. Snowflakes, shorelines, crystals are some of the examples exhibiting fractal structures. DFA is a modification of root-mean-square analysis of random walks applied to non-stationary signals (Acharya et al. 2007). In a log-log plot the root-mean-square fluctuation of an integrated and detrended time series is measured at different scales and plotted against the size of the scale. Let's consider an RR time series of length N. The RR series is integrated using

$$y(k) = \sum_{i=1}^{k} [RR(i) - RR_m] \tag{2.4}$$

Where y(k) is the kth value of the integrated series, RR(i) is the i th IBI, and RR_m is the average IBI (RR interval) for the entire time series. The integrated time series is separated into segments of length n and then a least squares line is fit to the data in each segment to define the local trend denoted by $y_n(k)$. After that the integrated time series is detrended by subtracting the local trend, $y_n(k)$ from each segment. The root-mean squared fluctuation of the integrated and detrended time series is calculated by

$$F(n) = \sqrt{\frac{1}{N}(\sum_{k=1}^{N} [y(k) - y_n(k)])^2} \tag{2.5}$$

where n represents the window or scale size. F(n) is computed on a user defined range of time scales. We have used m= 4 to 100, with a break point at 13. The scaling exponent, α, of the IBI time series represent the linear relationship between log(F) and log(n). Two linear regions on the log-log plot are used to describe the short term scaling, $\alpha 1$, and the long term scaling, $\alpha 2$ (Peng et al. 1995). These two regions are separated by a breakpoint as mentioned already, at 13 in our analysis.

2.0.7 Correlation Analysis

Given a pair of random variables (X,Y), the formula for Pearson's correlation coefficient, ρ, is given by:

$$\rho_{X,Y} = \frac{cov(X,Y)}{\sigma_X \sigma_Y} \tag{2.6}$$

where: cov is the covariance, $\sigma_X$is the standard deviation of X ,
$\sigma_Y$is the standard deviation of Y

We performed a correlation analysis between cardiac (RR) and respiratory signals. The RR signal was acquired using the methods explained in 2.0.6 and shown in the equation 3.1. The RR signal and the Respiration signal were resampled to a common frequency. The signals were then z-score normalized. When measurements involve data collected asynchronously by multiple sensors, cross correlation can be applied to synchronize their timings. We aligned the RR and Respiration signals using the cross correlation

function. The next step was to use the MATLAB *corrplot* function. corrplot (Data) creates a matrix of plots showing correlations among pairs of variables in "Data". The "Data" variable will contain (RR and Respiration signals). Histograms of the variables appear along the matrix diagonal and scatter plots of variable pairs appear in the off diagonal. The slopes of the least-squares reference lines in the scatter plots are equal to the displayed correlation coefficients. The correlation plots (corrplots) are shown in 3.13 and in 3.14.

2.0.8 Wavelet Coherence Analysis

Cross wavelet power reveals areas with high common power. Similarly, the cross-wavelet transform tells us how coherent the cross wavelet transform is in time frequency space (Grinsted et al. 2004). The wavelet transform has been used previously for analysing cardiovascular signals in the time–frequency domain (Keissar et al. 2009). Coherence between RR-Resp has been reported previously (Indic et al. 2008). It is also a powerful and robust tool for the analysis of transient phenomena of the Autonomic Nervous System (Pichot et al. 1999; Davrath et al. 2003) . From the works of Torrence and Compo (1998) the wavelet coherence of two time series can be defined as:

$$\frac{|S(C_x^*(a,b)C_y(a,b)))^2|}{S(|C_x(a,b)|^2).S(|C_y(a,b)|^2)} \tag{2.7}$$

Where: $C_x^*(a,b)$ and $C_y^(a,b)$ denote continuous wavelet transforms of x and y at scales a and position b. * is the complex conjugate and S is the smoothing operator in time and scale. We have used the Matlab default coherence computation parameters that uses the analytic Morlet wavelet, 12 voices per octave and smooths 12 scales.

We used the matlab function *wcoherence(x,y)* that returns the magnitude-squared wavelet coherence, which is a measure of the correlation between signals x and y in the time-frequency plane. For jointly stationary time series, the standard techniques for characterizing correlated behavior in time or frequency are cross-correlation, the (Fourier) cross-spectrum, and coherence. However, many time series are non-stationary, meaning that their frequency content changes over time. For these time series, it is important to have a measure of correlation or coherence in the time-frequency plane. Wavelet coherence can be used to detect common time-localized oscillations in non-stationary signals. Another advantage of wavelet coherence is that in situations where one time series is influencing another, as we can see how cardiac signal is influenced by respiratory signal, the phase of the wavelet cross-spectrum can be used to identify the relative lag between the two time series as shown in Figure 3.16. The coherence function can be used to assess the strength of linear coupling between two signals in the frequency domain (Kay 2013).

2.0.9 Statistical Analysis

The focus of data analyses was on the primary dependent variables HR, Heart Rate variability, Galvanic skin response, Respiration Rate, Visual Analogue Scale for Stress. The Independent Variables were: Experimental conditions (Baseline1, Stress, Intervention, Baseline2). We computed a repeated measures mixed ANOVA for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). If the Experimental condition $\times$ Intervention group interaction was significant, we did individual comparisons between Intervention groups and also between subsequent conditions (Baseline1, Stress, Intervention, Baseline2) collapsed across groups. The Greenhouse-Geisser statistic was used, as appropriate, to control for the sphericity effects. We also performed Spearman rank correlations between GSR and subjects' self-ratings and also between different stress ratings for each subject across all Experimental conditions for both the groups (Music, Breathing).

Chapter 3

Results

We analyzed Heart Rate Variability (HRV), Galvanic Skin Response (GSR) and Respiratory Rate to measure the effects of the stress and to compare the effects of the interventions (Breathing, Music). Similarly, we calculated the correlation and coherence between Respiratory and Cardiac signals to examine cardio-respiratory synchrony. We further analysed Visual Analog Scales (VAS) self-report measure. We also measured correlations between self-report and physiological measures (GSR and Respiration and HRV) and between various physiological measures. The details of the analysis results are discussed in the subsequent sections.

3.1 Heart Rate Analysis

We calculated the Heart Rate (HR) for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using HR as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within subjects Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$. Statistical Analysis was conducted using SPSS and R.

The main effect of Intervention group was not significant, but there was significant main effect of Experimental condition, F (3, 90) = 7.286, p <.001, $\eta_p^2 = .195$, with post hoc pairwise comparisons indicating differences in HR in the Intervention condition than during Baseline1 ($p < .05$) or the Stress condition ($p < .01$) (Figure 3.1 B). There was also an interaction between Experimental condition and Intervention group , F (3,90) = 4.169, p <.01, $\eta_p^2 = .122$. Post hoc pairwise comparisons (Figure 3.1 A) examining the interaction revealed that only during the Intervention condition did HR differ significantly between groups (p<.05) (Figure3.15) A). All post hoc tests were conducted using Bonferroni correction. Fuchs et al. (2018) found a reduction of HR in a slow breathing condition (6 breath/minute) where as Weippert et al. (2015) found an increase of HR during a 0.1Hz metronome guided Breathing condition. Moreover, Engel and Chism (1967) also found that there may be some tendency for Breathing to increase

HR. We also found that HR was not significantly increased from Baseline1 to the Stress condition. It is possible that some people enjoy stressful movies and their heart rate might not change. It is also possible that people "freeze" during the stressful movie, leading to a slower HR. One previous study also found no significant difference in HR between a baseline condition and watching a film (Palmer 2008).

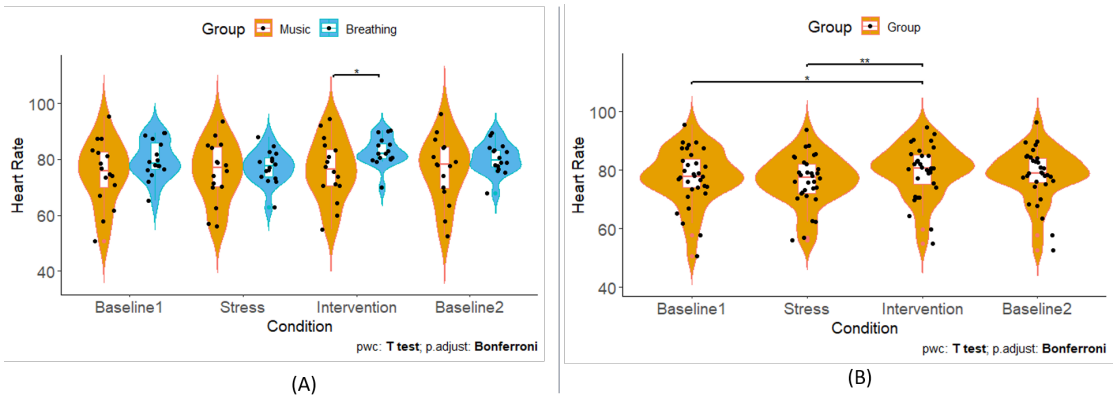

FIGURE 3.1: Pairwise comparisons for Heart Rate in bpm. Plot A shows Pairwise comparisons between Intervention groups. Plot B shows comparisons across Experimental conditions collapsed across groups(Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

3.2 Respiration Signal Analysis

We calculated the Respiration Rate for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using Respiration Rate as the dependent variable, we performed 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within-subject Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

The main effect of Intervention group was significant $F(1, 30) = 14.05$, $p=.001$, $\eta_p^2 = .319$. There was also a significant main effect of Experimental condition, $F(2.394, 71.809) = 91.964$, $p<.001$, $\eta_p^2 = .754$, and an interaction between Experimental condition and Intervention group, $F(2.394, 71.809) = 23.351$, $p<.001$, $\eta_p^2 = .438$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that the Respiration Rate was faster in the Stress condition than in the Baseline1 ($p < .001$) and Intervention ($p < .001$) conditions. It also revealed that the Intervention condition differed significantly from the Baseline1 ($p<.001$), Stress ($p<.001$) and Baseline2 ($p <.001$) conditions Figure 3.2 B). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did the Respiration Rate differ significantly between groups ($p<.05$) (Figure 3.2) A), with, not surprisingly, a slower Respiration rate

for the Breathing group than the Music group. All post hoc tests were conducted using Bonferroni correction. The results are shown in Figure 3.2.

Thus, Respiration increased during the Stress condition and decreased during both interventions, but it decreased more in the Breathing condition, presumably because participants were instructed to breathe slowly.

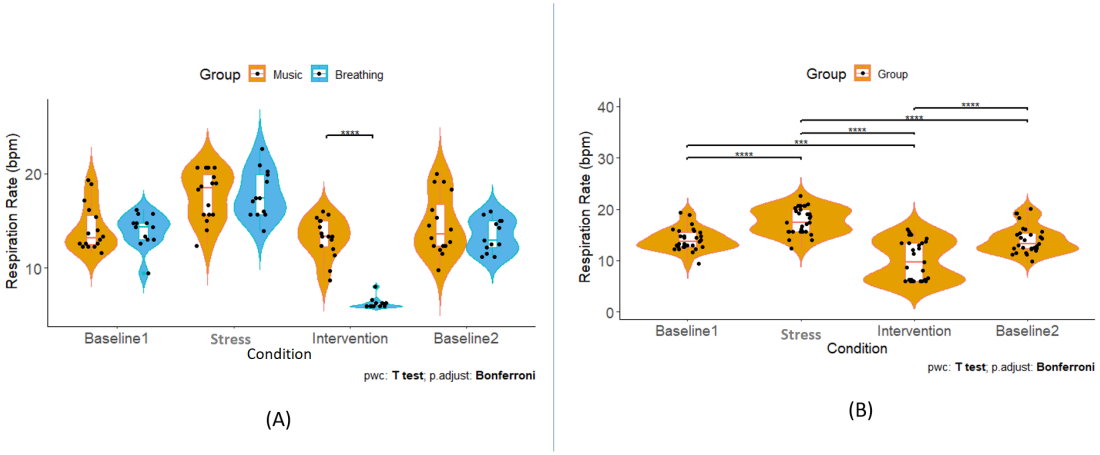

FIGURE 3.2: Pairwise comparisons for Respiration Rate in bpm. Plot A shows Pairwise comparisons between Intervention group. Plot B shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

3.2.1 Sample Entropy of Respiratory Patterns

We further explored the non-linear dynamics of the respiratory signal. Previously Caldirola et al. (2004) found that baseline respiratory patterns were higher in entropy in patients with panic disorder than controls, which indicates a higher level of irregularity and complexity in their respiratory signal. They also mentioned that greater respiratory entropy could be a factor in vulnerability to panic attacks. We hypothesized that Breathing and Relaxing Music would both result in a lower entropy value of the respiratory signal compared to Stress condition. Our results are in accordance with this hypothesis.

We calculated the Sample Entropy for the Respiration signal for each Intervention group (Breathing, Music) by Experimental condition (Baseline1, Stressful Movie, Intervention, Baseline2). Using Sample Entropy as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor, Intervention group (Breathing, Music) and within-subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

The main effect of Intervention group was not significant but there was a significant main effect of Experimental condition, F(2.174,65.218) = 22.419, p <.001, η_p^2 = .428, and an interaction between Experimental condition and Intervention group, F(2.174,65.218) = 6.407, p =.002, η_p^2 = .176, Post hoc pairwise comparisons of the main effect of Experimental condition revealed that Sample Entropy was lower in the Intervention condition compared to the other three conditions (p's <.001) (Figure 3.3 B). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did the Sample Entropy differ significantly between groups (p <.001) (Figure 3.3) A) with lower Sample Entropy in the Breathing than Music group. All post hoc-tests were conducted using Bonferroni correction.

In sum, the sample entropy analysis indicates that the Stressful movie increased the complexity of the Respiratory Signal and that both Breathing and Music decreased complexity, but that Breathing decreased complexity to a greater extent.

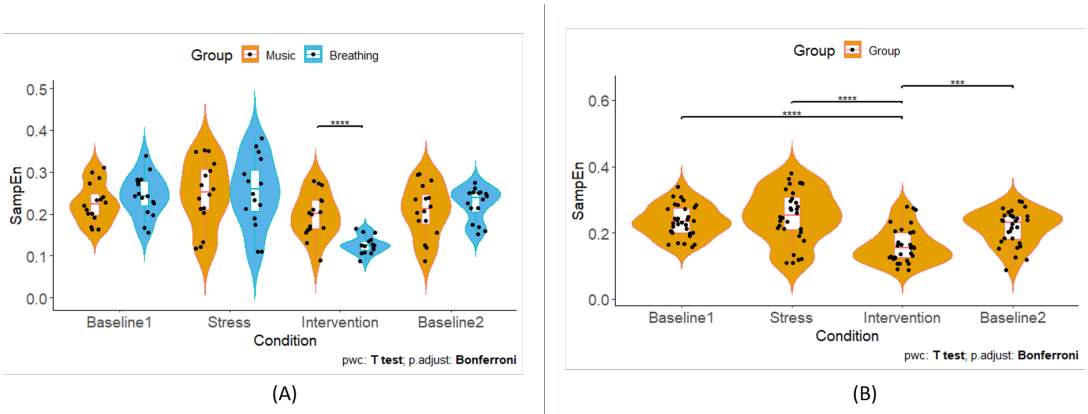

FIGURE 3.3: Pairwise comparisons for Sample Entropy Index. Plot A shows Pairwise comparisons between Intervention group. Plot B shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

3.3 Heart Rate Variability (HRV) Analysis

We analyzed the standard deviation of NN intervals (SDNN), low frequency (LF) power and high frequency (HF) power. LF band power (0.04-0.15 Hz) reflects both sympathetic and parasympathetic nervous system activity. HF band power (0.15 - 0.4 Hz) is used as an estimate of cardiac vagal tone. It also typically reflects heart rate changes related to breathing or respiratory sinus arrhythmia (RSA). In the case of slow breathing rates which was used in this study as guided breathing intervention, RSA falls within the LF band. Slow breathing should increase LF HRV power, which would indicate increased vagal outflow (Quintana and Heathers 2014). We also analysed the non linear measures

of HRV that includes Sample Entropy, Detrended Fluctuation Analysis and Poincaré Plot Analysis.

3.3.1 Time Domain Analysis of HRV

We measured the HRV metric SDNN for each Intervention group (Breathing, Music) by each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using SDNN as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within-subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

The main effect of Intervention group was not significant, but there was a significant main effect of Experimental condition, F (2.533, 75.99) = 10.950 p <.001, $\eta_p^2 = .267$, and an interaction between Experimental condition and Intervention group, F (2.533, 75.99) = 10.459, p <.001, $\eta_p^2 = .259$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that Intervention condition significantly differed from the Baseline1 (p < .01) and the Stressful movie conditions (p <.05). (Figure 3.4 B). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did the SDNN differ significantly between groups, and was higher in the Breathing than Music group (p <.05) (Figure 3.4) A). All post hoc tests were conducted using Bonferroni correction.

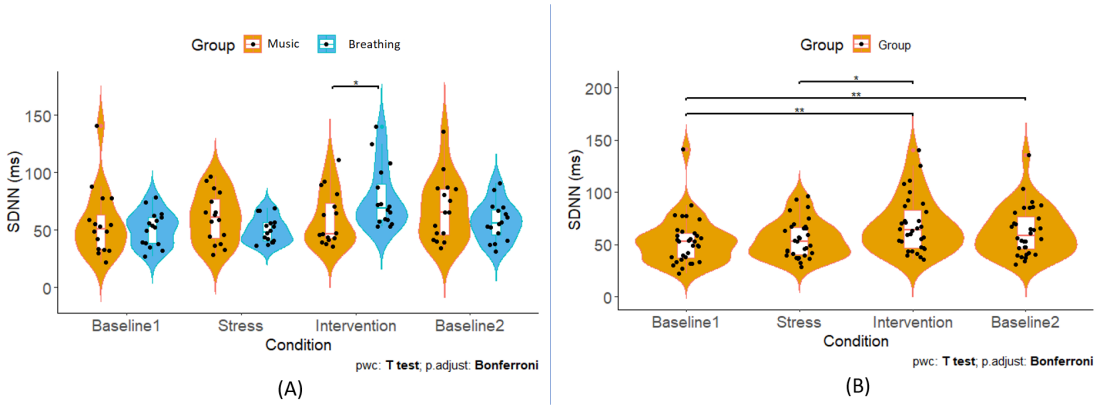

FIGURE 3.4: Pairwise comparisons for HRV measure SDNN (ms). Plot A shows Pairwise comparisons between Intervention group. Plot B shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

This increase in SDNN during slow breathing was expected as slow breathing has been shown to increase LF power (Fuchs et al. 2018) which correlates with SDNN (Otzenberger et al. 1998). Slow breathing is also associated with parasympathetically mediated RSA, marked by an increase in SDNN (Russo et al. 2017). However, SDNN differences between

groups disappeared immediately following the intervention, with no difference during Baseline2.

Previous studies also found that the primary source of variation in SDNN is parasympathetically mediated RSA in slow paced breathing protocols (Shaffer et al. 2014). As slow breathing increases the LF power hence it should increase the SDNN as we have shown in the present study. Our results corroborates previous findings and it can be said that slow breathing protocol is capable of inducing parasympathetic response marked by an increase in SDNN.

3.3.2 Frequency Domain Analysis of HRV

We calculated HRV for low frequency power LF (.04-.15 Hz), high frequency power HF (.15-.4 Hz) and the LF/HF ratio. The slow breathing paradigm guides the subjects to breathe at a rate of 0.1 Hz which shifts the RSA to the low frequency range. At slow respiration rates, vagal activity can easily generate oscillations in the heart rhythms that cross over into the LF band (Shaffer and Ginsberg 2017). We therefore expect that there should be a increased LF power in the Slow Breathing compared to Music Intervention condition.

Power spectral density (PSD) plots are shown in Figure 3.5. The windowed Lomb-Scargle periodogram (LSP) for each segment is computed as shown in Figure 3.5. We calculated the Frequency domain HRV measure Low Frequency (LF) for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using LF as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

The main effect of Intervention group was not significant. There was also a significant main effect of Experimental condition, F (1.549,46.45) = 13.571 p <.001, $\eta_p^2 = .311$, and interaction between Experimental condition and Intervention group, F (1.549, 46.45) = 10.164, p =.001, $\eta_p^2 = .253$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that the Intervention condition significantly differed from the Baseline1 condition ($p < .05$), the Stressful movie ($p<.01$) and the Baseline2 conditions ($p < .05$). As well, the Stressful movie condition differed significantly from the Baseline2 condition ($p<.05$) (Figure 3.6 B). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did the LF differ significantly between Intervention groups, being larger in the Breathing group as expected ($p <.01$) (Figure 3.6) A). All post hoc tests were conducted using Bonferroni correction.

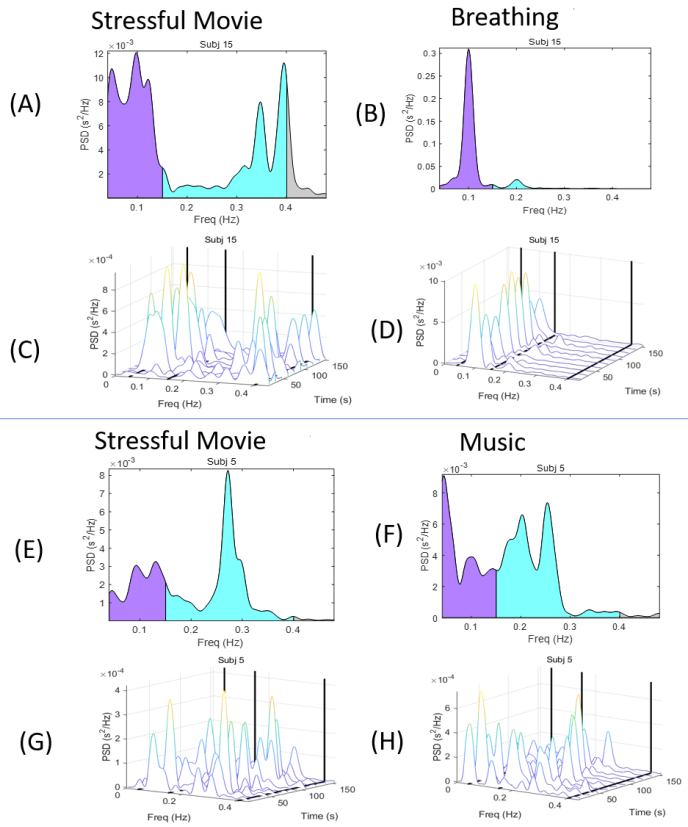

FIGURE 3.5: Spectral power density and time frequency plots. A and B show spectral density plots, and C and D show time frequency plots for the Stressful Movie and Intervention conditions for one participant (Subj 15) in the Breathing group. Similarly, for E, F, G , and H show the same plots for one participant (Subj 5) in the Music group. Clear peaks in plots B and D are visible at around the breathing rate of 0.1 Hz. In the plots, purple represents LF (0.04-0.15 Hz) and arctic represents HF (0.15-0.4 Hz).

We also performed a 2×4 mixed repeated measures ANOVA on the LF/HF ratio with between-subjects factor Intervention group (Slow Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

The main effect of Intervention group was significant, F (1, 30) = 25.11 p <.001, $\eta_p^2 = .456$, with an overall higher LF/HF ratio in the Breathing group compared to the Music group. There was also a significant main effect of Experimental condition, F (1.570,47.08) = 21.115 p <.001, $\eta_p^2 = .413$, and a significant interaction between Experimental condition and Intervention group, F (1.570,47.08) = 19.547, p <.001, $\eta_p^2 = .395$. Post hoc pairwise comparisons of the main effect of Experimental condition

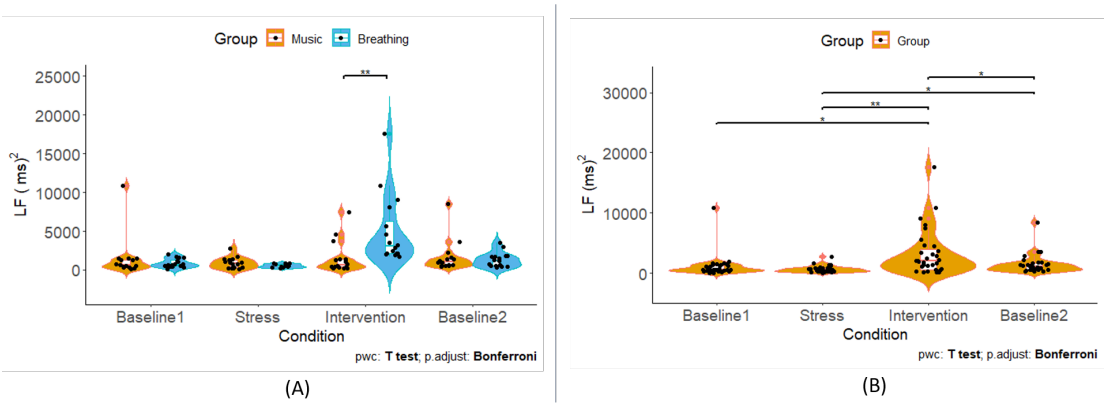

FIGURE 3.6: Pairwise comparisons for LF measure. Plot A shows Pairwise comparisons between Intervention group. Plot B shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

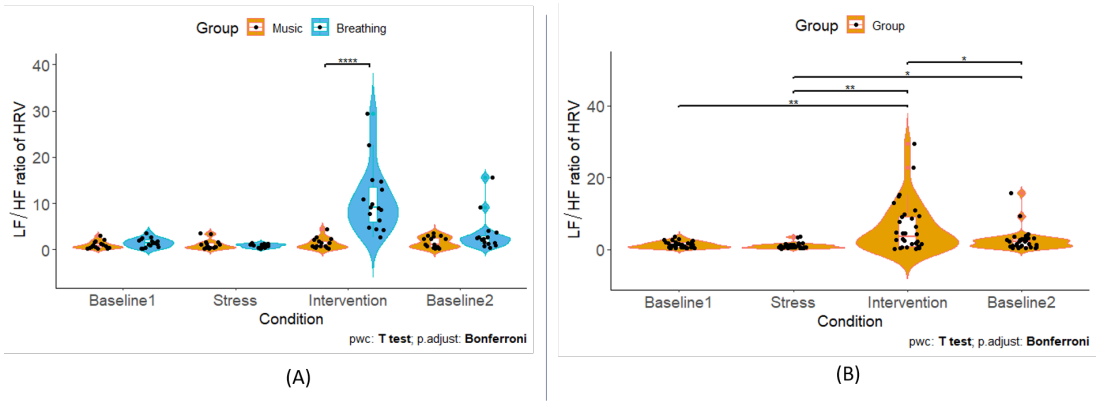

FIGURE 3.7: Pairwise comparisons for LF/HF ratio. Plot A shows Pairwise comparisons between Intervention group. Plot B shows comparison across Experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

revealed that the LF/HF ratio was significantly higher in the Intervention condition than in the Baseline1 ($p < 01$), Stress Movie($p < .01$), and Baseline2 ($p < .05$) as shown in (Figure 3.7 B). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did the LF/HF differ significantly between groups, being higher in the Slow Breathing than Music group ($p < .01$) (Figure 3.7) A). All post hoc tests were conducted using Bonferroni correction.

We have shown from the frequency domain analysis of HRV that LF power is higher

during the Intervention condition in Breathing group compared to Music group. Moreover LF/HF ratio was also significantly higher. During periods of slow respiration rates, vagal activity can generate oscillations in the heart rhythms that inflates the LF band power (Ahmed et al. 1982; Tiller et al. 1996). Also respiratory-related efferent vagally mediated influences can be seen in the LF band when respiration rates are low (Tiller et al. 1996) as in our case (0.1 Hz). Usually, HF is said to represent the parasympathetic tone because it reflects RSA as normal breathing falls in this HF range. When breathing is slowed down, RSA shifts to LF band. The effect did not carry over to the final baseline. We discussed why the effect did not carryover in the section 4.5.

3.3.3 Non-Linear Analysis of HRV

To examine the effects of Music and Slow Breathing We also investigated several non-linear measures of HRV. We hypothesized that there will be significant differences between Music group and the Breathing group during the Intervention condition.

3.3.4 Sample Entropy

Sample Entropy measures the complexity or irregularity of the signal. Large values of Sample Entropy indicate high irregularity and smaller values indicate more regular signal.

We calculated the HRV non-linear measure 'Sample Entropy' for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using Sample Entropy as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$

The main effect of Intervention group was significant $F(3, 90) = 15.366$, $p < .001$, $\eta_p^2 = .248$, with lower entropy in the Slow Breathing compared to Music groups. There was also a significant main effect of Experimental condition, $F(1,30) = 9.90$, $p < .01$, $\eta_p^2 = .339$, and an interaction between Experimental condition and Intervention group, $F(3, 90) = 3.820$, $p = .013$, $\eta_p^2 = .113$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that Entropy was significantly lower in the Intervention condition compared to the Baseline1 ($p < .01$) and Stressful movie conditions ($p < .001$) (Figure 3.8 B). Entropy was also lower in the Baseline2 condition compared to the Baseline1 ($p < .01$) and Stress movie conditions ($p < .001$). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did the Sample Entropy differ significantly between groups, with lower Entropy for the Slow Breathing than Music group ($p < .001$) (Figure 3.8) A). All post hoc tests were conducted using Bonferroni correction. Our results are in agreement with the findings of (Porto et al. 2018; Weippert et al. 2015).

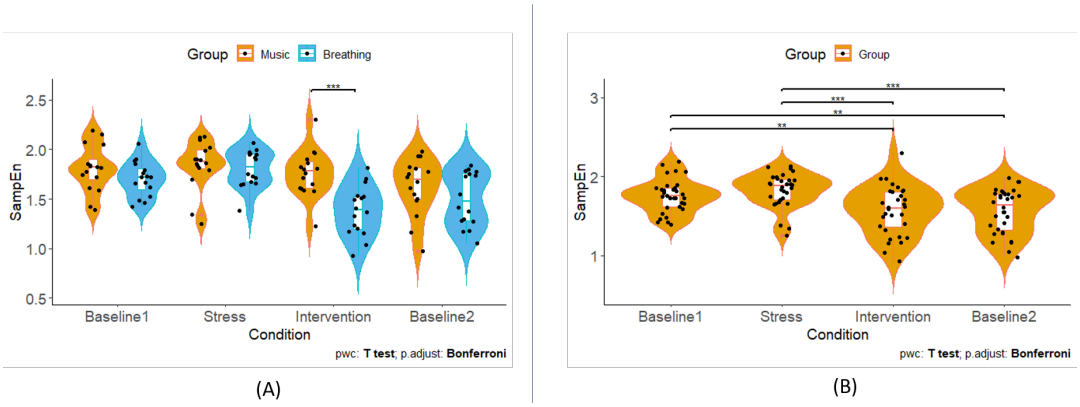

FIGURE 3.8: Pairwise comparisons for Sample Entropy. Plot A shows Pairwise comparisons between Intervention group. Plot B shows comparison across experimental conditions collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

3.3.5 Detrended Fluctuation Analysis: DFA $\alpha 1$ and DFA $\alpha 2$

The DFA plots are shown in Figure 3.9 for both Music and Breathing group. We calculated the HRV non-linear measure DFA $\alpha 1$ for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using DFA $\alpha 1$ as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Slow Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$

The main effect of Intervention group was significant F (1, 30) = 8.03, p<.01, $\eta_p^2 = .211$, with DFA $\alpha 1$ being larger in general for the Slow Breathing compared to Music group. There was also a significant main effect of Experimental condition, F (3, 90) = 29.102, p <.001, $\eta_p^2 = .492$, and an interaction between Experimental condition and Intervention group, F (3, 90) = 17.275, p <.001, $\eta_p^2 = .365$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that DFA $\alpha 1$ was higher in the Intervention condition than in the Baseline1 (p < .001) and Stressful movie conditions (p < .001). As well, DFA $\alpha 1$ was higher in the Baseline2 condition than in the Baseline1 and Stressful movie conditions (Figure 3.10 B) . Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did the DFA $\alpha 1$ differ significantly between groups, being higher in the Slow Breathing than Music group (p <.001) (Figure 3.10). All post hoc tests were conducted using Bonferroni correction. Our results supports the findings of (Weippert et al. 2015).

Similarly, we calculated the HRV non-linear measure DFA $\alpha 2$ for each Intervention group (Slow Breathing, Music) for each Experimental condition (Baseline1, Stressful

movie, Intervention, Baseline2). Using DFA $\alpha 2$ as the dependent variable, we performed a 2 × 4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

The main effect of Intervention group was not significant, but there was a significant main effect of Experimental condition, F (3, 90) = 8.368, p <.001, $\eta_p^2 = .218$, and an interaction between Experimental condition and Intervention group, F (3, 90) = 5.044, p <.001, $\eta_p^2 = .144$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that DFA $\alpha 2$ was lower in the Intervention condition than in the Stressful movie (p< .01) for Baseline2 conditions. (Figure 3.10 B). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did DFA $\alpha 2$ differ significantly between groups, being lower in the Slow Breathing than Music group (p <.001) (Figure 3.10) A) . All post hoc tests were conducted using Bonferroni correction. Our DFA analysis results are also in agreement with the existing

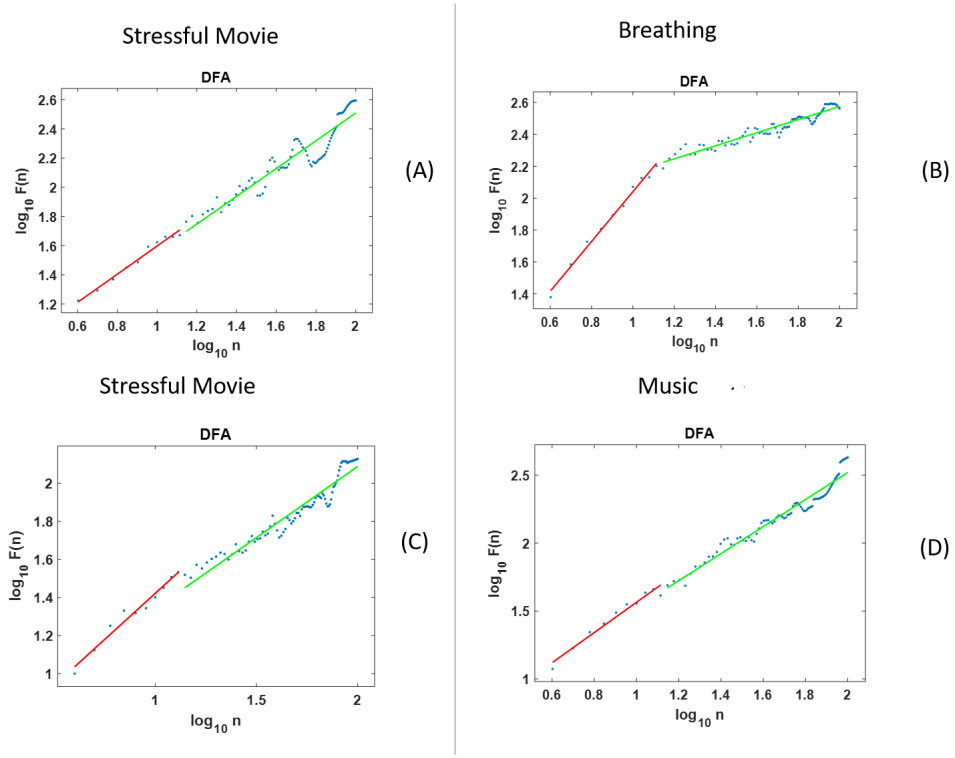

FIGURE 3.9: Detrended Fluctuation Analysis (DFA) plots. Slope of red line represents DFA $\alpha 1$ and slope of green line represents DFA $\alpha 2$. Plot A and Plot B represent DFA analysis for Breathing group for subject 15. Similarly, Plot C and Plot D represent DFA analysis for Music group subject 5.

literature (Weippert et al. 2015). Authors in Weippert et al. (2015) reported an increase

of DFA $\alpha 1$ and a decrease of DFA $\alpha 2$ in slow breathing condition. DFA plots are shown in Figure 3.9 and results are shown in 3.10. We found that in Intervention condition in Breathing group the DFA $\alpha 1$ was significantly higher than the Music group. On the other hand, DFA $\alpha 2$ was significantly lower for the Breathing group as compared to the Music group in the Intervention condition.

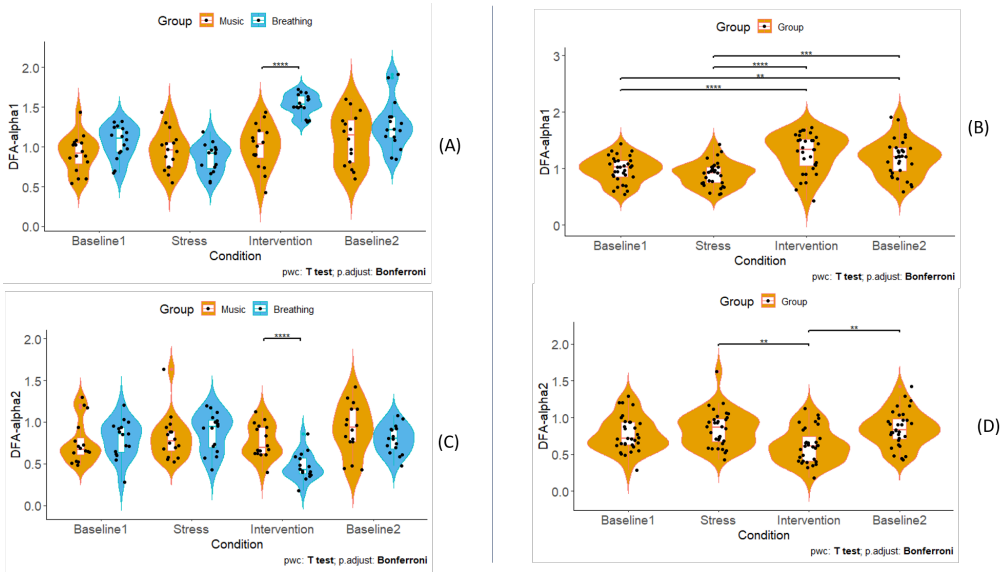

FIGURE 3.10: Pairwise comparisons for measures DFA $\alpha 1$ and DFA $\alpha 2$. Plot A shows Pairwise comparisons between Intervention group and Plot B shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie,Intervention and Baseline2) for DFA $\alpha 1$. Plot C shows Pairwise comparisons between Intervention group and Plot D shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2) for DFA $\alpha 2$. Comparisons are Bonferroni corrected

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3.3.6 Poincaré Plots

Poincaré plot is another commonly used nonlinear method that is easy to interpret. This plot is a graphical representation of the correlation between successive RR intervals, consisting of a plot of RR_{n+1} as a function of RR_n. An ellipse is fitted to the plot as shown in Figure 3.11. The orientation of the ellipse is according to the line-of-identity ($\mathrm{RR}_n = \mathrm{RR}_{n+1}$). The standard deviation of the points perpendicular to the line-of-identity is denoted by SD1 and describes short-term variability whereas the standard deviation of the points along the line-of-identity denoted by SD2 describes long-term variability.

We calculated the HRV non-linear measure SD1 for each Intervention group (Slow

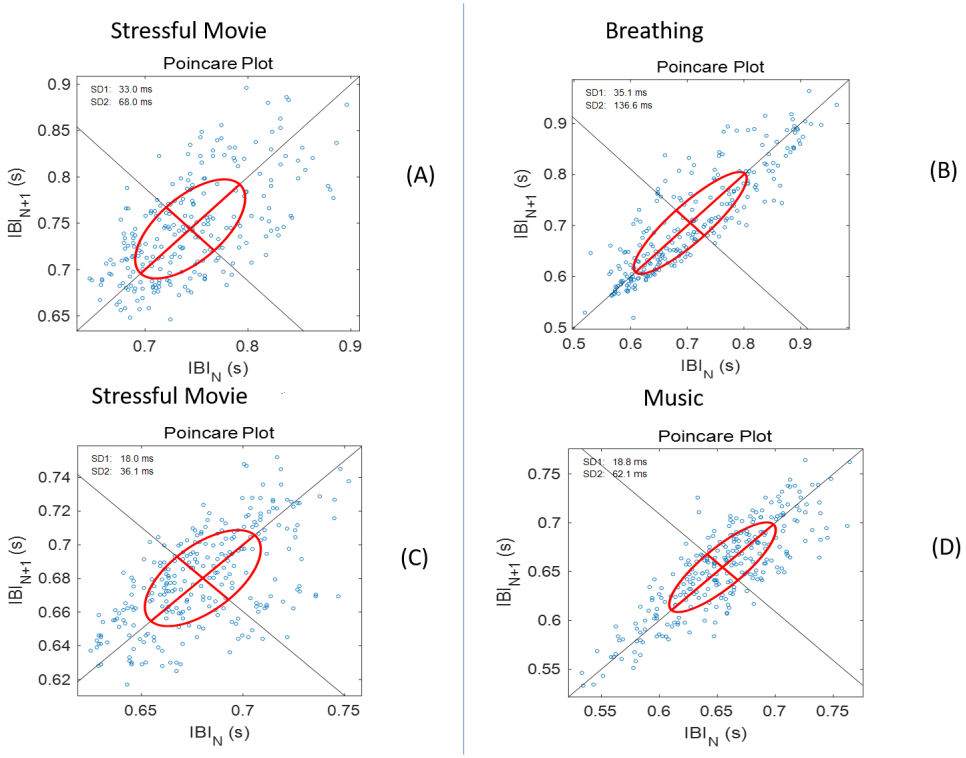

FIGURE 3.11: Poincaré plot analysis. SD1 and SD2 are the standard deviations perpendicular to and along the line-of-identity $\mathrm{RR}_n = \mathrm{RR}_{n+1}$, respectively. Plot A and B corresponds to Stressful movie condition and Intervention condition for subject 15 in Breathing group, similarly Plot C and D corresponds to Stressful Movie condition and Music condition for subject 5 in Music group.

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Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using SD1 as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Slow Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

Only the interaction between Experimental condition and Intervention group was significant, F (3, 90) = 1.150, p >.05, $\eta_p^2 = .037$. However, post hoc pairwise comparisons of the interaction revealed no significant differences (Figure 3.12 A) All post hoc tests were conducted using Bonferroni correction.

We calculated the HRV non-linear measure SD2 for each Intervention group (Slow

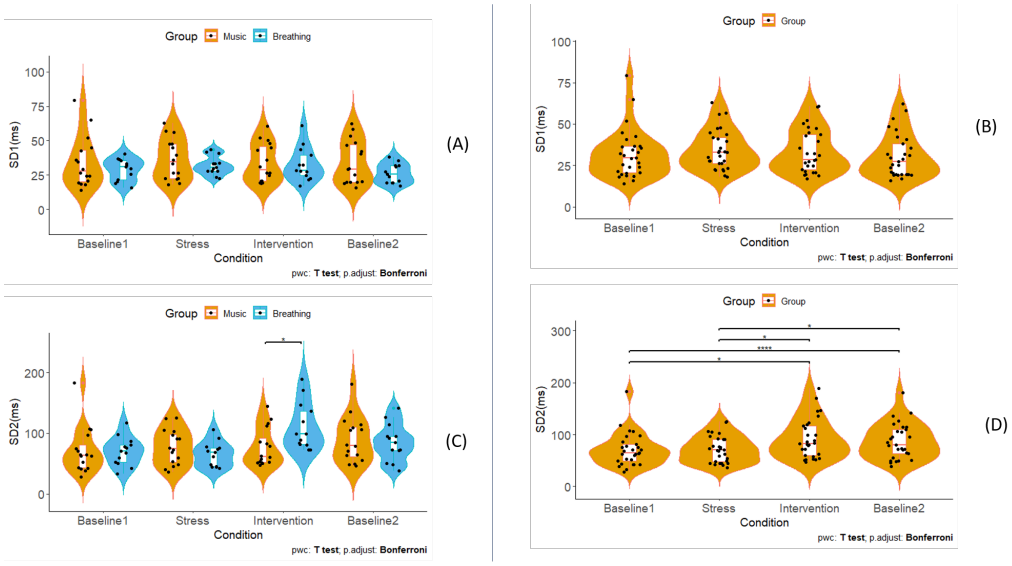

FIGURE 3.12: Pairwise comparisons for measures SD1 and SD2. Plot A shows Pairwise comparisons between Intervention group and Plot B shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2) for SD1 . Plot C shows Pairwise comparisons between Intervention group and Plot D shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2) for SD2 . Comparisons are Bonferroni corrected.

Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using SD2 as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Slow Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

There was a significant main effect of Experimental condition, F (3,90) = 11.707, p <.001, $\eta_p^2 = .281$, and an interaction between Experimental condition and Intervention group, F (3,90) = 9.062, p <.001, $\eta_p^2 = .232$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that SD1 was significantly higher in the Intervention condition than in the Baseline1 (p <.05) and Stressful movie conditions (p <.05). (Figure 3.12 D). As well, SD1 was higher in the Baseline2 condition than in the Baseline1 (p < .001) and the Stressful movie conditions (p < .05). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did SD2 differ significantly between groups, with higher SD2 in the Slow Breathing than Music group (p <.05) (Figure 3.12) C). All post hoc tests were conducted using Bonferroni correction.

Thus, Slow Breathing produced different effects from Music for SD2 but not for

SD1, but these differences did not carry over to Baseline2. Authors in Porto et al. (2018) concluded that Poincaré plot was not sensitive to detect changes which the DFA, symbolic analysis and entropy identified in HRV during slow breathing. Authors did not see visual differences in the Poincaré plot patterns during spontaneous breathing and during slow breathing. In our analysis we investigated the quantitative characteristics of Poincaré plots for the slow Breathing and Relaxing Music condition as well as for other Experimental conditions. We found that SD2 is significantly effected by slow breathing intervention.

TABLE 3.1: HRV measures for Music and Breathing group for all the experimental conditions.

Variable	**group**	**Baseline1**	**Stress**	**Intervention**	**Baseline2**
HR	Breathing	79.85 ± 6.81	77.30 ± 6.7	82.7 ± 5.15	80.31 ± 5.42
HR	Music	75.15 ± 11.69	75.58 ± 10.81	76.28 ± 11.07	76.37 ± 11.64
SDNN	Breathing	51.21 ± 15.01	49.96 ± 11.03	77.97 ± 26.89	57.47 ± 16.89
SDNN	Music	55.95 ± 29.32	60.63 ± 21.84	59.67 ± 23.19	68.01 ± 27.68
LF	Breathing	884.48 ± 560.11	523.7 ± 220.40	4983.46 ± 4400.29	1293.8 ± 938.6
LF	Music	1362.45 ± 2572.4	896.9 ± 717.15	1514.7 ± 2034.5	1642.2 ± 1997.1
LF/HF	Breathing	$1.52 \pm .956$	$.87 \pm .39$	10.79 ± 7.09	3.4 ± 3.84
LF/HF	Music	$1.0139 \pm .778$	1.09 ± 1.02	1.303 ± 1.154	1.599 ± 1.151
SampEn	Breathing	$2.05 \pm .26$	$2.3 \pm .22$	$1.5 \pm .32$	$1.8 \pm .45$
SampEn	Music	$2.3 \pm .42$	$2.4 \pm .50$	$2.1 \pm .40$	$2.0 \pm .34$
DFA1	Breathing	$1.06 \pm .20$	$.85 \pm .18$	1.5 ± 1.2	$1.2 \pm .30$
DFA1	Music	$.91 \pm .23$	$.95 \pm .24$	$.99 \pm .27$	1.09 ± 1.324
DFA2	Breathing	$.79 \pm .22$	$.87 \pm .23$	$.46 \pm .16$	$.79 \pm .17$
DFA2	Music	$.75 \pm .25$	$.81 \pm .26$	$.77 \pm .20$	$.91 \pm .30$
SD1	Breathing	28.19 ± 7.66	31.86 ± 7.088	31.90 ± 11.24	27.11 ± 6.74
SD1	Music	34.27 ± 18.38	36.23 ± 14.82	33.95 ± 13.92	34.34 ± 15.68
SD2	Breathing	67.18 ± 38.02	62.72 ± 19.90	104.76 ± 36.99	80.12 ± 29.25
SD2	Music	70.88 ± 38.02	77.10 ± 28.94	76.73 ± 31.14	80.128 ± 29.25

3.3.7 Summary of HRV measures

Summarizing the findings of the HRV analyses we can conclude that slow breathing affects HRV differently from relaxing music, with no significant differences between the Slow Breathing and Music conditions during the Baseline2 condition. The time domain and frequency domain HRV results are also consistent with previous findings which suggest that slow breathing can reduce stress, indicated by high HRV (Fuchs et al. 2018). We have also found that nonlinear matrices of HRV are significantly affected by slow breathing as compared to relaxing music intervention. The complexity and unpredictability of the heart rhythm reduces during slow breathing. These findings are also in line with previous research (Porto et al. 2018; Weippert et al. 2015). To interpret our results we have to consider the fact that Variability and complexity are two different concepts. Variability is measured by variance and related statistical metrics. Two signals

may have similar degree of statistical variability but very different complexity properties (Goldberger et al. n.d.). We discussed this in details in the discussion section 4.3. Heart rate variability describes the beat-to-beat changes in cardiac inter-beat intervals and indexed by SDNN in our work. Even two heart rate sequences having nearly identical mean values and variances for a given observation period can have different complexity measures such as entropy which is a measure of unpredictability and reflects complexity (Goldberger et al. n.d.). Detailed discussion can be found in section 4.3.

3.4 Cardio respiratory synchronization

3.4.1 Correlation Analysis

Heart rate increases with inspiration and decreases with expiration. This phenomenon is called Respiratory Sinus Arrhythmia (Yasuma and Hayano 2004). In order to investigate the synchrony of heart and respiration rhythms we performed correlation analysis between cardiac (RR) and Respiratory signals. The correlation plots are shown in Figure 3.14. We tested if we could replicate the findings of Lagos et al. (2008) that have shown a very high correlation between Respiration and Heart Rhythm during slow breathing. Other studies also reported that breathing at resonant frequency maximizes heart rate oscillations by creating a zero degree shift between heart rate and respiration (Vaschillo et al. 2006). Our results corroborate their findings. The respiratory and heart signals were acquired via different sensors, so we aligned the signals using cross correlation.

The normalized Respiration and RR signals for Breathing and Stress movie condition is shown in Figure 3.13. The x axis represents the time and y axis represents the normalized amplitude (Plot A and B). Similarly, the correlation matrix is shown in Plot C and Plot D for Breathing and Stress Movie condition respectively. A correlation as high as ($\rho = 0.68$) can be seen in Intervention condition compared to Stress Movie condition ($\rho = 0.26$) in Breathing group. Similarly, the normalized Respiration and RR signals for Music and Stress movie condition is shown in Figure 3.14. The x axis represents the time and y axis represents the normalized amplitude (Plot A and B). Similarly, the correlation matrix is shown in Plot C and Plot D for Music and Stress Movie condition respectively.

We calculated the correlation between the RR time series and the Respiration signal for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using correlation as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within-subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$

The main effect of Intervention group was not significant, but there was a significant main effect of Experimental condition, $F(3, 90) = 9.780$, $p < .001$, $\eta_p^2 = .246$, and an interaction between Experimental condition and Intervention group, $F(3, 90) = 10.726$,

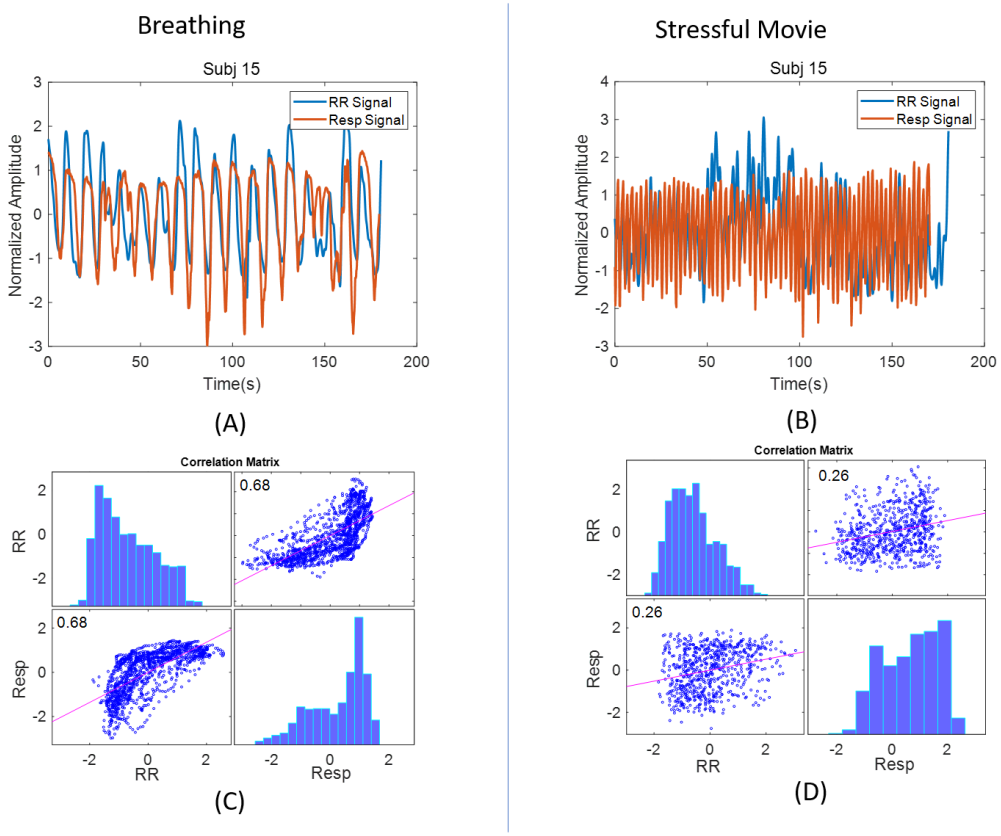

FIGURE 3.13: Correlation plot. Plot A and Plot B shows the normalized RR intervals and Respiration signals for subject 15 from the Breathing group in Intervention condition and Stressful movie condition respectively. Plot C and Plot D shows correlations between RR and Respiration. Histograms of the variables and scatter plots of variable pairs are displayed along with Pearson correlation coefficient. Correlation coefficient is higher for the Slow Breathing condition as compared to the Stress Movie condition.

p <.001, $\eta_p^2 = .263$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that the correlation was higher for the Intervention condition than the Baseline1 (p < .01), Stressful Movie (p < .01) and Baseline2 (p < .01) condition. (Figure 3.15 B). Post hoc pair-wise comparisons examining the interaction revealed that only during the Intervention condition did the correlation between RR and respiration differ significantly between groups, being higher in the Slow Breathing than Music group (p <.001) (Figure 3.15) A). All post hoc tests were conducted using Bonferroni correction.

Previous studies have reported that the modulation of heart rate by respiration is strongest at low breathing frequencies of approximately 0.1 Hz (6 respiratory cycles/min) (Berntson et al. 1993; Bernardi et al. 2000; Stark et al. 2000). In our study we have found a higher correlation during Breathing group compared to music listening, consistent with

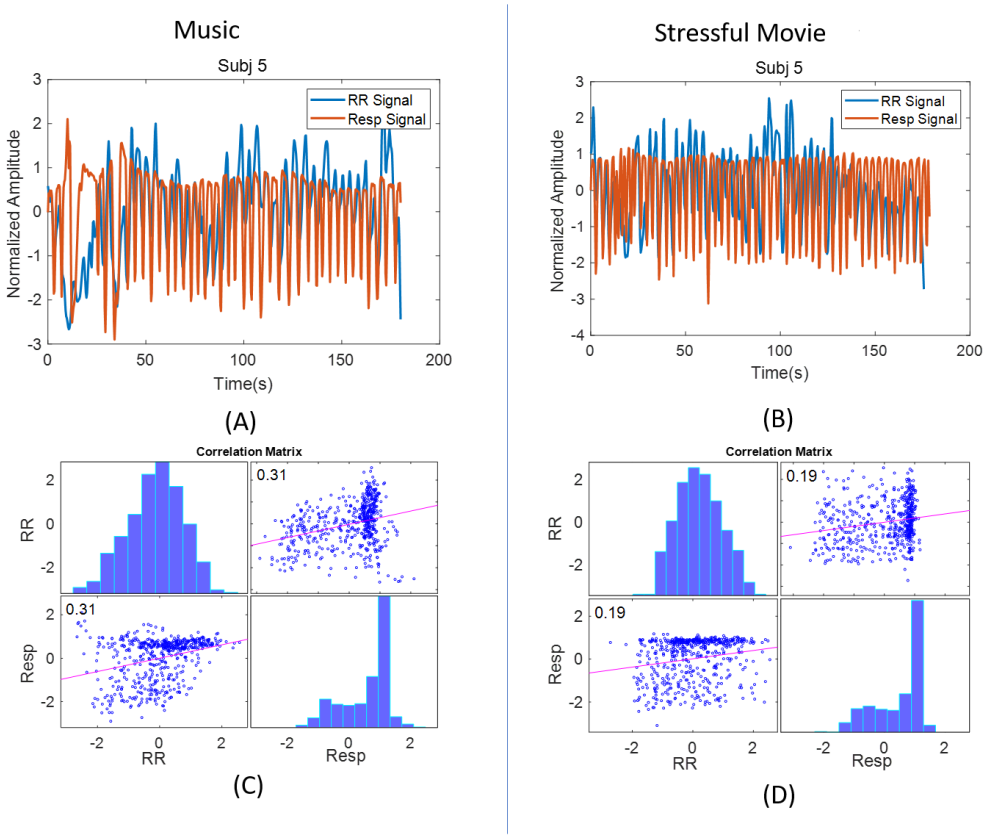

FIGURE 3.14: Correlation plot. Plot A and Plot B shows the normalized RR and Respiration signals for subject 5 from the Music group in Intervention condition (Relaxing Music) and Stressful movie condition respectively. Plot C and Plot D shows correlations between RR and Respiration. Histograms of the variables and scatter plots of variable pairs are displayed along with Pearson correlation coefficient. Correlation coefficient is higher for the Slow Breathing condition as compared to the Stress Movie condition.

this. Figure 3.15 shows Pairwise comparisons for correlation.

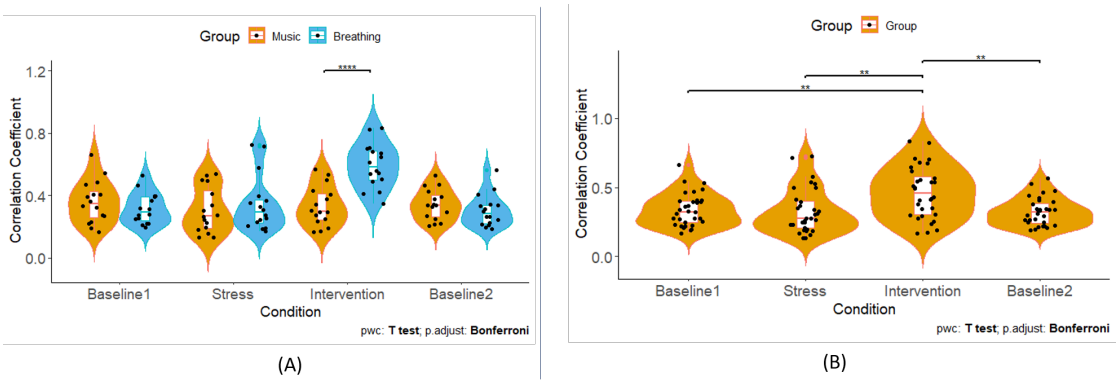

FIGURE 3.15: Pairwise comparisons for the correlation analysis. Plot A shows Pairwise comparisons between the two Intervention groups by Experimental condition. Plot B shows comparison across experimental conditions collapsed across groups Comparisons are Bonferroni corrected.

3.4.2 Coherence Analysis

In addition to correlation we also investigated heart rate-respiration synchronization using wavelet coherence technique in order to know at which frequency bands the synchronization between RR and Respiration is maximal. Coherence between the cardiac and respiratory signals has been found within the normative HF band (0.15–0.4 Hz) throughout the supine rest task in (Keissar et al. 2009). The coherence plots are shown in Figure 3.16. We hypothesized that this coherence would be shifted to LF band during slow breathing and there should be a high coherence in the HF band during the relaxing music condition compared to other conditions.

We calculated the wavelet coherence between the RR time series and the Respiration signal for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2) in LF (0.04-0.15 Hz)) and HF (0.15-0.40 Hz) bands.

Using HF coherence as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

The main effect of Intervention group was not significant, but there was a significant main effect of Experimental condition, $F(2.32,69.8) = 9.28$, $p < .001$, $\eta_p^2 = .0.23$, and an interaction between Experimental condition and Intervention group, $F(2.32,69.8) = 3.8571$, $p < 0.05$, $\eta_p^2 = .114$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that HF coherence was higher in Baseline1 than in the Stressful movie ($p < .05$), Intervention ($p < .05$) or Baseline2 ($p < .05$) conditions (Figure 3.17 B). Post hoc pairwise comparisons examining the interaction revealed that

only during the Intervention condition did the coherence between RR and respiration at HF band differ significantly between groups, with higher HF coherence in the Music than in the Breathing group (p <.001) (Figure 3.17) A) , All post hoc tests were conducted using Bonferroni correction.

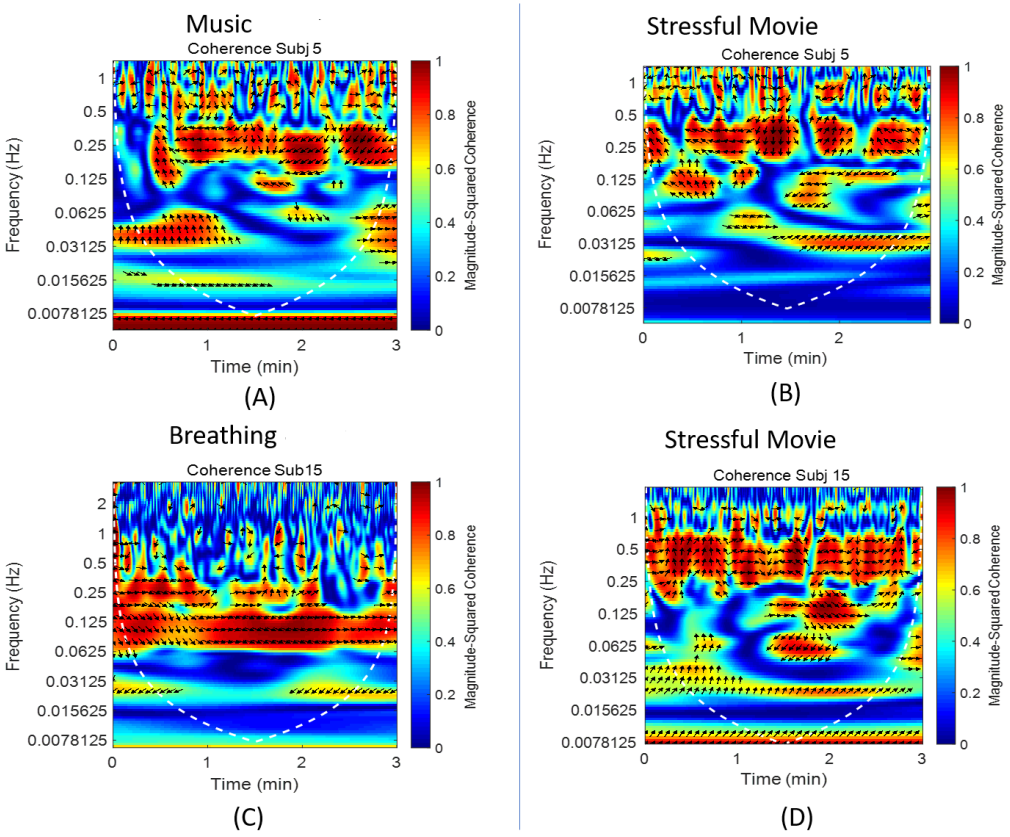

FIGURE 3.16: Wavelet transform coherence analysis between RR and Respiration. Plot A and Plot B show the time-frequency wavelet coherence for subject 5 in the Music group. Plot A corresponds to Music condition and Plot B to stressful movie condition. Plot C and Plot D show the time-frequency wavelet coherence for subject 15 in the Breathing group. Plot C corresponds to Breathing condition and plot D corresponds to Stressful Movie condition. High coherence is visible in Plot C around 0.1 Hz. The relative phase relationship is shown as black arrows. In-phase pointing right, anti-phase pointing left and vertical arrows pointing towards a 90 degree phase difference between RR and Respiration Signal.

We also investigated coherence in the LF band. The main effect of Intervention group was not significant, but there was a significant main effect of Experimental condition F (3, 90) = 21.35, p <.001, η_p^2 = .0.416, and an interaction between Experimental condition and Intervention group, F (3, 90) = 20, p< 0.001, η_p^2 = .4. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that coherence was

higher for the Intervention conditions than for the Baseline1 (p< .01), Movie (P< .01) and Baseline2 (p<.001) conditions (Figure 3.18) A). Post hoc pairwise comparisons examining the interaction revealed that only during the Intervention condition did the coherence between RR and respiration at LF band differ significantly beween groups, with higher LF coherence for the Slow Breathing than the Music group (Figure 3.18) B). All post hoc tests were conducted using Bonferroni correction.

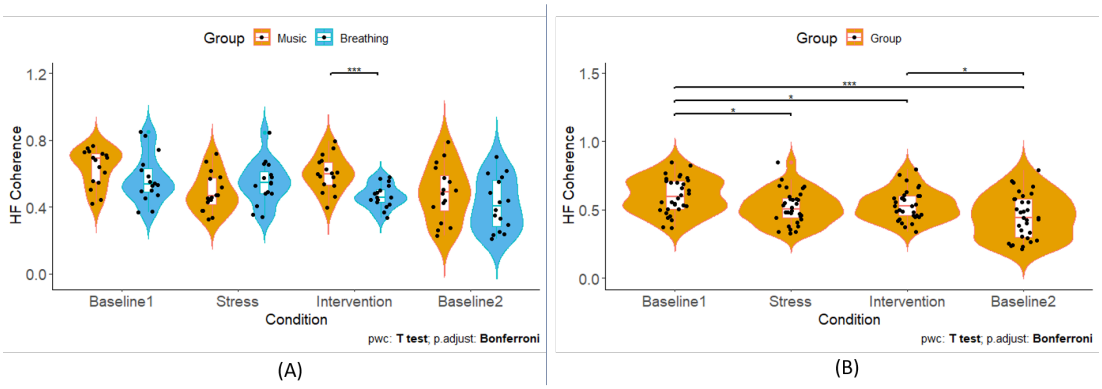

FIGURE 3.17: Pairwise comparisons for Coherence in the HF band. Plot A shows pairwise comparisons between Intervention groups for each condition. Plot B shows comparisons across experimental conditions collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

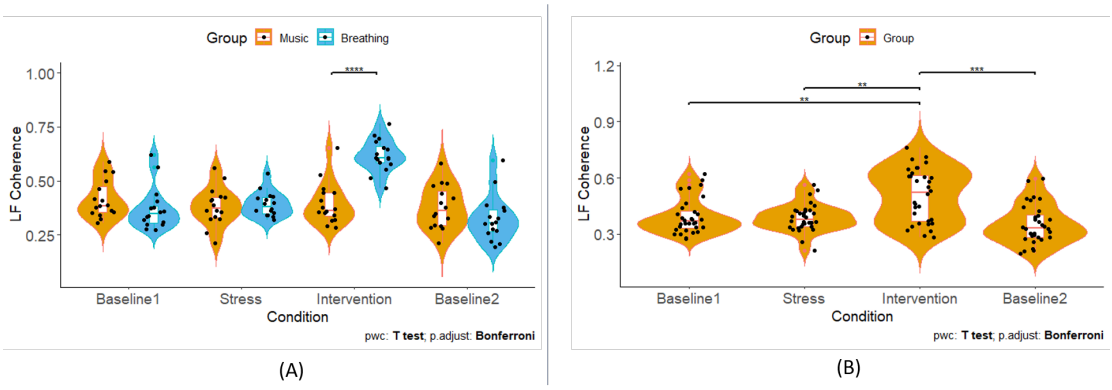

FIGURE 3.18: Pairwise comparisons for Coherence in the LF band. Plot A shows pairwise comparisons between Intervention groups for each condition. Plot B shows comparisons across experimental conditions collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

We have seen from Post hoc pairwise comparisons examining the interaction, that only during the Intervention condition did the coherence between RR and respiration at

LF and HF band differ significantly. Authors in Vickhoff et al. (2013) did not specifically looked at the HF and LF bands. We found in our analysis that the coherence was maximum in the HF band during Intervention condition (Figure 3.17) for Music group. where as LF coherence was maximum in the Intervention condition in the Breathing group. HF HRV oscillations are thought to be para sympathetically mediated, and LF HRV oscillations are thought to be both sympathetically and para-sympathetically mediated (Malliani et al. 1994; Pomeranz et al. 1985).

In summary, the larger HF coherence during the Music than the Slow Breathing condition is consistent with relaxing music influencing cardio vagal activity. Previous studies indicate that during slow breathing, the LF band reflects parasympathetic activity (Shaffer et al. 2014; Lehrer 2007). We could therefore interpret that parasympathetic activity was higher during the slow breathing condition compared to the music condition.

3.5 Galvanic Skin Response (GSR) Analysis

The GSR signals decomposed to tonic and phasic components are shown in Figure 3.19.

We calculated the Mean Tonic GSR for each Intervention group (Slow Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using Mean Tonic GSR as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Slow Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$

The main effect of Intervention group was not significant but there was a significant main effect of Experimental condition, $F(1.481,39.99) = 44.713$, $p < .001$, $\eta_p^2 = .623$ and no interaction between Experimental condition and Intervention group, $F(1.481,39.99) = .558$, $p = .623$, $\eta_p^2 = .020$. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that Tonic GSR was lower during Baseline1 than during the Stress Movie ($p < .001$), Intervention condition($p < .001$) and Baseline2 conditions ($p < .001$). In addition Tonic GSR was higher during the Stress Movie than during the Intervention ($p < .001$) and Baseline2 ($p < .001$) conditions. (Figure 3.20 B) significantly differed from Stressful movie condition ($p < .001$), Baseline1 condition also differed significantly from Stress Movie condition ($p < .001$). Post hoc pairwise comparisons examining the interaction revealed no significant difference between the groups 3.20 A). All post hoc tests were conducted using Bonferroni correction.

Results are in line with previous papers (Brouwer and Hogervorst 2014). We have decomposed the signals to tonic and phasic components and only considered the tonic response using the algorithm described in (Greco et al. 2015).

In Figure 3.19 the raw GSR signals are shown. Plot A,B,C,D represents the GSR activity for the whole duration of the experiment. The normalized signal is represented

by blue color, phasic component is represented by red color, tonic component is represented by yellow color. During the time period between 5 and 10 minutes, stressful movie condition increased the GSR amplitude marking a sympathetic arousal.

In summary, the GSR reveals the fact that stressful movie could change the intensity of emotional state. GSR signal does not represent the type of emotion, it only represents the intensity. There is no significant difference between GSR activity in slow breathing condition vs relaxing music condition. Stress stimuli resulted in an increase in arousal and thus an increase in skin conductance has been observed. More Details of the findings are discussed in the Discussion section 4.6.

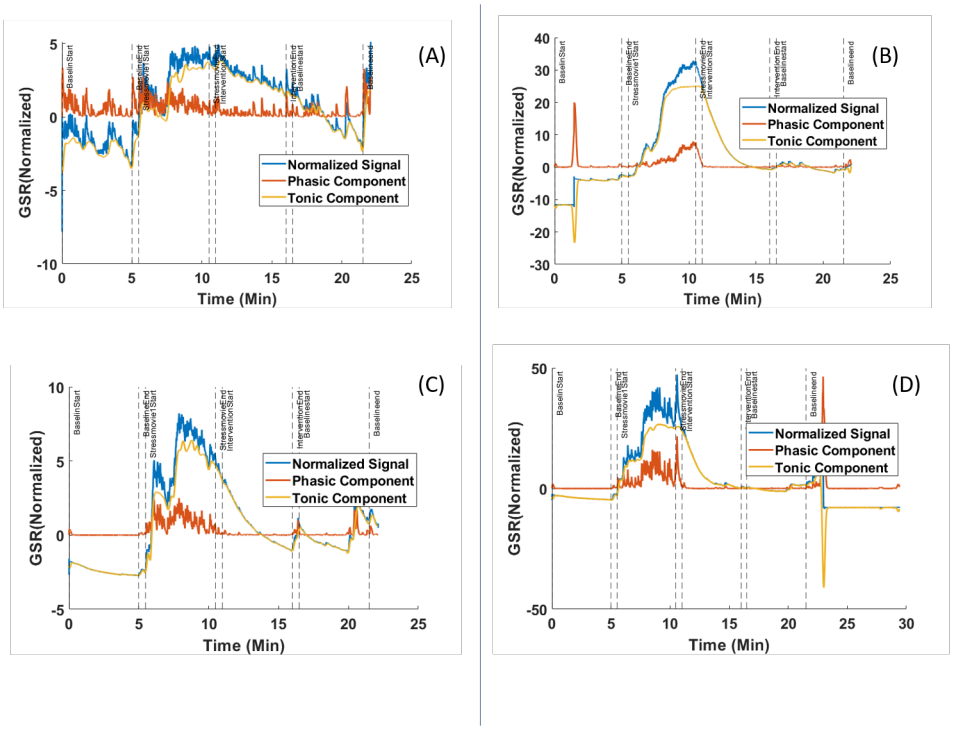

FIGURE 3.19: Galvanic Skin Responses. Blue color represents normalized amplitude. Red color represents Phasic activity and Yellow color represents Tonic activity of GSR. Plot A, B, C, D represents GSR activity for the whole duration of the experiment for subjects 13, 6, 24 and 30. Higher activity is seen in the stress movie condition. The vertical dashed lines represent Baseline1 start, Baseline1 end, Stress Movie start, Stress Movie end, Intervention start, Intervention end, Baseline2 start and Baseline2 end respectively.

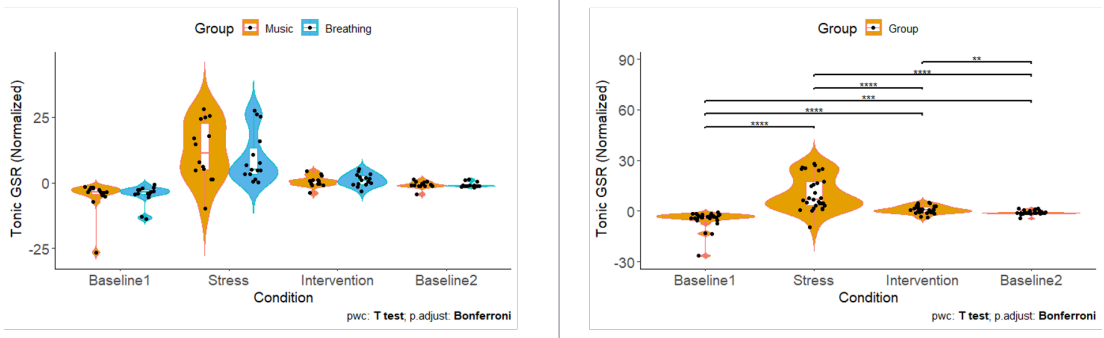

FIGURE 3.20: Pairwise comparisons for Tonic GSR activity. Plot A shows Pairwise comparisons between Intervention group. Plot B shows comparison across experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

3.6 Visual Analog Scale (VAS) for stress

We examined the Stress VAS for each Intervention group (Breathing, Music) for each Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). Using VAS as the dependent variable, we performed a 2×4 mixed repeated measures ANOVA with between-subjects factor Intervention group (Breathing, Music) and within subject factor Experimental condition (Baseline1, Stressful movie, Intervention, Baseline2). In cases where sphericity was violated, the Greenhouse Geisser adjustment was applied. Type-I error probability was set to $\alpha = .05$.

The main effect of Intervention group was not significant but there was a significant main effect of Experimental condition, F(1.481,39.99) = 44.713, p <.001, $\eta_p^2 = .623$, and no interaction between Experimental condition and Intervention group, F(2.187, 65.614) = .1861, p=.160. Post hoc pairwise comparisons of the main effect of Experimental condition revealed that Stress VAS was significantly higher for the Stress condition than Intervention (P < .001) and Baseline2 (p < .001) conditions (Figure 3.21 B). All post hoc tests were conducted using Bonferroni correction. VAS ratings are shown in Figure 3.21.

To summarize, VAS stress ratings confirm that Subjects in both the intervention group(Music,Breathing) felt a higher level of stress during stressful movie condition. Moreover, the perceived stress reduced in the Intervention and Baseline2 condition.

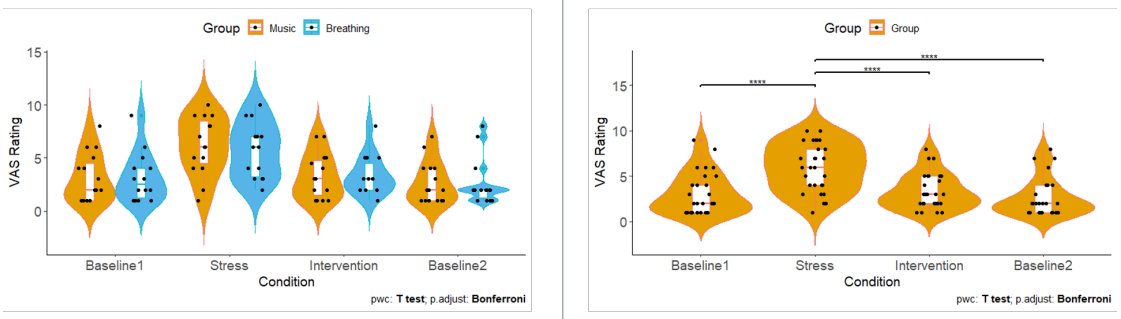

FIGURE 3.21: Pairwise comparisons for VAS Rating. Plot A shows Pairwise comparisons between Intervention group. Plot B shows comparison across Experimental condition collapsed across groups (Baseline1, Stressful movie, Intervention and Baseline2). Comparisons are Bonferroni corrected.

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3.7 Correlation between Stress Ratings and Physiological Measures

To examine how GSR and Respiration Rate related to perceived stress, we calculated correlations with the Participant's VAS ratings. We performed Spearman rank correlations between GSR and subjects' self-ratings for each subject across all conditions. The results showed that GSR activity was significantly correlated with perceived stress ($r_s(60) = 0.38, p = 0.0042$) for Music group and ($r_s(56) = 0.33, p = 0.0098$) for the Breathing group. We also investigated spearman rank correlation between Respiration and stress ratings. The correlation were not significant (p=.088 for Music and p=.5 for Breathing group). None of the HRV parameters measures correlated significantly with VAS stress ratings. We found a significant correlation p<.01 in both Music and Breathing groups in self reported perceived stress with skin conductance as shown in Figure 3.22.

We also found that Ln (LF) and Respiration in the Music group and also in Breathing group are negatively correlated. Respiration and GSR are not significantly correlated in any of the groups. We found that GSR and SDNN are not significantly correlated in any of the groups also GSR and LF not significantly correlated in any of the Groups. We found that respiration is negatively correlated with heart rate variability (3.23). We used Spearman rank correlation coefficient to measure the correlation strengths. In Gąsior et al. (2016) authors have found that the coefficients of correlation between Respiration rate and HRV decreased for all parameters such as in RMSSD, SDNN, LF, LF/HF etc. Our findings are also in line with their findings. Also, we did not see any significant correlation between GSR and HRV measures discussed above. Also, Resp and GSR are

non significantly but positively correlated. These findings suggest that respiration is a strong modulator of HRV and probably slow respiration rate is responsible for vagal stimulation that results in a high HRV and parasympathetic activation.

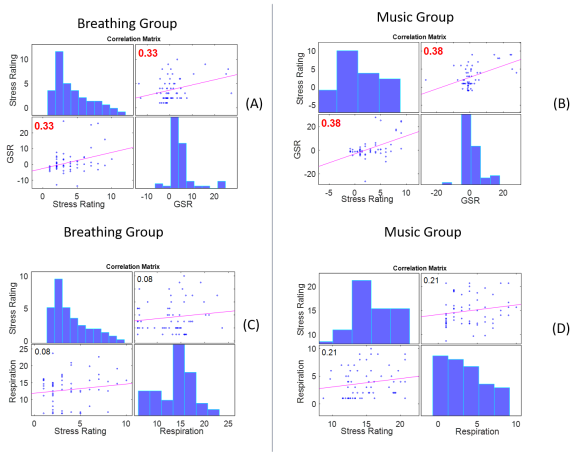

FIGURE 3.22: Spearman rank correlation matrix. The correlation between stress ratings of subjects in Breathing group and Music group with GSR activity is shown in plot A and B. The correlation between stress ratings of subjects in Breathing group and Music group with Respiration is shown in plot C and D. Histograms of the variables and scatterplots of variable pairs are displayed. Spearman Rank correlation coefficients (rs) of the significant correlations are shown in red.

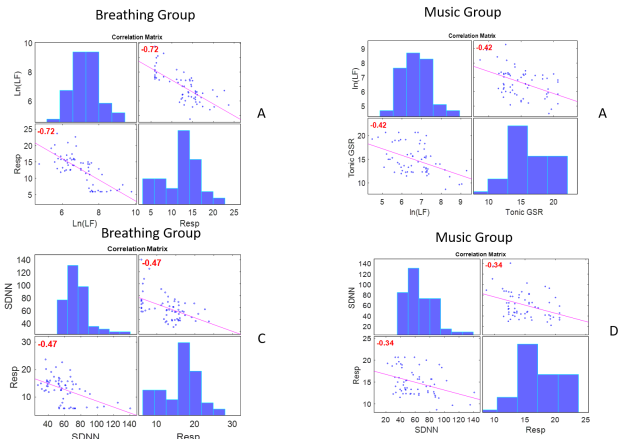

FIGURE 3.23: Spearman rank correlation matrix. The correlation between ln(LF) of subjects in Breathing group and Music group with Respiration is shown in plot A and B. The correlation between SDNN in Breathing group and Music group with Respiration is shown in plot C and D. Histograms of the variables and scatterplots of variable pairs are displayed. Spearman Rank correlation coefficients (rs) of the significant correlations are shown in red.

Chapter 4

Discussion

The purpose of this thesis was to investigate the efficacy of two widely used interventions (Breathing, Music) on psychological stress reduction. The main findings showed that (1) both the Music and Slow Breathing conditions affected perceived stress and (2) slow breathing had a larger effect than music on physiological measures of stress such as in HRV. Surprisingly, HRV was not affected significantly by the stressful movie, and it increased during the following Intervention condition only in the Slow Breathing group, not in the Music group. On the other hand, the amplitude of the GSR response increased significantly during the stressful movie. During the interventions, slow breathing reduced and music also reduced GSR amplitude. We also found that HRV and GSR changes during the Intervention conditions did not carry over to the immediately following baseline, suggested that they did not have lasting effects within the context of the present study.

4.1 Music Effects Perceived Stress not HRV

In this work we sought to understand the effects of Relaxing Music and of Slow Guided Breathing on perceived stress and psychophysiological measures including Respiration, HRV and GSR. We found that the Relaxing Music Intervention resulted in a lowered perceived stress in the Music group. Some previous studies have found reductions in perceived levels of psychological stress or altered levels of perceived relaxation after listening to music (Allen et al. 2001; Burns et al. 1999). Our findings from self-reports are in line with these studies. Not all previous studies have found anxiety reductions in all contexts, however. For example, Evans (2002) found that the music reduced anxiety of patients during normal care deliver, but had no impact on the anxiety of patients when undergoing different surgical procedures. They also found that music improved the mood and tolerance of patients but had little effect on vital signs. In our work, using a visual analogue scale, we found that both music and slow breathing reduced reported stress levels after watching a stressful movie. There was also a non-significant trend for a greater reduction with music than with slow breathing, perhaps because participants were not familiar with slow breathing techniques.

We found that HRV measure LF was higher in the Intervention condition for Music group compared to Baseline1 and Stress Condition although changes are not statistically significant. The LF/HF also increased during Intervention compared to Initial Baseline and remained high during the final Baseline, the changes are not significant after Bonferroni correction. Also, Post Hoc analyses revealed that during intervention, HRV measures SDNN, LF, LF/HF in the Music and Breathing group differed significantly. We also did not see significant changes in HR in the Music group. The effects of music on physiological measures are not consistent across studies. Some studies found that sedative music decreased HR and blood pressure (BP) (Fuchs et al. 2018; JONG et al. 1973; Knight and Rickard 2001) whereas other studies report that music induced no changes in HR or BP (Davis and Thaut 1989; Strauser 1997; Vanderark and Ely 1994). Some studies have found that music does effect HRV. For example, Iwanaga et al. (2005) found that the LF component of HRV and the LF/HF ratio increased during both sedative and excitative music sessions, whereas HRV decreased when there was no music. Fuchs et al. (2018) found an increase in LF, HF and RMSSD during a music Intervention condition compared to a Stress condition.

In our study, relaxing music affected self-reported stress levels, but had minimal impact on HR and HRV. One possibility is that there are large individual differences in how people react physiologically to music (Davis and Thaut 1989; Harrer and Harrer 1977). It is also the case that we tested participants in groups, and this may have affected their physiological responses. Finally, our sample size of 32 participants was relatively small, so this study needs to be repeated with more participants and with a comparison of individual and group settings.

4.2 Slow Breathing Effects Perceived Stress as well as HRV

The self-report ratings revealed that slow breathing lowered participants' perceived stress from what they reported during the Stress condition, and that this effect was similar to that of the Music condition.

We found that HR increased from the stressful condition to the slow breathing Intervention. Previous literature on the effect of slow breathing on HR is inconsistent. Fuchs et al. (2018) found a decrease in HR with slow breathing, but Weippert et al. (2015) found an increase in HR during .01 Hz metronome guided slow breathing and Engel and Chism (1967) found a tendency for HR to increase during slow breathing. We conducted a pilot test prior to the LIVElab experiment with slow and fast breathing conditions. The pilot test had no Stress condition and no baseline condition, HR was significantly lower during the slow breathing compared to the fast breathing condition. The Results of that pilot test are shown in the Supplement section in the Figure A1.1. Thus effects of slow breathing on HR may depend on the context such as whether the measure is taken following a stressful situation. Further testing is needed to reach a conclusion about the effect of slow breathing on HR.

We selected SDNN as the time domain measure of HRV as SDNN can be considered as an index of physiological resilience against stress, with higher SDNN reflecting higher HRV (Kim et al. 2018). SDNN was significantly higher in the Breathing group compared to the Music group during the Intervention condition and SDNN increased from the Stress movie to the Slow Breathing condition, but this effect did not carry over to the following baseline period. Both SNS and PNS activity contribute to SDNN, and it is highly correlated with both LF band power and total power (Umetani et al. 1998). Our results are consistent with previous studies indicating that in slow breathing, SDNN increases (Shaffer et al. 2014; Guzik et al. 2007) largely due to a lower-frequency of para-sympathetically mediated RSA. and a higher SDNN reflects high HRV. Guzik et al. (2007) also found that increasing respiratory rate caused a significant reduction in SDNN ($p = 0.0136$). The SDNN value increases when HRV is large and irregular. So, there was a significant increase of SDNN from Stress to Intervention condition in the Breathing group ($p=.002$). Similar significant change is absent in the Music group ($p>.05$).

Our analysis of LF power and LF/HF ratio showed results that are consistent with previous studies (Fuchs et al. 2018). We found that LF power was significantly higher during slow breathing than music listening condition. This is likely because at the very low breathing frequency of 0.1 Hz the RSA resonates with the LF baroreflex that integrates frequency and Mayer waves (Julien 2006). Mayer waves are waves in arterial blood pressure caused by oscillations in baroreceptor and chemoreceptor reflex control systems (Julien 2006; Sleight et al. 1995; Akselrod et al. 1985; Lanfranchi and Somers 2002) , As well, when breathing is slowed to 0.1 Hz, RSA will fall into the LF band rather than the HF band, and so the LF measurement during slow breathing will reflect parasympathetic processes to a greater extent compared to when the breathing rate is faster.

Baroreflex modulates blood pressure fluctuations and when blood pressure increases, the baroreflex causes an immediate decrease in heart rate. Similarly, as blood pressure falls, the baroreflex causes an immediate increase in heart rate. The slow breathing stimulation causes maximal stimulation to the baroreflex and increases HRV (Lehrer and Gevirtz 2014). This baroreflex response is mediated through the nucleus tractus solitarius (NTS) which is located in the brain stem (Raven et al. 1997; Rogers et al. 2000). The NTS communicates with the amygdala and also extends to the insula (Volz et al. 1990). Lehrer and Gevirtz (2014) speculate that this is why HRV biofeedback has been effective in treating anxiety and depression (Patron et al. 2013; Zucker et al. 2009; Siepmann et al. 2008; Reiner 2008; Karavidas et al. 2007). Similar to LF power, we also found that the LF/HF ratio was higher during slow breathing than during music listening. Previous studies (Steffen et al. 2017) have reported higher positive mood and a significantly higher LF/HF HRV ratio in Resonance Frequency Breathing relative to the control group($p < 0.05$). Together, our analyses of frequency domain measures are consistent with previous studies.

The traditional techniques for Heart Rate Variability (HRV) analysis in the time and

frequency domains are often not sufficient to characterize the complex dynamics of the heartbeat that are influenced by nonlinear regulatory inputs. Moreover the nonlinear domain provides useful information for characterizing autonomic balance and nonlinear measures may provide more reliable markers of cardiovascular disease (Godoy 2016). We examined three nonlinear measures: Sample Entropy, DFA, and Poincaré plot indices. In Music group none of these measures revealed significant differences between the stressful condition and the music listening Intervention.

In the Breathing group, Sample Entropy decreased from the stressful condition to the slow breathing Intervention, and was significantly lower during slow breathing than during music listening. We found that the Poincaré plot index SD1 do not change significantly between the stressful condition, either slow breathing or Music group. On the other hand, the Poincaré plot index SD2 was significantly greater during slow breathing than during the stressful condition, and it was significantly greater during Intervention in Breathing group than in Music group.

The DFA analysis is a scaling analysis method to represent the correlation properties of a signal (Peng et al. 1995), that determines the the statistical self-affinity of a signal. The $\alpha 1$ index increased from the stressful condition to the Slow Breathing conditions and was significantly higher during slow breathing than music listening. The $\alpha 2$ index decreased significantly from the stressful condition to the Slow Breathing condition and was significantly lower during slow breathing than during music listening.

The interpretation of these nonlinear HRV effects are discussed in Section 4.3.

4.3 Slow Breathing and not Music reduces complexity of Heart and Respiration signal

The RR time series is generated by a network of biological oscillators that have nonlinear proprieties (Porta et al. 2006). It has been observed that a healthy cardiovascular system is associated with HRV of a chaotic nature that reflects adaptability and a capacity to respond to unpredictable stimuli (Beckers et al. 2006). Our work was consistent with that of Weippert et al. (2015) and Porto et al. (2018) in showing that slow breathing decreases the chaotic behavior of heart rate dynamics. The reasons for this are still under debate. One hypothesis is that in periods of restoration the complexity might decrease, while during dynamically changing challenges from the internal and external environment the complexity might increase. For analogy, HRV drops during exercise and recovers post-exercise (Michael et al. 2017). Similarly regular biofeedback training can improve resting HRV. The Vagal Tank Theory of Laborde et al. (2018) says that cardiac vagal tone can be depleted and replenished and that this provides an integrative psychophysiological index of self-regulation. Resting levels of cardiac vagal tone are considered different from reactivity and recovery levels. The theory hypothesize that depleting factors may decrease cardiac vagal tone momentarily, but replenishing factors may boost it at the reactivity level and might help build higher long-term baseline levels leading to improved psychophysiological self-regulation (Blum et al. 2019). Also, In the

loss of complex variability with exercise in heart rate dynamics is followed by a relaxation (recovery) period in which slowing of the rate is accompanied by reappearance of complex variability (Goldberger et al. n.d.). Perhaps we can consider the slow breathing/resonant breathing biofeedback, which has already been established as a treatment for stress-related disorders and symptoms (Wheat and Larkin 2010; Gevirtz 2013; Goessl et al. 2017), as a reactivity stage for (replenishing) or restoration which will be followed by a relaxation state. In this case, the reduction in complexity of the heart rhythm, marked by low Sample Entropy, might reflect a state of restoration and building reserves. In this context we can also discuss the unease modulation (UM) model (Arpaia and Andersen 2019). A reduction in subjective unease during practice is seen in patients who practice relaxation, mindfulness, and similar techniques and is also accompanied by reduction in sympathetic nervous system activation and an increase in para-sympathetic nervous system activation (Van Der Zwan et al. 2015). According to the UM Model, such autonomic changes will also increase reserves and increased reserves will tend to reduce levels of sympathetic nervous system activation. Thus, one interpretations of our results is that the reduction in HR complexity during slow breathing is actually increasing the reserves and will eventually tend to reduce the sympathetic dominance over time. We did not see any carryover effects from the Slow Breathing condition to the immediately following baseline condition, suggesting that longer slow breathing practice may be needed to see any long term benefit. It remains for future research to investigate this further.

The Poincaré plot is a scatterplot in which current RR is plotted as a function of previous interval. We did not see any changes to Poincaré plot index SD1, reflecting short-term changes in HRV, whereas Poincaré plot index SD2, reflecting long-term changes in HRV, was significantly higher in the Slow Breathing group compared to Music group during the Intervention condition. Our findings are in line with (Guzik et al. 2007) who found that SD2 decreased when the respiration rate increased from 6 to 12 breath per minute. The ratio of SD2/SD1 has been interpreted as a measure of the balance between long- and short-term HRV, by its analogy to its similarity to LF/HF. LF oscillations are thought to be responsible for long-term HRV variability (Kleiger et al. 2005). In sum, our findings of Poincaré Plot analysis suggest that a slow breathing pace will increase the long-term characteristics of HRV.

The scaling exponents of DFA represent the correlation properties of the heart signal. We have seen that $\alpha 1$ increased whereas $\alpha 2$ decreased significantly from the Stress condition to slow breathing. Similar results were were seen in the works of (Grigorieva et al. 2017) and (Weippert et al. 2015). Additionally, the scaling exponents of RR intervals differ between normal and pathological conditions (Grigorieva et al. 2017). Melillo et al. (2011) found reduced $\alpha 1$ under academic stress. Studies have also found that patients with higher $\alpha 1$ were associated with lower cardiac and total mortality (Chiang et al. 2016). We found a significant increase in $\alpha 1$ from stress to slow breathing but not from stress to music listening. One interpretation is that $\alpha 1$ is related to the balance of LF to HF heart rate fluctuations. Breathing frequency of 0.1 Hz inflates the power in the

LF range compared with the HF range, thereby it increases the DFA $\alpha 1$ value (Weippert et al. 2015). The relationship of autonomic regulation to DFA will require more investigation, which is beyond the scope of this thesis.

4.4 Respiration and Heart Rhythm synchronizes maximally in Slow Breathing group during Intervention.

The Respiration rate falls within the LF band during slow breathing whereas during music listening the Respiration rate falls within the HF band. We found that the Intervention condition showed a significantly higher correlation between RR and Respiration than Stressful Movie condition. Also, only in the Intervention condition correlation between RR and Respiration differ significantly. Studies have found that modulation of heart rhythm by Respiration is strongest at low breathing frequencies of approximately 0.1 Hz (Berntson et al. 1993; Bernardi et al. 2000; Stark et al. 2000). Our results are in line with the assumption of higher cardio respiratory synchronization during slow breathing compared to spontaneous breathing. The correlation was higher in the Breathing group vs Music group, although we did not quantify RSA, but a higher synchronization of respiratory and heart rhythm would reflect a high amplitude of RSA and also it will stimulate the baroreceptors maximally improving baroreflex. Slowed respiration to approximately 6 breaths/min can increase both HRV and Baroreflex sensitivity (BRS) (Bernardi et al. 2001). The resonance created by respiratory rate of 6 breaths/minute with the baroreflex loop and Mayer waves of arterial pressure is probably responsible for the increase of the respiratory sinus arrhythmia (Eckberg 2003; Cooke et al. 1998; Hirsch and Bishop 1981) and (Eckberg 2003; Cooke et al. 1998; Hirsch and Bishop 1981). At approximately 0.1 Hz the maximum heart rate oscillations occurs and at this frequency the heart rate oscillates with breathing at a 0 degree phase relationship producing both the highest amplitude of RSA and the most efficient gas exchange (Lehrer and Gevirtz 2014). Also, during periods of slow breathing, vagal activity can generate oscillations in the heart rhythms that cross over into the Low Frequency band of HRV (Lehrer et al. 2003; Ahmed et al. 1982). This might explain the high correlation of RR and Respiration signals in the slow breathing group during Intervention condition than the Music group.

Correlation analysis does not reveal information at which HRV frequency band the synchronization is maximal. To further evaluate this, we also conducted the wavelet coherence analysis. Our results confirm the hypothesis that the coherence between RR and Respiration would be shifted to LF band during Intervention condition in Breathing group and there should be a high coherence in the HF band during the Intervention condition in Music group. We found that only during the Intervention condition the coherence between RR and Respiration at HF band differed significantly. We also found that during the Intervention condition the coherence between RR and Respiration at LF band differed significantly. The significance of these findings are that it quantifies coupling and degree of synchronization between different oscillating systems (RR and Respiration). We found that body's two oscillatory systems (respiration and heart rhythms) become entrained and operate at the same frequency when subjects breathe

at a slower frequency. There are evidences that supports the claim of a strong coherence as a state of well being and health. Previous findings also reveal that even the experience of positive emotion can result in a sine-wavelike heart rhythm that too without any conscious control (McCraty et al. 1995). According to McCraty and Zayas (2014) during coherent states there is an increase in vagal afferent neuronal traffic which also inhibits thalamic pain pathways at the level of the spinal cord.

In the Music group the HF band coherence was increased in the Intervention condition compared to Stress condition. But similar significant increase is not seen in the Breathing group for HF band. This finding might suggest that Music can have relaxing effects as HF band is para-sympathetically mediated. In the Breathing group the LF coherence was increased in the Intervention condition compared to Stress condition. At a very slow rate of breathing (0.1 Hz) the LF band almost entirely represent parasympathetic dominance. Our results suggest that the coherence between cardiac and respiratory rhythm maximises in the LF band. The physiological interpretation of the coherence could be explored in depth in a future study building upon our present work.

4.5 Most effect of Intervention are not maintained past the time of the Intervention

We found that the effects of the Interventions, whether slow breathing or music listening, did not carry over immediately to Baseline2 condition with two exceptions. First SDNN was higher during the final compared to initial baseline prior to the stressful and Intervention conditions. Second, Sample Entropy was lower during the final baseline compared to the initial baseline. It is not clear whether we did not have enough power with our current sample size to see effects that were maintained past the intervention, or whether a longer intervention would be necessary to see more long lasting effects.

Future studies would also benefit from the inclusion of cortisol measures, which can indicate whether stress levels are reduced at time points after the interventions. Previous studies have shown that HRV biofeedback training significantly improve general emotional well-being and reduce anxiety (McCraty et al. 1998). Several studies also have reported in a reduction of cortisol with deep breathing (Perciavalle et al. 2017; Kim et al. 2013). Another hypothesis for lack of significant group differences in the final Baseline period can be accounted for the attrition due to study fatigue-which is a drawback of repeated measures design (Breach 2012).

In the Protocol for Heart Rate Variability Biofeedback Training (Lehrer et al. 2013) practitioners are instructed to breath at the resonance frequency for 20 minutes twice a day, which is a much longer duration than what we used in our study. So one possible reason why the effects of slow breathing (increase in HRV) was not maintained once the slow breathing ended, was the short duration's (5 min) of the slow breathing and music listening interventions in our protocol. Another possible fact was that participants unfamiliarity with the slow breathing protocol. The visual displays might also have been distracting as the subjects were instructed to watch the screen during the experiment

while listening to the auditory cue. It is possible that only auditory feedback or sonification may have reduced possible visual overload and fatigue. We did not measure cortisol in this study. We speculate that although the effects of intervention is not reflected by HRV post Intervention condition, cortisol levels will still remain low as found in Ma et al. (2017). It would be definitely worth investigating the levels of cortisol post intervention in both the groups (Music, Breathing) in future studies.

4.6 Intensity of emotional state reflected by GSR is affected by Stressful Movie.

Previous studies have shown the link between stress and GSR/EDA, with the amplitude of GSR increasing linearly with perceived arousal in participants given emotional stimuli (Winton et al. 1984; Manning and Melchiori 1974; Greenwald et al. 1993). Other studies have also reported a rise in GSR in stress inducing tasks like sing a song (Brouwer and Hogervorst 2014). Our results are in agreement with these previous findings. We found that the Stress movie significantly increased GSR. GSR did decrease from the Stress movie to the Intervention conditions, but there was no significant difference between the effects of slow breathing and music listening. From this we can infer that Slow Breathing is as effective as Music in lowering the stress.

It is noteworthy that the GSR signal does not represent the type of emotion, it only represent intensity. It has actually been reported that GSR increases in participants who practice breathing exercises. The reasoning provided was that deep breathing causes a phasic sympathetic release that increases sweating (Nida et al. 2014). The reason we did not see any statistically significant differences between Tonic GSR activity in the relaxing and slow breathing techniques can be explained from the facts that GSR intensity might also be effected by slow breathing which does not mean a higher sympathetic tone. A deep breath can elicit a sympathetic discharge that causes an increase in sweating, which increase the GSR response. Authors in GN et al. (n.d.) found that statistically highly significant increase in GSR was seen in subjects who practiced deep breathing (6 breath/ minute). The reasoning provided was that it is a sign of reduction of sympathetic tone and increase in parasympathetic tone following such breathing.

Moreover, GSR alone might not shed light on the effectiveness of relaxing music vs slow breathing in reducing stress. HRV analysis should be considered carefully along with GSR activity.

4.7 Correlation between GSR, Respiration and self Reports.

Previous studies have investigated correlation between behavioural scales and GSR. Najafpour et al. (2017) found that GSR is a reliable and valid measure for assessment of children's dental anxiety in the clinical context and may help to identify clinically anxious children before dental treatment so that appropriate Interventions can be provided.

To examine how GSR and respiration rate related to perceived stress, we examined correlations among participants' stress ratings, GSR and Respiration. We found that GSR activity was significantly correlated with perceived stress for both Music group and the Breathing group. The correlation between and Stress Rating was not significant but correlation of with HRV measures were significant.

We also found some significant correlations between personality traits and self reports of perceived stress (VAS). We found that PSS and VAS are significantly correlated. Moreover, we found PSS and STAI-T correlated significantly. Similarly, Yu and Ho (2010) also found a positive correlation between PSS and STAI-T ($r = 0.693$, $P < 0.01$) consistent with our results. We also found significant positive correlations between ISMA and PSS. and ISMA and STAI-T.

4.8 Limitations of the current study

One of the methodological limitations of the current study is its small sample size. We only had 16 subjects in each Intervention groups (Music, Slow Breathing). Previous research supports the efficacy of HRV biofeedback, and we have also replicated the same, but the effect did not carry over to the post intervention stage for the most part. Another limitation of our study is that our results may be limited to the sample characteristic of our population and may or may not generalize to individuals of other demographic or age. Although the effects of slow breathing have already been established with minimal individual variability with the exception of choice of individual resonant frequency which was fixed (0.1 Hz) in our study. Same can't be applied to Relaxing Music.

We asked the participants not to take caffeine 2 hours prior to the experiment. But consumption of caffeine outside that time window, engagement in exercise, food taken before the study, Body Mass Index etc, were not controlled for in the current study. We did not correct HRV for HR and also . Laborde et al. (2017) recommended researchers not to engage in routine correction of HRV for respiration in the case of spontaneous breathing. Previous researchers whose experimental design was much closer to ours such as (Fuchs et al. 2018) did not adjust HRV for any confounding variables such as age, gender, circadian rhythm, medication, diseases etc. Also, Geus et al. (2019) argued that adjustment approaches might remove meaningful variance in outcomes of interest which can be attributable to autonomic and neurophysiological phenomena. Future studies might address the knowledge gaps in our understanding of the meaning of HRV metrics and whether adjustment is required or not.

The ideal procedure for dealing with confounding factors is to get objective measures of these potentially confounding factors whenever possible. But it was not practically feasible in our study, which is another limitation. For instance to control for individual differences in blood pressure, it's recommended to measure the blood pressure directly (Geus et al. 2019). We also did not control for the influence of circadian rhythm. We had one group in the morning and one in the evening. It was not practically possible to

have consistent timings for the recording of PPG due to the limited availability of the recording space.

Another limitation of our study is that we did not measure cortisol which is a stress hormone. Some other studies have also found that breathing exercises can lower the levels of cortisol as mentioned above. In the work of Ma et al. (2017), authors found that Breathing Intervention group showed significantly increased sustained attention after training, compared to baseline. Also, there was a significant interaction effect of group and time in the diaphragmatic Breathing condition on cortisol levels. Authors found a lower cortisol level after training compared to control group. The conclusion was that diaphragmatic breathing (4 breaths/min) could improve sustained attention, affect, and cortisol levels.

We asked the participants to watch a screen where a circle was growing big or small and participants were instructed to breathe to that cue. Continuous watching of this stimulus might have been tedious and influenced self-reports. This might explain why participants reported a higher perceived stress in the Breathing group compared to Music group during the Intervention condition.

4.9 Future Direction

In our study there was neither a human interaction nor live played music in device-guided breathing. Future studies should employ music therapists in a group setting such as in a classroom before the exam starts and it could lead to interesting findings regarding HR and HRV. Another important suggestion for future research is to use real-time biofeedback as visual or auditory feedback to gain control over involuntary bodily functions including blood pressure or heart rate. In the current study, the subject is breathing to a paced cue with no conscious awareness of his or her bodily signals. We hypothesize that using a real time biofeedback will increase bodily awareness and increase focus or mindfulness, the part that is missing in our study.

Another possible direction would be to use user-specific resonant breathing frequency. We had to keep the resonant frequency fixed at 6 breath per minute. Researchers can pilot test with 4,5,6,7 bpm sound cues and find out the frequency at which maximum FFT amplitude of heart rhythm is achieved. Although, in a group study such as ours it's not possible to use individualized resonant frequencies.

The stressor we used was an excerpt from the movie 'the vertical limit'. Future studies might use Trier social stress test (TISS) and Stroop test and compare with Stressful movies in terms of the impact on HRV and GSR. Future investigations should also replicate the current study, with the addition of a larger sample size. Researchers are also encouraged to replicate our findings in actual real world settings such as in classroom setting for students, yoga classes, or in resilience training among public safety personnel who are exposed to Post-Traumatic Stress Injuries, etc.

Future studies could look at he gender differences, as well as differences between participants with prior training and familiarity with meditation, HRV biofeedback, meditative dancing, tai chi etc. Studies should explore different types of relaxing music besides western ones, such as Indian music melodic scale, healing Ragas like Raga Bhimpalas (Ubrangala et al. 2020), Raga Darbari-Kanhra, Raga Bageshvari (Sarkar and Biswas 2015) etc.

Chapter 5

Conclusion

The present study confirmed that slow breathing, also known as resonant frequency breathing, is an effective intervention for stress in a group setting. Moreover, subjective reports of perceived stress showed that listening to relaxing music also reduces stress. This study has shown the effects of interventions (Music, Breathing) on HRV, GSR and Respiration of subjects. This study also explored both the linear and non-linear domains of HRV and possible explanations were considered for increased HRV (marked by an increase in SDNN), increased synchronization between respiration and cardiac rhythms, and the loss of complexity of cardiac and respiratory rhythms during slow breathing. These findings can be used in association with current research on the use of resonance breathing techniques and relaxing music as instruments to guide training for stress resilience that would optimize overall health and performance of people both in an individual setting or in a group setting, such as in a yoga studio or classroom.

Appendix A

Supplement

A1 Pilot Test Results for Heart Rate

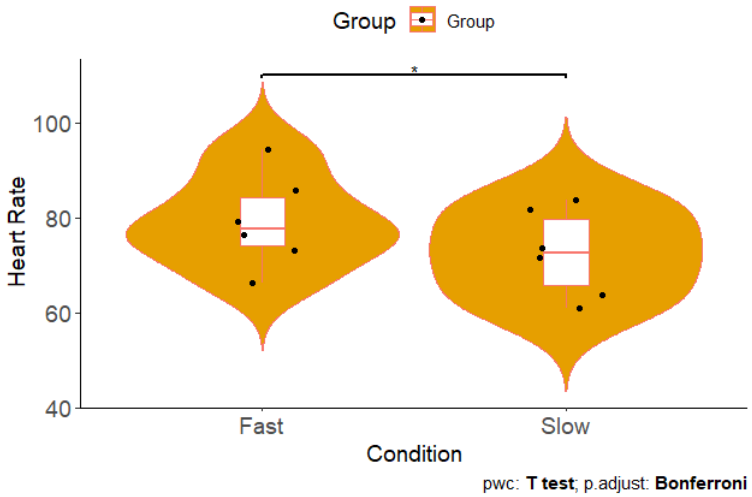

FIGURE A1.1: Fast and Slow Breathing comparison of HR. The plot shows the two Breathing Conditions Fast and Slow.

A2 Correlation analyses between personality traits and self reports of VAS stress

Correlation analyses between personality traits and self reports of VAS stress revealed some significant correlations. Significant correlations (p<.05) are shown by red color. The behavioural traits measured are ISMA Score, Perceived Stress Scale (PSS), STAI Form, Y-2(STAI), TIPI Extroversion(TIPIE), TIPI Agreeableness, TIPI Conscientiousness(TIPIC), TIPI Emotional Stability(TIPIE), TIPI Openness(TIPIO). As shown in figure A1.2 only PSS was significantly correlated with VAS stress scores ($r_s(64) = 0.44, p < .01$). The correlations are not corrected for multiple comparisons.

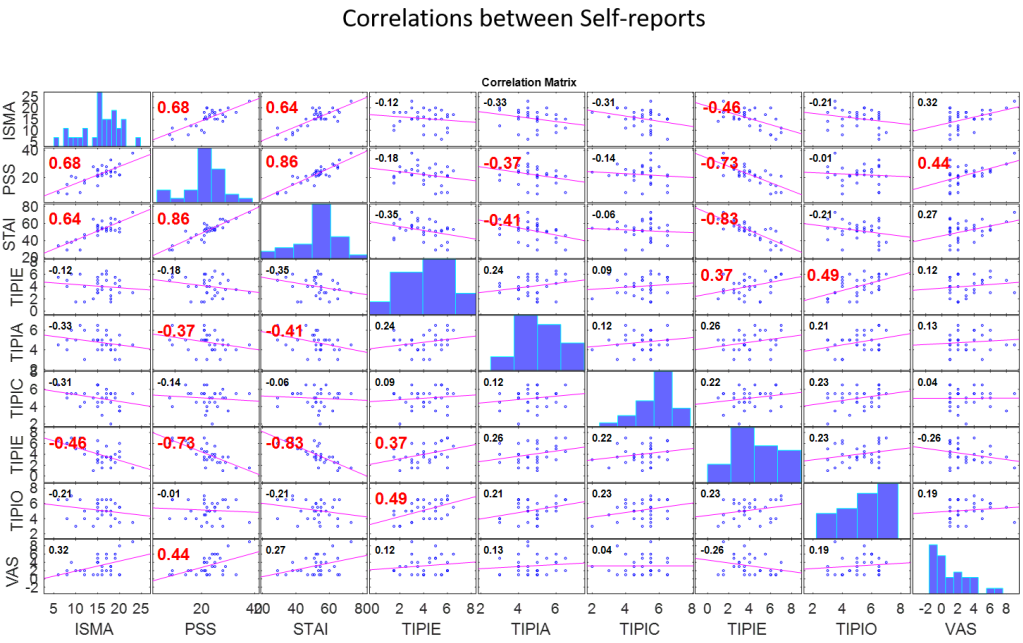

FIGURE A1.2: Spearman rank correlation. The correlation between personality traits and self reports of perceived stress is shown. Histograms of the variables and scatterplots of variable pairs are displayed. Spearman Rank correlation coefficients (rs) of the significant correlations are shown in red (p<.05)

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A3 VAS Ratings Table

TABLE A1.1: VAS ratings $Mean \pm SD$ of participant characteristics provided for both Breathing and Music group.

Categories	**Slow Breathing (16)**	**Music (16)**
Stress Rating 1	3.18 ± 2.22	3.00 ± 2.25
Stress Rating 2	5.25 ± 2.56	6.37 ± 2.70
Stress Rating 3	3.50 ± 1.86	3.21 ± 2.02
Stress Rating 4	2.62 ± 2.12	2.62 ± 1.89

A4 Questionnaires

Visual Analogue Scale for stress

Date: _____

ID: _____

Stress Rating 1

Are you feeling stressed?

No **1 2 3 4 5 6 7 8 9 10** **Severe**

Stress Rating 2

Are you feeling stressed?

No **1 2 3 4 5 6 7 8 9 10** **Severe**

Stress Rating 3

Are you feeling stressed?

No **1 2 3 4 5 6 7 8 9 10** **Severe**

Stress Rating 4

Are you feeling stressed?

No **1 2 3 4 5 6 7 8 9 10** **Severe**

3.9_Visual_Analog_Scale_v1 Feb-14-2020

FIGURE A1.3: Visual Analogue Scale for stress.

Ten-Item Personality Inventory (TIPI)

Here are a number of personality traits that may or may not apply to you. Please write a number next to each statement to indicate the extent to which *you agree or disagree with that statement*. You should rate the extent to which the pair of traits applies to you, even if one characteristic applies more strongly than the other.

Disagree strongly	Disagree moderately	Disagree a little	Neither agree nor disagree	Agree a little	Agree moderately	Agree strongly
1	2	3	4	5	6	7

I see myself as:
1. _____ Extraverted, enthusiastic.
2. _____ Critical, quarrelsome.
3. _____ Dependable, self-disciplined.
4. _____ Anxious, easily upset.
5. _____ Open to new experiences, complex.
6. _____ Reserved, quiet.
7. _____ Sympathetic, warm.
8. _____ Disorganized, careless.
9. _____ Calm, emotionally stable.
10. _____ Conventional, uncreative.

FIGURE A1.4: Ten-Item Personality Inventory

PERCEIVED STRESS SCALE

The questions in this scale ask you about your feelings and thoughts during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.**

Name _____ Date _____

Age _____ Gender (*Circle*): **M F** Other _____

	0 = Never	**1 = Almost Never**	**2 = Sometimes**	**3 = Fairly Often**	**4 = Very Often**
1. In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
7. In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
8. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9. In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

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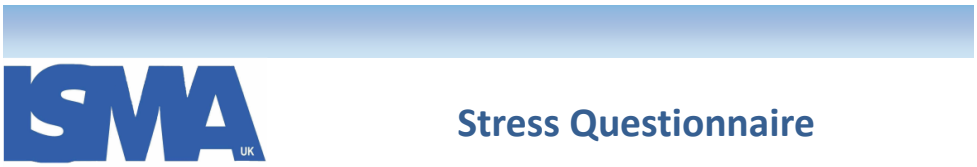
The PSS Scale is reprinted with permission of the American Sociological Association, from Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24,* 386-396.
Cohen, S. and Williamson, G. Perceived Stress in a Probability Sample of the United States. Spacapan, S. and Oskamp, S. (Eds.) *The Social Psychology of Health.* Newbury Park, CA: Sage, 1988.

FIGURE A1.5: Perceived Stress Scale



Stress Questionnaire

Because everyone reacts to stress in his or her own way, no one stress test can give you a complete diagnosis of your stress levels. This stress test is intended to give you an **overview** only. Please see a Stress Management Consultant for a more in depth analysis.

Answer **all** the questions but just tick one box that applies to you, either yes or no. Answer yes, *even if only part of a question applies to you*. Take your time, but please be completely honest with your answers:

		Yes	No
1	I frequently bring work home at night		
2	Not enough hours in the day to do all the things that I must do		
3	I deny or ignore problems in the hope that they will go away		
4	I do the jobs myself to ensure they are done properly		
5	I underestimate how long it takes to do things		
6	I feel that there are too many deadlines in my work / life that are difficult to meet		
7	My self confidence / self esteem is lower than I would like it to be		
8	I frequently have guilty feelings if I relax and do nothing		
9	I find myself thinking about problems even when I am supposed to be relaxing		
10	I feel fatigued or tired even when I wake after an adequate sleep		
11	I often nod or finish other peoples sentences for them when they speak slowly		
12	I have a tendency to eat, talk, walk and drive quickly		
13	My appetite has changed, have either a desire to binge or have a loss of appetite / may skip meals		
14	I feel irritated or angry if the car or traffic in front seems to be going too slowly/ I become very frustrated at having to wait in a queue		
15	If something or someone really annoys me I will bottle up my feelings		
16	When I play sport or games, I really try to win whoever I play		
17	I experience mood swings, difficulty making decisions, concentration and memory is impaired		
18	I find fault and criticize others rather than praising, even if it is deserved		
19	I seem to be listening even though I am preoccupied with my own thoughts		
20	My sex drive is lower, can experience changes to menstrual cycle		
21	I find myself grinding my teeth		
22	Increase in muscular aches and pains especially in the neck, head, lower back, shoulders		
23	I am unable to perform tasks as well as I used to, my judgment is clouded or not as good as it was		
24	I find I have a greater dependency on alcohol, caffeine, nicotine or drugs		
25	I find that I don't have time for many interests / hobbies outside of work		
A **yes** answer score = I (one), and a **no** answer score = 0 (zero).		**TOTALS**	

FIGURE A1.6: ISMA Stress Questionnaire

SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

Name_____ Date_____

DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

STAIP-AD Test Form Y
www.mindgarden.com

FIGURE A1.7: STAI-T self-evaluation questionnaire

PARTICIPANT BACKGROUND INFORMATION FORM

The following questions are optional, and you may choose to skip any that you would prefer not to answer. Any information that you do provide will be kept confidential.

Study ID: _____ Age: _____ Sex: _____

1. Do you **currently** play a musical instrument (including voice)?
   _____ Yes (go to question #2) _____ No (skip to question #3)

2. Please provide the following information for each instrument you **currently** play, starting with the one that you consider your primary instrument.

Instrument	Ages during which you have **played** this instrument	Ages during which you **took music lessons** on this instrument	Hours per week that you play this instrument **currently**

Please describe the situations in which you play (e.g., alone, in a small ensemble or band, in a large orchestra or choir, etc.)

_____

_____

3. Have you **previously** played an instrument (including voice) that you no longer play (e.g., as a child)?
   ____ Yes (go to question #4) _____ No (skip to question #5)

4. Please provide the following information for each instrument that you **used to play.**

Instrument	Ages during which you played this instrument	Ages during which you took lessons on this instrument	Hours per week that you played this instrument

Please describe the situations in which you played (e.g., alone, in a small ensemble or band, in a large orchestra or choir, etc.)

_____

_____

FIGURE A1.8: Demographic questionnaire

5. Do you have **dance** experience (lessons, amateur or professional experience)?
   _____ Yes (go to question #6) _____ No (skip to question #7)

6. Please provide the following information for each **dance style** you are familiar with.

Style of dance	Ages during which you danced this style	Ages during which you took lessons in this style	Hours per week that you dance(d) this style

Please describe the situations in which you dance(d) (e.g. alone, with family, in group classes)
_____
_____

7. Please indicate the highest formal music levels (instrumental/vocal performance, dance or theory) that you have achieved (e.g. Royal Conservatory, Theory, Suzuki Books, etc).

Instrument/Course/Subject	Level

8. Do you play music professionally? If so, please describe the situations in which you are paid to play music (e.g. performance, teaching, playing in bands, DJ, etc):
_____
_____

9. Describe your **current** recreational music and dance activities (e.g., "jam sessions" with friends, singing karaoke, dancing at nightclubs, etc.):
_____
_____

10. For how many years have you played any instrument (including voice) or danced regularly and consistently (e.g. at least 3x per week, most weeks of the year?) _____

11. How often do you attend musical or dance concerts or performances? _____

12. Have you had any formal ear training*? ___ Yes (____ years) ___ No ___ Not sure

*** In ear training or "aural skills" lessons, musicians learn to identify musical elements such as intervals, chords and rhythms, simply by hearing them.**

FIGURE A1.9: Demographic questionnaire

13. Do you play by ear*? ____Yes ____ No

*** playing or learning to play a piece of music by listening to a musical rendition, without the aid of printed material**

14. Do you have absolute/"perfect" pitch*? ___Yes ___ No ___ Not sure

*** absolute pitch is the ability to name notes without a reference, e.g. to hear a tone and immediately know it was a "C"**

15. Can you name a note if you are given a reference*? ___ Yes ____No ___ Not sure

*** e.g., if you heard two notes on the piano and were told the first one was a "C", could you name the other note?**

16. To the best of your knowledge, are you tone deaf*? ___ Yes ____No ___ Not sure

*** tone deafness is when you are unable to perceive differences of musical pitch accurately**

17. How many hours per week do you spend listening to music? _____ hours/week

18. Please describe your regular listening habits (e.g., listen to mp3/iPod on the bus, play stereo at home, etc.):

_____
_____

19. Do you pay close attention when listening to music? Please rank from 1 to 5
(music is background only) 1 2 3 4 5 (always pay close attention)

20. What styles of music do you listen to (e.g., rock, r&b, classical, traditional/folk, etc.)

_____
_____

21. Do any of your close friends or family members play a musical instrument (or did so in the past)? If so, please provide the following information.

Their relation to you	**Instrument that they play(ed)**	**How old were you (age range) when you heard them play?**	**Number of hours per week that you hear/heard them play**

22. Please briefly describe your other main activities or interests (e.g., sports, outdoor activities, art, reading, video game playing, etc.).

_____
_____

FIGURE A1.10: Demographic questionnaire

24. What is the highest level of education you have completed, or are currently completing?

____ High school / High school equivalency
____ College / skilled trade training program
____ University undergraduate (e.g. B.Sc., H.B.A, etc)
____ Graduate school – professional or academic (e.g. LLD, MD, Ph.D)
____ Other (please specify) _____
____ Prefer not to say

24. What is your current employment status?

____ Student
____ Employed – Full time
____ Employed – Part time
____ Unemployed
____ Retired
____ Other
____ Prefer not to say

25. Please indicate the range that reflects your annual household income

____ less than $30,000
____ $30,000 - $60,000
____ $60,000 - $90,000
____ $90,000 - $120,000
____ $120,000 - $150,000
____ greater than $150,000
____ Prefer not to say

23. Do you **currently** speak any other languages besides English? ___ Yes ___ No
If yes, please indicate which language(s) including English, the percentage of time that you use them, and the situations in which you speak each language.

Language	**Percentage (%) of time that you use this language**	**Situations in which you use the language**

FIGURE A1.11: Demographic questionnaire

24. Did you **previously** speak any languages other than English that you no longer speak? If yes, please list and describe the ages and situations in which you used these languages:

_____
_____
_____

25. Have you lived in North America for all your life? ____Yes ____No
If not, please describe where else you have lived, and for how long.

Location	**How old were you (age range) when you lived there?**

26. Do you have any hearing problems that you are aware of? If yes, please specify.

_____

27. Please indicate whether you are left or right handed when performing the following tasks:

	Left	Right	Both
Writing	_____	_____	_____
Drawing	_____	_____	_____
Using a Spoon	_____	_____	_____
Throwing	_____	_____	_____
Kicking	_____	_____	_____

28. Do you wear glasses or contacts? ____Yes ____No

29. Do you currently have a cold or other illness? ____Yes ____No

Thank you for your assistance!

FIGURE A1.12: Demographic questionnaire

24. Did you **previously** speak any languages other than English that you no longer speak? If yes, please list and describe the ages and situations in which you used these languages:

_____
_____
_____

25. Have you lived in North America for all your life? ____Yes ____No
If not, please describe where else you have lived, and for how long.

Location	**How old were you (age range) when you lived there?**

26. Do you have any hearing problems that you are aware of? If yes, please specify.

_____

27. Please indicate whether you are left or right handed when performing the following tasks:

	Left	Right	Both
Writing	_____	_____	_____
Drawing	_____	_____	_____
Using a Spoon	_____	_____	_____
Throwing	_____	_____	_____
Kicking	_____	_____	_____

28. Do you wear glasses or contacts? ____Yes ____No

29. Do you currently have a cold or other illness? ____Yes ____No

Thank you for your assistance!

FIGURE A1.13: Demographic questionnaire

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