# Physiological and psychological measurements during cognitive stress: comparing the effectiveness of two stress intervention techniques.

by

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**Abstract:**

**BACKGROUND:** A high level of stress can be detrimental to work performance and general health; in particular cardiovascular health. Work related stress has an effect on the autonomic nervous system and hence also can affect heart rate. By studying heart rate variability we can indirectly observe autonomic regulation in a convenient, non-invasive manner. Greater autonomic control and HRV are beneficial for managing work stress and could be beneficial for improving cardiovascular health particularly in an elderly population. HRV can be modified through changes in respiratory rate, and is enhanced greatly at slow breathing frequencies. **OBJECTIVE:** To establish meaningful relationships between physiological and psychological measures during a cognitive test; and to determine the effectiveness of short duration use of a beading system. The effects of age on autonomic regulation are also investigated. **METHODS:** Eighty-nine men and women were conveniently assigned into 3 intervention groups; beads, biofeedback and music. The participants completed a modified Stroop test before and after a 10 minute intervention with either of the devices listed above. Subjective questionnaires based on anxiety, relaxation, and medical history was completed before and after testing. Blood pressure, heart rate, breathing rate and perceived sleepiness were also recorded at specific intervals during testing. **RESULTS:** Age was significantly correlated with Stroop response time ($S_R$=0.2), and both LF ($S_R$=-0.2) and HF ($S_R$=-0.2) power in the cardiac spectrogram. Perceived mindfulness score correlated significantly with Stroop response time (r = -0.29). The participants in the beads group had significantly higher SDNN and LF power during the Intervention compared to the music group. Participants using the beads responded to the Stroop test more accurately, reported a significant increase in mental relaxation, and had quicker response times with a near significant decrease in word mistakes pre to post (p=0.06). Listening to classical music was rated as a more useful stress management tool, but did not result in reduction in mistakes. **CONCLUSIONS:** A short duration intervention with the beading system, leads to noteworthy improvement during a cognitive test, a more variable heart rate, and increased subjective relaxation. The beading system can be an effective and inexpensive tool for reducing stress and improving cardiovascular health.

Abbreviations: SDNN – standard deviation of time between RR intervals, LF – low frequency, HF –high frequency.

# Introduction and background:

## The History of HRV:

The first mention of heart rate variability (HRV) is frequently accredited to Stephen Hales (1677 – 1761). In 1733 Rev. S. Hales noticed a pattern which resembled the breathing frequency, while studying the heart rate (HR) and blood pressure (BP) of a horse (Parati *et al.* 1995, Bernston *et al.* 1997, Billman 2011). The history of the technology for recording HR began with the galvanometer. This then led to the invention of the kymograph, the polygraph, and finally these technologies were incorporated and the electrocardiogram (ECG) was created (Bernston *et al.* 1997). More specifically, early in the 19$^{th}$ century Luigi Galvani and Alessandro Volta created the galvanometer. The galvanometer records changes in voltages between two points via magnetic induction (Bernston *et al.* 1997). Thereafter, the invention of the kymograph by Carl Ludwig (1818-1895) allowed the movements of the galvanometer needle to be recorded (Bernston *et al.* 1997, Billman 2011). The development of the ECG late in 1895 by Willem Einthoven (1860 – 1927), incorporated the technology of the galvanometer with that of the polygraph (Bernston *et al.* 1997, Billman 2011). An ECG indicates the changes in ionic flow between two electrodes placed on the skin, and indicates periods of contraction and relaxation of the heart muscle (McSharry *et al.* 2003). Therefore the ECG is essential for studying HRV. As science progressed, our understanding of HRV grew and new ways of analyzing the ECG were discovered. In the 20$^{th}$ century non-linear techniques based on the theory of "chaos" were first introduced. This was done in order to investigate some of the non-linear properties of HRV (Billman 2011). Bernston *et al.* (1997) and Billman (2011) write very clear and comprehensive introductions to the techniques and technologies behind the ECG and HRV, and the reader is referred to these papers for more information.

The Autonomic Nervous System (ANS) is made up of the sympathetic (SNS) and parasympathetic (PNS) nervous systems. The SNS dominates during stress causing a "fight or flight" response by the body. This response causes increases in; blood pressure, breathing rate, heart rate and energy mobilization (Thayer and Brosschot 2005, Karim *et al.* 2011, Moore *et al.* 2011). The PNS causes the opposite effects, enhancing vegetation and restoration (Thayer and Brosschot 2005). At rest in a healthy individual the PNS and the SNS are in a dynamic balance; although both systems are constantly changing (dynamic), they are in balance and neither dominates for extended periods of time. This dynamic relationship has some degree of variability, and helps regulate the ANS when under stress. In a systems model (in this case the ANS is a system) greater regulatory variability is beneficial. This is because the time taken by the system (ANS) to reach equilibrium (or balance between the PNS and SNS activation) decreases. In so doing, the time taken to reach the least

energy demanding state is reduced. Without this dynamic relationship, the health and stability of the ANS could be compromised (Thayer and Brosschot 2005).

HRV is the change in instantaneous heart rate; or the time change between consecutive heart beats (Papaioannou 2007, Kaikkonen *et al.* 2008, Kaur *et al.* 2013). HRV is a pseudo-random event it is not strictly periodical in nature (Pagani *et al.* 1986). In simpler terms, the variability between each heart beat is not always fixed. Logically this is to be expected as heart rate is a biological process not a mechanical one. These pseudo-random features of HRV have made it possible to analyze HRV using a wide range of different techniques. Measures of HRV can be used to study the PNS, SNS and the dynamic HR regulation of the ANS by providing reliable, repeatable (Pagani *et al.* 1986), quantitative data in a non-invasive manner (Akselrod *et al.* 1981, Acharya *et al.* 2006). The link between HRV and the ANS was first observed during the mid 1960's when two clinicians (Hon and Lee) observed the disappearance of variability in foetal inter-heartbeat intervals during foetal distress; this was taken to be indicative of possible ANS dysfunction (Akselrod *et al.* 1981, Saul 1990, Parati *et al.* 1995, Task force 1996). This was the first finding that HRV was reflective of the functioning of the ANS (Bernston *et al.* 1997) and ANS influence on the heart (McCraty *et al.* 1995, Moore *et al.* 2011).

The role of the PNS is very important for the regulation of the dynamic PNS-SNS relationship (Thayer and Brosschot 2005). The PNS controls the sympathetic stimuli, and by inhibiting the pre-frontal cortex can decrease sympathetic firing (Brosschot *et al.* 2007). This is important because it ensures that a full sympathetic response is not mounted immediately after stimulation, and thus allows for everyday functioning (Thayer and Brosschot 2005). A poorly regulated ANS will not inhibit the SNS from mounting a full systemic response to each and every small threat, and if this persists chronically physical and physiological health problems could arise (Brosschot *et al.* 2007). If the SNS in uninhibited the resulting behavior of an individual will be reaction to the situation in which they are placed, rather than a clearly processed response (Thayer and Brosschot 2005). The difference between a response and a reaction may seem trivial, but it is not always be so. Reacting to a situation is sympathetically driven and aims at preserving life; this instinctive reaction prompts either a fight or flight response to a harmful situation. In contrast, responding to a potentially dangerous situation involves choosing the most sensible solution to the problem with a clear, calm mind. In many instances in life, whether it be work-related or non work-related, difficult or stressful situations will arise, which require decisions to be made. A better regulated ANS could ensure more appropriate responses during these difficult periods whereas. Changes in arousal and memory processing have been linked with changes in cardiovascular activity (Hansen *et al.* 2003); by studying HRV we can observe autonomic changes in the cardiovascular system and record changes before and after a cognitive test. HRV is not the only method which is used to

study autonomic control of HR, HR recovery, resting HR and baro-reflex sensitivity can also be used (Thayer and Brosschot 2005). Some of these other techniques will be explained in this short review.

## Heart beat activity and initiation:

Heart rate is regulated both intrinsically and extrinsically (Glass 2001, Verrier and Tan 2009). Intrinsic regulation occurs due to the self-generated current produced at the sino-atrial (SA) node (Verrier and Tan 2009), and can regulate the heart rate independently of extrinsic influences (Verrier and Antzelevitch 2004). The SA node is composed of a specialized type of neural tissue containing voltage dependent channels called "funny channels" which allow the generation of the pacemaker current ($I_f$) (Verrier and Tan 2009, DiFrancesco 2010) and the intrinsic generation of HR. The major control of heart rate is done extrinsically by the ANS by generating a "central drive". This "central drive" is generated within the medulla; wherein the areas involved are the central tegmental field, ventro-lateral medulla (VLM) and the raphe nuclei (Bernston *et al.* 1993, Hjortskov *et al.* 2004). Neuronal information initiating a heartbeat from the medulla is received first in the SA node (Opthof 2000, Karim *et al.* 2011); thereafter the depolarization signal spreads through the bundle of His and to the atrioventricular node (AV) (von Borell *et al.* 2007, DiFrancesco 2010). This depolarization wave is what causes the co-ordinated contraction of the heart chambers. Cardiac neuronal cells depolarize in the same manner as non-cardiac cells; however this change in polarity is mediated primarily by calcium ions ($Ca^{2+}$) instead of a combination of calcium and sodium ions (Ganong 2005). Cardiac depolarization is characterized by a pre-potential phase and depolarization phase. The pre-potential phase occurs prior to depolarization, and is controlled by Transient voltage dependant calcium (T-type $Ca^{2+}$) channels, that allow the slow influx of calcium ions from the sarcoplasmic reticulum into the cells (Berg 2002, Ganong 2005). Once the neural threshold is reached, long-lasting voltage dependant calcium (L-type $Ca^{2+}$) channels are opened causing depolarization (Berg 2002). This process of depolarization and repolarization at the SA node is illustrated below in Figure 1.1. Heart rate is generated intrinsically and extrinsically; however, the regulation of heart rate is dependent on many external and internal factors providing regulatory feedback to the heart eg. blood pressure (Glass 2001) and breathing rate. Consequently, HR is complex and its variation is multi-faceted (Glass 2001).

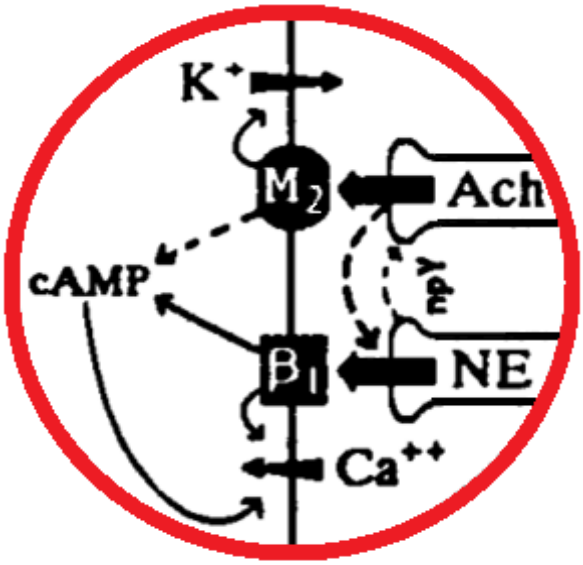


Figure 1.1: Representation of the autonomic control of heart rate at the SA node. Diagram copied with permission from Berston *et al.* (1993). $M_2$=Muscarinic receptor, $\beta_1$=Beta receptor, npY=Neuropeptide-Y, NE=Norepinephrine, cAMP=cyclic AMP, Ach=Acetylcholine.

Parasympathetic stimuli transmitted through the neurotransmitter acetylcholine (Ach) are directed towards the heart via cranial nerve 10 (vagal nerve) and cause a decrease in HR. The cardiac control centres in the medulla, the Dorsal Motor Vagal Nucleus (DMV) and Nucleus Ambiguus (NA) regulate parasympathetic control of HR via the vagus nerve (Green and Paterson 2008). Muscarinic (mainly $M_2$) receptors at the SA node receive the cardio-inhibitory signal from vagal nerve and have a dual effect. Firstly, the number of open potassium channels increases, thus prolonging repolorization, increasing hyperpolarization and decreasing $I_f$ slope (Verrier and Tan 2009), thereby delaying the time before the next impulse (Ganong 2005). Secondly, $M_2$ receptor stimulation also causes a reduction in the opening time of the calcium channels (particularly L-type $Ca^{2+}$ channels) by decreasing cyclic AMP, therefore further increasing time between consecutive heart beats (Ganong 2005). Vagal stimulation of the heart is directed primarily at the atria and SA node (Guyton and Hall 2006, von Borrell *et al.* 2007) and can cause HR to go as low as 20 beats per minute (McSharry *et al.* 2003). Sympathetic stimulation begins with glutaminergic neurons in the Rostral Ventro-lateral Medulla (RVLM) which stimulate spinal pre-ganglionic neurons, which in turn synapse with spinal ganglionic neurons and these relay the excitatory signals to the heart, blood vessels, kidneys and adrenal medulla (Guyenet 2006, Papaioannou 2007, Green and Paterson 2008, Mischel and Mueller 2001). At the SA node, SNS stimulation (mediated by $\beta_1$ receptors) affects HR by accelerating cardiac relaxation, reducing intracellular calcium and increasing spontaneous depolarization of the SA node. This is achieved through decreased hyperpolarization, increased $I_f$ slope (Verrier and Tan 2009), and increasing contraction

strength (Berg 2002). SNS stimulation on the heart causes a large increase in heart rate (chronotropic effect) and contraction power (ionotropic effect) (McSharry *et al.* 2003, Ganong 2005). Consequently, under sympathetic control, HR can increase from 70 to more than 200 beats per minute (bpm), with the possibility of contractile strength doubling (Guyton and Hall 2006). Removing the vagal influence (either through vagotomy or through the administration of atropine) increases HR to between 150-180 bpm as SNS activity is unopposed (Ganong 2005). Thus, HR appears to be controlled mainly by the vagal nerve (Spyer *et al.* 1988, Pal *et al.* 2004), yet is a balance between the SNS and PNS stimulations (Yasuma and Hayano 2004).

## The ageing cardiovascular system:

The work done in mammalian studies suggests that there is a link between heart rate and life expectancy. The human heart beats an average of 100 000 times per day and 2 billion times per lifetime (Opthof 2000), therefore if the maximum number of heart beats is fixed, decreasing HR might prolong life. The benefits of a slower resting heart rate cannot be denied, and people who have a lower resting heart rate live longer; however, the mechanism by which this slower heart rate helps increase life span is uncertain (Levine 1997). HRV may be used as a tool to study changes in autonomic regulation in elderly people, or people who are at risk of cardiac failure in order to monitor progression and increase longevity (Zulfiquar *et al.* 2010).

The intrinsic heart rate is between 100 and 110 beats per minute, and is the resulting heart rate after complete sympathetic and parasympathetic blockade (Jose and Collison in Opthof 2000). This intrinsic heart rate decreases with age (Kostis *et al.* 1982, Craft and Schwartz 1995, Opthof 2000), previous studies on autonomic blockade and intrinsic heart rate have shown that intrinsic HR decrease at a rate of (-0.6 to -0.8 beats yearly) (Jose and Collison, in Tanaka 2001). Intrinsic HR is not the only cardiovascular factor which changes with age. For instance, dysfunction of the hearts' electrical conduction system are usually seen after the geriatric years, whereas; cardiovascular complications (hypertension, valve dysfunction) begin while middle aged (Craft and Schwartz 1995). Another cardiovascular factor which increases with age is resting systolic blood pressure (Lakatta 2002). The primary reason for increased systolic blood pressure is increased arterial stiffness due to ageing. Diastolic blood pressure increases until middle age and thereafter decreases slightly (Lakatta 2002).

## How the ANS changes with age:

As a person gets older (without the influence of disease); the sensitivity of the heart to ANS stimulation decreases (Kostis *et al.* 1982, Stratton *et al.* 2003). SNS sensitivity decreases; this is manifested as a diminished response in blood pressure, heart rate, ejection fraction, after beta-adrenergic stimulation (Stratton *et al.* 2003) or exercise (Craft and Schwartz 1995). One of the

possible explanations for decreased responsiveness to beta adrenergic stimulation is that the aged heart has a decreased ability to cause $Ca^{2+}$ release (particularly in L-type calcium channels) (Lakatta 2002). As a consequence of sympathetic cardiovascular responsiveness decreasing with age, there is an increase in sympathetic nervous activity with age, this is to be expected. Baseline sympathetic activity is believed to increase with age (Dishman *et al.* 2000, Choi *et al.* 2006); this presents with increased norepinephrine and epinephrine levels in the circulation, more specifically; increased circulating levels, decreased plasma clearance and neurotransmitter reuptake (Lakatta 2002).

Baseline parasympathetic activity also decrease with age (Sloan *et al.* 1994, Levy *et al.* 1998, Dishman *et al.* 2000, Choi *et al.* 2006, Tolga Doğru *et al*. 2010), this effect is seen more on the heart rate rather than contractility (Stratton *et al*. 2003). The hallmark signs of this PNS decrease are a reduced HRV, baro-reflex response and sensitivity to atropine administration (Craft and Schwartz 1995, Levy *et al.* 1998, Lakatta 2002). Measures of HRV (discussed subsequently) also decrease with age, SDNN, RMSSD, pNN50 and SDANN were highest during the adolescent years and decreased thereafter until 80 years of age (Zulfiquar *et al.* 2010). The decrease in HRV with age can be attributed to decreased autonomic sensitivity, as differences in HRV between young and old participants were eliminated after autonomic blockade (Craft and Schwartz 1995). Other possible reasons for decreased HRV with age can include changes in cellular metabolism, decreases in the neuronal cells in the SA node, or changes in transmembrane potentials (Craft and Schwartz 1995). In summary; the ageing of the cardiovascular system causes a decrease in autonomic regulation which is dangerous for cardiovascular health, in particular cardiac health.

## The importance of HRV:

In both animals and humans, BP and HR have a degree of variability (Bernston *et al.* 1994). This dynamic variability (small fluctuations in HR or BP) is due to the interplay between the autonomic branches which influence them (Akselrod *et al.* 1981, Bernardi *et al.* 2001, von Borell *et al.* 2007, Min *et al.* 2009, Chen 2011, Karim *et al*. 2011, Paul and Garg 2012). Due to the complexity of these oscillations, this suggests that there are multiple biological systems influencing the regulation of BP and HR (Papaioannou 2007). The advantage of being influenced by so many systems is that higher numbers of regulatory systems provide a greater capacity for control (Vaschillo *et al.* 2002) and allow better adaptability. Oscillations in the neuronal control of HR and BP are part of a normal healthy cardiovascular system (Vaschillo *et al.* 2002, Lagos *et al.* 2008), and their function is to enable the cardiovascular system to respond to any external challenges (eg. stress) (von Borell *et al.* 2007, Paul and Garg 2012). Greater HRV, i.e. greater variability between successive heartbeats is associated with better cognitive performance, heart rate regulation and emotional responsiveness under pressure (Lagos *et al.* 2008). Therefore a more variable HR is

beneficial for coping in a stressful situation. A less variable HR is indicative of increased sympathetic control, or a decrease in parasympathetic control of HR (Xhyheri *et al.* 2012). A low HRV is a risk factor for mortality (Tsuji *et al.* 1994, Levy *et al.* 1998), and can be used as a predictor of cardiac and all cause mortality (Kors *et al.* 2007). A low HRV is also associated with hypertension and might be linked with coronary arthrosclerosis (Xhyheri *et al.* 2012). Changes in autonomic control of HRV can be studied by analysing HRV (Choi *et al.* 2006), and alterations in HRV have been studied particularly in cardiovascular disease patients (Choi *et al.* 2006). One of most important features of HRV is that it allows us to study the ANS during conditions which would be impossible to test directly (Malpas 2002).

## Calculating HRV:

On the ECG, normal cardiac depolarization will result in the recording of the P waves, QRS complexes and the T waves. The "R" wave in the QRS complex is the maximal upwards deflection on an ECG (Karim *et al.* 2011) and can be used to indicate a heartbeat. A tachogram reflects consecutive inter-beat intervals (IBI's) or R wave intervals plotted together (von Borell *et al.* 2007). In order to derive these R-R intervals (also NN intervals) from the ECG signal, a mathematical algorithm is used (Montano *et al.* 1994), where RR intervals are simply expressed as the reciprocal of HR (HR=1/RR) (McSharry *et al.* 2003, Karim *et al.* 2011). Thus from the tachogram we can either study changes in time between consecutive RR intervals, or changes in instantaneous heart rates. The time domain analysis of HRV is the simplest method by which HRV can be analyzed (Task force 1996, Billman 2011). Within the time-domain there are many mathematical models which are used to calculate HRV. These help reveal which are the important factors regulating HRV and what kind of affect these regulatory signals could have. To calculate time changes in RR intervals the standard deviation of consecutive NN intervals (SDNN) is frequently used (Task force 1996, Cohen and Benjamin 2006, Billman 2011). SDNN is believed to reflect overall variability (Tekelioglu *et al.* 2013). The square root of the mean squared differences between successive RR intervals (RMSSD) is also commonly used in time domain HRV analysis (Papaioannou 2007), and is indicative of vagal influence on HRV (DeGiorgio *et al.* 2010).

## Power spectral density (PSD)

In 1971 power spectral analysis first become used for HRV processing (Task force 1996, Billman 2011) and is now used in the research and clinical fields to determine ANS functioning (Acharya *et al.* 2006) as well as, PNS and SNS influence on the heart (McSharry *et al.* 2003). The results from the frequency domain analysis are comparable to those from the time domain analysis (Task force 1996). The difference being that one represents the data in the time domain, the other in the frequency domain. Spectral analysis of heart rate is done using either a Fast Fourier Transform (FFT) or Autoregressive Modeling techniques; this analysis gives us a quantitative understanding

of the frequency components, and how they contribute to the variability in HR (Stein *et al.* 1994, Pichon *et al.* 2006, Kaur *et al.* 2013). One of the results of spectral analysis is the frequency power, also referred to as the spectral variance (Parati *et al.* 1995). When studying HRV in the frequency domain the periodic elements of HRV can be grouped into several different frequency components, ranging from 0 – 0.5 Hz. The amplitudes and frequencies of HRV can be influenced by different environmental conditions such as sleep, stress, exercise and body position (Parati *et al.* 1995), so much so that no dominant frequency can be established (Saul 1990) thus ranges are used. Very low frequencies (VLF) are found at frequencies smaller than 0.04 Hz and are believed to reflect diurnal variation in hormone concentrations or temperature regulation (Paritala 2009). The branches of the ANS, although separate can be stimulated concurrently (Bernston *et al.* 1994) as low frequencies (LF) (0.04 – 0.15 Hz), reflect both sympathetic and parasympathetic activity, thermo-regulatory activity and can also reflect breathing rates (Parati *et al.* 1995). The predominant frequency of the LF component is linked with vasomotor drive and arterial blood pressure and is found at 0.1 Hz. High frequencies (HF) (0.15 – 0.40Hz) reflect parasympathetic dominance (Task force 1996, Perini *et al.* 2003, Acharya *et al.* 2006, Tekelioglu *et al.* 2013) and have a predominant frequency at 0.25 Hz which reflects respiratory rate (Montano *et al.* 1994, Acharya *et al.* 2006).

## Non-Linear Methods for calculating HRV:

HRV also has non-linear components (Glass 2001, Vuksanovic and Gal 2007) consequently these non-linear elements can be normalized. Logarithmic transformations are commonly used for this. Non-linear techniques allow a degree of complexity of the HRV to be studied rather than the variability itself (Billman 2011), and can be useful for revealing further information not yet recognized using linear methods (Task force 1996). Non-linear analysis of mathematical systems is well established; however the translation of these non-linear principles into biological systems is difficult (Glass 2001). For this reason, as well as for the complex nature of non-linear techniques, in this study they were not used and will not be discussed further.

## Factors affecting HRV:

HRV is higher in females (Levy *et al.* 1998, Acharya *et al.* 2006). Time of day and body position (for example supine or standing) also affect HRV (Pagani *et al.* 1986, Acharya *et al.* 2006). HRV parameters are highest at night, during the early morning hours (Bilan *et al*. 2005, Chen 2011) and lowest just before lunch (Kostis *et al.* 1982) as well as after dinner (Chen 2011). Drugs also can affect HRV; caffeine was found to reduce HRV (Sondermeijer in Rauh *et al.* 2006); whereas, cardiovascular drugs such as; statins, Beta-blockers and ACE-inhibitors will cause an increase in HRV (Bilchick and Berger 2006). Unhealthy lifestyles also affect HRV, smoking reduces overall HRV (Acharya *et al.* 2006), and studies have also shown that elevated levels of cholesterol and

body weight are associated with a reduced HRV (Thayer *et al.* 2010, Tolga Doĝru *et al.* 2010). Diabetes and hypertension also reduce HRV (Acharya *et al.* 2006); diabetic patients are found to have reduced RMSSD, NN50 and pNN50 all indicating a decrease in parasympathetic control of HR (Xhyheri *et al.* 2012).

## Emotions and how they affect HRV:

HRV is very sensitive to both emotional and physical/behavioural states in the body (Bernston *et al.* 1993, Bernardi *et al.* 2000). Emotions can also affect the dominant autonomic regulatory system; this is because emotions are partially regulated in the limbic system (Moore *et al.* 2011). Greater vagal tone is better for coping with stressful situations (von Borrell *et al.* 2007), and may also influence self-control and emotion regulation (Movius *et al.* 2005). Mental stimulation can also affect HRV; tasks which involve mental arithmetic and some measure of response time for example the Stroop test, cause parasympathetic withdrawal (Hjortskov *et al.* 2004, Movius *et al.* 2005) and sympathetic activation (Smirnov 2000, Berniston *et al.* 2004), hence reducing HRV. Positive emotions result in greater HRV and decreased systolic BP (Sloan *et al.* 1994, von Borrell *et al.* 2007), whereas negative emotions (anxiety or depression) result in increased diastolic BP (Sloan *et al.* 1994, von Borrell *et al.* 2007). Anxiety level also affects autonomic control; patients with higher trait anxiety had significantly lower baro-reflex control and heart rate, than low anxiety patients (Watkins *et al.* 1998). This relationship between anxiety and vagal control was found to be true in healthy men and women (Watkins *et al.* 1998). Depression also has an effect on HRV; in the study conducted by Carney *et al.* (1995) SDNN was found to be significantly smaller in depressed patients than controls, indicating that the overall variability is affected by depression.

## Heart Rate variability during exercise:

During rest vagal control dominates the activity of the heart, however as physical activity increases; there is an increase in sympathetic dominance (von Borell *et al.* 2007, Lagos *et al.* 2008). This is important because it causes physiological adaptations e.g. arterial dilation and venous contraction which allow a high HR, without hypertrophy, regardless of increased blood flow and blood pressure (Lakatta 2002). Physical activity affects vagal tone as people who take part in vigorous physical exercise, have a higher vagal tone and lower resting heart rate, when compared to those which participated in low intensity exercise (Thayer *et al.* 2010). This increased vagal tone and lower resting HR could be the reason why exercise is beneficial for longevity of life. As a consequence of 6 month endurance exercise intervention an increase in the HRV was found in both young and old groups (Levy *et al.* 1998). The decrease in sympathetic dominance of the ANS as a consequence of exercise is believed to be an indirect consequence of weight loss, reduced depression and increased insulin sensitivity (Curtis and O'Keefe 2002). The above studies show that exercise is beneficial for improving HRV.

## Stress and HRV:

Stress is when the demands of a task (whether at work or on the sports field) are greater than the person's perceived capacity to manage the task (Deshpande 2012). The stress felt as a consequence of work demands causes a reduction in vagal activity and is linked to increased SNS activation (Stein *et al.* 1994). This increased SNS activation can lead to increased levels of circulating chatecholamines (Chandola *et al.* 2010) which may indirectly cause health problems. Long-term work stress can also lead to harmful physiological, psychological and behavioural changes (Deshpande 2012). Physiological changes and health problems can arise if extreme, frequent or chronic stress are not managed (Pastor *et al.* 2008, Chandola *et al.* 2010). These physiological changes present as changes in systolic blood pressure (Vrijkotte *et al.* 2000), muscle tension, eye-strain, stiffness and increased injury risk (Pastor *et al.* 2008, Schultz *et al.* 2012), as well as altered cognitive function (Kofman *et al.* 2006, Sousa and Almeida 2012) and decreased work productivity (Deshpande 2012). Work stress has a negative effect on HRV, as participants with greater quantities of vagal tone are expected to perform better in tests involving attention and working memory (Kofman *et al.* 2006). Low levels of HF HRV have been shown to be indicative of poor autonomic flexibility and vagal tone (Berniston and Cacioppo 2004), therefore it is to be expected that a person suffering from high stress levels presents with low HF HRV. When studying the effects of mental stress and the cardiovascular response, changes in HR and HRV are very important (Hjortskov *et al.* 2004, Iwanga *et al.* 2005).

Coping with a stressful situation begins with the "fight or flight" response. The aim of this "fight or flight" centrally mediated response is to prepare the body for physical action. This SNS response presents as; redistribution of blood flow towards the brain and major muscle groups, increased heart rate (Iwanga *et al.* 2005, Karim *et al.* 2011), as well as an increase in blood sugar concentrations (Vogel 2006, Taelman *et al.* 2009). Pomerleau and Pomerleau (1991) describe a two stage response to stress or a stressful event; the first being increased sympathetic dominance. The second stage is seen if the stressor remains; the hypophyseal-adreno-cortical axis is activated resulting in the release of corticosteroids (cortisol) and some opioids. This secondary response to the stressor is thought to not only aid with managing the stress but also to reduce that activity of the first response, thereby maintaining some degree of homeostasis (Pomerleau and Pomerleau 1991). HRV decreases with stress because during a response to stress the SNS dominates; PNS regulation decreases and consequently so does HRV. A low HRV as would be seen after chronic activation of the "fight or flight" response means a diminished capacity to flexibly respond in a stressful situation, therefore greater rigidity and vulnerability (Horsten *et al.* 1999). How severely HRV is altered in response to a stress (acute or chronic), is dependent on the genetics of the person, his/her age and personality, as well as the environment they are in (Taelman *et al.* 2009). Type A personalities for example don't manage stress as well as type B (Kaur *et al.* 2013).The

negative consequences on health and work performance due excessive stress have been briefly discussed above. By increasing vagal control and HRV a greater degree of PNS influence on the ANS will result. With better ANS control the effects of a stressful situation can be handled more effectively, thus ensuring a relaxed response while under pressure (Prinsloo *et al.* 2011, Paul and Garg 2012).

The link between excessive stress and decreased HRV is probably due to the excess sympathetic nervous activity; other possible causes could be direct effects of stress on the neuro-endocrine system, or because of the unhealthy lifestyle (as a consequence of prolonged work stress) (Chandola *et al.* 2008). Some of the unhealthy lifestyle decisions include; a lack of exercise, excessive alcohol consumption and smoking (Chandola *et al.* 2008). The nicotine in cigarettes causes cortisol release (Pomerleau and Pomerleau 1991) as well as BP, HR increases immediately after, and significant increases in LF power and decreases in HF power 15 minutes after nicotine administration (Sjoberg and Saint 2011). The cortisol released as a consequence may not only be used to indirectly reduce stress, but in frequent smokers may actually be helping to maintain a level of homeostasis, as heavy smokers could have dampened cortisol sensitivity and tolerance (Pomerleau and Pomerleau 1991).

Job uncertainty, long work hours (Vogel 2006), lack of support and managerial supervision (Hjortskov *et al.* 2004) as well as information overload (Deshpande 2012) are the major ways that stress is perceived at the workplace. In the study conducted by Schultz *et al.* (2012) a sample South African employees (working in the financial sector) when asked on an online survey, responded yes to experiencing one or more repetitive strain injuries (RSI) symptoms. The RSI symptoms investigated in the survey included: muscle, neck and shoulder stiffness and were greatest in people who were classed as having a healthy view of work, but were exhausted. Psychological consequences of chronic stress include; exhaustion, behavioural problems, and problems with relaxation (Schultz et al. 2012).

That being said, stress at work is not always a negative thing, it ensures job productivity, and produces a form of accomplishment once the task is completed (Vogel 2006). A stressful experience may also be used to teach more efficient, and less demanding responses or actions to the stress in the future (Sousa and Almeida 2012). These benefits of stress are termed Eustress (Deshpande 2012). The stressful experience will only beneficial if the demands of the stressor are not so extreme that the person cannot adequately respond physiologically (Sousa and Almeida 2012).Thus in order to be effective at work a balance needs to be found, a balance between stress regarding task completion while avoiding anxiety due to impossible task demands. HRV can also be influenced by the following three systems; the baro-reflex, the respiratory rate and thermoregulatory control (Karim *et al.* 2011). The effects of the baro-reflex and the respiratory rate on HRV, are of particular importance in this research project, and will be subsequently discussed.

## The baro-reflex:

The baro-reflex is a neural feedback network through which BP is regulated (Hesse *et al.* 2007). The baro-reflex is thought to mediate short term changes in BP as would be seen with changes in posture or activity. Long term changes in blood pressure are believed to be regulated by the kidneys (Hesse *et al.* 2007). The baro-reflex is believed to be controlled by the PNS (Matsuka *et al.* 1996), and can be used to study cardiovascular regulation by the ANS (La Rovere *et al.* 2008). Baro-reflex sensitivity is the sensitivity with which the cardiovascular system responds to changes in BP, it increases with PNS dominance (Hesse *et al.* 2007, Papaioannou 2007), and sympathetic blockade (Pickering *et al.* 1972). The sensitivity of the baro-reflex is inversely proportional to heart rate; the higher the heart rate the lower the baro-reflex sensitivity (Hesse *et al.* 2007). Baro-reflex sensitivity also decreases with increasing mental activity (Conway *et al.* 1983). Thus baro-reflex sensitivity could be a useful tool along with HRV to study autonomic changes brought about by stress.

People who are constantly under stress or have had prolonged periods of sympathetic stimulation, will present with higher blood pressures and less effective baro-reflex systems. One of the possible explanations for this is that the baro-reflex sensitivity is decreased sensitivity (Frosman *et al.* 1983, Hesse *et al.* 2007). Decreased baro-reflex sensitivity causes changes in BP to no longer be recognized and thus BP remains elevated (hypertension). Baro-reflex sensitivity naturally decreases with age; this is believed to happen due to decreased stretch ability in the arteries (Gribbin *et al.* 1971, McCraty *et al.* 1995, Matsuka *et al.* 1996, Vaschillo *et al.* 2002, Billman 2011). This decrease in baro-reflex sensitivity with age is similar to the decrease in baro-reflex sensitivity with stress. This relationship is only natural as stress and age both reduce autonomic regulation.

With regards to the actual mechanism by which the baro-reflex works; an increase in blood pressure is detected by the arterial baroreceptors. The baro-receptors are primarily found in the carotid and the aortic arch (Vaschillo *et al.* 2002) and are essential for detecting changes in blood pressure. After stimulation, the baro-receptors relay sensory information to the CNS, more specifically the Nucleus of the Tractus Solitaris (NTS). Thereafter regulatory signals are released via the DMV and NA to stimulate changes in the autonomic regulation of HR and vascular tone, thereby maintaining BP (Vaschillo *et al.* 2002, Guyenet 2006, Taggart *et al.* 2011). However; because of the differences in the response times of the vagal and sympathetic arms, these time differences causes multiple oscillatory waves to be produced (Julien 2006, Papaioannou 2007). The vagal response time is much quicker than the sympathetic; therefore the majority of the baro-reflex regulation of HR is controlled by the PNS (La Rovere *et al.* 2008). These BP regulations are reflected within HRV, at 0.1 Hz. 0.1Hz falls within the LF range (discussed above), however the LF component of HR is a contentious issue, as there are still some differing beliefs on the

dominant autonomic branch/s regulating it. Nevertheless, it is commonly accepted that the LF range of the HR is reflective of blood pressure regulation.

## Effects of breathing rates on HRV:

Normal breathing rates affect BP and HR (Malpas 2002, Parati *et al.* 2013). During inspiration intra-thoracic volume increases, consequently the pressure inside the thorax decreases. With increased intra-thoracic volume, venous return and cardiac output decrease, resulting in an increased HR to maintain arterial blood pressure (Malpas 2002, Michard 2005, Papaioannou 2007). The breathing rate also affects BP through Respiratory Sinus Arrhythmia (RSA) (discussed subsequently) and through BP changes which are seen as a consequence of the mechanical actions of respiration (discussed previously) (Saul *et al.* 1991). By having a regulatory effect on HR, these processes also have a regulatory effect on HRV. As a consequence of the effects of breathing rate on HRV, these two physiological systems are frequently measured concurrently.

Unlike BP and HR, the breathing rate is under voluntary and involuntary control; thus in some regard by controlling breathing rate the actions of the ANS can be voluntarily and consciously controlled (Bernardi *et al.* 2001, Cerutti *et al.* 2006, Moore *et al.* 2011).This is important because better ANS regulation can lead to better decisions under stress and decrease the effects of chronic stress. Breathing rate is regulated by the limbic system, the ANS neural networks, the prefrontal cortex as well as the neuroendocrine system (Moore *et al.* 2011). Within the limbic system respiratory rate is regulated by the neural centres in the medulla; these being the ventral and dorsal respiratory groups (Bernston *et al.* 1993) and the pneumotaxic centres in the Pons (Guyton and Hall 2006). The dorsal group is believed to be responsible for regulating inspiration, ventral for expiration and the pneumotaxic centres are believed to be involved in frequency and depth of breath (Guyton and Hall 2006). The medulla is responsible for interpreting regulatory signals from peripheral and arterial baroreceptors and chemoreceptors (Green and Paterson 2008), thereafter regulatory signals leave the medulla and are directed via the spinal neurones to the heart, kidneys and arteries (Green and Paterson 2008).

Normal breathing rates (12-18 breaths per minute) have an influence on HF (Lehrer *et al.* 2000); respiratory rate influences only the PNS because the sympathetic response is too slow to affect high frequencies (Saul 1990). The PNS has a short latency, after stimulation of the vagal nerve the response of the heart is within a couple of hundred milliseconds (Akselrod *et al.* 1981, Bernston *et al.* 1993, von Borell *et al.* 2007). The quicker and more powerful response by the heart after PNS stimulation is also the reason why HRV is controlled by the PNS. The cardiac response to SNS stimulation is slower; this is due to the kinetics of the neurotransmitters used by the SNS (Saul 1990, Malpas 2002). The effects of sympathetic stimulation on the heart can be seen after 20 – 30s (Bernston *et al.* 1993, von Borell *et al.* 2007).

A faster, shallower breathing rate will reduce spectral power (Akselrod *et al.* 1981, Jorna 1992, Billman 2011), whereas a deeper slower breathing rate (4 – 8 breaths per minute) will produce a greater influence on the overall HRV (Engel and Chism 1967, in Jorna 1992), as well as increase HF (Kaikkonen *et al.* 2008) and enhance vagal activity on the heart (Bernston *et al.* 1993). The time spent inhaling and exhaling also affects the ANS (Pastor and Menéndez 2002, in Pastor *et al.* 2008). Slow breathing can be beneficial, some studies indicate that even short term practise of slow breathing rates can have a beneficial effect with regards to, increasing HRV and calmness as well as for reducing anxiety (Moore *et al.* 2011). It is believed that slow breathing exercises help manage stress by increasing energy, increasing mental arousal, increasing focus and performance (Moore *et al.* 2011).

## RSA (Respiratory Sinus Arrhythmia):

Carl Ludwig was not only credited for the invention of the kymograph (discussed previously) but he also is believed to be the first person to observe RSA (Yasuma and Hayano 2004). RSA is the momentary acceleration or deceleration of heart rate in synchrony with the breathing rate; as a consequence of the phasic changing of vagal outflow to the heart during inspiration and expiration, (Potter 1981, Bernston *et al.* 1993, Yasuma and Hayano 2004). RSA is believed to be controlled by the PNS (Bernardi *et al.* 2000), as it is greatly reduced after vagal blockade (Bernston *et al.* 1993). Therefore RSA activity can indicate a degree of vagal control on the heart (Ruediger *et al.* 2004) and can be used as measure of cardiac health (Verrier and Tan 2009). RSA is a naturally occurring process, and may enhance oxygen conservation and delivery to the respiratory exchange surfaces, during respiration (Potter 1981, Yasuma and Hayano 2004). RSA can be affected by many things such as; breathing rate, age, sleepiness, body position, and sex (Yasuma and Hayano 2004). Both RSA and HRV are markers of ANS influences on heart rate (Billman 2011). However; a change in HRV is not only brought about by respiratory changes, any bit of variability in HR is considered HRV, whereas the variability seen in RSA is due only to respiratory rate. For a clear illustration of RSA, please refer to the Figure 1.2 on the next page.

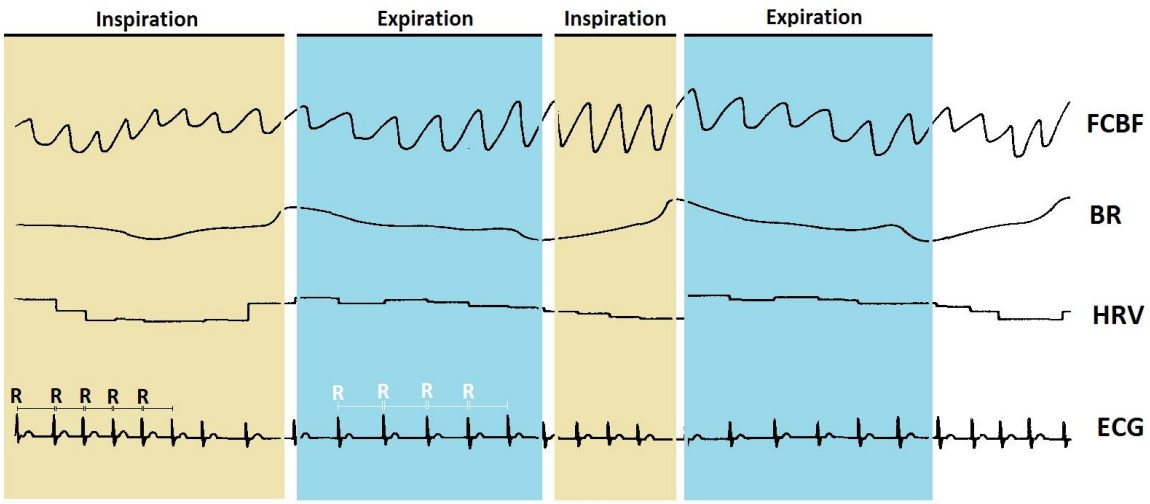


Figure 1.2: Edited figure from the study conducted by Ahmed *et al.* (1982), showing some cardiovascular changes while breathing at normal breathing rates (0.25Hz). Inspiration and expiration panels/phases are added for clarity. FCBF = finger capillary blood flow, BR = breathing rate, HRV = heart rate variability, R wave = part of the QRS complex found in an ECG and ECG = electrocardiograph. HR increases during inspiration (shorter distance between consecutive R-R intervals), and a decreases during expiration (big difference between R-R intervals).

RSA is maximal during slow breathing rates; this believed to be due to changes in blood gas composition and chronotropic changes of the breathing and heart-rate regulatory centres (Vaschillo *et al.* 2002). RSA is a biological phenomenon, occurring; either through direct vagal interruption by the pulmonary stretch receptors during lung inflation, or through regulatory changes in vagal influence of heart rate by the respiratory centres (Yasuma and Hayano 2004). During inspiration the lungs inflate; this is an active process controlled through the respiratory muscles. Central regulatory signals are sent down from the medulla (respiratory centres) through the anterior horn of the spinal column and out to the respiratory muscles via $C_3$, $C_4$, $C_5$, as well as to the diaphragm through the phrenic nerve and through $T_3$, $T_4$, $T_5$ and $T_6$ to the intercostals muscles (McNaught and Callander 1968). The muscles mentioned above contract during inspiration and cause air to enter the lungs. During inspiration phrenic nerves are active, consequently interactions between the respiratory centres and the cardiac centres in the brain cause vagal activity to be inhibited, more precisely cardiac vagal motor neurons (CVM) remain hyperpolarized, thus inhibiting the vagal nerve (Spyer *et al.* 1988, Bernston *et al.* 1993, von Borell 2007). Thus during inspiration vagal activity is almost completely abolished (Yasuma and Hayano 2004), and heart rate increases. At rest expiration is a passive process by which the inspiratory muscles relax; during expiration central drive is no longer needed, and the respiratory muscles simply relax. Therefore phrenic

activity is inhibited, and vagoexcitatory impulses are no longer inhibited (hence vagal activity is very near maximal), thereby decreasing HR (Bernston *et al.* 1993, Yasuma and Hayano 2004). This process was first described by Hering and Breuer in 1868 and consequently is called the Hering-Breuer reflex (Breuer, in MacDonald *et al.* 2009). This reflex is important because it demonstrates the direct effect of breathing rate on HR. In order to maximise RSA breathing rates need to fall within a range, the reason for this range and the average breathing rate at which RSA is maximal are discussed subsequently.

## Resonance and resonance frequency breathing:

Many processes in nature and in the human body have natural frequencies; circadian rhythms, breathing rate and heart rate (Glass 2001), other less known examples include; the metabolic rate, digestion and hormone release (Glass 2001). Resonance is when two separate systems are oscillating at the same frequency, leading to a resulting potentiating effect on both systems. Resonant frequency breathing is when the oscillations in the breathing rate match those of the heart rate, producing the greatest amplitude/variance in HR (Ahmed *et al.* 1982, Vaschillo *et al.* 2002, Gervitz 2003, Lehrer *et al.* 2003, Lagos *et al.* 2008, Lin *et al.* 2012). Most people will experience this resonant frequency breathing at ~ 0.1 Hz (6 breaths per minutes) (Lehrer *et al.* 2003). 0.1Hz is an approximation so some might need to take more and other fewer breaths. While breathing at this frequency HRV and baro-reflex oscillate together with the breathing rate, however at 180° out of phase of each other (Lehrer *et al.* 2000, Lehrer *et al.* 2003). Out of phase at 180° indicates that there are no differences between the two wave forms i.e. they are both displaying crests and both displaying troughs at the same time points. At normal breathing rates, inspiration causes heart rate increases (Hering-Breuer reflex) and BP decreases (discussed earlier); as a consequence of this change in BP the baro-reflex is activated and has a reducing effect on HR (Lehrer *et al.* 2003). Thus the breathing rate and heart rate have some measure of interference on each other. However, while breathing at the resonant frequency; inspiration still causes HR acceleration (Hering-Breuer reflex) and BP decrease, but because the breathing rate is slower (180° out of phase) it matches that of the baro-reflex, resulting in a potentiating effect on the HR. This potentiating effect on HR is seen as maximal HRV. Learning to breathe at the resonant frequency could be beneficial for the regulation of blood pressure, relaxation, and concentration while under pressure (Moore *et al.* 2011, Lin *et al.* 2012). Learning deep breathing techniques is easy to do and most people (regardless of culture, social and economic background) can learn it (Pal *et al.* 2004). Learning to breathe at the resonant frequency can be facilitated with a biofeedback device.

## Biofeedback:

A biofeedback device teaches a person to regulate one aspect of their physiology (for example breathing rate), and hence affect/influence other linked physiological processes e.g. heart rate (Lehrer *et al.* 2003, Paul and Garg 2012). HRV biofeedback devices give feedback on HRV and can be used to influence the amplitudes of both HR and BP. With sufficient HRV biofeedback training increases in variability are seen (Vaschillo *et al.* 2002, Lagos *et al.* 2012) and this may have beneficial effects with regards to treating depression and increasing cardiovascular control (Siepmann *et al*. 2008). A typical biofeedback device displays or produces feedback as a performance measure; for example a 0.1 Hz wave in the HRV spectrum, while the person is regulating his/her breathing rate (Nolan *et al*. 2005). There are a whole host of techniques/biofeedback devices which can help improve ANS regulation: yoga (Telles *et al.* 2011), deep breathing, visualization, meditation and tai-chi are a few examples (Yasuma and Hayano 2004). This inherent ability to influence ANS regulation with breathing occurs in part due to the fact that the ANS is regulated by many small interlinked systems, thus changes in one small component could have profound effects on the system as a whole (Papaioannou 2007).

## The limitations of biofeedback training:

These limitations were indicated by Lagos *et al.* (2008); the first limitation to biofeedback training being that not all athletes/users using biofeedback will learn how to translate the techniques learnt in the lab, to the sports field or while at work (Hjortskov *et al.* 2004). The problem here is the translation of the biofeedback technique (in this case deep breathing) out of the practise setting and into real life situations, rather than actually learning how to control breathing. The second limitation is that the long term benefits of HRV biofeedback training are currently unknown; the immediate beneficial effects could be short lived, or could also be enhanced with repeated use. Therefore further research needs to be conducted (Lagos *et al.* 2008, Prinsloo *et al.* 2011). Teaching of a biofeedback technique is also very dependent on the trainer, if he or she has an extensive knowledge and practical experience, the trainee will likely learn the biofeedback technique correctly and quickly (Pastor *et al.* 2008). In my personal experience, knowledge and familiarity with teaching biofeedback techniques is crucial, each person will respond differently to the training and being able to work around these difficulties (dizziness and hyperventilation or performance anxiety) in the shortest time requires experience.

## How music can affect emotions:

Hyde and Scalapino (in 1918) were the first to report the physiological effects of music on the cardiovascular system (Cervellin and Lippi 2011, Trappe 2012). The physiological consequences of listening to music have not been consistent in the literature (Iwanga *et al.* 2005). None the less; listening to classical has relaxing effects and is recognized universally for this. This is evident as

people are known to listen to classical music after a stressful day at work (Trappe 2012). Classical music is believed to have beneficial effects for patients suffering from: anxiety, stress, depression and sleep disturbances (Trappe 2012), most of which can be seen as a consequence of chronic work stress. Music can also affect mood, stimulate endorphin release, and influence task performance; hence, have an effect on the ANS (McCraty *et al.* 1998, Sohadze 2007). Taken together these suggest that music could have some beneficial effects with regards to stress management. In order for music to be an effective stress management tool, a decrease in HR (a sign of relaxation) while listening to the music, should be noted (Iwanga *et al.* 2005). These benefits are attributed to the fact that classical music (in particular Bach's music), does not have too many sudden changes in music structure, presenting with almost perfect mathematical periodic repetition (Trappe 2012). Mozart's music is also frequently used when investigating brain-music effects or music therapy (Cervellin and Lippi 2011). A minor limitation of music as a stress management tool is that not all people will respond to the music played to them; culture, personality, age, appreciation, and previous exposure to the music, as well as the genre and type of music all influence how the person responds (Ellis and Brighouse 1952, McCraty *et al.* 1998, Sohadze 2007, Cervellin and Lippi 2011, Trappe 2012). Regardless of these factors, the universally recognized usefulness of classical music to aid in relaxation cannot be disputed.

## Stress management:

With regards to stress management, evaluating stress levels before and after a management intervention is an efficient way to determine the effectiveness of the stress management tool (Van Zyl 2002). Deshpande (2012) writes about the importance of starting stress management (within a work context) from the top town. Senior managers are the ones who recognize stress can then put plans into action so as to reduce this for themselves, and their employees. Introducing techniques like yoga, meditation and humour might also be effective tools at reducing work stress (Deshpande 2012). Practises such as yoga, meditation and humour if done frequently enough could have a positive effect on HRV; thus studying HRV is a good way to objectively observe changes. Self evaluation questionnaires (for example Spielbergers State and Trait Anxiety Inventory) are reliable tools for subjectively evaluating stress level, and can be used to asses anxiety before and after a stress management intervention. The advantages of using self-evaluation questionnaires are that they are easy to complete: cost effective, easily comparable and they present an objective self-reported measure of stress (Van Zyl 2002). Disadvantages could be; ambiguous answers, and subjective feelings at that current moment (Van Zyl 2002). While self evaluation questionnaire can be used to study psychological changes as a consequence of stress; HRV and baro-reflex sensitivity can be used to observe some of the physiological changes due to stress (Sloan *et al.* 1994). In the study conducted by Lucini *et al.* (2002) HRV and other measure of stress (cortisol, inter-leukin 1, 2 and tumour necrosis factor-alpha) were also used. HRV can not only be used as a

tool to measures changes in autonomic control, but may also be an effective stress management tool.

## Models for ageing:

The advancement of science in particular with regards to the health sciences has ensured that people live longer (Levine 1997); by the year 2035 approximately 25% of individuals worldwide will be older than 65 years (Lakatta 2002). In Europe in 2005, the average number of young people (0 – 14 years), was equal to the average number of elderly (65+years) (World Population Prospects, in Dlugosz 2011). This figure puts incredible pressure on the working individuals to support an ever growing elderly population. According to Dlugosz (2011) the reasons why the numbers of young people and older people are so similar in Europe is due to lower birth rates, and higher immigration rates. Life expectancy is also on the rise; between 2000 and 2005 the average life expectancy was lower than 70 in Europe; yet, if current trends are maintained, between the years 2040 and 2045 the average life expectancy will increase to more than 80 in most European countries. Thus in Europe in particular, there is a need for cost effective tools which could increase health status, without causing too much of a financial burden on the economy. South Africa does not have such a severe problem; however, South Africans (in particular the working class) are suffering from epidemics like: HIV/AIDS, tuberculosis and malaria. This intensifies the demands on the working South African; to drive the economy and support the elderly. As was the case in Europe, a cost effective tool which can increase health status is needed in Africa.

## Rationale for conducting this study:

The natural process of ageing may seem completely unrelated to stress but in fact both have very similar effects on the ANS. Both stress and ageing decrease autonomic control of heart rate. By improving HRV, ANS regulation increases thereby ameliorating some of the negative consequences of stress and ageing on the human body.

This current study uses a protocol set up in our laboratory and is based on research previously conducted and published by Prinsloo *et al.* (2011). The study conducted by Dr. Prinsloo found beneficial effects of using an HRV biofeedback device (the StressEraser), with regards to reducing stress in stressed businessmen. The investigators in the above mentioned study found that the StressEraser device significantly increased perceived relaxation scores (SRSI3), decreased perceived anxiety scores; and significantly sped up reaction times during a Stroop test. The protocol described in this current thesis uses the same protocol previously used by Prinsloo *et al.* (2011), however with additional intervention groups. The aim of this thesis is to see if a cheaper locally produced “beads feedback device”, can have greater beneficial effects (on stress management) as compared to a 10 minute classical music intervention. In the current research

project the sample population (stresses business people) remains the same (as in Prinsloo *et al* 2011); however, women and a wider age range of participants are included in our study. As a consequence of having a larger and older sample size, the effects of ageing on the cardiovascular system, in particular HRV will also be studied. Participants will still be tested at the work place or retirement villages as the effects of stress on the autonomic balance may be greater if stress were to be measured at work (Sloan *et al.* 1994)

# CHAPTER 2: Materials and Methods

## Introduction:

Eighty-nine (89) participants were recruited for this study from various local businesses and retirement homes in and around Cape Town, South Africa. Information brochures/invitations were sent out to invite stressed middle management employees and healthy independent living residents to attend the testing sessions. The study protocol was approved by the University of Cape Town Research and Ethics Committee (Rec ref: 296/2005) in accordance with the Declaration of Helsinki. Participants were recruited as a random sample and were conveniently allocated into their intervention categories. Informed consent was first read and signed by all the participants. Participants were excluded if they were taking any heart altering medication or if they frequently participated in meditation. A total of 9 participants were excluded; 5 working and 4 retired participants were excluded from this study for not fulfilling the criteria. Volunteers were asked via email to abstain from smoking and drinking alcohol up to 4 hours before the start of the testing sessions. 52 males and 28 females, between the ages of 24 and 85 years old, were assigned into 3 groups; beads, biofeedback and music. Participants were conveniently assigned into each group; 31 into the beads group, 23 to the biofeedback group and 26 to the music group. The study was divided into two testing sessions, a Familiarization trial lasting 30 minutes and an Experimental trial (80 minutes). The methodology in chapters 3 and 4 are the same; the difference is that some participants are not included in Chapter 4 and the focus of the investigation shifts. Chapter 3 presents the combined results of the test when all three intervention devices were used. Chapter 4 compares and contrasts the beads and the music groups only, the details about the participants for this second study are described in Chapter 4.

## Familiarization (30 minutes):

All the testing was done at the various business offices or retirement residences with whom we were testing. The testing procedure was as follows; participants first completed a Spielbergers' TRAIT Anxiety questionnaire (STAI-T) where after instantaneous blood pressure (BP) and heart rate (HR) readings were taken. BP was measured using an electronic sphygmomanometer on the brachial artery of the left arm; this measurement was repeated twice and the average taken. The participants were then connected to a Biopac system (MP150WSW, Biopac Systems, Goleta, CA) ECG was recorded using a 3 lead ECG system; lead 1 was attached medially to the left shoulder and beneath the level of the clavicle. Lead 2, near the proximal end of the sternum and lead 3, between rib 9 and 11 on the left hand side underneath the heart. In order to measure breathing rates, a strain-gauge transducer (TSD101C, Biopac systems) was strapped around the chest at the level of the proximal end of the sternum. To calculate average breathing rates, average breaths per minute were taken over 3 separate minutes during the recording. The ECG and breathing rate were

sampled at a rate of 1,000Hz and digitized with a 16-bit analog-to-digital converter. Throughout the test for all HRV analyses 5 minute recording segments were used, the same 5 minutes were used for each test stage i.e. R1, S1, INT, R2 and S2 AcqKnowledge 4.2 software (Biopac Systems) was used in order to remove artefacts and ectopic beads from the 5 minute ECG recording. Once HRV recordings had been inspected and edited the tachograms were analyzed further with HRV Analysis (Biomedical Signal Analysis Group, The University of Eastern Finland, Finland). The Stroop task was programmed using E-Prime v1.1 computer software.

Participants were then asked keep quiet and sit still during the 10 minute baseline recording. Once this was completed, the leads and chest strap were removed and the participants underwent familiarization with the intervention tool. The intervention tools were listening to classical music, using the biofeedback device or using the beading system. Participants were taught how to use the beads and biofeedback devices. After connecting the Biopac to the participants again they were then required to complete at least 10 minutes of correct usage, of the bead system and biofeedback device. ECG and breathing rate were recorded during this time. The participants listening to music were instructed to simply sit and listen to the music; ECG and breathing rate were recorded. The track of music which was selected for this intervention was Frederic Chopin's Piano Sonata in E minor. Once completed the 10 minute intervention leads were removed and participants were reminded about the abstinence of alcohol and cigarettes before their next testing session.

## The Stroop task (80 minutes):

The second part of the study was also conducted at the participants' workplaces/residences. The participants did the Experimental trail approximately one week after the Familiarization trial. The Experimental trail began with participants completing the following questionnaires; Spielbergers' STATE anxiety questionnaire (STAI-S) and Smith's Relaxation Inventory questionnaire (SRSI3), a short health questionnaire (smoker, exercise hours per week, caffeine intake and medication use), as well as a reading of BP and HR with the same sphygmomanometer used previously. Thereafter participants were reminded how to use their devices, this was short; lasting a couple of minutes. The participants in the music group listened to 2 minutes of the music track previously played. ECG leads and the breathing transducer chest strap were attached. Participants were then explained how the modified Stroop task works, and were guided through a modified shorter version of the Stroop task (2 minutes in length). Once completed, the participants performed a familiarization Stroop task (5 minutes in length); ECG and breathing rate were recorded during this time. After the familiarization Stroop task, the participants had 5 minutes of rest, ECG and breathing rates were recorded, this was recorded as rest 1 (R1). Thereafter, the Stroop task was completed for a second time, ECG and breathing rates again, this was recorded as Stroop task 1 (S1). The participants were then asked to use the intervention tool (either using the beads,

StressEraser Biofeedback, or listening to the music track) for 10 minutes, ECG and breathing rates again were recorded. This time period was noted as the intervention (INT). After the intervention, instantaneous HR and BP were recorded once more; participants were also asked to complete a Visual Analogue Scale (VAS 1 – How sleepy do you feel at present?). This was followed by another recorded 5 minute rest period while ECG and breathing rates were recorded (R2). There after instantaneous BP and HR were measured, and VAS scale 2 (VAS 2 – How sleepy do you feel at present?) was also completed. The third and final Stroop task was completed; while data from the ECG and the chest strap were recorded (S2). Thereafter, instantaneous BP and HR readings were recorded, as well as two questions from the VAS scale (VAS 3 - How sleepy do you feel? & 4 – How helpful do you think the intervention tool was? - Explain). Participants then completed the final STAI-S and SRSI3 questionnaires. Please refer to the figure underneath for a graphical illustration of the Stroop test section during our protocol.

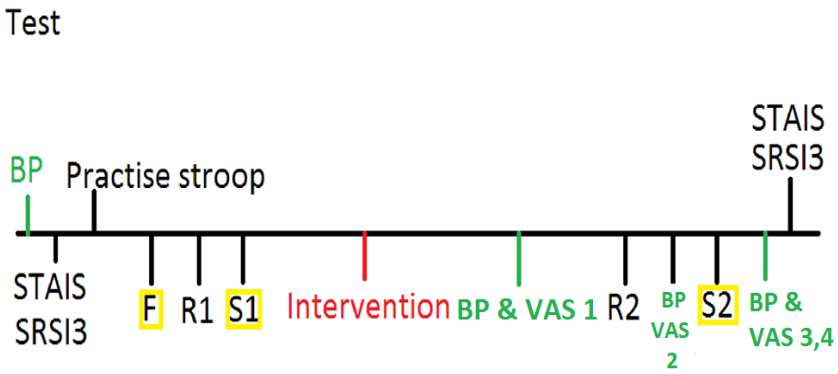


**Figure 2.1: Time line representation of the events during the Stroop test part of our testing protocol.**

## Statistical analysis:

All statistical analyses were conducted using Statistica 11(StatSoft), p-values smaller than 0.05 were considered to be statistically significant. Where relevant, data is presented as the mean plus or minus the standard deviation. In certain cases the data was normalized using a log transformation. Data was tested for normality using the Kolmogorov-Smirnov test for normality. Independent t-tests as well as ANOVA's were used for comparisons between the beads and the music groups. Tukey post hoc analyses were done to see where the significant differences were.

If the data was not normally distributed non-parametric tests were used. The Mann-Whitney U test was used for comparing two variables. The Pearson r index was used for correlating normally distributed data and the Spearman r index for non-parametric data sets.

## What is the beading system?

The beading system is a tactile feedback device that teaches a person to slow their breathing rate down; it consists of a bracelet made up seven brown beads separated by 2 black beads (see picture above). These beads are roughly the same size as your thumb and can fit comfortably in your hands. The brown coloured breads are smooth to the touch; the black ones are rough and have deep grooves in them. Users are asked to place one of the black beads in-between their thumb and index fingers, and to inhale fully. Once the users' lungs feel comfortably full, begin to slowly exhale. As they begin exhaling users "flick" or "cycle" through the brown beads so that by the time they had returned to the black bead, their lungs had comfortably emptied. One entire inhalation/exhalation cycle takes roughly 10 seconds. Thereafter, the breathing cycle begins again. Users are encouraged to take deep, slow inhalations and slow controlled expirations. Users are instructed to breathe in as they normally would but in a slower and deeper manner. As a consequence of using the beads; breathing rates are slower and the exhalation phase is extended. The principle behind the beading system is that the user can track his/her breathing rate visually and/or by simply feeling the different beads; while indirectly breathing at a much slower rate and with greater depth. Either hand could be used to "flick" through the beads.

## The music intervention:

Frederic Chopin's Piano Sonata in E minor was played during the intervention; this is a piece of classical music lasting approximately 10 minute. The track of music was 10 minutes in length so as to correspond to the intervention time allocation during the testing protocol. Music was played to the participants through laptop speakers in a quiet isolated room. During the familiarization and the intervention period, participants were asked to listen to the music, keep their eyes open and were encouraged to sit back and relax.

## The StressEraser Biofeedback device:

The biofeedback device displays the users' HR trace on a small screen and prompts the user when to breathe in and when to breathe out to thereby maximise HRV (Prinsloo *et al*. 2011).

## Chapter 3: Changes in physiology during the test:

In Chapter 3 participants were not differentiated into their specific intervention groups, rather the relationships between physiology, psychology, age and measures of HRV were presented graphically. First the effects of age on human physiology and Stroop task performance were presented; thereafter the change in HRV with age and systolic blood pressure was illustrated. The remainder of the chapter was used to illustrate some of the possible links between psychological measures (mental relaxation) and HRV as well as how breathing rate can affect both selected measures of HRV, and perceived anxiety and sleepiness. The aim of this chapter was to visually portray some of the factors which can affect HRV, as well as some of the statistical relationships between HRV and emotional state. In so doing, finding ways to enhance HRV or combat the decline of HRV with age or stress.

## The influence of ageing on physiology, psychology and Stroop task performance:

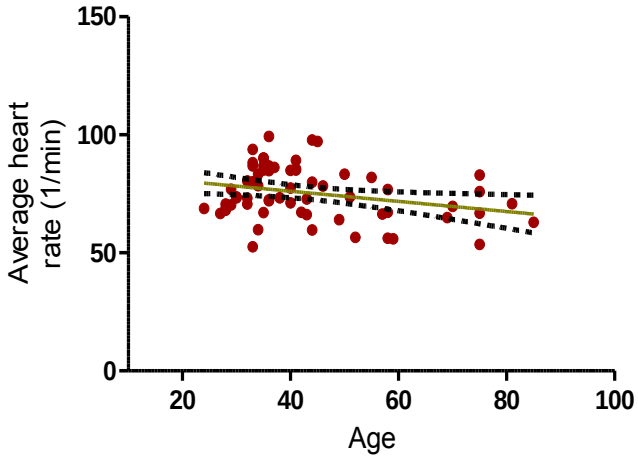


**Figure 3.1: The effects of age on heart rate and breathing rate. Average heart rate is presented from Rest 1 (R1).**

There was no significant correlation between average heart rate and age ($p=0.1$, $S_R=-0.19$). Heart rate is known to decrease with age; this figure illustrates the slow decline in HR with age.

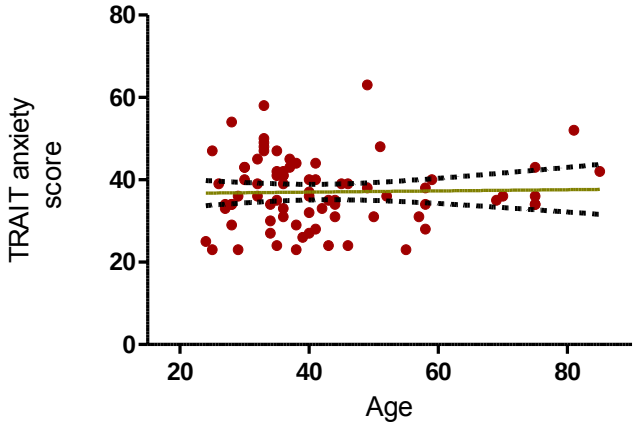


**Figure 3.2: The effect of age on TRAIT anxiety score. TRAIT anxiety as evaluated using Spielbergers' TRAIT anxiety questionnaire, evaluated before the Familiarization.**

Trait anxiety remained constant with ageing ($p>0.05$, $S_R$=-0.06) (Figure 3.2). There also was no correlation with STATE anxiety and age, this was seen both before the start of the Stroop testing session and at the end (not shown).

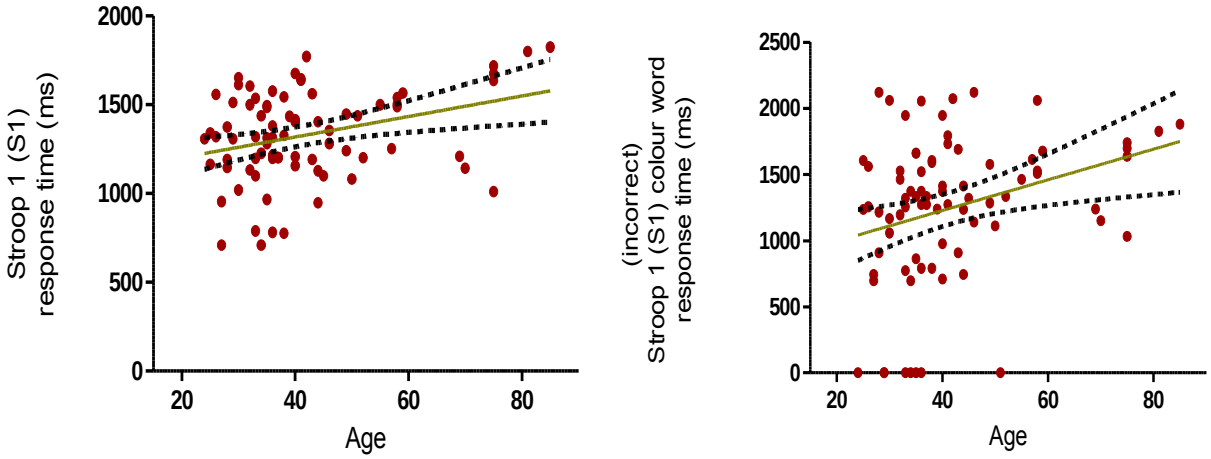


**Figure 3.3: The effect of age on reaction time during Stroop task 1 (S1). The figure on the left shows the effect of age on response time during S1 before either of the three 10 minute interventions. The figure on the right illustrates the effect of age on the time taken to respond (incorrectly) to colour word prompts during S1 before either of the three 10 minute interventions.**

S1 response time is the time taken to key a response during the Stroop task, this time is evaluated immediately after being shown the prompt on the screen. S1 response time was positively correlated with age ($p=0.02$, $S_R$ =0.25, 95% confidence interval= 0.02 – 0.4, $R^2$=0.1); this positive relationship suggests that with increasing age, there will be slower Stroop response time (Figure 3.3). Stroop colour word mistakes also correlated positively with age ($p=0.004$, $S_R$ =0.31, 95% confidence interval= 0.09 – 0.5, $R^2$ =0.08). The incorrect Stroop colour word response time is the

time taken (when responding incorrectly) to colour words (red, blue, green and yellow) during the Stroop task (Figure 3.3). This figure may seem confusing, however; the relevant and interesting information is on the x-axis itself. The only participants which made zero incorrect colour responses were found between the ages of 20 and 60; this further highlights the decrease in Stroop response accuracy with age.

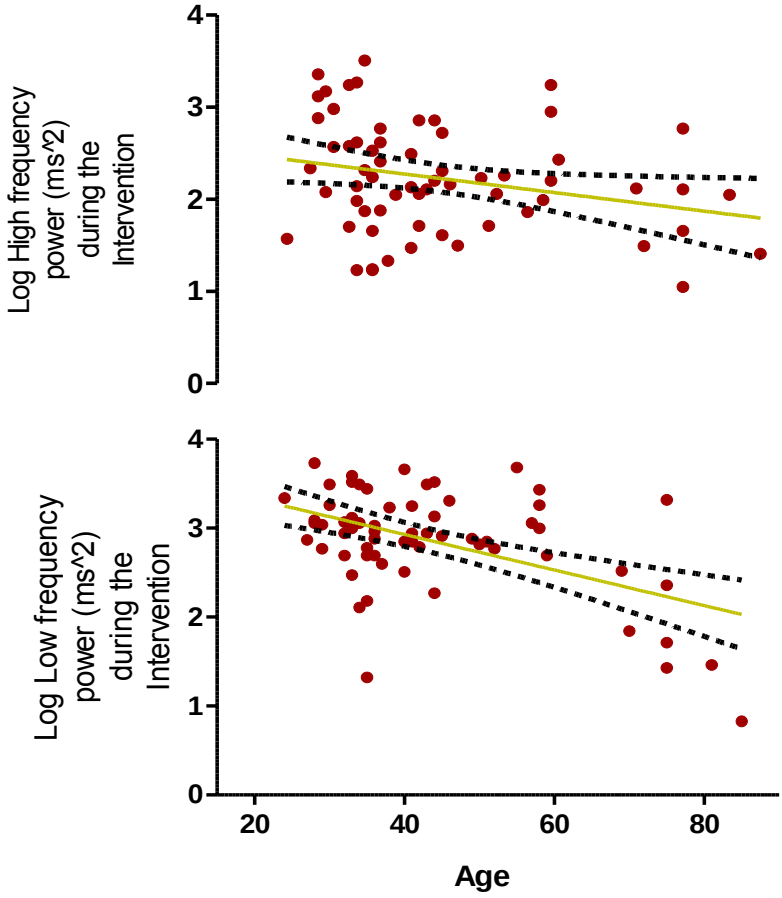


**Figure 3.4: The effects of age on Low and High frequency power (HRV) while using either of three intervention devices.**

Both LogLF (p=0.01, $S_R$ =-0.29, $R^2$ = 0.25) and LogHF (p=0.02, $S_R$ =-0.27, $R^2$ = 0.06) correlated significantly with age (Figure 3.4). Both figures indicate that regardless of the intervention tool used, LogLF and LogHF power decreases with age. One noteworthy observation from these graphs is the ages at which the LogLF and LogHF power values are highest, these are not casual and are discussed in Chapter 5.

## The effect of blood pressure and mental relaxation on HRV:

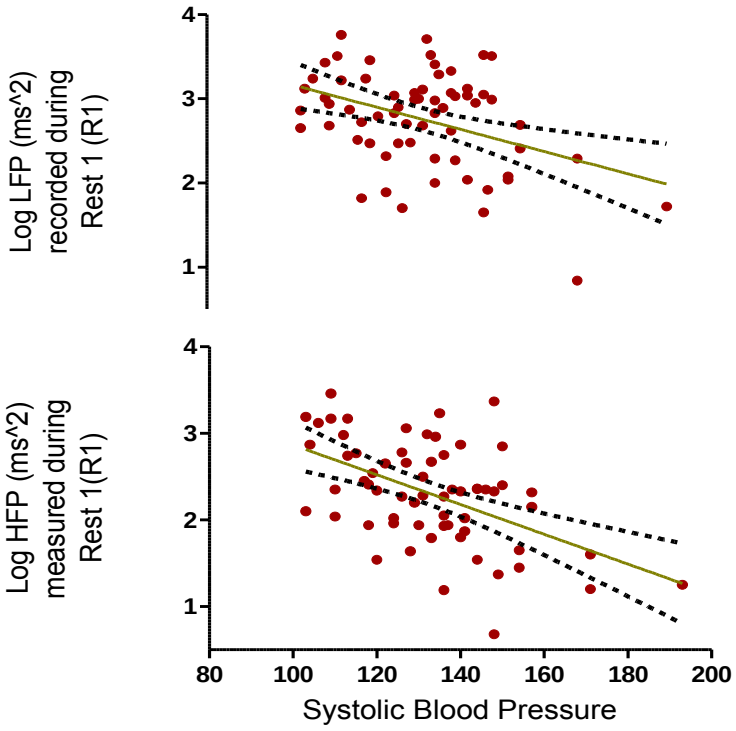


**Figure 3.5: The relationships between systolic BP measured at the start of the Stroop testing session and frequency domain measures of HRV (LogLF and LogHF power) during Rest 1.**

Systolic BP at the start of the Stroop task was negatively correlated to Log LF ($p=0.001$, $r=-0.39$, $R^2= 0.154$) and Log HF ($p<0.0001$, $r=-0.51$, $R^2= 0.259$) recorded during R1 (Figure 3.5). Systolic BP was also significantly correlated with LogTP (Total Power) ($p = 0.0003$, $r = -0.43$) (not shown), recorded during the same test stage.

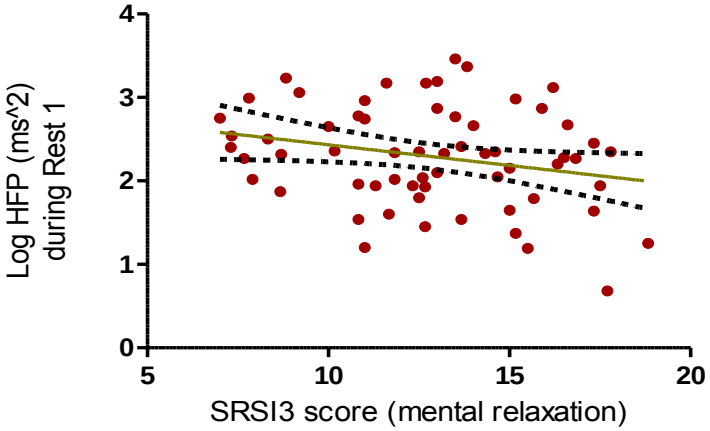


**Figure 3.6: The relationship between perceived mental relaxation at the start of the Stroop testing session and LogLF power during rest 1.**

There was a significant negative correlation between mental relaxation at the start of the test and R1 Log HF (p=0.04, r =-0.25, $R^2$=0.06) (Figure 3.6). Mental relaxation was evaluated using the Smith's Relaxation Inventory questionnaire.

## The effects of breathing rate on HRV and the significance of varying breathing rates on HRV and perceived sleepiness.

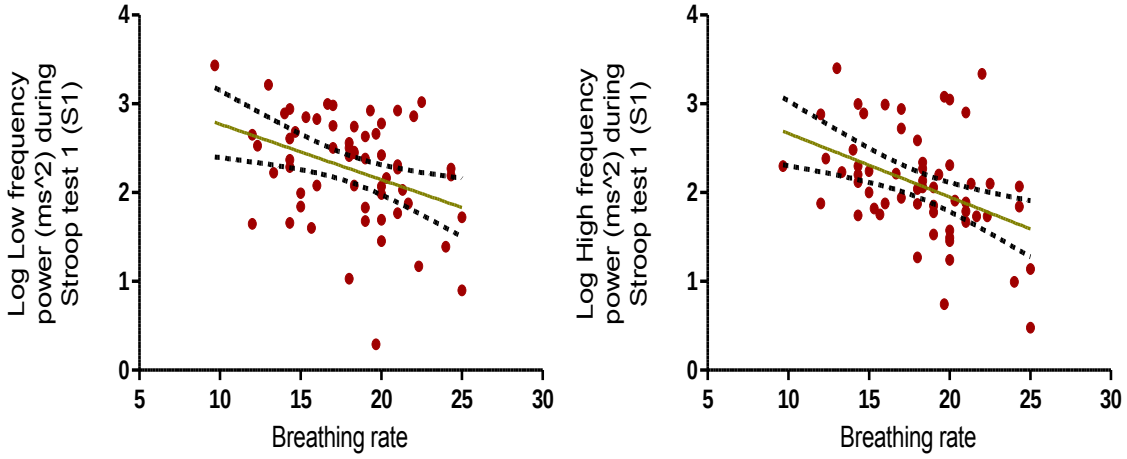


**Figure 3.7: The above figure illustrate the effect of breathing rate on LogLF and LogHF power, both measures were recorded during Stroop task 1 (S1).**

In the above figures the breathing rate during Stroop 1 is compared to some of the frequency domain measures of HRV also recorded during S1. The breathing rates during S1 correlated negatively with both Log LF (p=0.004, r = -0.36, $R^2$= 0.132) and S1 Log HF (p=0.0009, r = -0.42, $R^2$= 0.174) power at the same test stage.

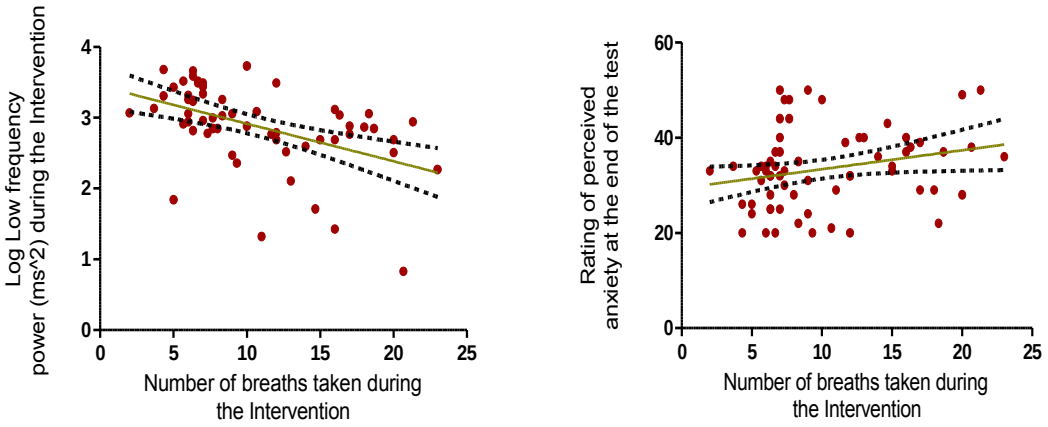


**Figure 3.8: In the above figures the breathing rate during the intervention (INT) is compared to Log LF power during the INT and a subjective measure of anxiety, evaluated at the end of the testing protocol.**

Breathing rate during the intervention correlated significantly with Log LF power ($p<0.0001$, $S_R$ =-0.56, $R^2$= 0.231), both measures were recorded during the intervention. Although this relationship is strongly significant, another important aspect to note is where the Log LF power is maximal; the fact that Log LF power is highest at breathing rates below 10 is to be expected. Breathing rate during the INT also correlated negatively with SDNN ($p=0.002$, $S_R$ =-0.38) (not shown). Both of these results are discussed in Chapter 5. STATE anxiety score at the end of the testing protocol, as evaluated by using Spielbergers' STATE anxiety questionnaire is positively correlated with the breathing rate during the intervention ($p=0.01$, $S_R$ =0.30, $R^2$= 0.06) (Figure 3.8). The significance of this relationship will also be discussed in Chapter 5.

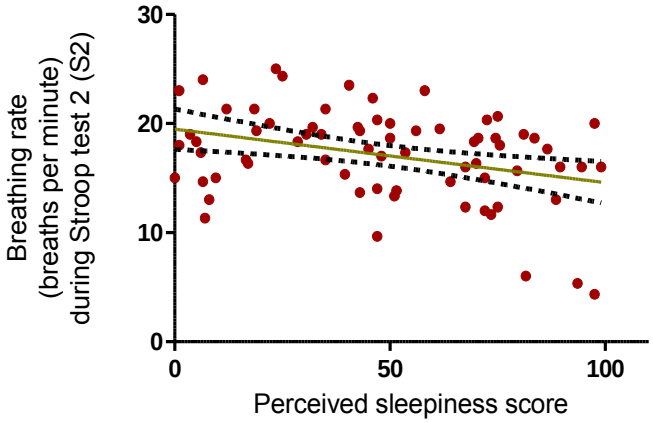


**Figure 3.9: The above figure illustrates the correlation between the breathing rate during Stroop task 2 (S2) and ratings of perceived sleepiness at the end of the test.**

The breathing rate during S2 was negatively correlated with perceived sleepiness after Stroop 2 ($p=0.01$, $S_R$ = -0.28, $R^2$= 0.116) (Figure 3.9). Perceived sleepiness was subjectively evaluated by using Smith's Relaxation Inventory questionnaire; this was evaluated at the end of the testing protocol. The significance of this relationship will be discussed during Chapter 5.

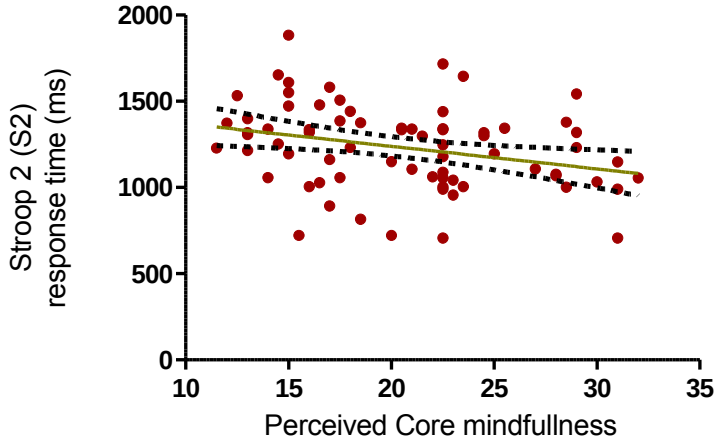


**Figure 3.10: The relationship between core mindfulness at the end of the testing protocol and the Stroop task response time during the second Stroop test (S2).**

A negative correlation between core mindfulness at the end of the test and average Stroop 2 response time was found ($p=0.01$, $r = -0.29$, $R^2= 0.09$). Core mindfulness is one of the elements of the Smith's Relaxation Inventory questionnaire, thus core mindfulness is subjectively evaluated in this context. Participants who felt more mindful at the end of the test had quicker responses during the second Stroop task (S2).

## Chapter 4: Beads and music interventions

27 beads (9 ♀) and 22 music (9 ♀) participants were included for this chapter. The participant ages ranged from 25 to 58 (mean 38 years) and 25 to 59 (mean 35 years) for the beads and music groups respectively. Participants were recruited as per the methods in Chapter 3 and had the same exclusion criteria as previously described. Only the beads and the music participants were included for this chapter because a comparative study between the effectiveness of the StressEraser and the music data has already been conducted by another colleague from our research group.

## Questionnaire Results:

There were no significant changes in average number of white squares counted PRE to POST for both the beads (19±1.6 – 19.4±1.9) and music (18.7±3.3 – 19.3±2.9) groups. There were no significant differences between STAI-T scores (TRAIT anxiety) (35.1±6 vs. 39.3±9.1, p=0.09), for beads and music groups respectively. No significant differences were seen in PRE to POST STAI-S (State anxiety) for both the beads (35.5±10.5 – 34.6±8.9, p=0.7) and the music groups (35.5±8.1 – 34.4±7.7, p=0.6). Both music and beads groups showed a decrease in STATE anxiety from PRE STAI-S to POST STAI-S. There were no significant inter-group differences when comparing beads and music groups during either PRE STAIS (35.5±10.5 vs. 35.5±8.1, p=0.9) or POST STAIS (34.6±8.9 vs. 34.4±7.7, p=0.6). There were no significant between group differences in any of the 4 categories of both the PRE and POST SRSI3 questionnaires.

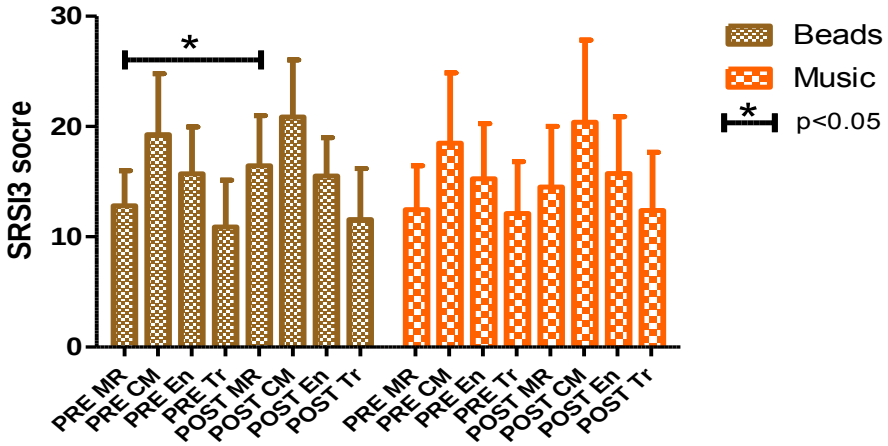


**Figure 4.1: Changes over time in the Smith's Relaxation Inventory questionnaire scores. PRE=prior to the intervention, at the start of the test, POST=after the intervention, the final questionnaire at the end of the test. MR=mental relaxation, CM=core mindfulness, En = energized/positive feelings, Tr=Transcendence.**

Some significant within group differences were found (Figure 4.1). In the beads group there was a significant increase in mental relaxation PRE to POST (12.825±3.168 – 16.439±4.559, p<0.05). In

the music group no significant change was seen PRE to POST (12.468±3.981 – 14.5±5.520, p=0.18).

## Physiological differences:

No significant differences in age and sex were found between the groups. Beads participants smoked an average of 3.3±7.7 cigarettes per day and did 3.7±3 hours of exercise a week. Music participants smoked an average of 2±4.8 cigarettes per day and did 3.4±2.3 hours of exercise per week. No significant differences in number of hours spent exercising and cigarettes smoked daily were found between the groups. Before the start of the test there were no significant inter-group differences between: measures of HRV (including HR, frequency domain measures and time domain measures).

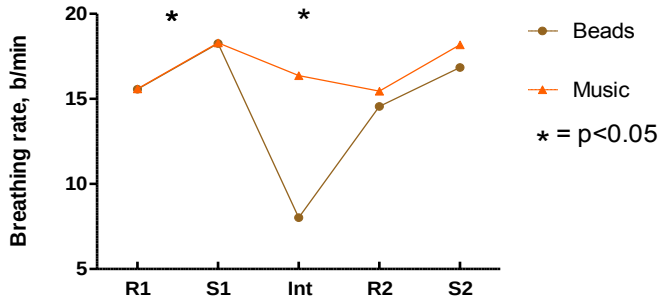


**Figure 4.2A: The change in average breathing rate during the test.**

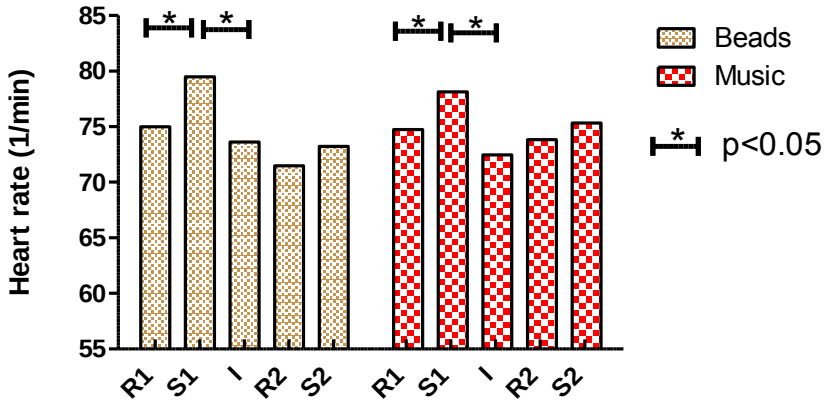


**Figure 4.2B: Change in heart rate during the different test stages.**

Figure 4.2A illustrates the changes in breathing rate during the different stages of the test. There was a significant increase breathing rate from R1 to S1 in both the beads and music groups. A significant decrease in breathing rate was seen between S1 and Int (18.2±4.3 - 8±3, $p<0.05$) in the beads group only. A significant increase from Int to R2 (8±3 - 14.5±4.4, $p<0.05$) was also seen only in the beads group. There was no significant decrease during the intervention in the music group. At the level of the intervention, there was a significant difference in the breathing frequency comparing beads (8±3 vs. 16.3±3.3, $p<0.05$) and the music groups. No other significant differences were seen during any stage of the test.

Figure 4.2B illustrates the changes in HR during the test. The increase in HR from R1 to S1 was significant in both beads (74.5±10.3 beats per minute (bpm) – 79.4±12.6bpm, p=0.02) and music groups (74.7±9.7 – 78.1±10.9 bpm, p=0.01) respectively. From S1 to INT HR decreased in beads (79.4±12.6bpm – 73.6±8.7 bpm, p=0.003) and music groups (78.1±10.9 – 71.4±10.5 bpm, p=0.001). There was no significant change in heart rate from INT to R2 in either the beads (73.6±8.7 – 71.4±9 bpm, p=0.13) or the music groups (71.4±10.5 – 72.4±11 bpm, p=0.35). The participants listening to the music showed a near significant increase in heart rate from R2 to S2 (72.4±11 – 75.3±9 bpm, p=0.06), the beads group also increased but not significantly (71.4±9 – 73.4±8.7 bpm, p=0.19).

## HRV (time domain changes) during the test:

With regards to HRV analysis both in the time domain and in the frequency domain, technical errors resulted in the exclusion of 7 beads participants for R1, S1, I and S2 and 8 for R2. Music participants were also excluded; 5 were excluded for R1 and S1 and 4 were excluded for INT, R2 and S2.

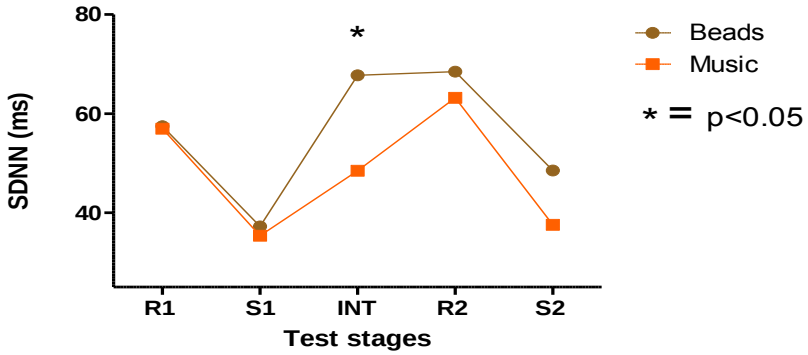


**Figure 4.3A: Changes in SDNN during the test.**

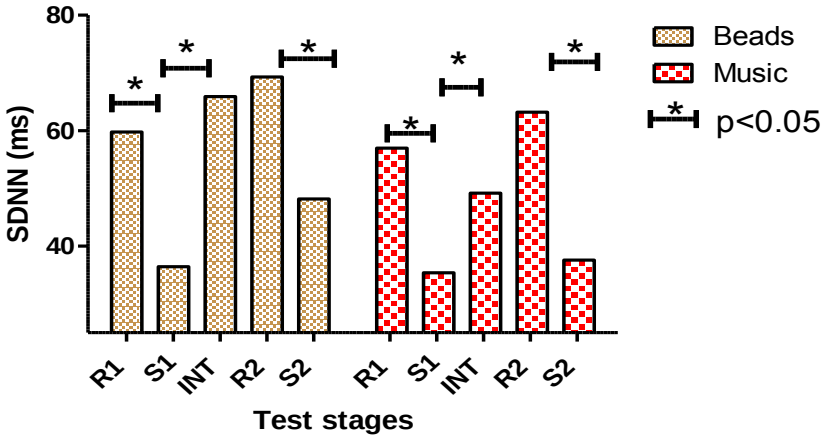


**Figure 4.3B: Change in SDNN during the test.**

INT SDNN was significantly different between beads and music groups (67.7±34.2ms vs. 49.1±17.6 ms, p=0.03) (Figure 4.3A). Within group changes in SDNN from R1 to S1 were significant in both the beads (57.4±25.2ms – 37.2±21.3 ms, p=0.009) and the music groups (56.9±33ms – 35.3±19.7 ms, p=0.02) (Figure 4.4B). There was also a significant increase in SDNN from S1 to the INT for the beads group (37.2±21.3ms – 67.7±34.2ms, p=0.001). The music group showed similar increases (35.3±19.7ms – 49.1±17.6ms, p=0.04). SDNN increased in the music group from INT to R2 (49.1±17.6 ms – 63.1±31.8ms, p=0.09) as well as in the beads group (67.7±34.2ms – 68.4±29.5ms, p=0.9). From R2 to S2 a significant decrease in SDNN was seen in both beads (68.4±29.5ms – 48.1±22.7ms, p=0.02) and music groups (63.1±31.8ms – 37.5±16.4ms, p=0.004).

## Spectral analysis of HRV:

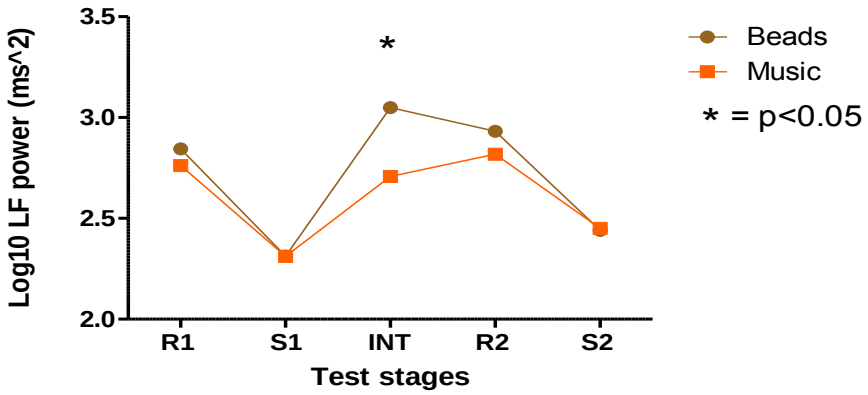


**Figure 4.4: Log10 low frequency power during the test.**

With regards to LogLF during R1 and S1 the beads and the music groups had similar values, however during the intervention there was a significant difference between the beads and the music groups (3.04±0.5 vs. 2.70±0.2, p=0.018) (Figure 4.4). During R2 and S2 there were no significant differences between the two groups.

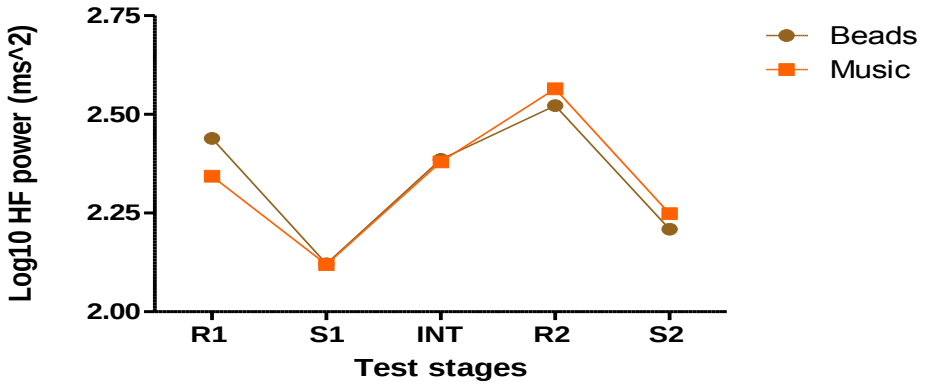


**Figure 4.5: Log10 high frequency power during the test.**

There were no significant inter-group differences in LogHF at any stage of the test (Figure 4.5).

## Stroop Task results:

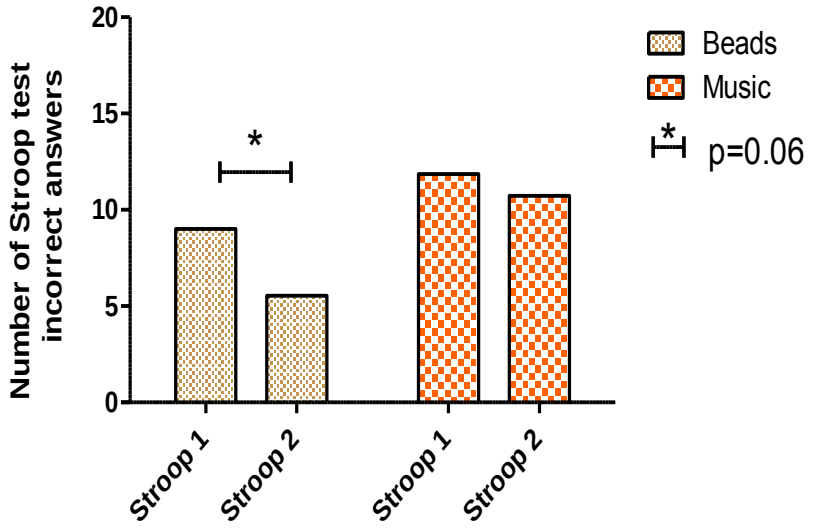


**Figure 4.6A: The change in incorrect word selections during the Stroop tasks, before and after the 10 minute intervention.**

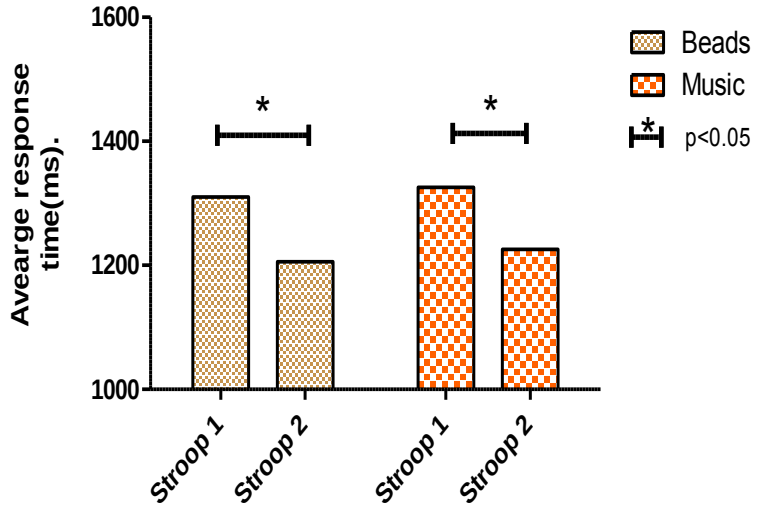


**Figure 4.6B: The change in Stroop response time before and after the intervention.**

There were no significant changes in the number of word mistakes made from S1 to S2 for both beads and music groups. However, the beads did show a near significant decrease (Figure 4.6A). Both beads and music groups decreased the number of mistakes made from S1 to S2; these changes were (9±16.0 – 5.5±13.3, p=0.06) and (11.8±17.4 – 10.7±18.0, p=0.4) respectively.

S2 response times were significantly quicker than S1 in both the beads (1310.2±216.2ms – 1205.5±228.4ms, p=0.002) and music groups (1325.9±282.4ms – 1225.8±242.7ms, p=0.001) (Figure 4.6B). There were no significant intergroup differences either during S1 response time (1310.2±216.2ms vs. 1325.9±282.4ms, p=0.8) or S2 (1205.5±228.4ms vs. 1225.8±242.7ms, p=0.7). Data from 1 beads participant was excluded from the analysis as the Stroop Task was not completed.

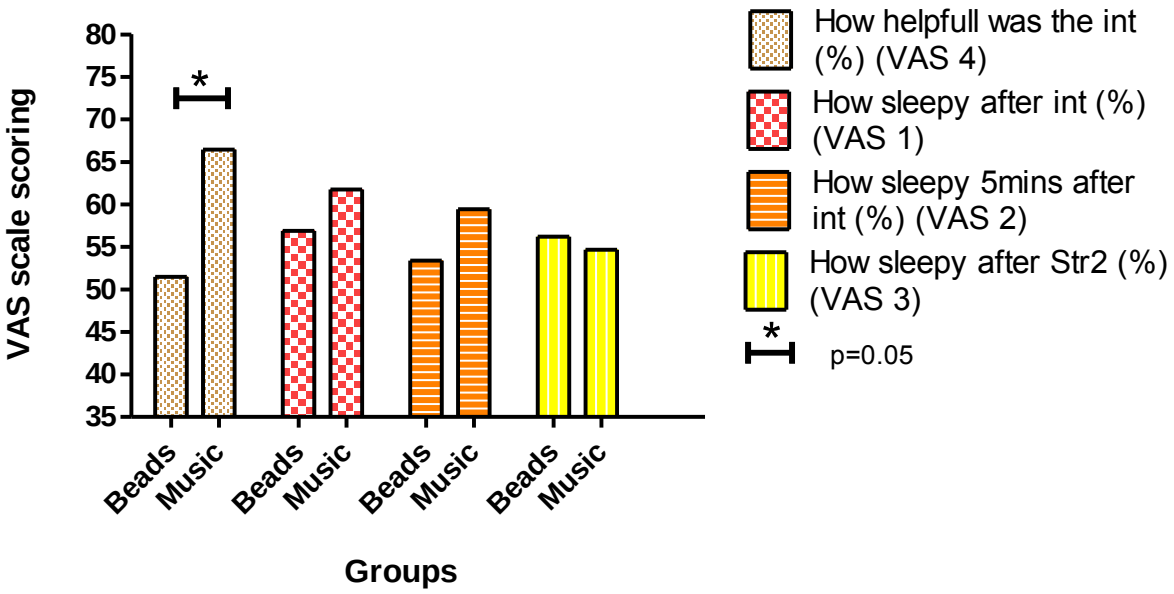


**Figure 4.7: Visual analogue scale scoring during the different test stages.**

For a complete description of the visual analogue scale please refer to Chapter 2. Briefly, participants were asked 4 questions during the test, three on perceived sleepiness and one on the usefulness of the intervention device. When comparing how helpful the beads and the music interventions were (VAS 4); beads were rated as 51.4% useful and music 66.4% (p=0.05) (Figure 4.7). How sleepy do you feel immediately after the intervention? (VAS 1) The beads felt on average 56.8% asleep and the music group felt on average 61.7% asleep. How sleepy do you feel after the rest period? (VAS 2) The beads group felt on average 53.4% asleep and the music group felt 59.4% asleep. How sleepy do you feel after Stroop 2? (VAS 3) was the most similar between the groups (56% asleep for beads compared to 54% of music). No significant differences were found for VAS 1, VAS 2 or VAS 3.

# Chapter 5: Discussion

This chapter begins with a description of some of relationships between ageing and HRV. Cognitive performance decreases with age and consequently the decrease in Stroop task performance with age was described for both Chapters 3 and 4. Thereafter some of the factors which are known to affect HRV are discussed, the benefits of regulating your breathing rate, and the potential benefit of consciously regulating your breathing rate is then described. We found that by regulating your breathing rate, the amount of sympathetic nervous control directed towards your heart is reduced, thus resulting in greater HRV and this is beneficial for performance in a cognitive task. Finally, the relationships between psychological measures (mental relaxation and core mindfulness) and HRV are described and highlight the potential use of HRV as a physiological tool to measure psychological state.

**The effects of unhealthy habits on physiology:**

Habitual smokers have reported that they tend to smoke when in a stressful situation; this leads to the assumption that smoking could be a method for coping with excessive stress (Pomerleau and Pomerleau 1991). Smoking is an unhealthy lifestyle choice which has detrimental effects on health and HRV; these were seen in our investigation. The number of cigarettes smoked daily correlated significantly with the average breathing frequency ($p=0.01$, $S_R = 0.30$) and heart rate ($p=0.04$, $S_R = 0.22$) (results not show), both values were taken at the start of the test. The increases in breathing rate and heart rate at baseline are to be expected as nicotine is a central nervous system stimulant and causes increases in HR (Sjoberg and Saint 2011).

Exercising weekly has beneficial effects on breathing rate and TRAIT anxiety; hours spent exercising correlated negatively with breathing rate ($p = 0.01$, $S_R = -0.29$) and TRAIT anxiety ($p = 0.04$, $S_R = -0.22$) (not shown), both measures were taken at the start of the test. This suggests that weekly exercise could be beneficial for managing certain aspects of anxiety. Breathing rate and TRAIT anxiety are complex variables which cannot be simply explained by the number of exercise hours done weekly. Consequently, the small Spearman R indices for both graphs (in particular TRAIT anxiety) highlight this. Hours spend exercising was not significantly correlated with SDNN or RMSSD (not shown) but in both cases with greater hours spent exercising; greater values of SDNN and RMSSD were seen. This relationship between exercise and HRV was to be expected as the benefits of exercise on HRV have been previously shown (Levy *et al.* 1998).

**The physiological consequences of ageing:**

In figure 3.1A the effects of age on HR are illustrated; no significant correlations were found but the HR decreased with age, indicative of the decreasing intrinsic HR (Kostis *et al.* 1982, Craft and Schwartz 1995, Opthof 2000). Similarly to the relationship between age and HR, TRAIT anxiety

did not correlate with age (p = 0.6) (Figure 3.2). This is important as it shows that TRAIT anxiety remained constant with age during our investigation; thus reducing the effect age could have on perceived TRAIT anxiety in our study.

Age is known to affect Stroop test performance (MacLeod 2005); this is related to a decrease cognitive performanc*e* typically seen after the age of 60 years (MacLeod 2005). In our study Stroop performance also decreased with age; age correlated negatively with Stroop 1 response time (p= 0.02, $S_R$ = 0.23) (Figure 3.3). Age also correlated significantly with the number of colour word mistakes made during the first Stroop task (p=0.004, $S_R$ = 0.31) (Figure 3.3). This second correlation indicates that an older person will respond less accurately to the colour words (red, blue, green and yellow) during the Stroop task. This is to be expected as the colour words in the Stroop task require a measure of cognitive function. Another interesting observation is that no participants over the age of 51 were able to make zero colour word mistakes (Figure 3.3). Taken together these results indicate that the younger participants had more accurate answers and better cognitive performance than the older ones during the Stroop task. The investigators also noted that time spend teaching Stroop task, and the amount of practise needed to understand the Stroop task was greater for the elderly participants. Elderly participants also more frequently made comments like "I cannot do this test"; these differences are purely observational but to be expected.

With regards to the white squares –flashed during the Stroop tasks. In total 20 white squares were displayed, no differences were found between the number of white squares selected for the beads and music groups. Both beads and music groups learned how to correctly answer the Stoop task (Figure 4.6). Stroop word mistakes decrease from S1 to S2; the change in the number of mistakes made was approaching significance (p=0.06) in the beads group. Suggesting that the participants using beads are better at correctly answering the Stroop task. Beads and music groups both showed significant decreases in response time from S1 to S2; however no significant differences were found. I suggest that these differences in Stroop task performance were due to the increases in LogLF power (Figure 4.4) and overall HRV. As well as due to the reduced feeling of anxiety and higher mental relaxation. These results indicate that having a higher HRV (as a consequence of using the beads), has some benefit with regards to managing all aspects of the cognitive Stroop task (Hansen *et al.* 2003).

Age correlated significantly with SDNN (p = 0.002, $S_R$ = -0.37) during rest 1. LogLF power, LogHF power and Log total power (p <0.0001, $S_R$ = -0.53) measured during rest 1 also correlated significantly with age (graph not shown). Neither of these findings are novel, and the decrease in autonomic control with age was to be expected (Choi *et al.* 2006, Zulfiquar *et al.* 2010). This significant relationship between ageing and HRV was also seen during the 10 minute intervention,

both LogLF ($p = 0.02$, $S_R = -0.29$) and LogHF ($p = 0.01$, $S_R = -0.27$) power were negatively correlated with age (Figure 3.4). One of the possible explanations for the decrease in HRV with age (and chronically elevated blood pressure), could be that the ability of the arterial walls to stretch decreases (Gribbin *et al.* 1971), therefore the responsiveness of the baro-reflex decreases, resulting in the control of heart rate (particularly vagal control of HR) decreasing.

The sympathetic nervous system has an important role to play particularly with regards to bringing about physiological changes after a stressor; these stressors include mental arithmetic tasks, hand grip exercise etc. (Joyner et al. 2008). A sympathetic response, as a consequence of a stressor will have an effect on the blood pressure. In our study the systolic BP recorded at the start of the Stroop task was negatively correlated with both LogLF ($p = 0.001$, $r = -0.39$, $R^2 = 0.154$) and LogHF ($p < 0.0001$, $r = -0.50$, $R^2 = 0.259$) power during rest 1 (Figure 3.5). Arterial blood pressure is one of the cardiovascular factors which are known to be particularly sensitive to a stressful situation (Nogawa et al. 2006). Sympathetic activation (as a consequence of a stressor) will not only cause an increase in HR but blood pressure as well, resulting in a decrease in HRV. HRV can also be influenced by breathing rate; breathing rate has a significant positive correlation with muscle nerve sympathetic activation (Narkiewicz et al. 2006). This positive correlation suggests that with slower breathing rates, greater reductions in sympathetic nerve activation will be seen. In our study breathing rates during the first Stroop task correlated negatively with both LogLF ($p=0.004$, $r = -0.36$, $R^2 = 0.132$) and LogHF ($p=0.0009$, $r = -0.42$, $R^2 = 0.178$) power, also recorded during Stroop task 1 (Figure 3.7). This relationship between HRV and breathing rate is to be expected as participants breathing more frequently are doing so as a consequence of the systemic effects of sympathetic activation, thus presenting with a lower HRV.

**The benefits of regulating your breathing rate:**

During the 10 minute intervention the breathing rate was positively correlated with the anxiety score at the end of the test ($p = 0.012$, $S_R = 0.30$) (Figure 3.8). The breathing rate was also related to the rating of perceived sleepiness. Respiratory frequency recorded during the second Stroop task correlated negatively with perceived sleepiness score at the end of the test ($p = 0.01$, $S_R = -0.28$) (Figure 3.9). These correlations can be explained because mental state can be associated with breathing rate; anxiety or anxious feelings present as faster heart rates and breathing rates (Moore *et al.* 2011). A faster breathing rate is also indicative of greater sympathetic activity (Narkiewicz *et al.* 2006); resulting in greater arousal and feelings of wakefulness (Vincente *et al.* 2011). The opposite also holds true, slower breathing rates can be indicative of sleepiness (Figure 3.9). With regards to the beads and music groups, the heart rate followed similar patterns for both groups (Figure 4.2B). HR increased during S1 and S2, and decreased during the intervention. The Stroop task had a dual effect on the physiology of our participants, HR and breathing rate both increased

during the tasks. These increases are brought about by greater sympathetic activity and are expected as the Stroop task is relatively difficult.

Heart rate is regulated both intrinsically and extrinsically (Glass 2001, Verrier and Tan 2009), however, this regulation is sub-consciously driven therefore impossible to consciously control. Conversely, breathing rate is under both voluntary and involuntary control; thus in some regard by controlling breathing rate the actions of the ANS can be voluntarily and consciously controlled (Bernardi *et al.* 2001, Cerutti *et al.* 2006, Moore *et al.* 2011). As discussed above, breathing rate is related to mental state, therefore managing your breathing by doing yoga, or breathing exercises (for example) can lead to relaxation, and a reduction in sympathetic nerve activation (Narkierwicz *et al.* 2006), hence resulting with a greater HRV. This increase in HRV is important because greater HRV is associated with better cognitive performance (Kofman *et al.* 2006), heart rate regulation and emotional responsiveness under pressure (Lagos *et al.* 2008). Slower breathing rates may be more beneficial for managing the stressful demands of the Stroop task as feelings of anxiety are reduced.

The beads beading system are a tool which indirectly teaches a slow breathing technique. During the intervention, the beads group had a significantly slower breathing rate compared to the music group (Figure 4.2A). Thus a greater degree of perceived sleepiness would have been expected as well some possible differences in perceived anxiety. Perceived anxiety was unfortunately not measured immediately after the intervention, however the beads group showed a significant increase in subjective mental relaxation (as evaluated by the Smith's Relaxation Inventory questionnaire) at the end of the test as compared to the music group (Figure 4.1). This difference may have been as a consequence of the different breathing rates during the intervention. With regards to perceived sleepiness, after the intervention both groups reported moderately high levels of perceived sleepiness, the participants in the music group however reported feeling more asleep (not statistically significant) than the beads (Figure 4.7). I suggest that one of the principal reasons for this difference is due to the fact that LogLF power was significantly higher in the beads group during the intervention. Greater LogLF power is indicative of somatic sympathetic regulation of HR, with greater sympathetic regulation of the heart higher levels of perceived arousal are expected (Vincente *et al.* 2011).

The breathing rate during the intervention correlated negatively with LogLF ($p < 0.0001$, $S_R = -0.56$) power during the same testing stage (Figure 3.8), this correlation points to a relationship between slow breathing rate and LogLF power (Figure 4.4). This negative correlation is to be expected as slower breathing frequencies can be reflected in the LF power range (discussed earlier in Chapter 1). SDNN ($p = 0.002$, $S_R = -0.38$) and Log total power ($p = 0.0004$, $S_R = -0.44$) during the intervention both correlated negatively with breathing rate during the intervention (results not

shown). During the intervention; SDNN, LogLF and LogHF power values were highest while breathing at ranges between 4 and 10 breaths per minute. This is evident from the large cluster of points within this breathing range (Figure 3.8). SDNN, LogLF and LogHF are maximal within this range as a consequence of increased parasympathetic activation and synchronization of the baro-reflex and HRV, as can be seen while breathing at the resonant frequency (6 breaths per minute) (Ahmed *et al.* 1982, Vaschillo *et al.* 2002, Gervitz 2003, Lehrer *et al.* 2003, Lagos *et al.* 2008, Lin *et al.* 2012). While breathing at your resonant frequency the breathing rate is synchronized into the LF range; however, this is vagally mediated rather than sympathetically (Aysin and Aysin 2006). This increase in LF power could be beneficial for performance in the cognitive test as LF power is also indicative of sympathetic control. Without this increase in sympathetic activity physical performance increases eg. response time and physical arousal would not be seen. The significantly higher LogLF power and SDNN during the intervention in the beads group is presumably one the reasons why performance measures changed during the second Stroop task. The second reason for the performance differences during the second Stroop task could also be that participants rated themselves as feeling more awake after the intervention compared the music group (Figure 4.7). A reason to why participants may feel more awake when using the beads could be that they remain active and actively engaged in using the beads. The participants using the beads have to concentrate on both the movements of their fingers as well as their breathing, thus decreasing the likely hood of feelings of sleepiness.

Using the beads in our experiment clearly caused a significant increase in LogLF power (Figure 4.4) and SDNN as illustrated in Figure 4.3, this is brought about by resonant frequency breathing (described above). The average breathing frequency for the beads group during the intervention was 8 not 6, and that for the music group was 16 (Figure 4.2A). Presumably if all the participants were breathing at their resonant frequency (+/- 6 breaths per minute), there would be a greater increase in HRV (in particular LF power and SDNN) and possibly even clearer differences in cognitive performance. In future investigations, longer time should be spent teaching the use of the beads. With greater instructor experience, and training time with the participants, breathing with a frequency of 6 breaths can easily be achieved.

Listening to 10 minutes of classical music was rated by the music participants as being significantly more useful as a stress management tool compared to the beads participants. This was not entirely unexpected as classical music is known around the world to be a tool for relaxing. The participants listening to the music had a significantly faster response times, though there were no significant improvements in the number of mistakes from Stroop 1 to Stroop 2. Therefore listening to 10 minutes of classical music clearly had some beneficial effect. However this beneficial effect may have been dampened by the fact that listeners felt sleepy after listening to the music, only beginning to feel more awake after the music was turned off (VAS 3) and after the stimulation of

the second Stroop task (VAS 4). This increased feeling of sleepiness may have played a role in their being only a small non-significant decrease in incorrect Stroop answers, as compared to the beads group. Feelings of sleepiness may not always be beneficial for a cognitive test, as previously discussed

**Mental relaxation and HRV:**

Mental relaxation (MR) is one of the components of the Smith's Relaxation Inventory questionnaire. MR is made up of questions on feeling rested/refreshed and mental relaxation. The higher the score, the more mentally relaxed the participant is perceived to be. MR at the start of the test was negatively correlated with LogHF ($p = 0.04$, $r = -0.25$) power during rest 1 (Figure 3.6). Elevated levels of HF power can be indicative of decreased mental arousal, fatigue or a sleepy state (Michail *et al.* 2008); however, if HF power is too abundant mental relaxation could lead to feelings of sleepiness, resulting in impairments in cognitive performance (Nava *et al.* 2004). With regards to the beads and music groups in Chapter 4, the levels of perceived sleepiness from the intervention to the second rest period decreased for both beads and music groups, thus the participants felt more awake during the second rest stage. Comparatively during the second rest stage, the music group felt sleepier, and had slightly higher LogHF power, whereas the beads group were more awake and had slightly less LogHF power. This relationship is minor; however it is used solely to highlight the interaction between perceived sleepiness and HF power. Taken together, these suggest that in order to perform at your best ability in a cognitive test, optimal mental relaxation must be present so as to feel relaxed. HF power needs to remain low thereby reducing the feelings of drowsiness, but still present so as to allow some measure of relaxation, and LF power maximal so as to ensure optimal alertness

## Core Mindfulness and HRV:

Core mindfulness (CM) is another one of the components of the Smith's Relaxation State Inventory (SRSI3) questionnaire. The higher the CM score; the greater the level of perceived mindfulness. Mindfulness training aims to increase the user's awareness to different mental states and processes in a non-judgemental, non-emotional perception state within each situation they find themselves (Grossman *et al.* 2004). The benefits of mindfulness training are believed to include; improved management of both chronic illnesses and chronic stress states (Grossman *et al.* 2004). Mindfulness based interventions may also be useful for the treatment of anxiety, both trait and state anxiety (Vøllestad *et al.* 2011). In our investigation CM at the end of the test correlated significantly with the second Stroop task average response time ($p = 0.01$, $r = -0.29$) (Figure 3.10). Participants who felt more mindful at the end of the test had quicker Stroop response times, in this manner being more mindful could have reduced the felt stress of the participants, thereby allowing for improvements in our cognitive task.

**Conclusion:**

Age had a significant effect on the autonomic nervous system and on performance measures of the Stroop task. Measures of HRV including SDNN, LogLF and LogHF power decreased with age, correlated significantly with age at baseline. Age also was negatively correlated with LogLF and LogHF during the intervention, suggesting that the responsiveness of the cardiovascular system to autonomic control also decreases with age. Breathing rate correlated negatively with SDNN suggesting that a higher breathing rate is not as beneficial as a slow breathing frequency, with regards to increasing SDNN. A breathing rate of between 4-10 breaths per minute was found to be most beneficial for increasing HRV. Core mindfulness at the end of the test correlated positively with the response time during Stroop task 2; greater core mindfulness could be beneficial for improving Stroop task response time, thus highlighting the potential benefits of mindfulness training in the work place.

The participants using the beads had significantly lower breathing rates during the intervention, consequently a significantly higher SDNN and LogLF power compared to the music group. The changes resulted in overall better performance during the Stroop task, and a subjective increase in MR. Using the beads therefore is useful for increasing HRV, for teaching slow breathing and for causing some cognitive performance improvements.

**Study limitations and possible future research:**

I would consider one of the major limitations in this study is the lack of instructor experience with regards to teaching how to use a feedback device, and problem solving around that. With better knowledge on this topic participants would have learnt the technique more effectively and differences in the effectiveness of the devices might have been easier to see. This problem could have been avoided with more instructor practise, before the start of the testing process. This is what I would suggest if any future research is to be done with any of the techniques described in this thesis. A second study limitation is that the number of participants between the ages of 60 – 90 was not enough; a larger sample size with participants within this range would be useful for any future research. Future research from this thesis could use the already well established testing protocol and investigate the effectiveness of other biofeedback devices. The effectiveness of the devices already studied could also be applied to sportsmen and women and their effectiveness could be studied with elite athletes. A final thought on future research includes investigating the long term benefits of using any of the stress management devices, and what an appropriate training period would be before these techniques become second nature.

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