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THE EFFECT OF SPONTANEOUS VERSUS PACED BREATHING ON EEG, HRV, SKIN CONDUCTANCE AND SKIN TEMPERATURE

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A dissertation submitted in fulfilment of the requirements for the degree Master of Science in Engineering, in the Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg.

January 2017
Johannesburg

DECLARATION

I hereby declare that the dissertation entitled **The effect of spontaneous versus paced breathing on EEG, HRV, skin conductance and skin temperature**, which I hereby submit for the degree Master of Science (Electrical Engineering) at the University of the Witwatersrand, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE **DATE**

Brett Alan Klette

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RESEARCH ABSTRACT

It is well known that emotional stress has a negative impact on people's health and physical, emotional and mental performance. Previous research has investigated the effects of stress on various aspects of physiology such as respiration, heart rate, heart rate variability (HRV), skin conductance, skin temperature and electrical activity in the brain. Essentially, HRV, Electroencephalography (EEG), skin conductance and skin temperature appear to reflect a stress response or state of arousal. Whilst the relationship between respiration rate, respiration rhythm and HRV is well documented, less is known about the relationship between respiration rate, EEG, skin conductance and skin temperature, whilst HRV is maximum (when there is resonance between HRV and respiration i.e. in phase with one another).

This research project aims to investigate the impact that one session of slow paced breathing has on EEG, heart rate variability (HRV), skin conductance and skin temperature. Twenty male participants were randomly assigned to either a control or intervention group. Physiological data were recorded for the intervention and control group during one breathing session, over a short initial baseline (B1), a main session of 12 minutes, and a final baseline (B2). The only difference between the control and intervention groups was that during the main session, the intervention group practiced slow paced breathing (at 6 breaths per minute), while the control group breathed spontaneously. Wavelet transformation was used to analyse EEG data while Fourier transformation was used to analyse HRV.

The study shows that slow-paced breathing significantly increases the low frequency and total power of the HRV but does not change the high frequency power of HRV. Furthermore, skin temperature significantly increased for the control group from B1 to Main, and was significantly higher for the control group when compared to the intervention group during the main session. There were no significant skin temperature changes

between sessions for the intervention group. Skin conductance increased significantly from Main to B2 for the control group. No significant changes were found between sessions for the intervention group and between groups. EEG theta power at Cz decreased significantly from Main to B2 for the control group only, while theta power decreased at F4 from Main to B2 for both groups. Lastly, beta power at Cz decreased from B1 to B2 for the control group only.

This significant effect that slow-paced breathing has on HRV suggests the hypothesis that with frequent practice, basal HRV would increase, and with it, potential benefits such as a reduction in anxiety and improved performance in specific tasks. Slow-paced breathing biofeedback thus shows promise as a simple, cheap, measurable and effective method to reduce the impact of stress on some physiological signals, suggesting a direction for future research.

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
ANS	Autonomic nervous system
BVP	Blood volume pulse sensor
CAN	Central autonomic network
CAT	Computerised axial tomography
CWT	Continuous Wavelet Transform
DFT	Discrete Fourier Transform
DHEA	Dehydroepiandrosterone
DWT	Discrete Wavelet Transform
ECG	Electrocardiogram
EDA	Electrodermal activity
EDR	Electrodermal response
EEG	Electroencephalography
FFT	Fast Fourier Transform
fMRI	Functional magnetic resonance imaging
HF	High frequency
HRV	Heart rate variability
IBI	Interbeat interval
ICA	Independent Component Analysis
LF	Low frequency
MRI	Magnetic resonance imaging
PET	Positron emission tomography
PPG	Photoplethysmographic
PSTD	Post-traumatic stress disorder
RF	Respiration frequency
rRCB	Regional cerebral blood flow
RSA	Respiratory sinus arrhythmia
SMR	Sensorimotor rhythm
SC	Skin conductance
STFT	Short time Fourier transform
TP	Total power
VLF	Very low frequency

CHAPTER 1: INTRODUCTION

1.1 Problem statement

The interplay between respiration rate, heart rate variability (HRV), brain activity, skin conductance and skin temperature is an intriguing subject within physiological studies. It is well documented [1] [2] that breathing has a direct impact on heart rate through the mechanism of respiratory sinus arrhythmia (RSA), where RSA is the heart rate variability in synchrony with respiration [3]. HRV has also been described as an index of autonomic function correlating with longevity [4], and a possible marker for stress and relaxation due to being partially influenced by both the sympathetic and parasympathetic nervous system [5]. HRV biofeedback is a process whereby a participant deliberately increases HRV through matching respiration rate and rhythm typically guided by equipment [6]. An increase in HRV is considered a sign of good health and reflects the cardiovascular systems ability to adapt to external and internal demands [7-10].

Skin conductance and skin temperature are further markers for stress and relaxation. Skin conductance is a term used to describe the electrical characteristics of the skin. Both psychological and physiological arousal influences the sympathetic branch of the autonomic nervous system, which in turn controls sweat glands [11]. Skin conductance increases with an increase in psychological and physiological arousal [12] [13] or stress response [14-16]. Skin temperature is negatively correlated with stress response. During stress, blood flow moves from the skin and body's extremities towards the heart and muscle tissues. The decrease in blood flow in fingers causes a decrease in skin temperature during an emotional arousal or a stress effect, while an increase in blood flow and skin temperature is caused during relaxation, and is therefore considered an indicator of stress or relaxation [17-20].

The power spectrum of spontaneous electroencephalography (EEG) offers a useful instrument for the assessment of cortical arousal [21]. The waking state is mostly characterised by power in the alpha (8-12Hz) and beta (13-30Hz) ranges in healthy

humans. A dominance of alpha waves indicates comparatively low activation and relaxed awareness without concentration [22]. An increase in beta activity reflects an increase in cortical activity [22], [23].

In summary, HRV, EEG, skin conductance and skin temperature appear to reflect a stress response or state of arousal. Whilst the relationship between respiration rate, respiration rhythm and HRV (and therefore potentially the sympathetic and parasympathetic nervous systems) is well documented, less is known about the relationship between respiration rate, EEG, skin conductance and skin temperature, whilst HRV is maximum (when there is resonance between HRV and respiration i.e. in phase with one another).

1.2 Aim and objectives

The aim of this research project is to investigate HRV, skin conductance and electroencephalography (EEG) patterns in response to breathing techniques. This aim will be met through the following objectives:

- To replicate existing research to determine the impacts of slow paced breathing on EEG and various aspects of physiology;
- To investigate the impact of slow paced breathing on stress and relaxation as inferred by HRV, skin temperature, skin conductance and EEG;
- To investigate the relationship between respiration rates, HRV, skin temperature, skin conductance and brain state as inferred by EEG [24, p. 66]; and
- To use advanced signal processing techniques such as Wavelet transforms to evaluate EEG.

1.3 Hypothesis

To achieve the aim and objectives of this study, the following hypotheses have been set out:

- **Hypothesis 1:**

  H1: Slow paced breathing initiates resonance in the baroreflex system, and therefore increases heart rate variability.

  H0: Slow paced breathing does not initiate resonance in the baroreflex system, and therefore does not increase heart rate variability.

- **Hypothesis 2:**

  H1: Slow paced breathing increases relaxation and therefore increases efferent vagal flow represented by increases high frequency HRV.

  H0: Slow paced breathing does not increase relaxation and efferent vagal flow, and therefore high frequency HRV stays constant or decreases.

- **Hypothesis 3:**

  H1: Slow paced breathing increases relaxation, which results in decreased peripheral blood vessel constriction and higher peripheral blood flow, and therefore increases finger temperature.

  H0: Slow paced breathing does not increase relaxation, blood vessel constriction and peripheral blood flow remains constant, and therefore finger temperature remains constant or decreases.

- **Hypothesis 4:**

  H1: Slow paced breathing increases relaxation, which reduces the sweating response, and therefore decreases skin conductance.

  H0: Slow paced breathing does not increase relaxation, keeping the sweating response constant, and therefore skin conductance remains constant.

- **Hypothesis 5:**

  H1: Slow paced breathing increases relaxation and therefore theta and alpha power of EEG measurement increase, while beta power decreases.

  H0: Slow paced breathing does not increase relaxation and therefore theta and alpha power of EEG measurement remain constant or decrease, while beta power remains constant or increases.

1.4 Project outline

This research project is divided into seven chapters. The above chapter presents an introduction to the project with a brief overview of HRV, EEG and the impacts of stress on these, giving context to the project. The aim of the project is presented with the hypotheses that are used to test the aim. The second chapter presents a review of literature on HRV, HRV biofeedback and EEG. Chapter 3 focuses on a literature review on the techniques used to measure and analyse HRV and EEG. Chapter 4 outlines the methodology followed to test the above hypotheses based on the existing literature. Chapter 5 presents the results produced following the described methodology which is followed by a discussion and interpretation of these results in Chapter 6. Research limitations and recommendations for future research are also provided. Finally, Chapter 7 highlights the main conclusions drawn and implications of this research.

CHAPTER 2: BACKGROUND AND PREVIOUS RESEARCH TO HRV, SKIN CONDUCTANCE, SKIN TEMPERATURE AND EEG

2.1 Introduction

This chapter presents an introduction and literature review on the physiological concepts of this research. First, the concepts and mechanisms of respiration, heart rate and heart rate variability (HRV) is investigated in depth, as well as how HRV relates to stress and anxiety. Second, HRV biofeedback is discussed in terms of it being an intervention to change HRV. Third, the concept of electroencephalography (EEG) is introduced and also investigates its relationship with stress and anxiety.

In general, this chapter investigates the above concepts and their relation to one another. Previous research on the influence of stress and anxiety on the heart and brain, as well as mechanisms to measure HRV, skin temperature, skin conductance and EEG are discussed, which form the basis of the methodology followed in this research. The word physiology generally refers to the study of life or biological systems, however, in this dissertation it will be used specifically to refer to respiration, heart, HRV, skin temperature, and skin conductance.

2.2 Physiology background

2.2.1 Respiration

Respiration is the process of moving air in and out of the lungs with the main function to facilitate gas exchange of i.e. deliver sufficient amounts of oxygen and remove excess carbon dioxide from body tissue [25]. The neuronal circuits responsible for respiration are located in the medulla oblongata and the dorsolateral pons. During inspiration, pulmonary stretch receptors are activated sending afferent flow via the vagus nerve to the nucleus tractus solitaries (NTS). The NTS in turn inhibits the medullary respiratory centre

inhibiting inspiration with reduced efferent flow via the phrenic and intercostal nerves [26]. With the exception of rapid breathing, expiration occurs when intercostal muscles are relaxed. Central and peripheral chemoreceptors also monitor blood chemistry and respond to changes in pH and carbon dioxide levels, stimulating the respiratory centre, in turn altering rate and depth of respiration [26].

2.2.2 Heart rate

Heart rate refers to the frequency of complete cardiac cycles and is determined by the firing of the sinoatrial (SA) node in the right atrium. The SA node initiates an electrical impulse at the beginning of each heart beat while the atrioventricular (AV) node spreads the electrical signal from the SA node through the ventricles of the heart [3]. This is controlled and modulated from the cardiovascular centre in the medulla oblongata via efferent sympathetic and parasympathetic flow to the heart [27]. The cardiovascular centre receives information from four main receptor groups: pressure receptors in the heart; chemoreceptors in the heart; thermoreceptors in muscles and stretch receptors in muscles. The received information is then processed and heart rate is altered via the autonomic nervous system (ANS) as required [28].

Respiratory and cardiovascular rhythms are regulated synergistically to ensure optimum respiratory gas exchange with respiratory sinus arrhythmia being a classical example of cardiorespiratory coupling [26].

2.2.3 Heart rate variability

Heart rate variability (HRV) is defined as the natural variations in heart rate [29], [30] and reflects the ability of the cardiac system to cope with situational stress demands [7]. The clinical implications of HRV was first acknowledged in 1965, when [31] observed changes in interbeat intervals preceding fetal death. The clinical importance of HRV has since become widely recognised as low basal HRV has been associated with mortality following

Myocardial Infarction [29], hypertension [32], depressive symptoms [33], anxiety symptoms [34], disorder [35], panic disorder [36] and post-traumatic stress disorder (PTSD) [37]. Higher basal HRV has been found after successful treatment for depression using psychotherapy and anti-depressants [38], [39]. Thayer et al [40] found evidence of a relationship between higher resting basal HRV and improved performance on tasks that use executive functions.

Heart rate is regulated by both the sympathetic and parasympathetic branches of the ANS, and therefore HRV can be considered a useful marker of stress and relaxation [5]. An increase in HRV is considered a sign of good health and reflects the cardiovascular systems ability to adapt to external and internal demands [7-10].

HRV is a dynamic process of bidirectional communication between the central nervous system and the cardiovascular system [40-42], that is particularly influenced by the ANS and central autonomic network (CAN) [7], [43]. There is interconnection in almost all structures of the CAN, with bi-directional communication occurring between the brain and body [44]. Stress signals are therefore sent from the body to the brain and vice versa.

Elghozi et al. [1] classifies HRV into seven types depending on the speed and mechanism that affects the oscillation. From slowest to fastest: age related; seasonal changes; daily rhythms; hourly rhythms; minute changes of between 0.5 to 5 minute periods (very low frequency (VLF)); medium waves (also referred to as low-frequency (LF)) with periods of between 7 to 15 seconds; and high frequency (HF) waves with periods of approximately 2 to 5 seconds. The frequency bands for VLF are <0.04Hz; LF is between 0.04-0.15Hz; and HF 0.15-0.4Hz [29]. Due to the nature and recording length of this research project, only VLF, LF, and HF oscillations fall into relevant time scales. VLF is also excluded from the analysis as there is much debate whether any physiological process causes fluctuations to this frequency band [29]. Changes in HRV in the LF and HF bands are clearly influenced by the activity of the ANS [1].

2.2.4 Low and high frequency heart rate variability

Low frequency HRV oscillations are typically generated by resonance in the baroreflex system [45-50]. Baroreceptors sense changes in blood pressure, which in turn, send afferent information in combination with information from higher centres that combine at the NTS [51]. An increase in blood pressure triggers an inhibition of sympathetic outflow from the vasomotor centre to the vasculature, reducing vasomotor tone and blood pressure [52-54]. Vagal flow to the heart is simultaneously increased, decreasing heart rate, and therefore increasing HRV [55] [56]. The time delay between one blood pressure cycle to the next causes oscillations that occur at approximately 0.1 Hz [46], [57]. This resonant blood pressure oscillation is known as the Mayer wave and primarily caused by the baroreflex action [46], [57].

Respiratory sinus arrhythmia (RSA) is the natural variation in heart rate largely modulated by the inhalation, exhalation, amplitude and frequency of respiration [1], [2]. RSA is also known as HF HRV, as it is synchronous with respiration [58], with a normal period in resting adults between 2 – 5 seconds, and hence a power spectral peak usually observed around the 0.25Hz band. One exception to RSA influencing HF HRV is during paced breathing, where slower breath rates could influence LF HRV [59]. Resonance between respiration and baroreflex oscillations occur at a respiration frequency of around 0.1 Hz, or approximately 4.5 to 6.5 breaths per minute [60] [61] [45] [62] [63] [64]. RSA and therefore HRV is maximal at resonant frequency [60].

A respiration rate of 6 breaths per minute (0.1 Hz) results in a HRV spectral peak at 0.1 Hz and therefore resonance [65] between respiration rate, HRV and blood pressure wave (with Mayer wave at 180 degrees out of phase) [61]. Peak HRV occurs at this resonant frequency, which differs between 4.5 to 6.5 breaths per minute, depending on the individual [66].

HF HRV oscillations are regulated by efferent vagal flow [67-70]. Cyclical vagal discharge from the medulla is another generator of RSA [69] [71] [67] [72] and therefore contributes to both LF and HF HRV oscillations depending on respiration frequency. RSA increases

as cardiac vagal activity increases [73] [74] and decreases as vagal activity decreases [69] [73] [74] [50]. HF HRV power is thought to reflect cardiac parasympathetic activity [73] [75] [70] [76] [50] and RSA [77] when breathing is in this frequency range. LF HRV power was initially thought to represent sympathetic activity only [77-79], although subsequently, LF HRV is thought to be directly regulated by the parasympathetic nervous system (PSNS) [70] [49] [76] [67] [72] [68], and indirectly regulated by the sympathetic nervous system [49] [76] [72] [69].

Central oscillators in the cardiovascular and respiratory centres contribute to both LF HRV oscillations [69] [77] [80] [81] as well as HF HRV oscillations [77] [82].

In summary, the control and regulation of HRV is a complex system that depends on both peripheral and central factors [45] [57] [47].

2.3 HRV and depression, emotion and meditation

Low basal HRV has been linked to symptoms of depression [83] [84] [85] [86] and reduced baroreflex sensitivity [86], possibly due to a continuous state of sympathetic arousal, decreased parasympathetic activity and a dulled sympathetic response to stressors [87]. Geisler et al [88] showed that basal HF HRV was positively correlated with subjective feelings of well-being. High resting state HRV has been associated with overall physical health [89], emotional resilience and stress vulnerability [40].

HRV patterns are not only reflective of emotional state, but are also thought to influence emotional experience and processing [30]. This is due to the neurological connection and influence from the heart to subcortical regions of the brain (such as the amygdala, thalamus and hypothalamus) involved in emotional processing [90], [91]. Previous studies have demonstrated that activity in the amygdala is synchronised with the cardiac cycle [90], [91].

It is interesting to note the influence of meditative and relaxation practices on respiration and thus heart rate. For example, Lehrer et al [92] showed that respiration slowed during

Zen meditation and, as expected, RSA tracked breathing rate even at low breath rates. It has also been observed, that chanting of the Pali mantra and the recital of the catholic rosary are both recited at 6 breaths per minute and elicit an increased baroreflex sensitivity [93].

Researchers should however, be cautious when interpreting HRV recordings as to not attribute changes in HF HRV and LF HRV to differences in breathing rate and RSA [94].

2.4 HRV biofeedback

The definition of biofeedback has seen numerous unofficial definitions since 1971 [95], creating confusion among practitioners and patients, alike. In 2007, a task force was commissioned by the Association for Applied Psychophysiology and Biofeedback, the Biofeedback Certification Institute of America and the International Society for Neurofeedback and Research to develop an official and professional definition for biofeedback [6]. In 2008, this process was completed with a final definition approved by these institutions, as below:

"Biofeedback is a process that enables an individual to learn how to change physiological activity for the purposes of improving health and performance. Precise instruments measure physiological activity such as brainwaves, heart function, breathing, muscle activity and skin temperature. These instruments rapidly and accurately ''feed back'' information to the user. The presentation of this information – often in conjunction with changes in thinking, emotions, and behaviour – supports desired physiological changes. Over time, these changes can endure without continued use of an instrument." [6, p. 90]

HRV biofeedback is performed by either matching breathing rate to a breathing pacer set to a specified rate [66], [41]; or by matching breathing to the individuals increase and decrease in heart rate, therefore maximising the chances of achieving resonant frequency and highest possible HRV power [96]. In both cases, breathing at a rate of between 4.5-6.5 breaths per minute is practised, as this is where there is a maximum increase in HRV due to resonance.

Karavidas et al [97] suggests that HRV biofeedback stimulates the vagus nerve during practice sessions as well as in between sessions, while Lehrer et al [10] suggests that HRV biofeedback produces acute and chronic increases in total HRV power. LF HRV increases recorded during HRV biofeedback sessions are thought to reflect resonance between baroreflex gain and respiratory sinus arrhythmia as well as an increase in vagus nerve activity [10] [61] .

Numerous studies show that HRV biofeedback may be effective in reducing symptoms relating to depression, anxiety and optimal performance [97-101] and is discussed in greater detail in the following sections.

2.4.1.1 HRV biofeedback mechanisms of effect

Gevirtz [102] suggests HRV biofeedback training derives its many benefits from two main possible mechanisms. The first mechanism is a restoration of autonomic balance due to the improved response of disorders such as asthma [103] [104], functional gastrointestinal disorders [105], fibromyalgia [106] [107], hypertension [61] [108] and chronic muscle pain [109], using HRV biofeedback training. The second suggested mechanism is the impact of vagal afferent stimulation on central effects [102].

Vagal nerve stimulation, a technique using an implanted electrical device to stimulate the vagal afferent pathways, has been used in several studies [110-113] to successfully treat depressive symptoms. These techniques show promise, even though they have not been used in large, randomised and controlled studies. Various authors [114] [115] [9] suggest that certain breathing methods, which in turn stimulates sub-diaphragmatic vagal afferent signals, impact these same afferent pathways possibly causing an impact on central effects, and therefore, on depressive and anxiety symptoms. The theory for these central effects is supported by studies investigating the effects of interoception (the ability to sense the physiological condition of the body), heartbeat detection and slow paced breathing on the brain using heart evoked potentials techniques [116-118].

Due to a combination of pharmacological and neuroimaging studies, as well as a neural basis. Thayer et al [40] [119] proposes that "HRV may serve as a peripheral index of the integrity of CNS networks that support goal directed behaviour". They suggest their findings support the theory of an association between higher basal HRV and improved performance on tasks that use executive function. Lane et al [120] and Ahs et al [121] found positive correlations between HRV and cerebral blood flow in the medial prefrontal cortex and right supra genual anterior cingulate cortex and other cerebral areas responsible for emotion processing.

The connection between the brain and the body is extremely complex, with the above section offering some descriptions of the possible mechanisms in which HRV biofeedback seems to alter stress and anxiety.

2.4.1.2 HRV biofeedback and disease and emotion

In patients with fibromyalgia, HRV biofeedback has been associated with a decrease pain and depression, increase overall functioning [106] and improve asthma severity [122].

It has been shown that six sessions of HRV biofeedback, over a two week period, was linked with a decrease in anxiety in patients with moderate to severe depression [99], and one brief session of HRV biofeedback was correlated with a decrease in heart rate and anxiety, when compared to passive relaxation techniques [123]. Karavidas et al [97] showed a significant improvement in the Hamilton depression scale after session four of HRV biofeedback and an increase in LF HRV. Nineteen individuals showed significantly reduced insomnia, PTSD symptoms and reduced substance craving for individuals with PTSD [101].

HRV biofeedback has also been associated in decreasing alcohol and drug cravings [41]; food cravings and eating and weight concerns in individuals frequently experiencing lack of control over eating [124].

2.4.1.3 HRV biofeedback and performance

HRV biofeedback is suggested to improve the performance of physical activities, such as dance performance [125] and golf performance, due to a decrease in anxiety [126].

Prinsloo et al [127] demonstrated that a single session of biofeedback training was associated with improved reaction times, while making fewer mistakes on a modified stroop test when compared to a control group. The intervention participants were also more relaxed and awake.

There is some controversy on the efficacy of biofeedback. During a triple-blind randomised study, Raaijmakers et al [128] found no differences in treatment effect and no correlation between HRV and cognitive variables between two groups.

2.5 Stress and physiology

2.5.1 Skin conductance biofeedback

Skin conductance (SC), electrodermal response (EDR), and electrodermal activity (EDA) are a few of the commonly used terms used to describe the electrical characteristics of the skin. Psychological or physiological arousal, a dimension of an emotional response, influences the sympathetic branch of the ANS that in turn controls sweat glands [11], [129]. As arousal [12], [13] or stress response [14-16] increase, so does SC, and is therefore considered to be an indicator of an emotional and sympathetic response [130], [131].

2.5.2 Skin temperature biofeedback

During stress, blood flow moves from the skin and body's extremities towards the heart and muscle tissues. The decrease in blood flow in fingers causes a decrease in skin temperature during an emotional arousal or a stress effect, while an increase in blood flow

and skin temperature is caused during relaxation, and is therefore considered an indicator of stress or relaxation [17-20] Lin et al. [132] demonstrated that a percentage increase in finger temperature was most sensitive to indexing stress and depression, followed by a percentage increase in skin conductance, heart rate, and respiration rate.

2.6 Electroencephalography (EEG)

EEG equipment measures the electrical potential between different scalp positions and a reference point, produced by the summation of postsynaptic electrical field of similarly aligned neuronal dendrites and other intra cranial electrical activity [133] [22]. Anywhere between one and more than 300 electrodes [24, p. 68] can be used during a recording; however, often the 10-20 international system is used that has 19 active electrodes that cover different areas of the scalp, with an additional electrode that provides a reference. The reference electrode is often placed over the mastoid bone behind the ear or on the earlobe. The captured data/signal is a waveform that has an electrical potential at a specific frequency measured as a voltage over time. Each electrode is brought into high conductivity (below 5kΩ) with the scalp by gently rubbing the scalp through a hole in the back of the electrode with a stick to remove excess dirt, followed by inserting conducting gel between the electrode and scalp. The voltage from each electrode is fed into a differential amplifier, to enhance the difference between each active electrode and the reference electrode. This electrode voltage is then digitised and saved for artefacting and analysis at a later stage.

Evaluations of the brain using EEG have mixed reviews, with advantages being its simplicity, relatively low cost, ease of use and temporal resolution. EEG has been widely accepted and used to determine brain state as inferred by frequency analysis [24, p. 66], however some critics suggest that it gives mere insight into the working of the brain [134].

2.6.1 Cerebral cortex, EEG frequency and state of consciousness

The human brain can be separated into three main divisions: 1) the brainstem that relays sensory and motor signals between the spinal cord and higher brain centres; 2) the cerebellum that is responsible for aspects of cognition and fine control of muscle movement; and 3) the largest part of the brain, the cerebrum, with the cerebral cortex as the outer layer [133]. The thickness of the cerebral cortex varies between 2 – 5 mm with cortical neurons densely covered and strongly interconnected. Each area of the cortex has a different function to fulfil, with the prefrontal cortex being largely responsible for executive functions [22].

A person's state of consciousness is reflected by the frequency and shape/form of the EEG signal. The states are grouped into delta, theta, alpha, sensorimotor rhythm (SMR), beta and gamma. Delta waves are usually considered to be between 1-4Hz; theta between 4 – 8 Hz; alpha between 8 – 12 Hz (however alpha is often sub-divided into alpha 1: 8 – 10 Hz and alpha 2: 10 – 12 Hz); SMR between 12 – 15 Hz, however, only when measured over the sensorimotor cortex; beta 1 between 13 – 20 Hz, beta 2 between 20 – 30 Hz; and gamma 30 – 50 Hz [23], [22].

Each wave is characterised by various states of consciousness and functions. Delta waves are usually associated with deep sleep [23], while theta waves have been linked to creativity, meditation, access to unconscious material, drowsiness [135], tasks requiring memory [136], [137], mental imagery, enhanced internalised attention and monitoring of internal processes [23], [138]. Alpha waves often take a sinusoidal morphology and traditionally indicate cortical idling or relaxed awareness without concentration [22, p. 71]. This understanding is correct for simple sensory-motor tasks [23], however does not hold true for all circumstances. Alpha 1 appears to indicate internalised attention, expectancy, and alertness, while alpha 2 appears to indicate activity of brain modules used in task performance [139]. Beta waves are associated with focussed executive processing, active thinking, attention, problem solving and outward focus, and is considered as the waking

state of the brain [22], [23]. Various other EEG waves exist, however these are not used in this research and are therefore beyond the scope of further discussion.

2.6.2 Link between EEG, PET, MRI and HRV

During Zen meditation, Takahashi et al [139] found an increase in theta and alpha power in frontal areas suggesting enhanced internalised attention; and an increase in HF HRV and decrease in LF HRV suggesting increased parasympathetic activity and decreased sympathetic activity. A negative correlation between normalised LF HRV power and frontal alpha power was also found.

Using positron emission tomography (PET), Lane et al [120] showed that there was a positive correlation between HF HRV and emotion specific regional cerebral blood flow (rRCB) in the medial prefrontal cortex, the thalamus, the caudate nucleus and the left mid-insula. It was concluded that, as brain activity increases, the braking action on the heart increases. Gianaros et al [140] also used PET to correlate HF HRV to ventral AAC during a memory task, and found that an increase in task difficulty decreases HF HRV, indicating decreased cardiac parasympathetic activity. During an fMRI study, Critchley et al [141] showed a correlation between HRV and dorsal anterior cingulate cortex (ACC) during mental and physical stress.

The above pharmacological and neuroimaging studies [120] suggest a link between prefrontal neural function and HRV. Thayer et al [40] found evidence of a relationship between higher resting basal HRV and improved performance on tasks that use executive functions.

2.6.3 EEG and meditation

Meditation can be defined as a practice regime for the cultivation of well-being, relaxation and emotional balance [142]. Lutz et al [142] categorise the majority of meditation

techniques into two styles: focused attention meditation that entails the voluntary focus on a particular object and open monitoring meditation that involves non-reactive observing of current experience. A third category, automatic self-transcending, is added by [23], where techniques are used to transcend the practitioner's own activity. Slow paced breathing could be considered closest to focused attention meditation.

Focused attention meditation techniques such as, loving-kindness-compassion [143], Qigong [144], and Zen [145], have a positive correlation with an increase in EEG gamma power (30-50Hz) and EEG beta 2 power (20-30Hz). Open-monitoring techniques such as Vipassana [146], Zen [147], Sahaja Yoga [148], have a positive correlation with an increase theta; while Automatic Self-Transcending techniques such as Transcendental Meditation [149], [150], have a positive correlation with an increase in frontal alpha power and coherence.

Yu et al [151] evaluated a form of Zen meditation, where the practitioner used focussed attention on their abdomen to control their breathing at a rate of roughly 3-4 breaths per minute. They found an increase in alpha band and a decrease in theta band during and after focused attention on abdominal breathing. EEG results correlated with blood (peripheral) serotonin (5-HT) levels. While these results suggest that the activation in the prefrontal cortex and/or peripheral 5-HT levels may be responsible for EEG changes, this cannot be concluded definitively as there was no control group involved. It is also noted that peripheral 5-HT levels are entirely different from 5-HT neuro transmitter levels in the brain (central).

Wang et al [152] used the Fast Fourier Transform (FFT) method to analyse long term Zen meditators versus a control group with no meditation experience. It was found that the long term meditators have a slower mean frequency, greater theta and alpha power at rest, when compared with novices.

2.6.4 EEG and breathing

Prinsloo et al [127] and Park et al [153] demonstrated that slow paced abdominal breathing is correlated with a brain state that is calm and relaxed while awake and alert. Prinsloo's research showed an increase in relative theta power and in theta/beta ratio, with a decrease in relative beta power, while Park showed that paced breathing was correlated with an increase in low frequency alpha and decreased theta. In a separate study, Prinsloo et al [154] showed that HRV biofeedback, which is a method that causes participants to breathe at a rate of between 4.5 to 6 breaths per minute, was positively correlated with participants' cognitive performance during a modified stroop test, with both speed and accuracy. Fumoto et al [155] demonstrated that abdominal breathing at 3-4 breaths per minute was positively correlated with vigour-activity with a tendency of anxiety reduction.

Various research efforts have focused on the effects of breathing on the EEG, with each applying different breathing techniques and methodologies. These differences are likely to account for some of the inconsistencies in results. A summary of this research follows in the section below.

Fumoto et al [155] compared EEG information of 20 minutes of rest to 20 minutes of rhythmic breathing (eyes open and closed on different days) at Cz, C3, C4, Fz, and Pz at 3- 4 breaths per minute. High and low alpha increased significantly more during paced breathing then autonomic breathing at all locations. Participants had an increased feeling of vigor-activity, with a reduction in anxiety during and after paced breathing, which correlates to the increase in high alpha [156].

Park et al [153] compared EEG information of 15 minutes of rest to 15 minutes of paced breathing at (eyes closed condition) at F3, F4, T3, T4, P3, and P4. In general, an increase in low and high alpha was found with a local decrease in theta, indicating internal and external awareness and alertness.

Other studies found only marginal increase in alpha brain frequency [157] and [158], or a decrease in beta brain frequency [127]. The incongruences in results might be attributed to differences in methodologies, such as duration of slow paced breathing (only three minutes for [158]), the number and placement of EEG electrodes on the skull and methodology to calculate the power in different frequency bands.

2.6.5 EEG and stress and anxiety

There are numerous studies that examine the effect that stress and anxiety have on EEG signals. According to a study by Knyazev et al [159], state and trait anxiety scores were negatively correlated to relative delta power across all sites.

An increase in frontal midline theta reflects relief from anxiety [160-162], mental concentration [163] and correlates with HF HRV from relaxation training [129]. An increase in theta, rather than alpha, is often seen as a reliable marker for relaxation [164]. During a breathing intervention designed to reduce stress in participants, relative theta power and theta/beta ratios increased while relative beta power decreased during the intervention [127]. A decrease in alpha reflects relaxation [161], while an increase in relative alpha has been shown to correlate to state and trait anxiety scores [159]. In other research, beta power increased with an increase in stress [165], [166], and paying attention [166] while decreasing in response to relaxation stimuli [167], [168], [127]. Total absolute power has been shown to increase with anxiety [165], [169].

2.7 Summary

The above chapter firstly presents an introduction and literature review on the concepts and mechanisms relating to respiration, heart rate, HRV, biofeedback, skin conductance, skin temperature and EEG. Furthermore, previous research relating to the relationship between these concepts and breathing, stress, relaxation, meditation and emotion are investigated.

In summary, both positive emotion and breathing at a rate of 6 breaths per minute has the ability to increase HRV. In turn, resonance may be induced between respiration, HRV and

blood pressure at the body's natural resonant frequency of approximately 0.1 Hz. This resonance potentially impacts brain state and may have the benefit of reducing stress and increasing performance. Chapter 3 provides information on the technical aspects of measuring and analysing the concepts introduced in this chapter.

CHAPTER 3: BACKGROUND AND PREVIOUS RESEARCH ON SIGNAL PROCESSING

3.1 Introduction

In Chapter 2, various physiological concepts are introduced and their known relationships to each other are investigated. This chapter presents an introduction and literature review on the technical aspects of this research, namely signal processing. The analysis of HRV, breathing, skin conductance, skin temperature and EEG is described. The practical aspects of EEG recordings and EEG signal processing techniques are also discussed.

3.2 HRV analysis

Heartbeat, and therefore the interbeat interval (IBI) time necessary to calculate HRV, can be measured by either an electrocardiogram (ECG) or blood volume pulse (BVP) sensor, also known as a photoplethysmographic (PPG) sensor. An ECG sensor measures the electrical activity of the heart that provides a more precise signal with fewer movement artifacts when compared to a BVP sensor, however it is not without its drawbacks. An ECG sensor is more cumbersome, obtrusive and more time consuming to apply [170] than a BVP sensor. The BVP sensor measures changes in blood volume in the tissue, capillaries and arteries by shining infrared light through the tissues, and then measuring the amount of light that is reflected. The amount of reflected light measured by the BVP sensor is proportional to the volume of blood in the tissue [170]. Using a BVP sensor has an advantage in that the shape of the signal reflects cardiovascular variables such as blood pressure; while the amplitude indicates an individual's response to physical and emotional stressors, and vasodilation (therefore peripheral temperature) [171] . Speckenbach et al [171] demonstrated that BVP sensors for biofeedback training were highly reliable.

HRV is commonly evaluated using time, frequency domain, rhythm pattern analysis and non-linear techniques, with each technique reflecting different neural regulatory changes [29]. Only frequency domain analysis is evaluated in this research study. Frequency domain analysis can be performed using either parametric or non-parametric

methods, with both methods providing similar results [29]. Non-parametric methods such as the FFT have advantages such as ease of implementation and high processing speeds; while parametric methods provide smoother spectral components and an accurate estimation of power spectral density with a small number of samples.

Heartmath [30] is more concerned with the heart rhythm pattern, rather than the heart rate or change in heart rate. An increase in HRV coherence is defined as a large increase in power band around 0.1 Hz (low frequency) and a decrease in the 0.0033 - 0.04 Hz (very low frequency) and 0.15 - 0.4 Hz (high frequency) bands [172]. Interbeat-intervals are firstly measured from the heartbeat (either acquired from an electrocardiogram (ECG) or BVP sensor), storing them every 500 ms, and then calculating coherence with the following technique for the latest 64 seconds of data. The frequency where the maximum power peak in the low frequency range (0.04 - 0.26 Hz) is found and the integral in a 0.03 Hz window centred on this peak frequency is calculated (see Figure 3.1). The total power is calculated by taking the integral of the entire spectrum and the coherence ratio is calculated using the following equation:

$$Coherence\ ratio = \left(\frac{peak\ power}{total\ power - peak\ power}\right)^2 \tag{3.1}$$

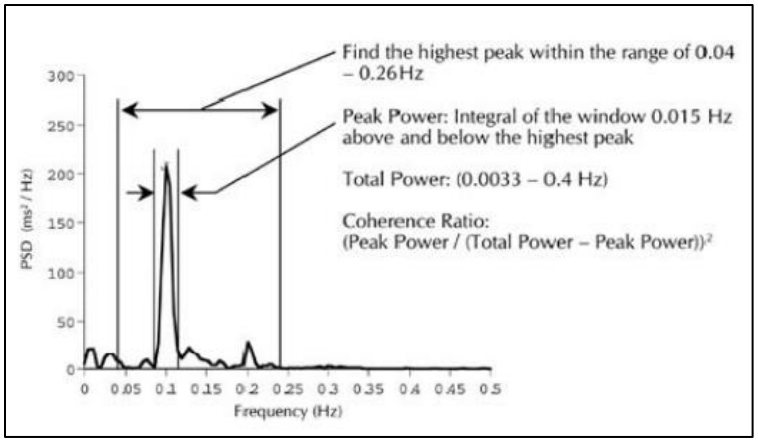

Figure 3.1: Illustration by Heartmath demonstrating how coherence ratio is calculated [30].

The approach that this research applies for the HRV calculation is based on the work from the task force of The European Society of Cardiology and The North American Society of

Pacing and Electrophysiology [29]. FFT transforms provide information on how much power or heart rate variance is distributed as a function of frequency [1]. Spectral power density of the HRV is calculated in each fixed frequency band (VLF <0.04 Hz; LF between 0.04 – 0.15 Hz; and HF between 0.15 – 0.4 Hz), over a 64 second moving average and stored every 62.5 ms. These values are averaged over a whole session to obtain the final value.

The units for LF HRV and HF HRV are in ms^2, and these can be normalised using the following formulae [29]:

$$LF\ norm = \frac{LF\ Power}{Total\ Power - VLF\ Power} \times 100 \quad (3.2)$$

$$HF\ norm = \frac{HF\ Power}{Total\ Power - VLF\ Power} \times 100 \quad (3.3)$$

HRV movement artifacts are manually removed using BioGraph infinity version 6.0.2 before any calculations are processed.

3.3 Respiration analysis

As far as the author is aware, no previous studies have assessed breathing quality by evaluating the respiration percentage power in the LF band (0.04 - 0.15 Hz). One representation for the percentage respiration power in the LF band is the quality (smoothness and rhythm) of a participant's breathing.

This is evaluated in the same way as calculating HRV LF norm [29], except calculating the power spectral density of respiration inhalation and exhalation (from a strain gauge) instead of from HRV signals. The Fourier transform of the participants' respiration was determined, and the percentage power in the LF range over the total range was calculated.

3.4 Equipment and Electroencephalography (EEG)

Electroencephalography (EEG) is the process and technology of measuring and recording the electrical signals of the brain, in order to analyse a person's brain state [22].

Surface EEG is considered non-invasive [24], quiet, relatively tolerant of patient movements, does not require exposure to high intensity magnetic fields, and hardware costs are significantly less than other methods to study brain function.

Some disadvantages of EEG are listed below:

- EEG is most sensitive to post-synaptic potentials that are generated from within the cerebral cortex (the outer crust of the cortex), on the crests of the gyri adjoining the skull. Neural activity deeper within the cortex, within the sulci, or that produce currents at a tangent to the skull have less contribution to the EEG signal.
- EEG has a relatively poor spatial sensitivity when compared with other methods of studying brain function.
- As some neuron electric potentials cancel each other out, it is mathematically impossible to reconstruct an intracranial current source from given EEG recordings. This is known as the inverse problem, however, much work has been done to generate good estimates.
- EEG takes a large amount of time to connect electrodes to the scalp and establish good, low impedance connections.

3.4.1 Alternative commonly used equipment

Some of the most common equipment used for assessing brain anatomy and physiological function are briefly discussed below.

Cerebrospinal fluid, brain tissue, blood and bone have different densities to one other. CAT scan usually takes 9 or 12 slices of the brain using X-ray technology, and show brain anatomy and possible haemorrhage.

Magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI) can be used for imaging brain anatomy and brain physiological function respectively. They use three magnetic fields to distort the behaviour of protons, and record the amount of time it takes for the protons to recover. This information is then used to create an image of the anatomy of the brain. As the MRI scan uses magnetic fields, individuals with pacemakers and metal within the body not connected to hard tissue cannot be subjected to a MRI scan. fMRI uses the knowledge that cerebral blood flow and blood oxygenation are accompanied by neuronal activity [22, p. 65], which infers different levels of activity in different brain areas.

Like MRI, PET examines the metabolic activity of the brain. Its main advantages are that it allows doctors and researchers to first measure an amount of a specific compound used by different areas of the brain, and second provides absolute levels of brain metabolism due to blood flow, oxygen use, and glucose metabolism.

3.4.2 EEG equipment

3.4.2.1 EEG electrodes

EEG electrodes are made from a variety of different metals, the most common being silver-silver chloride (Ag/AgCl) due to its low dc offset variability and its ability to quickly establish and maintain stable electrochemical potentials against biological tissue. Ag/AgCl electrodes are also considered to be non-polarisable, that is, the potential between the scalp, conducting solution, and metal does not change significantly when current passes through the electrode. Other metals used are tin, stainless steel, gold plated silver, and pure gold or silver. Using electrodes made from different metal for the EEG and reference electrode can result in altered EEG recordings, as each type of metal has different electrochemical

properties. It is therefore important to use the same type of electrodes for all scalp locations [173, p. 119].

Electrodes can either be passive (the scalp potential is only amplified at the EEG amplifier) or active (the scalp potential is pre-amplified using electronic circuitry at the electrode). Due to high impedance matching properties between the scalp and electrode amplifier's input, active electrodes are inherently more stable over a broader range of scalp impedances and have a larger tolerance to impedance differences between different scalp electrodes. They also have a better cable noise immunity between electrode and amplifier due to the electrode amplifier's low output impedance [133, p. 29]. Should passive electrodes be used, scalp to electrode impedances should be kept below 5 kΩ to ensure good stability and noise immunity. Similarity between the different electrodes to scalp impedances is also important. Achieving good EEG recordings is largely due to proper scalp preparation (slight mechanical abrasion to remove dead skin cells), conducting gel application and impedance checking.

3.4.2.2 EEG amplifier

The amplifier circuitry amplifies the potential difference between each electrode and the reference electrode (typically around 30 – 50 μV) using differential amplifiers. This analogue voltage is then converted into a digital number for storage and future processing [174].

3.4.3 EEG protocols and montage

3.4.3.1 Electrode placement

In the 1950's, the first standards in EEG were defined and among them was an electrode placement system known as the international 10-20 system, named as each electrode [175] is either 10% or 20% to the adjacent electrode of the total distance between the nasion and

inion [175]. Since then, alternative systems have been developed that allow for a greater number of electrodes to be used if required [133, p. 31].

When using the 10-20 system, electrodes placed over the left and right hemisphere are labelled with odd and even numbers respectively, while electrodes placed over the midline are labelled a - z. The uppercase letter is an abbreviation for the skull bones that underlie the cerebral cortex, namely the: frontal, parietal, temporal and occipital lobes [24]. For example, P_4 is off centre towards the right hemisphere over the Parietal lobe, as illustrated in Figure 3.2.

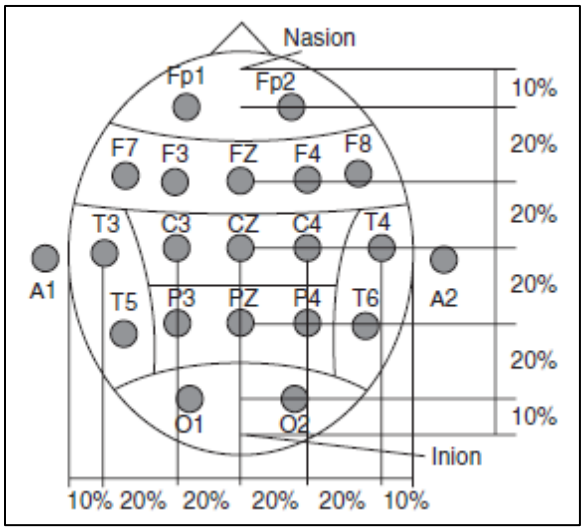

Figure 3.2: Demonstration of electrode placement using the 10-20 International placement system [173].

3.4.3.2 Montage

EEG machines use differential amplifiers, therefore the output recorded potential is the difference in voltage between the measuring electrode and a reference. The reference could be another electrode or the average of a group of electrodes. Montage is the rule which states the relationship between the measuring electrode and reference.

Often when recording digital EEG, the reference electrode is connected to either one or two earlobes (mastoids, chin, or tip of nose can also be used) in the linked ear montage. The montage and filter setting can then be changed when the EEG is reviewed [173].

Ideally, the reference is electrically neutral and stable; however, as all sites on the head are at least slightly electrically active, there is no ideal and therefore the recorded voltage is influenced by a non-ideal reference. The typical montage attempts to deal with this by averaging the potentials over all the electrodes and using this as a reference for each channel. Another common montage is the local average montage, in which a unique reference is calculated for each measuring electrode by averaging the potential of a number of electrodes in the vicinity of the measuring electrode. The bipolar or sequential montage is calculated by finding the difference between two measuring electrodes located on the scalp. For example, F4/C4 or F7/T3 [176].

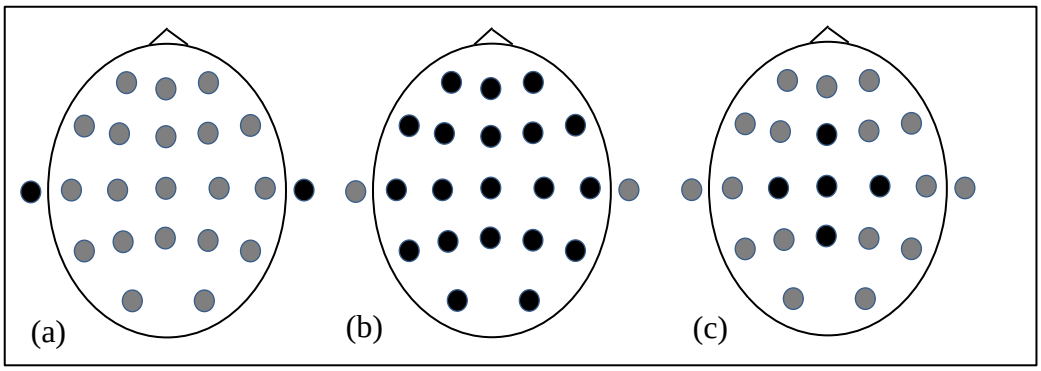

Figure 3.3: Montages (a) linked ear, (b) common average, and (c) local average. Adapted from [173].

3.4.4 Artifacts

EEG artifacts are unwanted electrical potentials of non-brain origins that are merged with the electrical potentials of the brain during EEG recording and prevent accurate analysis of brain activity [177].

An artifacts origin is either non-physiological (faulty electrodes or electrode pop, or noise from external sources such as AC lines) or physiological (eye or tongue movement, teeth grinding, swallowing, breathing, ECG, sweat, and electromyography/muscle movement) [177], [178]. Figures 3.4 – 3.7 demonstrate the impact of different artifact on EEG signals.

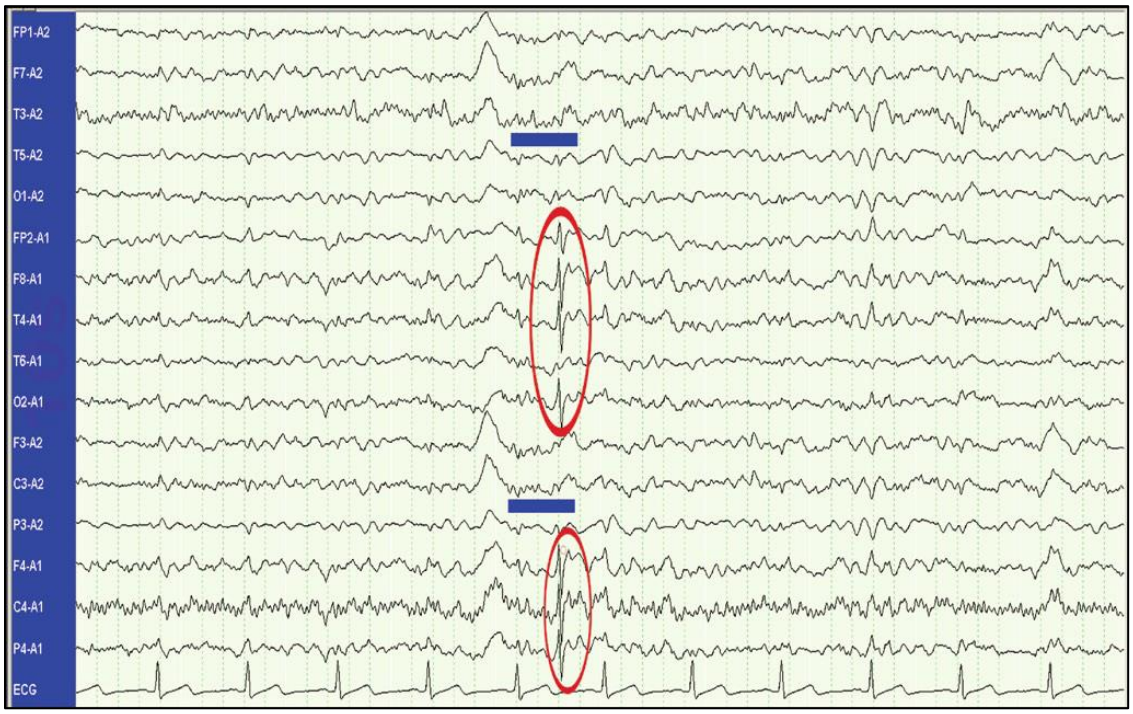

Figure 3.4: Red circles indicate electrode pop. Reproduced with permission from [177].

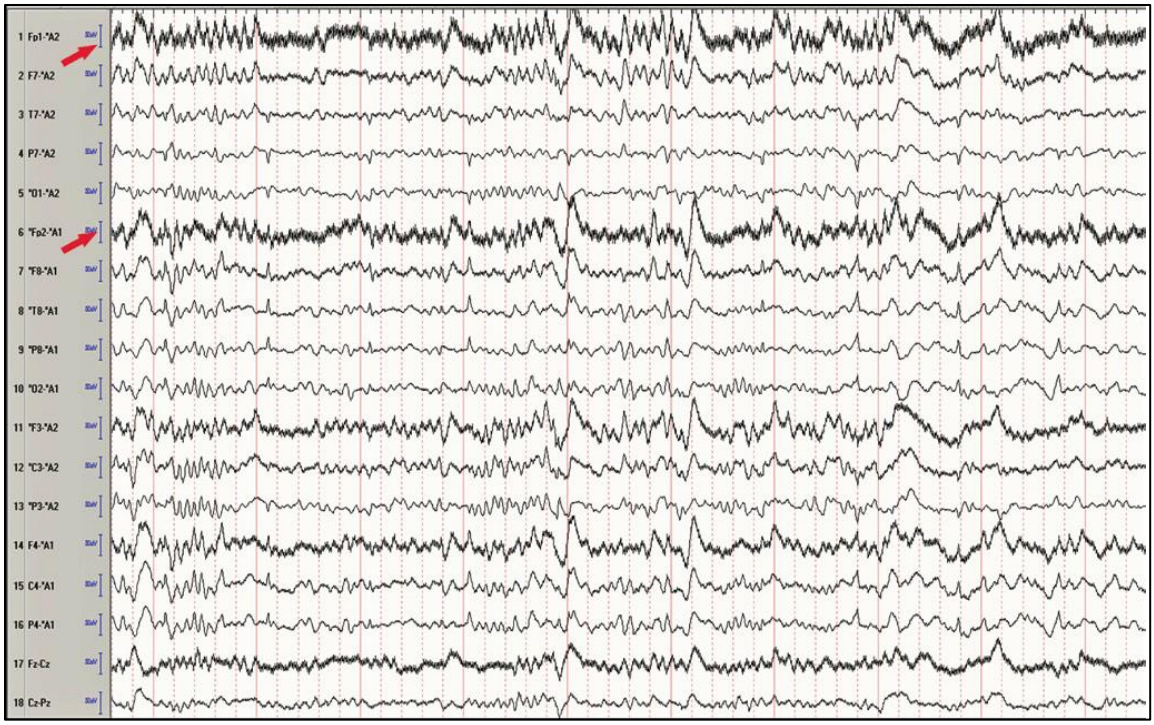

Figure 3.5: Red arrows indicate 60Hz AC line artifact. Reproduced with permission from [177].

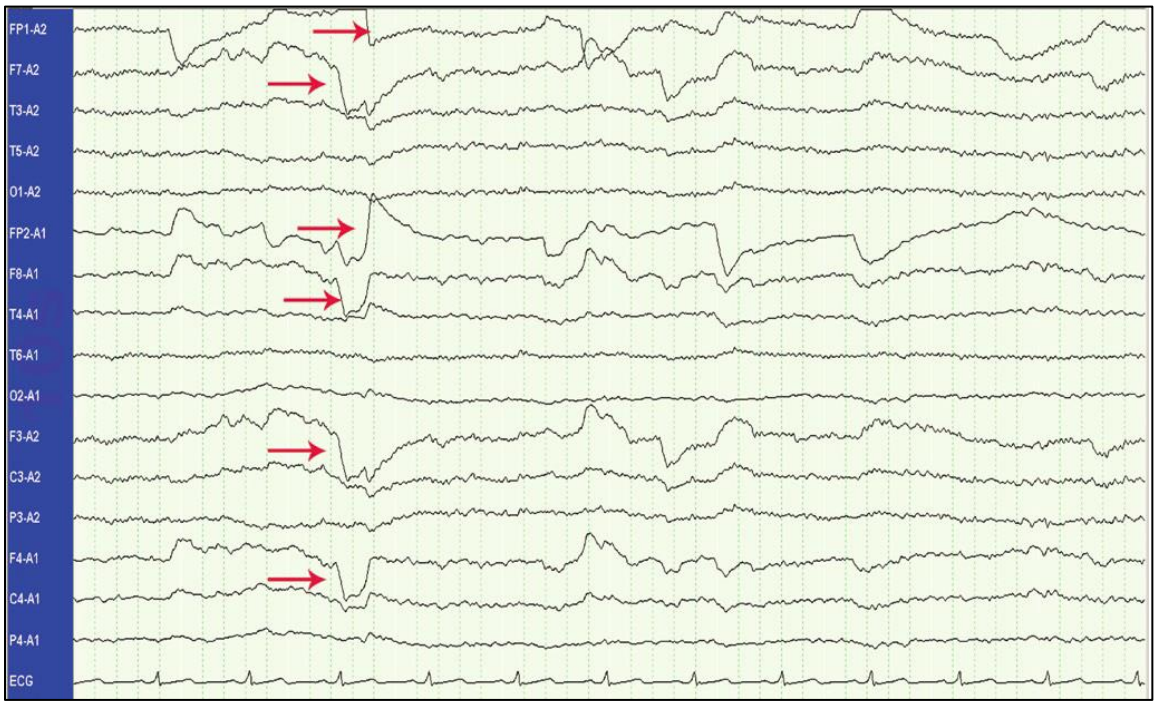

Figure 3.6: Red arrows indicate eye movement artifact. Reproduced with permission from [177].

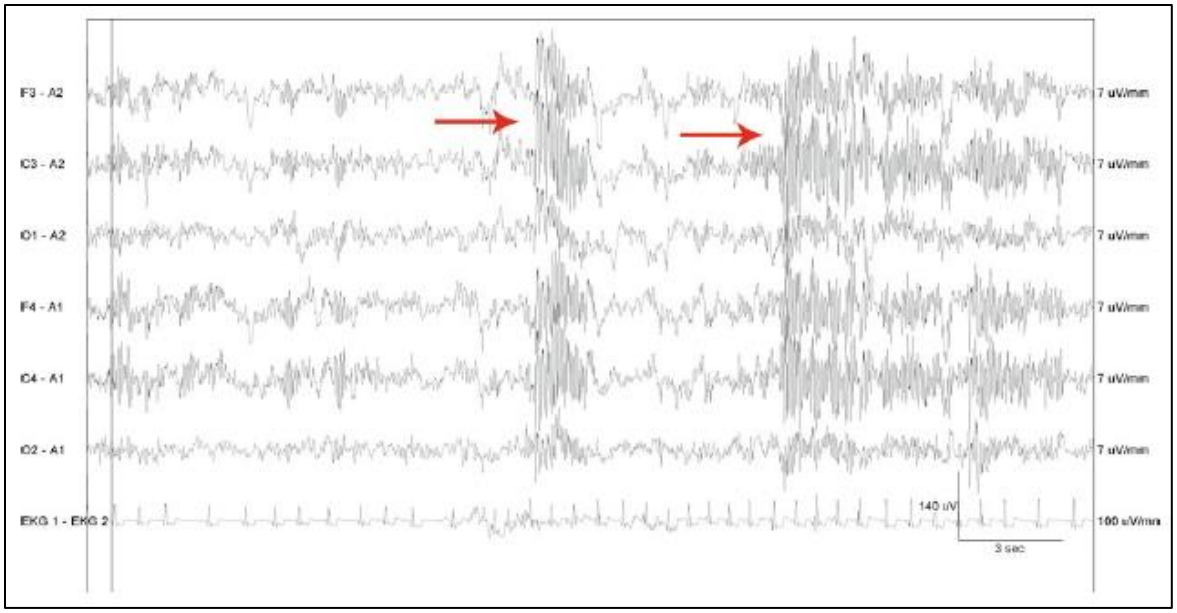

Figure 3.7: Red arrows indicate teeth grinding artifact. Reproduced with permission from [177].

Several techniques have been developed for artifact removal, with the majority removing the entire segment of contaminated data. The most common of these methods are: simple amplitude threshold, min-max threshold, gradient criterion, spectral distribution, standard deviation and joint probability [133], [179]. The rejection of all epochs contaminated by artifact can result in a large amount of data loss, therefore methods based on filtering, Principal Component Analysis, regression analysis and Independent Component Analysis (ICA) have been developed for correcting the artifacts rather than deletion [177], [180].

ICA is a processing method that decomposes a multichannel signal into statistically independent components [178], [133], and is based on the assumption that artifacts and brain activities are generated by independent processes [179]. There is a possibility that part of the EEG signal may also be lost when removing the artifact component [178].

Delorme et al [181], [182] developed a program (EEGLAB) for semi-automated artifact rejection. It includes routines to identify artifacts such as improbable data, extreme values, abnormal trends, abnormal spectra and routines to identify and remove independent components.

3.5 EEG signal processing techniques

3.5.1 About the EEG signal

The human brain is not a fully stochastic or deterministic system otherwise it would not be able to create anything new or learn and repeat thoughts and tasks [183]. The EEG signal is generated by both nonlinear and linear neuronal dynamics [184-188]. The brain could therefore be considered a complex nonlinear system and one of its outputs, the EEG signal, a nonlinear, nonstationary (or quasi-stationary up to 0.25 seconds), and noisy signal [135], [183] that contains useful information regarding brain state [189], [190].

3.5.2 Linear analysis of non-stationary signals

Signals that are statistically stationary are easy to characterise in either the frequency or time domain. Often signals are analysed in the frequency domain using linear transforms such as the Discrete Fourier Transform (DFT), or the Discrete Cosine Transform. As EEG signals are non-stationary (amplitude or frequency varies over time), other techniques such as the Short Time Fourier Transform (STFT) and the Wavelet Transform have been used to analyses signals in the time frequency domain [135].

3.5.3 Short time Fourier transform (STFT)

The STFT can be used to find the distribution of energy in the time-frequency space by applying the STFT to extracted successive short pieces of signal using a window function [135].

$$X(n,\omega) = \sum_{\tau=-\infty}^{\infty} \chi(\tau) w(n-\tau) e^{-j\omega t} \tag{3.5}$$

Where $\chi(\tau)$ is the signal to be transformed and $w(t)$ is the window function [135, p. 56].

There is always a trade-off between time or frequency resolution when using the STFT. From the equation above, it can be seen that the shorter the duration of the time window, the better the temporal resolution and poorer the frequency resolution. As the time window increases, the frequency resolution increases and temporal resolution decreases [190].

3.5.4 Wavelet analysis

Wavelet analysis refers to a type of multiresolution analysis that uses wavelets and wavelet packets to decompose nonstationary signals providing exceptional joint time-frequency resolution [191].

Wavelet transforms have better accuracy then FFT, however a larger ambiguity in signal decomposition. In addition, wavelets have a faster computational speed compared to the STFT [192], and have been shown to offer better spectral characteristics and detect brain diseases more effectively than FFT [193]

3.5.4.1 Background to Wavelet Transform

Wavelets are oscillating amplitude functions of time with requirements to satisfy certain conditions: a wavelet ψ must have a zero mean amplitude, with small lower frequency energy compared to its higher frequency energy and finite energy over its time course [191]. A mother wavelet is the type of wavelet chosen to perform the wavelet transformation, while a wavelet family is created by a process of scaling and translation of the mother wavelet in order to create an infinite number of wavelets in the family. Scaling with parameter s and translating with u results as equation 3.6.

$$\Psi_{u,s}(t) = \frac{1}{\sqrt{s}}\Psi\left(\frac{t-u}{s}\right) \tag{3.6}$$

The term translation refers to the location of the wavelet in time, while scaling or dilation refers to the process of stretching or compressing a wavelet. Stretching a wavelet increases

the scale, making it less localised in time and shifting its spectrum to lower frequencies with a higher magnitude. The converse holds true for compressing a wavelet. Wavelets therefore obey the Heisenberg Uncertainty principle due to the trade-offs between time and frequency localisation as the wavelet is stretched or compressed. Wavelet analysis has an advantage over the STFT in that it can give good time resolution (with poor frequency resolution) at high frequencies and good frequency resolution (with poor time resolution) at low frequencies [194].

The Continuous Wavelet Transform (CWT) for a one-dimensional signal $f(t)$ can be calculated using (3.7), where * denotes the complex conjugate, $\Psi(t)$ is the analysing wavelet, s is the scale parameter that is inversely proportional to frequency and u is the translation parameter [135].

$$F(u,s) = \frac{1}{\sqrt{s}} \int_{-\infty}^{\infty} f(t)\, \Psi^* \left(\frac{t-u}{s}\right) dt \tag{3.7}$$

The CWT scales and translates the mother wavelet by infinitely small steps in relation to a continuous signal while computing the wavelet coefficient at each step. The above equation shows that the wavelet transform produces an infinite set of wavelet coefficients $F(u,s)$ when s and u are varied, for a given signal $f(t)$. Due to the unnecessary redundancy for most applications, the CWT is inefficient and time consuming to compute. Mallat [195] developed a much more efficient and computationally simpler wavelet analysis technique called the Discrete Wavelet Transform (DWT) that provides a highly efficient wavelet representation that can be implemented with a recursive filter system.

The DWT decomposition, as seen in figure 3.8, is obtained by passing the original signal through successive half band low and high pass filters with an impulse response determined by the chosen wavelet. Due to the frequencies being halved, and according to the Nyquist rule, half of the samples can be eliminated by discarding every other sample (subsampling). This subsampling process doubles the scale of the signal, while the filtering process halves the amount of frequency information in the signal [196]. The filtering and subsampling together constitute one level of decomposition that outputs both detail and approximation

coefficients and can be repeated for further levels of decomposition. Each level of decomposition halves the time resolution (since the number of samples that characterise the signal is halved) and doubles the frequency resolution (since the frequency band is half the previous level). The coefficients represent a correlation between the original signal and the chosen wavelet at different scales and translation [190].

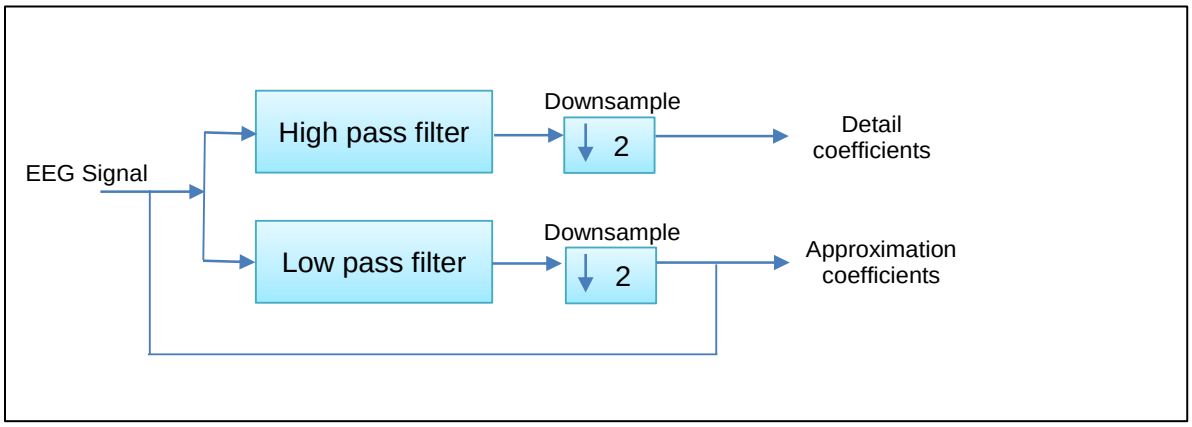

Figure 3.8: DWT recursive filter and decomposition scheme.

An advantage of the DWT is that the original signal, or any level of decomposition, can be reconstructed using a filter system as per figure 3.5. The process is reversed by upsampling the coefficients, filtering and summing them together.

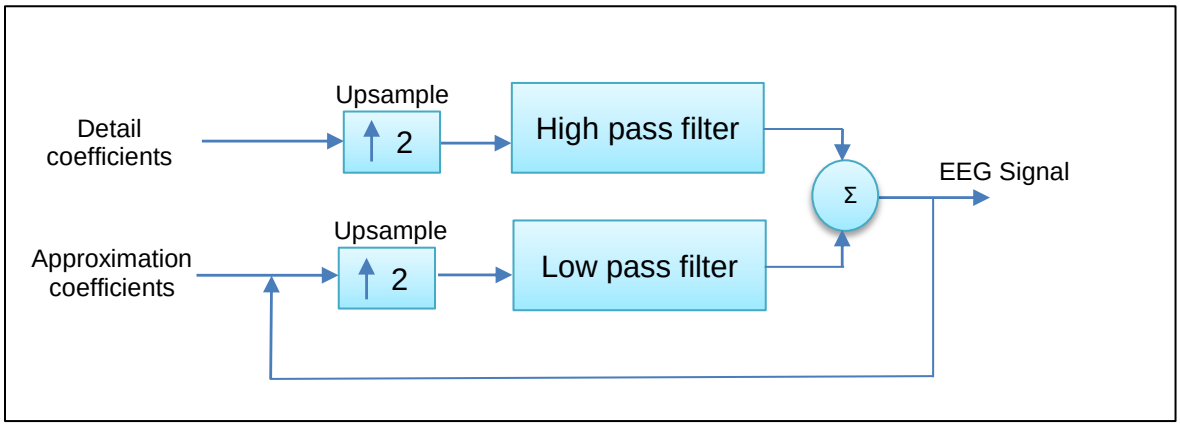

Figure 3.9: DWT reconstruction scheme.

3.5.4.2 Wavelet packets

A wavelet packet is a group of signals found at the end of each decomposition level derived from a single mother wavelet. The difference between conventional DWT and wavelet packet decomposition, is that only the approximation coefficients are decomposed into deeper levels during the conventional method; whereas both approximation and detail coefficients can be decomposed for the latter method. Wavelet packet decomposition is especially useful when evaluating neuro electric waveforms such as EEG signals, as previous literature specifies distinctions in frequency bands (e.g. beta 1 and beta 2) that are found in deeper levels of the detail bands. Figure 3.6 demonstrates this advantage when evaluating EEG signals comparing the two methods using a four level decomposition tree.

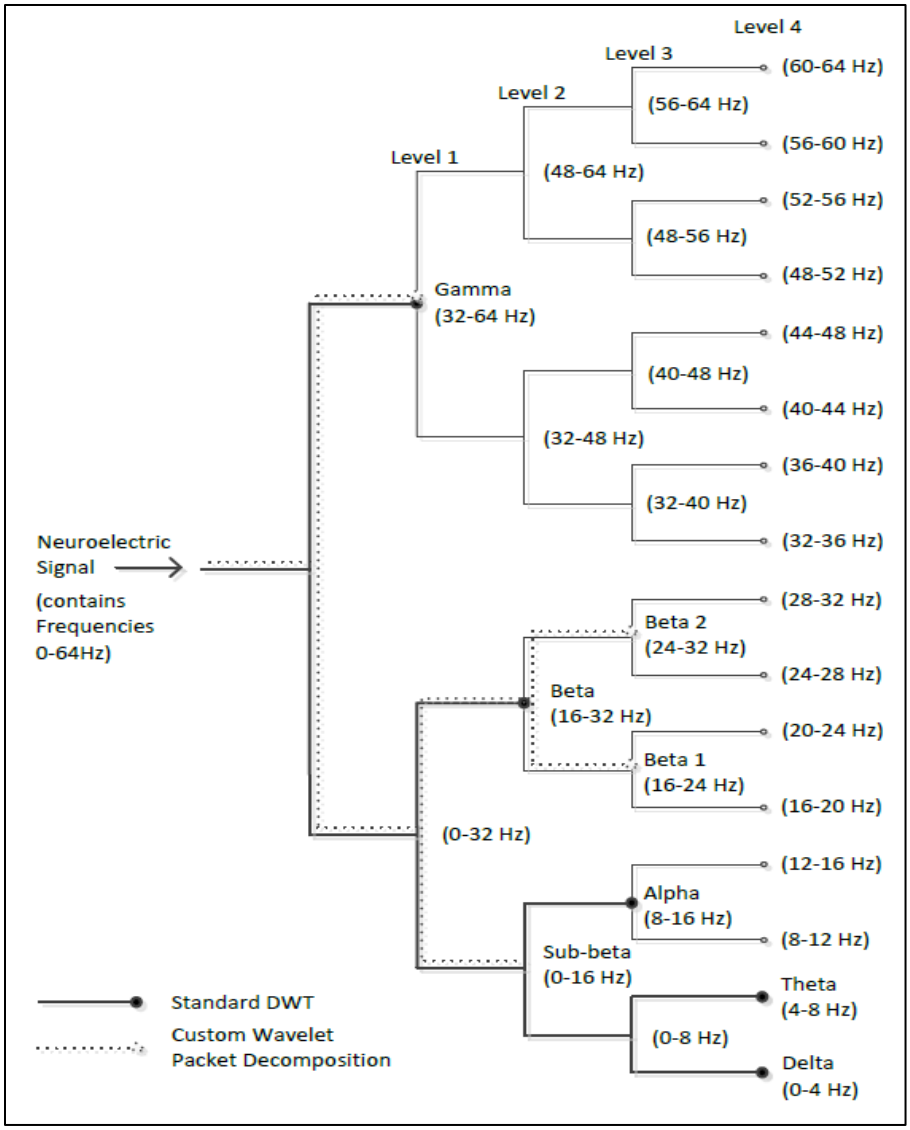

Figure 3.10: Comparison between the standard DWT (bold line) and wavelet packet decomposition (dashed line). Adapted from [191].

3.5.4.3 Choosing a Wavelet

The type of application and signal being analysed should determine the type of wavelet used. The ability of the wavelet transform to choose a wavelet with a morphology as close as possible to the shape of the neuroelectric events being analysed, gives a large advantage over traditional Fourier techniques. A wavelet can be designed to match the shape of the

signal of interest using a matching pursuit technique, or the direct design technique by matched Meyer wavelets.

3.6 Summary

This chapter has provided relevant background information for this study relating to the techniques used to measure and analyse HRV, respiration and electrical brain signals. A detailed description of EEG equipment has also been included. Furthermore, EEG signal processing techniques (Wavelet Transforms) are discussed in detail which forms the basis of the methods used to analyse the EEG data. The study methodology is discussed in greater depth in Chapter 4.

CHAPTER 4: METHODOLOGY

4.1 Introduction

This chapter deals with the methods and materials used in the undertaking of this dissertation. It describes the scope of research design, equipment and analyses situated amongst existing research strategies that were used to test the hypotheses of the project.

4.2 Sampling strategy

Twenty male participants from the psychology and electrical engineering department at Wits University were invited to volunteer for the research study through the Wits University psychology website. The subject information sheet and consent form (Appendix A and Appendix B, respectively) were placed on the Wits psychology notice website through which potential volunteers were able to contact the research investigator to volunteer.

Participant exclusion criteria that was stated were as follows: previously diagnosed anxiety, cardiac, psychiatric or epilepsy disorders; current use of heart rate altering medications; or current use of stimulants or recreational drugs. Women were also excluded from this study as men and woman may have different EEG [197] and cortisol [198] responses to stress, and therefore may also have different physical responses to breathing at different rates.

The following ethical guidelines were put into place for the research period:

- The dignity and wellbeing of students was protected at all times;
- Ethics was approved by the Wits University Human Research Ethics committee (Protocol number: M130611, Appendix C);
- All participants read and signed the consent form;
- No participant was subordinate to the researcher;
- Recording EEG data is considered non-invasive [24]; and

- The research data remained confidential throughout the study and the researcher obtained the students' permission to undertake the assessment.

Of the volunteers that started the study, there was one dropout due to work commitments. The mean age of the participants was 26.8 (± 6.31) years. The participants' ethnicity was divided into 10 black, 8 white and 2 coloured. Participants were randomly assigned to either a control or intervention group.

4.3 Procedure

4.3.1 General

All participants attended two sessions. The first was an information, preparation and training session; while the second session that took place approximately one week later was the actual experiment where measurements were recorded and questionnaires filled out.

There was no literature found on fainting due to slow paced breathing, however, some participants may occasionally experience slight dizziness due to breathing too deeply and hyperventilating [35]. Participants were advised both verbally prior to the study and in the patient information sheet to revert to their normal breathing pattern and abandon the study should dizziness occur. Participants were seated in a comfortable chair with arm rests and the researcher was present at all times, so that there was no foreseeable risk of injury in the unlikely event of fainting.

Five EEG sensors, BVP, temperature, skin conductance and respiration sensors were connected to participants during the second session to record EEG readings and physiological responses of participants.

A successful session was defined as one that contained small enough artifact that could be removed, and enough useful information extracted from the physiology and EEG signals.

4.3.2 First session: Training

During the first session, the procedure for both the intervention and control groups was as follows:

1. Welcoming and introduction to the venue;
2. Reading and signing of the consent form;
3. Reading the participant information sheet;
4. Advice provision on what stimulants and food to abstain from for 2 hours before the second experiment as per the participant information sheet;
5. Explanation of what the experiment was about, including the function of sensors that was to be connected in the second session;
6. Questions and answers.

The control and intervention groups had respiration and BVP sensors connected to familiarise the participants with the sensors and software, however, the intervention group was also taught pursed lipped abdominal breathing (as per the below instructions), whereas the control group was not.

4.3.3 Second session: Experimental

The actual experiment consisted of connecting all sensors to participants (approximately 20 min), followed by 3 recording sessions: Baseline 1 (B1), the Main session (M), and Baseline 2 (B2). B1 was a 3 minute eyes open baseline recording (to establish participants' regular EEG and physiology patterns) where subjects were required to look at a blank screen as seen in Figure 4.1. The intervention group breathed at approximately 6 breaths per minute (established by synchronising their breathing to a visual stimuli) for 12 minutes, while the control group breathed at a spontaneous rate for 12 minutes. All participants then had another 3 minute eyes open baseline recording (B2), followed by removal of equipment. Participants were asked to answer a short written

questionnaire at the start and end of the procedure. The whole experiment took less than 1 hour and 10 minutes.

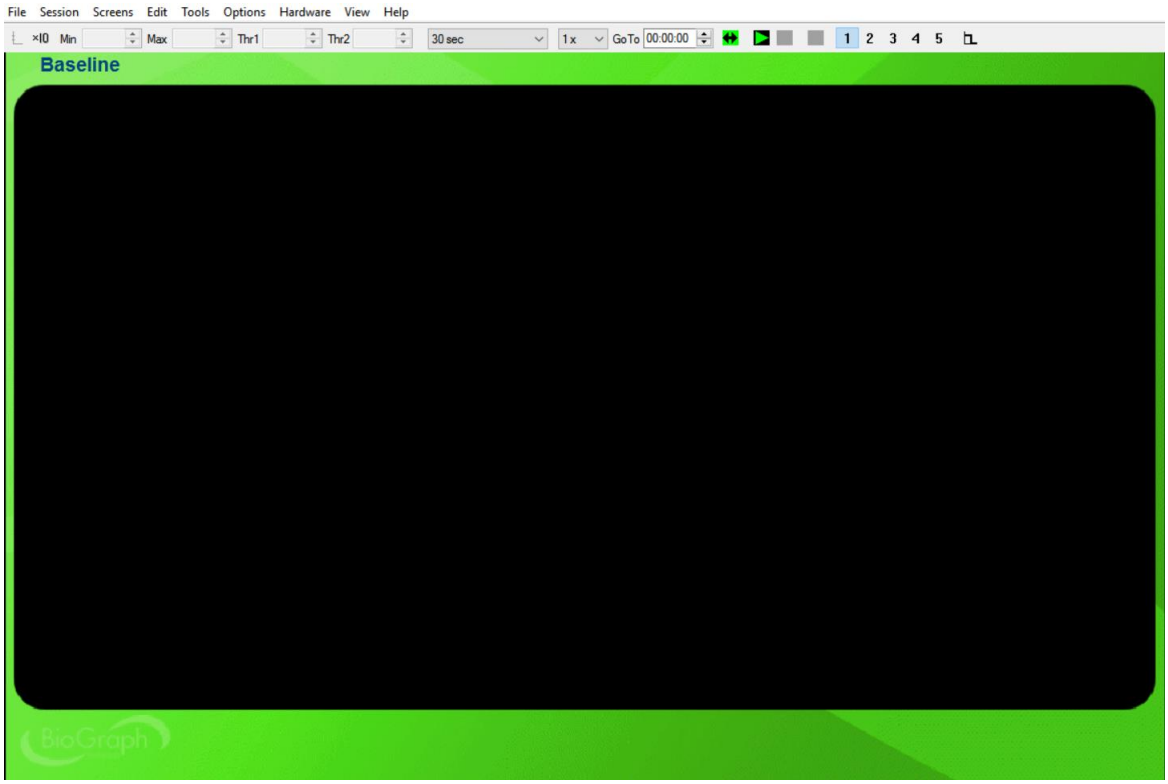

Figure 4.1: Blank screen on BioGraph Infiniti software that all participants looked at during Baseline 1 and 2.

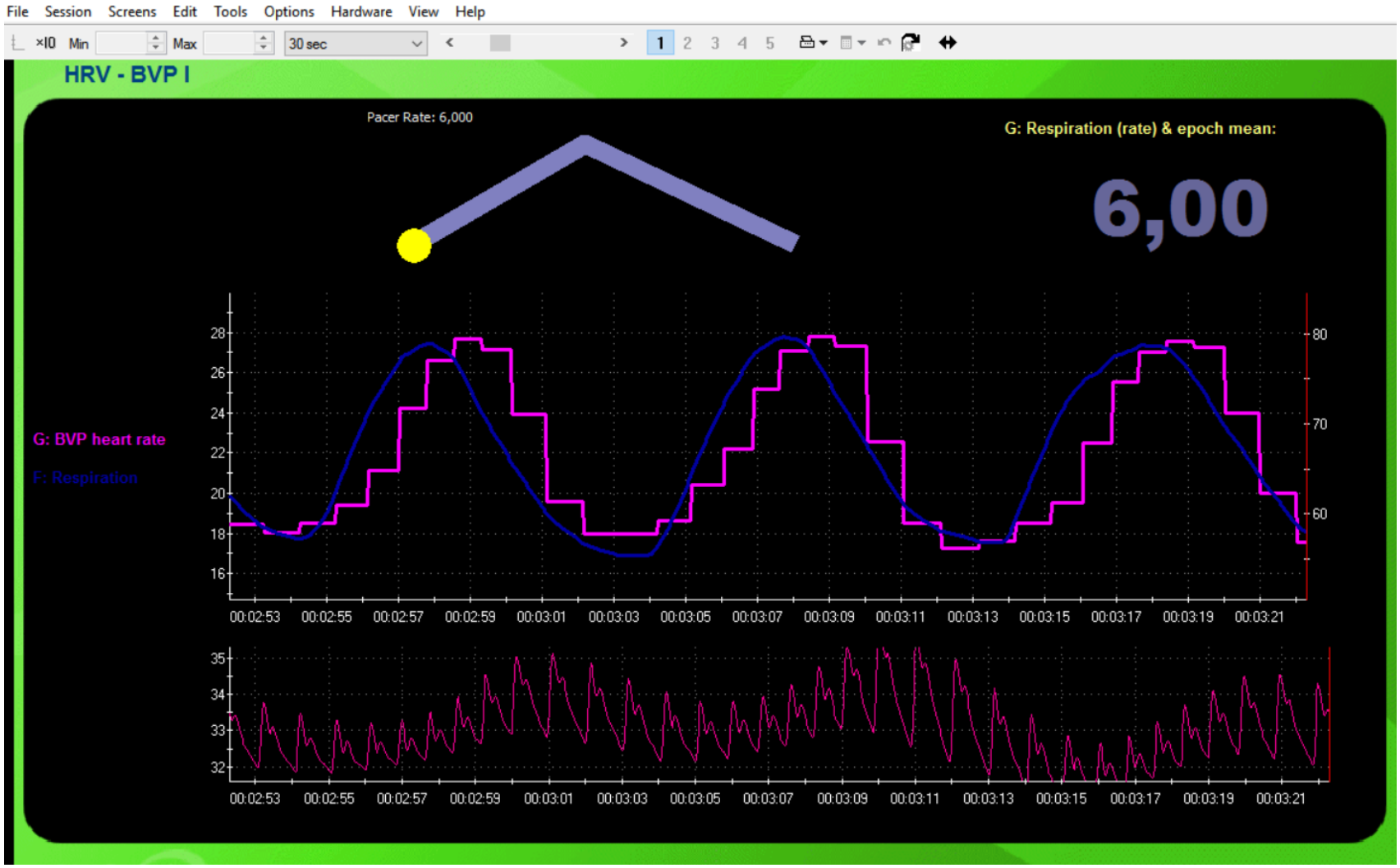

Figure 4.2: Screen on BioGraph Infiniti software that participants from the intervention group looked at during the main session. Participants matched their breathing with that of the yellow marker which moved up and down along the grey line at a rate of 6 cycles per minute. In the top graph, the blue line represents participants' inhalation and exhalation measured using a respiration belt and the pink line represents increase and decrease in heart rate. The bottom graph illustrates the participants' heart beat taken measured using a BVP sensor. The respiration rate is indicated by the figure in the top right screen.

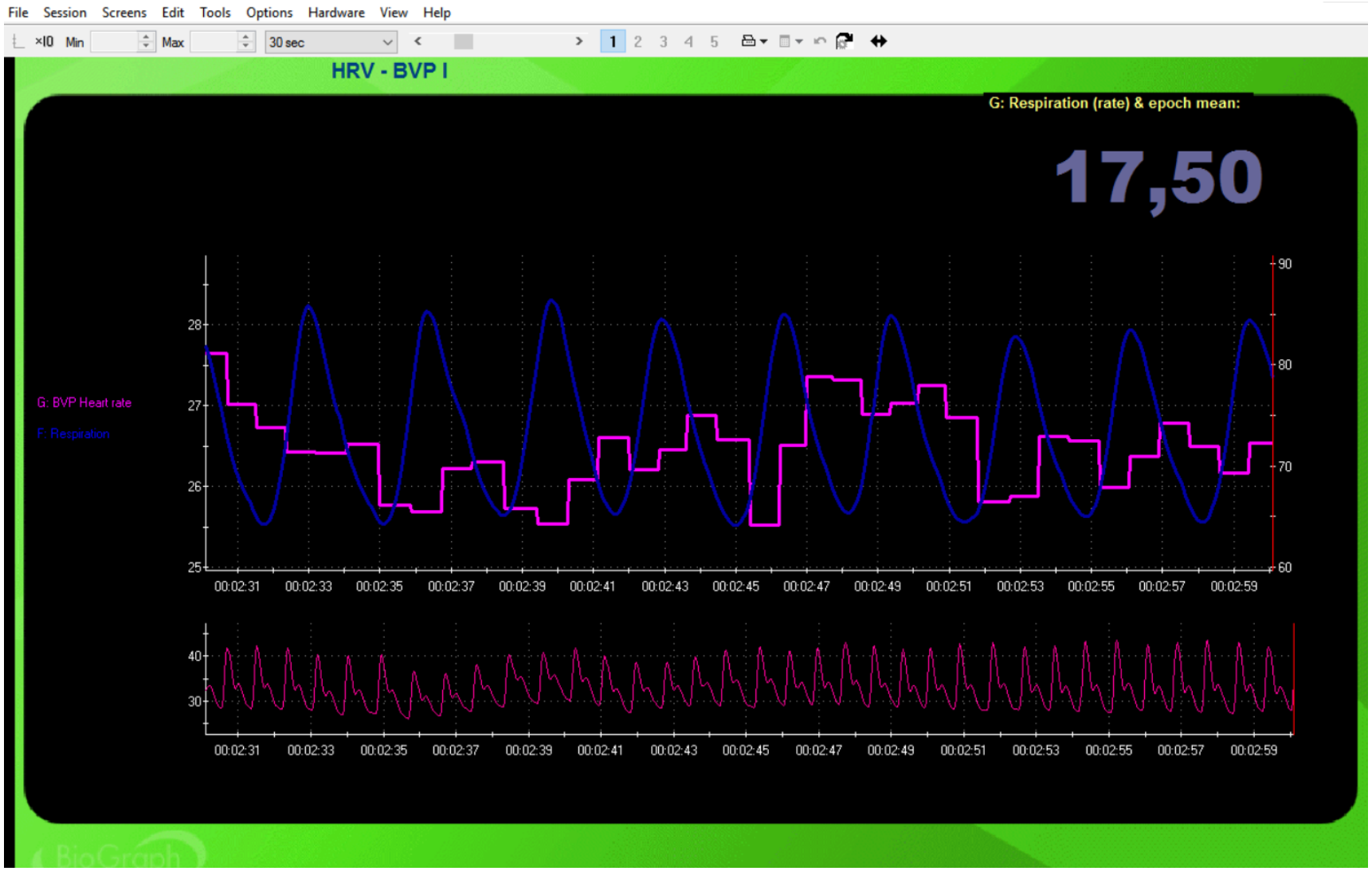

Figure 4.3: Screen on BioGraph Infiniti software that participants from control group looked at during the main session. In the top graph, the blue line represents participants' inhalation and exhalation measured using a respiration belt and the pink line represents increase and decrease in heart rate. The bottom graph illustrates the participants' heart beat measured using a BVP sensor. The respiration rate is indicated by the figure in the top right screen.

4.3.4 Instructions for pursed lipped abdominal breathing

A five-minute training session with verbal instructions, modified from Lehrer et al. [96], was given to the intervention participants during the first session to assist them in following a visual breathing pacer that guided them to breathe at 6 breaths per minute. During this session, a respiration strain gauge belt and BVP sensor were connected to participants.

The instructions provided to the intervention participants were as follows:

- Place one hand on your stomach and the other on your chest,
- Breathe into stomach and feel the hand on the stomach moving outwards during the in breath, and inwards during the out breath. Try to keep the hand on your chest still,
- Let the rate of your in-breath and outbreath be gently guided by synchronising your breathing with the yellow dot on the EZ-Air program.

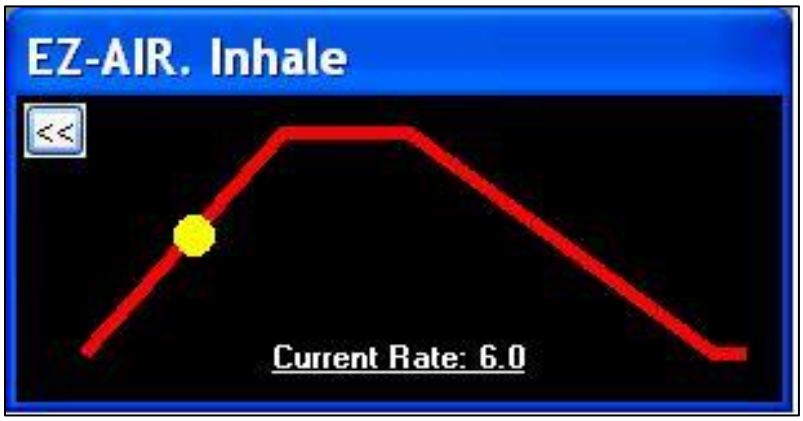

Figure 4.4: EZ-Air breathing pacer.

4.3.5 Questionnaires

Two questionnaires' (Appendix D) were filled out by each participant at the beginning and end of the second session. The first two were the Smith Relaxation States Inventory (SRSI3) to measure various aspects of relaxation, stress, meditation and mindfulness in the participants' general state at that specific point in time. Participants repeated one of the SRSI3 questionnaires at the end of the experiment so that a "before and after" intervention comparison on the effects of the intervention could be made. The second questionnaire was aimed at recording the participants' subjective experiences of the experiment.

The questionnaires were used to assess any major discrepancies in participants' EEG and physiological measurements, and for future research.

4.4 Hardware and sensor placement

EEG and physiological signals were recorded using the 10 channel Flexcomp Infinity made by Thought Technology. Five EEG electrodes (Model: 9305M) were placed at international 10-20 system [175] locations F_{P1}, F_{P2}, F_3, F_4, and C_z with linked ear references. Another four channels were used to record respiration information, inferred from abdominal movement from a respiration strain gauge belt (Model: SA9311M) worn over the abdominal area; heart rate from a BVP sensor (Model: SA9308M) lightly clamped over the middle finger; skin conductance (using a small sensor in a Velcro strap wrapped around the ring and index finger, Model: SA9309M), and skin temperature (using a sensor lightly strapped to the baby finger. Model: SA9310M). All finger sensors were connected to the left hand of each participant. All channels data was recorded at a rate of 2048 samples per second.

4.5 Sensor technical specifications and equipment used

BVP, respiration and skin temperature bandwidth were not specified by the manufacturer, however, the type of sensors used should be more than adequate due to the low frequency waveforms recorded by these sensors.

EEG (Thought Technology T9305M)

Input impedance	10GΩ in parallel with 10pF
Signal input range	0 –200µV
Sensitivity	<0.1µVRMS
CMRR	>130dB
Channel bandwidth	2Hz – 1kHz
Accuracy	±0.3µVRMS, ±5% of reading @10°C to 40°C

BVP (Thought Technology SA9308)

Input range Unit less quantity displayed as 0% – 100%

Accuracy	±5%

Respiration (Thought Technology SA9311M)

Range	30% – 65%

Skin conductance (Thought Technology SA9309M)

Input Impedance	$>10^{12}\Omega$ in parallel with 10pF
Operating Input Bias	~ 1.0 to 2.0 V above sensor ground
Signal Input Range	± 40 mV
Channel Bandwidth	0.05 Hz - 1 kHz
Signal Output Range	± 2.0 V (+ 2.8 V if used with Sensor Isolator)
Input / Output Gain	50
Supply Voltage	7.26 V (±0.05 V)
Current Consumption	< 1.5 mA
Accuracy	±5%

Skin temperature (Thought Technology SA9310M)

Temperature range	10°C - 45°C
Accuracy	±1.0°C between 20°C – 40°C

4.6 Software

All data was initially captured in Thought Technology's BioGraph Infiniti software (version 6.02). The EEG data was exported to EEGLAB (version 13.4.4b) for pre-processing, and then to MATLAB (version R2012a), where the Wavelet Transform was used to decompose the signal into five different frequency bands at all five locations of the EEG signal. Physiology data was first artifacted in the Biograph Infinity software, and then exported into MATLAB where further processing and averaging was implemented.

EEG pre-processing consisted of reducing the sampling rate to 256 Hz, using a FIR filter to low pass filter the signal with an upper edge of 60 Hz, and high pass filter the signal with a lower edge of 1 Hz; visually and manually artifacting "one of a kind" noise activity from the signal, and finally using ICA to further artifact the signal from eye blinks and movement, and temporal muscle activity.

The artifacted and averaged absolute EEG signal was normalized using the natural logarithmic transform ($\ln(x)$) [199-203], as well as finding the normalised relative value ($\ln(x/1-x)$) as per [199-204]. In both cases, *x* is a dimensionless version of the value being transformed.

4.7 Statistical analysis

The data were analysed using IBM SPSS Statistics (version 22), with a significance level of $p < 0.05$ set for the tests. The independent variable was considered to be respiration frequency, whilst the dependant variables were EEG, HRV, skin conductance and skin temperature.

Normality of EEG, HRV, skin conductance and skin temperature was evaluated by assessing skewness and kurtosis. HRV, skin conductance and skin temperature were all determined to be non-normally distributed. Although more normal than the absolute EEG data, the normalised EEG data were still found to be non-normally distributed. As such, non-parametric tests were used to analyse EEG and physiology data. Friedman's analysis of variance for repeated measures [205] was first used to determine significant differences between tests for each group. The Wilcoxon matched pairs test [206] was then used to determine the specific differences. Due to multiple comparisons being conducted on the Wilcoxon matched pairs tests, a Bonferroni correction was applied that resulted in a significance level set at $p < 0.017$ being set. The Mann–Whitney U test [207] was used to determine the differences between groups.

4.8 Summary

In conclusion, this chapter describes the methodology and instrumentation used in collecting and analysing the data for this research. The sampling strategy and procedure followed are discussed. Aspects relating to the hardware used for executing the research, as well as software used for data processing and analysis are also described. The results of the data analysis is presented in the following chapter.

CHAPTER 5: RESULTS

5.1 Introduction and Overview

This chapter presents the results of the data analysis performed following the methodology that was set out for the project.

5.2 Physiology results

Physiological parameters examined include respiration frequency (RF), heart rate, heart rate variability, skin temperature and skin conductance (results shown in Table 5.1).

Table 5.1: Comparison of physiological median, quartile 1 (Q1) and quartile 3 (Q3) results during the B1, Main and B2 sessions for the control (N=8) and intervention group (N=10).

		Baseline 1			Main				Baseline 2			
		Percentiles			Percentiles				Percentiles			
Variable	**Group**	**Median**	**25th**	**75th**	**Median**	**25th**	**75th**		**Median**	**25th**	**75th**	
Respiration	Int	0,23	0,17	0,28	0,12	0,11	0,13	##,***	0,21	0,15	0,25	+,**
frequency (hz)	Con	0,28	0,26	0,30	0,27	0,26	0,28		0,27	0,26	0,29	
Resp - % power	Int	11,64	2,68	38,51	89,10	84,78	92,28	##,***	24,44	7,76	64,00	++,*
in LF band	Con	6,62	5,80	8,61	10,09	6,89	12,09		5,42	3,09	17,07	
Heart rate	Int	67,39	61,20	71,90	69,76	62,92	76,79		69,35	62,41	75,93	
(b/min)	Con	66,57	59,47	74,15	64,10	58,59	73,97		65,00	55,95	76,77	
HRV - LF band	Int	214,33	135,57	473,38	1877,14	1594,87	2348,14	##,***	300,42	164,69	1303,69	++
(ms^2)	Con	112,85	59,49	289,05	223,04	75,34	329,93		147,66	116,55	373,15	
HRV - HF band	Int	113,80	44,54	312,74	180,92	47,61	353,76		173,03	63,96	281,18	
(ms^2)	Con	212,33	88,04	476,70	186,55	127,54	504,61		192,03	83,36	387,99	
HRV - Total	Int	681,09	278,43	1003,79	2262,52	1887,42	2697,04	##,***	623,01	371,65	1597,42	++
power (ms^2)	Con	607,59	229,49	743,80	519,46	354,47	1184,02		426,34	365,52	1052,86	
HRV - LF norm	Int	55,15	42,85	85,92	93,00	85,83	96,25	***	71,17	59,25	77,80	+
(%)	Con	41,92	30,71	56,97	56,73	33,94	66,32		61,36	36,57	69,86	
Skin temp	Int	28,97	24,57	34,00	28,74	26,36	34,45	*	29,44	26,09	34,51	
(deg C)	Con	33,90	30,61	35,37	34,73	32,94	35,49	#	33,79	32,71	35,61	
Skin temp delta	Int				0,30	-0,01	1,00					
(deg C)	Con				0,33	-0,18	0,96					
conductance	Int	0,90	0,23	1,54	0,75	0,41	1,30		0,64	0,43	1,66	
(µS)	Con	0,91	0,72	2,95	0,83	0,62	2,60		1,26	0,70	3,47	+
conductance	Int				-0,10	-0,54	0,04					
delta (µS)	Con				-0,14	-0,43	0,00					

Time effect between B1 and Main (Wilcoxon matched pairs test)

# p<0,017	CON-ST
## p<0,01	INT-RF; INT-RLF%P; INT-HRV-LF; INT-HRV-TP

Time effect between B1 and B2 (Wilcoxon matched pairs test)

^ p<0,017	
^^ p<0,01	

Time effect between Main and B2 (Wilcoxon matched pairs test)

+ p<0,017	INT-RF; INT-HRV-LFNorm; CON-SC
++p<0,01	INT-RLF%P; INT-HRV-LF; INT-HRV-TP

Group effect (Mann–Whitney U test)

* p<0,05	INT B2 RLF%P vs CON B2 RLF%P; INT Main ST vs. CON Main ST
** p<0,01	INT B2 resp vs. CON B2 resp;
***p<0,001	INT Main resp vs. CON Main resp; INT Main RespLF%P vs CON Main RespLF%P; INT Main LF-HRV vs CON Main LF-HRV; INT Main HRV-TP vs CON Main HRV-TP; INT Main HRV-LFN vs CON Main HRV-LFN

RF significantly decreased in the intervention group from B1 to Main ($z = -2.8$, $p<0.01$), and then significantly increased from main to B2 ($z = -2.49$, $p<0.017$), as breathing was altered by matching breathing to the breathing pacer (Figure 5.1). No significant differences were found in RF between the three sessions for the control group. In comparing the control and intervention group, RF was significantly lower ($z = -3.67$, $p<0.001$) during Main and during B2 ($z = -2.94$, $p<0.01$) for the intervention group. No other significant differences were found for RF between the control and intervention group (Table 5.1).

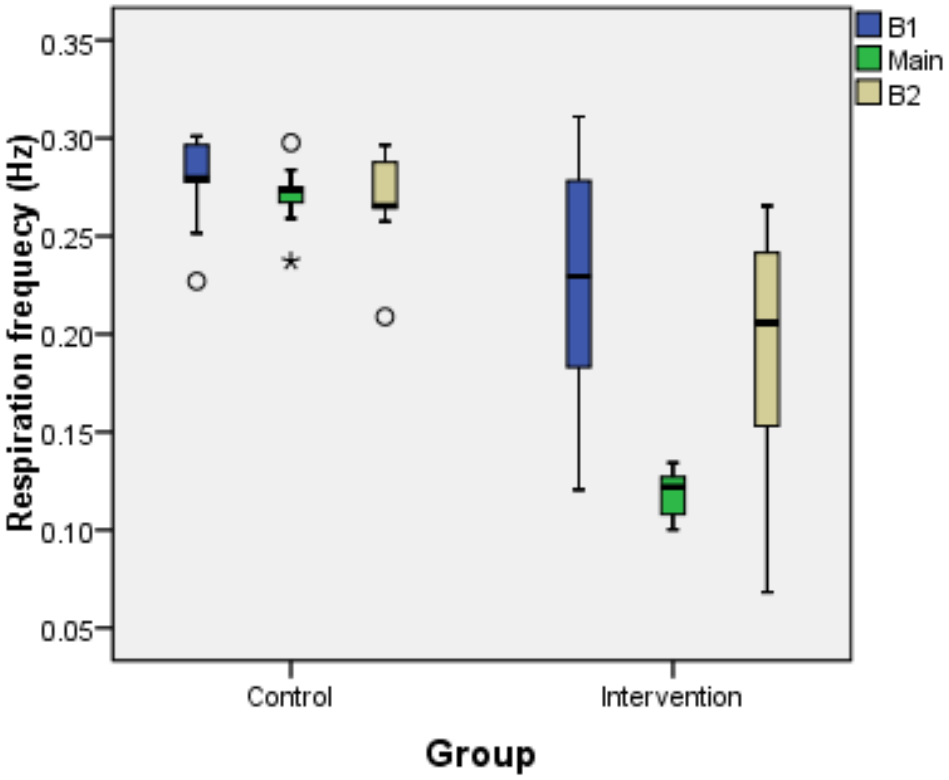

Figure 5.1: Box and whisker diagram showing distribution of mean respiration frequencies (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

The respiration percentage power in the LF band increased significantly (z= -2.8, p<0.01) from B1 to Main, and then significantly decreased from Main to B2 (z= -2.7, p<0.01) for the intervention group. There was a significant increase of the intervention group during both the Main (z= -3.67, p<0.001), and B2 (z= -2.04, p<0.05) sessions when compared to the control group.

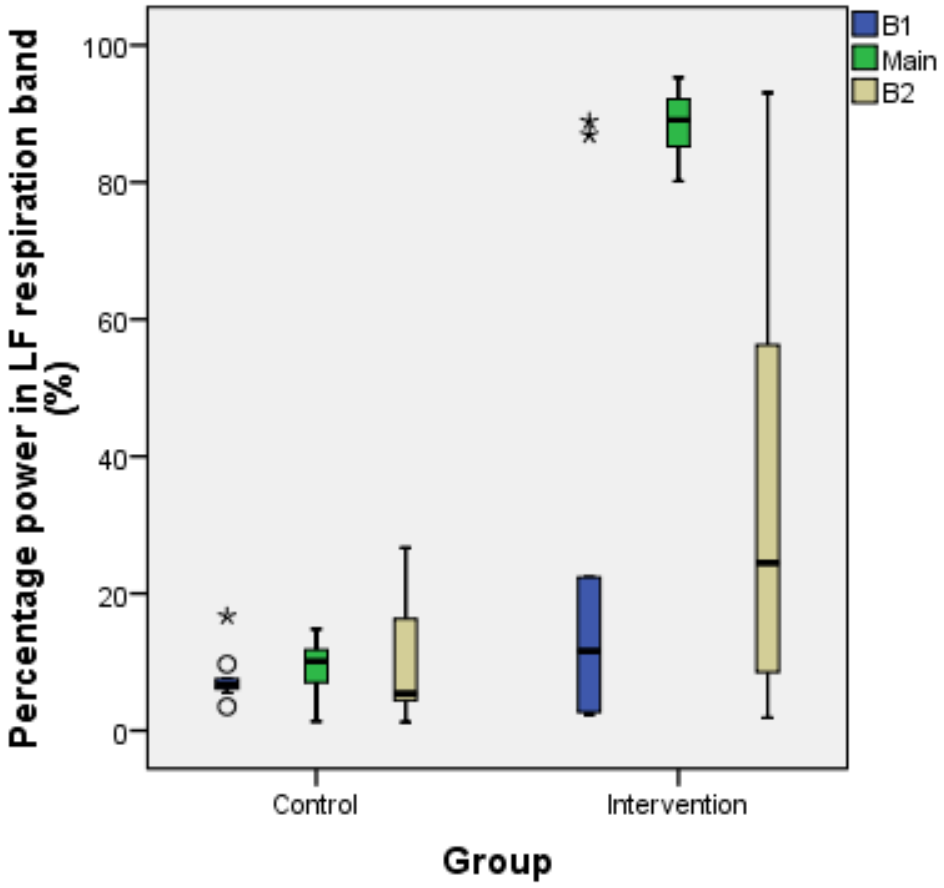

Figure 5.2: Box and whisker diagram showing distribution of mean respiration LF percentage power (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

LF HRV power significantly increased (z= -2.8, p<0.01) from B1 to Main and then significantly decreased from Main to B2 (z= -2.8, p<0.01) for the intervention group (Figure 5.3). No significant differences were found in LF HRV power between the three sessions for the control group. There was a significant increase (z= -3.59, p<0.001) for the intervention group when compared to the control group during the Main session. Furthermore, there were no significant differences for either group for HF HRV (Table 5.1).

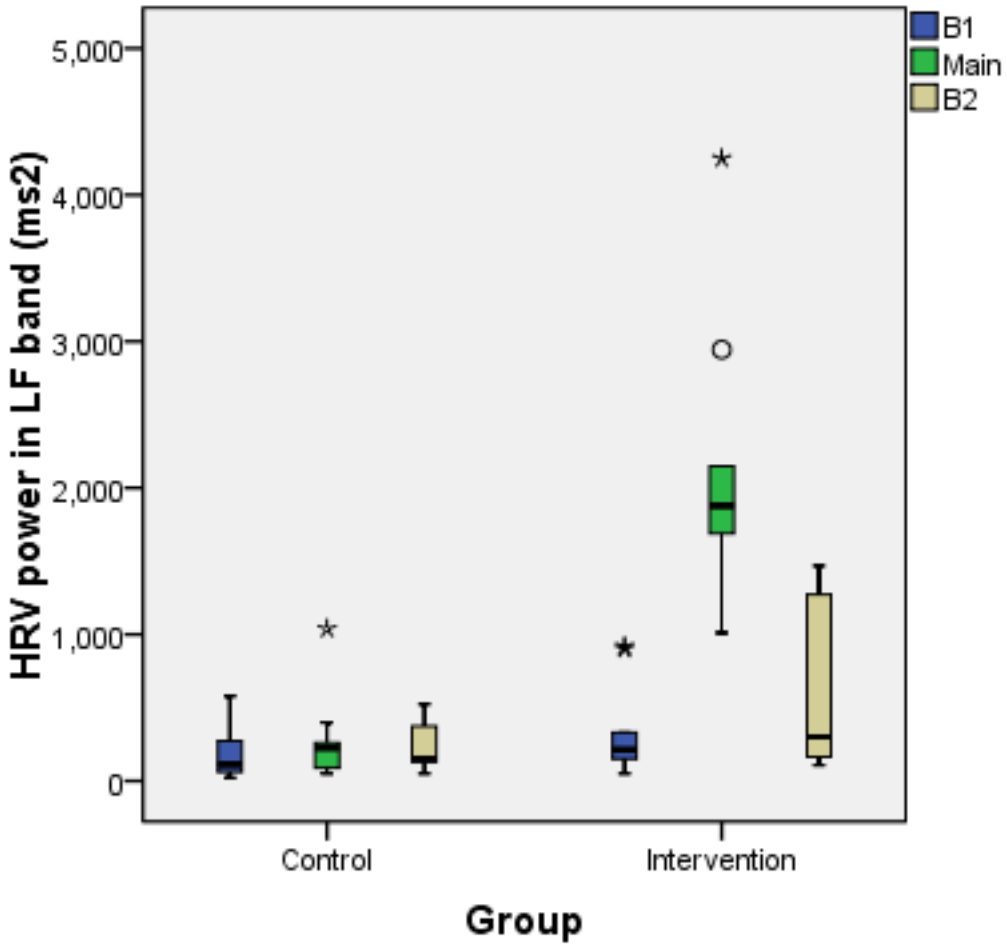

Figure 5.3: Box and whisker diagram showing distribution of mean heart rate variability power in the LF band (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

HRV total power increased significantly (z= -2.8, p<0.01) from B1 to Main, and then decreased significantly (z= -2.8, p<0.01) from Main to B2 for the intervention group, showing no carry over effect (Figure 5.4). No significant differences were found in HRV total power between the three sessions for the control group. There was a significant increase (z= -2.94, p<0.001) in total power of the intervention group when compared to the control group during the Main session (Table 5.1).

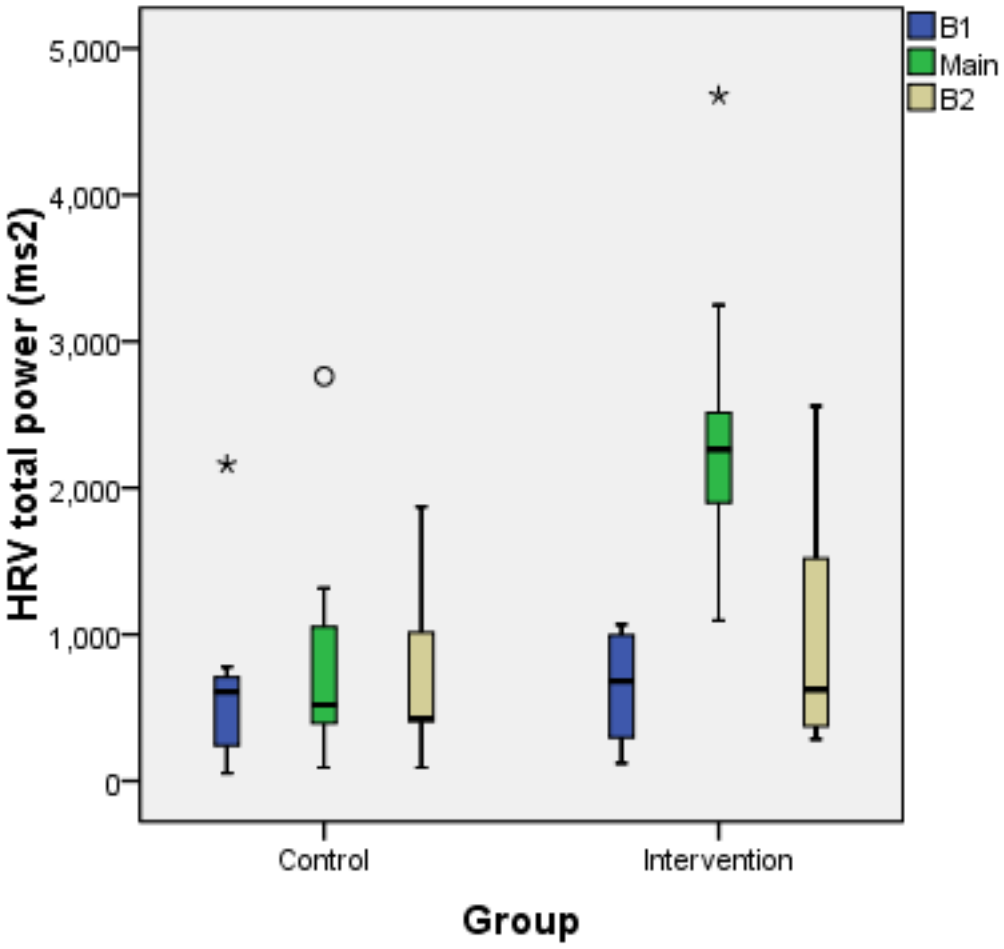

Figure 5.4: Box and whisker diagram showing distribution of mean heart rate variability total power (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

HRV LF norm for the intervention group (Figure 5.5 and Table 5.1) was significantly higher (z= -3.4, p<0.001,) in comparison to the control group during the main session.

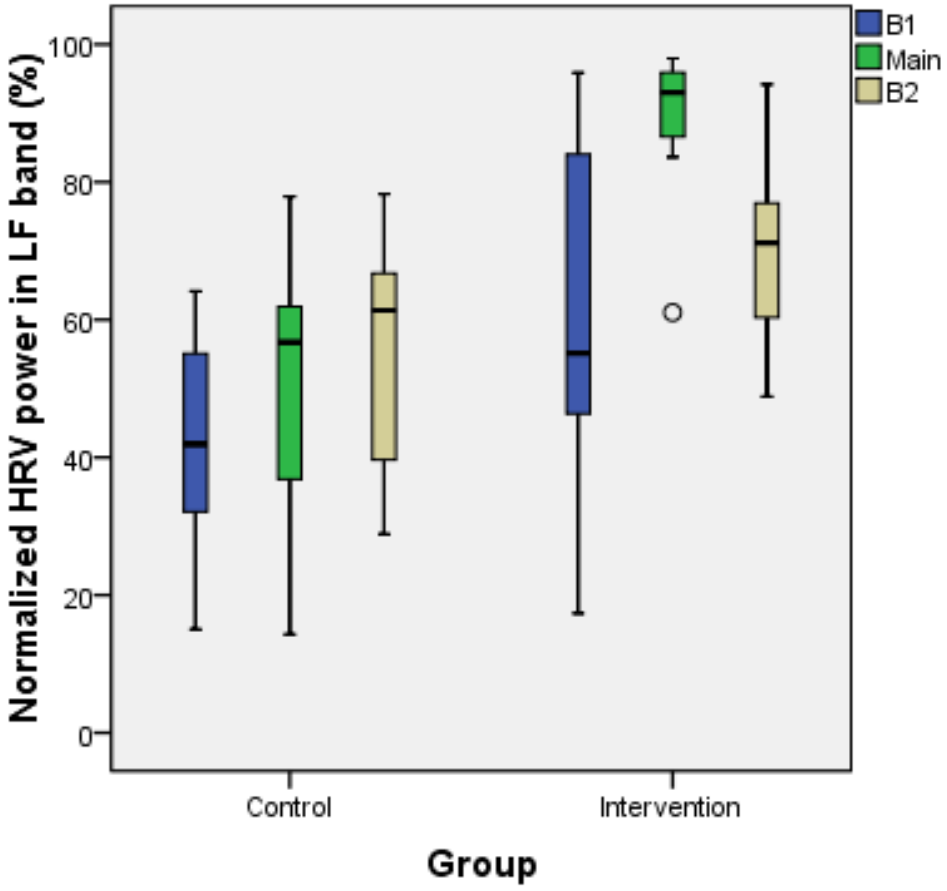

Figure 5.5: Box and whisker diagram showing distribution of mean normalised low frequency heart rate variability power (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

Skin temperature significantly increased (z= -2.42, p<0.017) for the control group from B1 to Main (Figure 5.6 and Table 5.1). There were no significant skin temperature changes between sessions for the intervention group. The skin temperature of the control group was significantly higher (z= -2.04, p<0.05) when compared to the intervention group during the Main session.

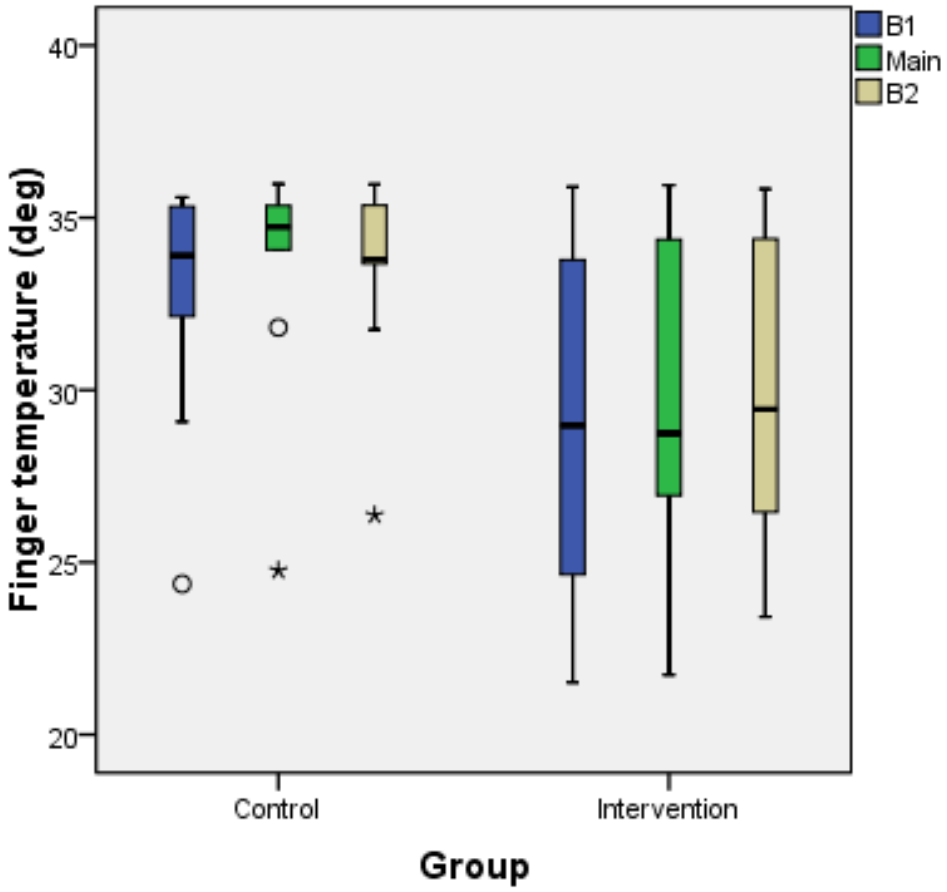

Figure 5.6: Box and whisker diagram showing distribution of mean finger skin temperature (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

Skin temperature delta was defined as the change in the average skin temperature of the first 1.5 minutes from the average skin temperature of the last 1.5 minutes during the Main session. No significant differences in skin temperature delta was found between groups (Table 5.1).

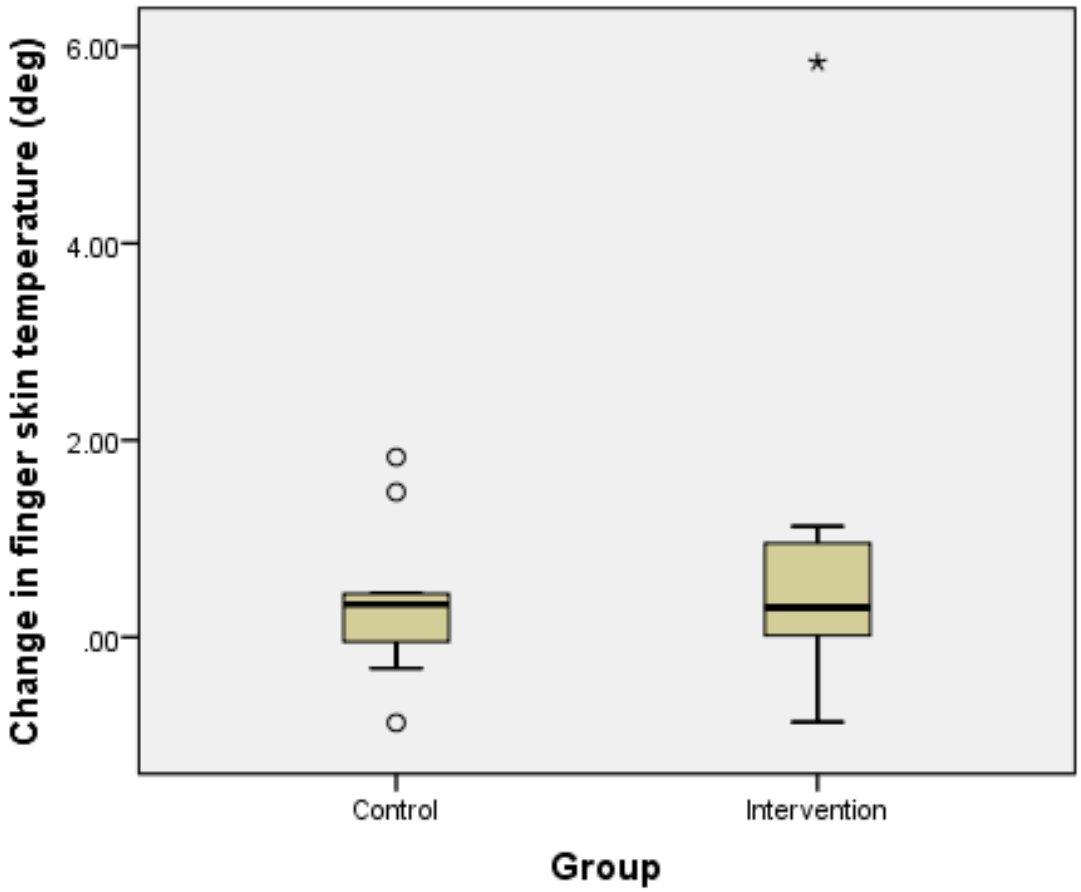

Figure 5.7: Box and whisker diagram showing distribution of mean change in finger skin temperature (per participant) between the first and last 1.5 minutes of the main session for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

Skin conductance increased significantly (z= -1.71, p<0.017) from Main to B2 for the control group. No significant changes were found between sessions for the intervention group and between groups (Table 5.1).

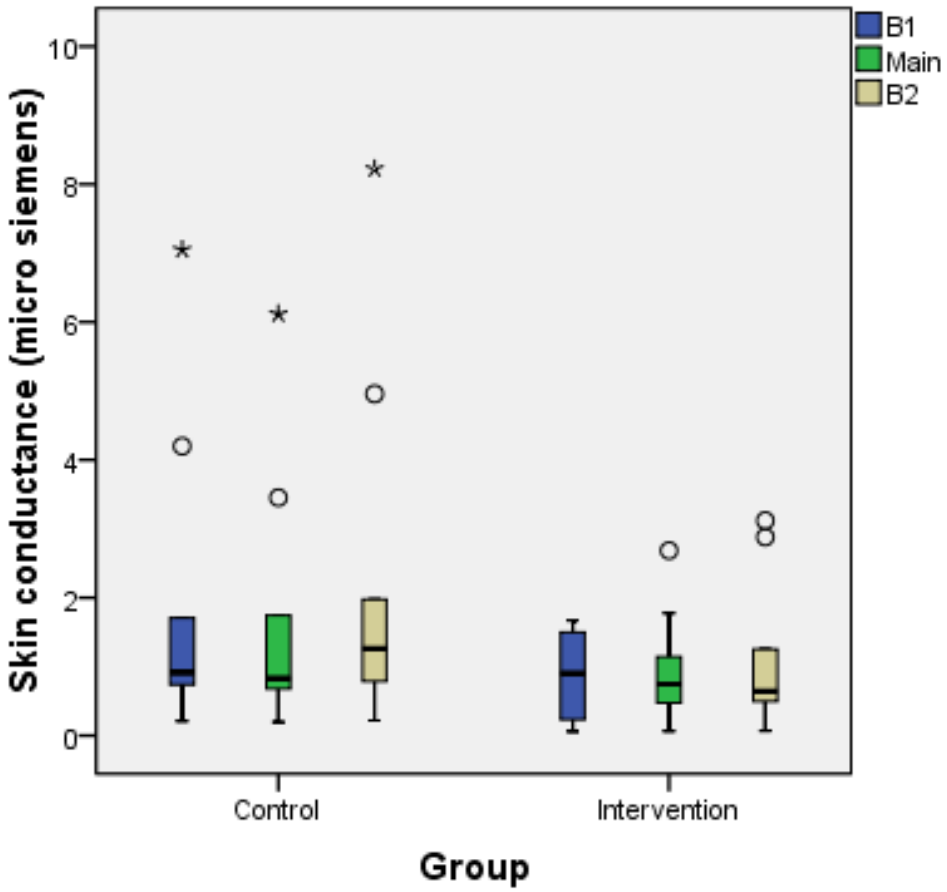

Figure 5.8: Box and whisker diagram showing distribution of mean skin conductance (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

Skin conductance delta was defined as the change from the average skin conductance of the first 1.5 minutes to the average skin conductance of the last 1.5 minutes during the Main session. There were no significant differences in skin temperature delta between the sessions for the control and intervention group, as well as between groups.

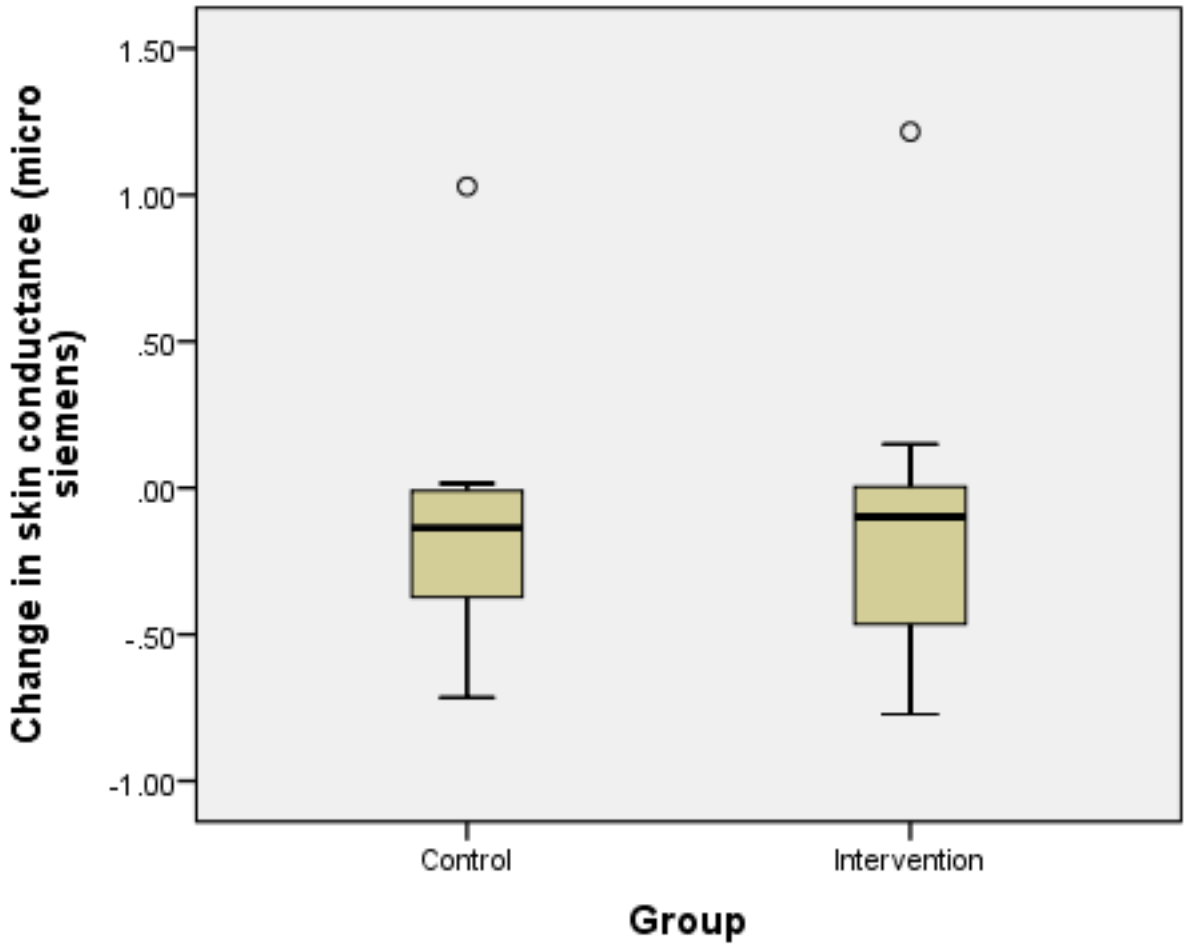

Figure 5.9: Box and whisker diagram showing distribution of mean change in skin conductance (per participant) between the first and last 1.5 minutes of the main session for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

5.3 EEG results – natural logarithm

EEG bands examined include theta, alpha and beta (results shown in Table 5.2). Two methods of normalising data exist: natural logarithm and relative, and it was decided to evaluate the EEG results using both methods. This section shows those EEG results using natural logarithm. Ten participants were used in the intervention group and nine participants were used in the control group; however, one participant's EEG recording in the control group data was unusable due to noise.

Table 5.2: EEG median, quartile 1 (Q1) and quartile 3 (Q3) results (normalised using natural log) during the B1, Main and B2 sessions for the control (N=8) and intervention group (N=10).

			Baseline 1			Main			Baseline 2			
			Percentiles			Percentiles			Percentiles			
	Site	Group	Median	25th	75th	Median	25th	75th	Median	25th	75th	
Theta Power (4-8Hz)	Cz	Int	1,115	0,998	1,222	1,134	0,961	1,234	1,044	0,924	1,188	
	Cz	Con	1,051	0,868	1,093	1,018	0,862	1,044	0,950	0,753	0,986	+
	F3	Int	0,945	0,872	1,143	0,934	0,824	1,186	0,939	0,815	1,109	
	F3	Con	0,880	0,839	1,079	0,838	0,785	0,976	0,837	0,627	0,920	
	F4	Int	0,909	0,725	1,010	0,928	0,691	1,037	0,906	0,735	1,012	
	F4	Con	0,821	0,675	0,945	0,793	0,656	0,886	0,714	0,527	0,855	+
Alpha Power (8-16Hz)	Cz	Int	1,246	1,127	1,382	1,265	1,067	1,353	1,249	1,123	1,495	
	Cz	Con	1,066	0,989	1,189	1,043	0,963	1,123	0,993	0,816	1,091	
	F3	Int	1,126	0,977	1,291	1,137	0,963	1,317	1,175	0,999	1,379	
	F3	Con	1,014	0,931	1,087	0,899	0,822	1,096	0,856	0,739	1,086	
	F4	Int	1,019	0,866	1,212	1,083	0,892	1,242	1,126	0,911	1,266	
	F4	Con	0,877	0,826	1,035	0,837	0,682	1,076	0,797	0,600	1,021	
Beta Power (16-32Hz)	Cz	Int	1,314	1,021	1,555	1,351	1,014	1,523	1,410	1,022	1,663	
	Cz	Con	1,011	0,829	1,193	0,980	0,755	1,176	0,948	0,709	1,130	^
	F3	Int	1,095	0,876	1,466	1,130	0,960	1,431	1,123	0,984	1,581	
	F3	Con	0,898	0,754	1,192	0,878	0,663	1,140	0,890	0,609	1,169	
	F4	Int	1,016	0,817	1,358	1,093	0,839	1,346	1,072	0,908	1,502	
	F4	Con	0,802	0,671	1,112	0,822	0,571	1,085	0,875	0,488	1,100	

Time effect between B1 and Main (Wilcoxon matched pairs test)

# p<0,017 None

p<0,01 None

Time effect between B1 and B2 (Wilcoxon matched pairs test)

^ p<0,017 Con-Beta_Cz

^^ p<0,01 None

Time effect between Main and B2 (Wilcoxon matched pairs test)

+ p<0,017 Con-Theta_Cz; Con-Theta_F4

++p<0,01 None

Group effect (Mann–Whitney U test)

* p<0,05 None

** p<0,01 None

In terms of theta power, significant differences were only found at Cz and F4 locations (Table 5.2). Theta power at Cz decreased significantly from Main to B2 (z= -2.521, p<0.017) for the control group (Figure 5.10) only. Theta power at F4 decreased significantly from the Main to B2 (z= -2.521, p<0.017) for the control group (Figure 5.11), with no other significant results being found between sessions and between groups.

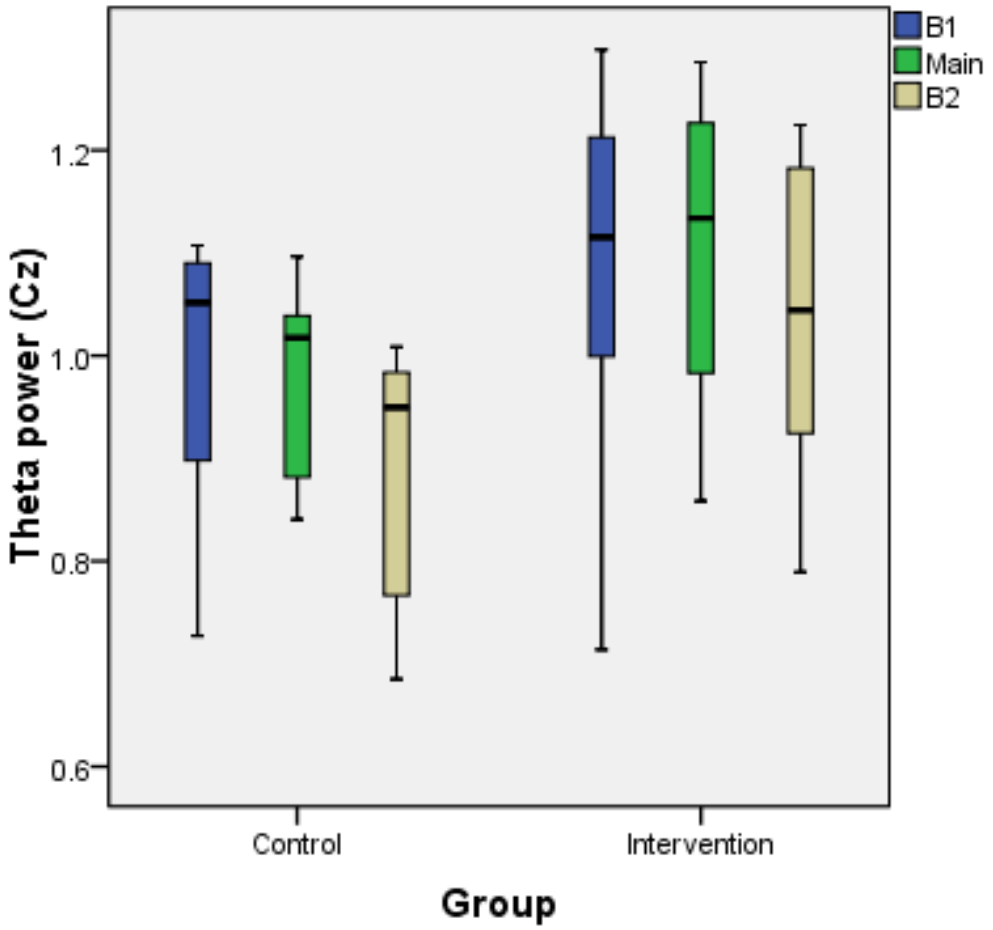

Figure 5.10: Box and whisker diagram showing distribution mean theta power at Cz (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

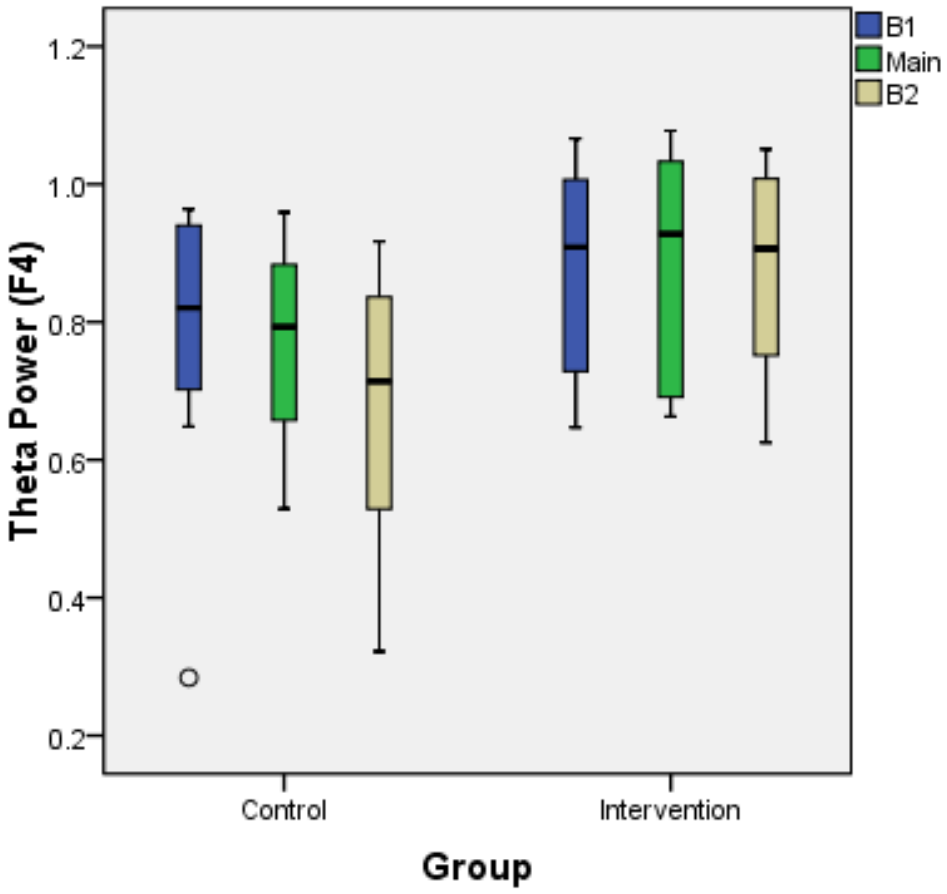

Figure 5.11: Box and whisker diagram showing distribution of mean theta power at F4 (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

No significant results were found between sessions and between groups for Alpha power (Table 5.2).

In terms of beta power, significant differences were found only at the Cz location (Table 5.2), where beta power decreased significantly from B1 to B2 at Cz (z= -1.54, p<0.017) for the control group (see Figure 15.12).

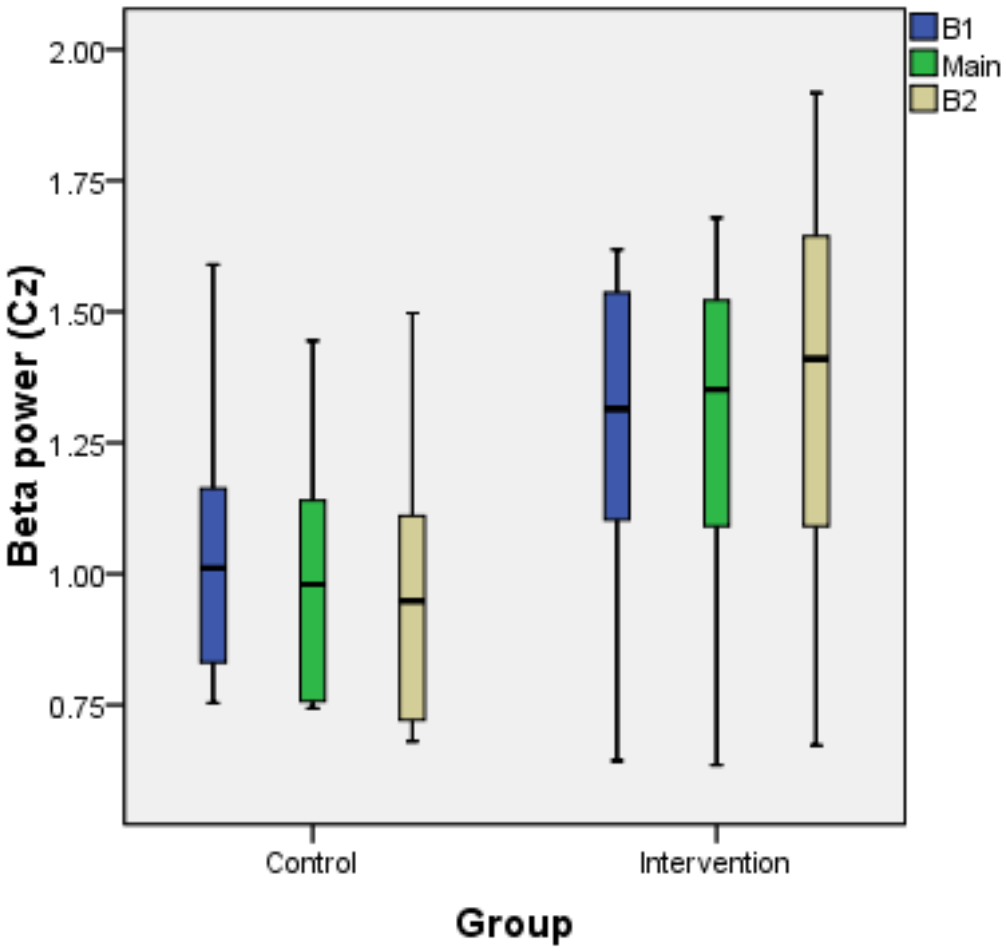

Figure 5.12: Box and whisker diagram showing distribution of mean beta power at Cz (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

5.4 EEG results – Relative

This section shows those EEG results using the relative method. Ten participants were used in the intervention group and nine participants were used in the control group; however, one participant's EEG recording in the control group data was unusable due to noise.

Table 5.3: EEG median, quartile 1 (Q1) and quartile 3 (Q3) results (normalised using relative method) during the B1, Main and B2 sessions for the control (N=8) and intervention group (N=10).

			Baseline 1			Main			Baseline 2			
			Percentiles			Percentiles			Percentiles			
	Site	Group	Median	25th	75th	Median	25th	75th	Median	25th	75th	
Theta Power (4-8Hz)	Cz	Int	0,189	0,173	0,207	0,184	0,172	0,218	0,181	0,149	0,202	
	Cz	Con	0,193	0,156	0,204	0,192	0,171	0,210	0,191	0,160	0,199	
	F3	Int	0,181	0,170	0,204	0,180	0,162	0,206	0,174	0,148	0,200	
	F3	Con	0,180	0,142	0,202	0,181	0,156	0,197	0,172	0,142	0,197	
	F4	Int	0,180	0,167	0,203	0,181	0,158	0,207	0,178	0,144	0,196	+
	F4	Con	0,180	0,137	0,195	0,180	0,150	0,198	0,168	0,143	0,189	+
Alpha Power (8-16Hz)	Cz	Int	0,216	0,196	0,233	0,217	0,193	0,227	0,216	0,205	0,222	
	Cz	Con	0,204	0,191	0,227	0,205	0,188	0,226	0,202	0,192	0,226	
	F3	Int	0,211	0,195	0,227	0,214	0,193	0,227	0,208	0,195	0,221	
	F3	Con	0,195	0,165	0,224	0,193	0,179	0,219	0,195	0,169	0,211	
	F4	Int	0,211	0,194	0,224	0,212	0,194	0,227	0,204	0,198	0,219	
	F4	Con	0,193	0,170	0,220	0,191	0,180	0,213	0,190	0,173	0,217	
Beta Power (16-32Hz)	Cz	Int	0,230	0,186	0,248	0,225	0,186	0,254	0,246	0,190	0,264	
	Cz	Con	0,196	0,168	0,203	0,191	0,180	0,201	0,191	0,185	0,214	
	F3	Int	0,204	0,183	0,248	0,211	0,185	0,234	0,221	0,185	0,250	
	F3	Con	0,187	0,157	0,194	0,183	0,169	0,193	0,183	0,170	0,212	
	F4	Int	0,208	0,183	0,248	0,213	0,187	0,228	0,223	0,183	0,241	
	F4	Con	0,183	0,160	0,203	0,178	0,172	0,199	0,182	0,173	0,215	

Time effect between B1 and Main (Wilcoxon matched pairs test)

# p<0,017 None

p<0,01 None

Time effect between B1 and B2 (Wilcoxon matched pairs test)

^ p<0,017 None

^^ p<0,01 None

Time effect between Main and B2 (Wilcoxon matched pairs test)

+ p<0,017 Int-Th_F4; Con-Th_F4;

++p<0,01 None

Group effect (Mann–Whitney U test)

* p<0,05 None

** p<0,01 None

In terms of theta power, a significant difference was found only at the F4 locations (Table 5.3), where relative theta power at F4 decreased significantly from the Main to B2 ($z= -2.38$, $p<0.017$) for the control group and deceased significantly ($z= -2.38$, $p<0.017$) intervention group (Figure 5.13). Furthermore, no other significant results being found between sessions and between groups at other locations (Table 5.3).

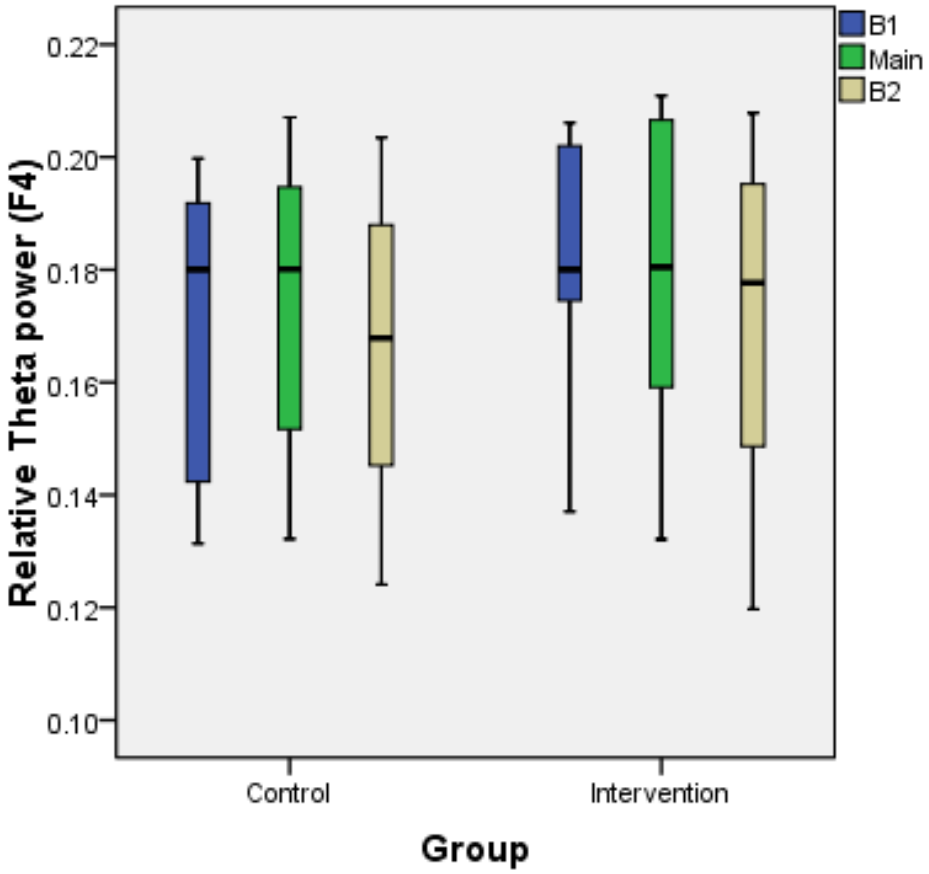

Figure 5.13: Box and whisker diagram showing distribution of mean relative theta power at F4 (per participant) during the B1, Main and B2 sessions for both control and intervention groups. Mild and extreme outliers are represented by the O and * symbols, respectively.

5.5 Summary

Chapter 5 presents the results following the methodology discussed in the previous chapters. Important findings and analyses have been highlighted and are discussed in the following chapter. Chapter 6 provides a detailed discussion based on the findings of the results presented in this chapter. The chapter then revisits the hypotheses tested to reach the objectives of the study, as well as highlights some key limitations of this research project and where scope for future research should be directed.

CHAPTER 6: DISCUSSION

6.1 Introduction

This chapter presents a discussion on the results presented in Chapter 5. Firstly, the impacts of respiration on HRV, skin conductance, skin temperature and EEG, and the implications thereof are discussed. Thereafter, the hypotheses are revisited based on the results. Finally, a discussion is provided on the limitations of this research and where future research should be focused.

6.2 Respiration and HRV

As expected, the intervention group was able to follow the breathing pacer and alter breathing frequency to around 0.1Hz (6 breathes per minute) during the main session. Even though the intervention groups breathing frequency increased from main to B2, it stayed below B1's frequency, and was significantly lower than the control group's B2 result. This indicates that slow paced breathing has an extended impact on the participants' breathing patterns.

Some researchers [77-79] suggest that LF HRV power represents sympathetic activity only, while others [69], [208] a combination of sympathetic and vagal influences, making the LF/HF ratio a representation of the sympo/vagal balance [29]. Furthermore, it is well know that respiration (known as RSA) has a modulating impact on HRV [1], [2] and influences LF HRV during slow paced breathing, as they are in the same frequency band [59]. Therefore, LF HRV does not accurately reflect sympathetic or vagal activity for this research and will not be evaluated further.

Vagal activity [29] and parasympathetic activity [77] [73] [75] are considered as the main contributors towards the HF component of HRV, with an increase indicating a more relaxed state, and a decrease indicating a more stressful state [209] [210]. There was no

significant difference in either group for HF HRV indicating that one session of slow paced breathing was not enough to alter HF HRV such that it becomes a marker for relaxation.

Low basal HRV is linked with hypertension [32], depressive symptoms [33], anxiety symptoms [211], [34] and disorder [35], panic disorder [36] and PTSD [37], while higher basal HRV has been found after successful treatment for depression using psychotherapy and anti-depressants [38], [39] and overall physical health [89]. HRV total power increased significantly during the main session for the intervention group only, indicating that slow paced breathing has a positive impact on HRV. Basal power did not increase from B1 to B2 which was unexpected, and would have shown a carry-over effect from slow paced breathing. It may take more breathing sessions to show a carry-over effect.

6.3 Respiration and physiology

Skin temperature has been known to increase with an increase in relaxation and decrease with an increase with stress as blood moves from the body's extremities towards major organs [17-20]. Peculiarly, the control group's skin temperature increased from B1 to main whereas the intervention group did not, suggesting that the control group relaxed more than the intervention group. A possible reason might be that the first session of slow paced breathing for the intervention group actually took concentration and induced some level of stress [66], [96], [98] and it may take more sessions for the benefits of slow paced breathing to show in participants' physiology.

Skin conductance is another method shown to reflect stress [14-16] and arousal [12], [13]. As stress increases so does moisture on the hands, and with it an increase in skin conductance. The control group's average skin conductance increased significantly from main to B2, indicating an increase in stress during B2. A possible reason might be participants' agitation or boredom sitting in the chair for the time period and desire to finish the session. It is interesting to note that the increase in stress from Main to B2 was only seen in the control group. It is possible that the breathing exercise for the intervention group allowed them to be more relaxed.

Skin temperature and skin conductance delta reflects the average of the first 1.5 minutes compared to the average of the last 1.5 minutes and indicates an increase or decrease of either skin conductance or skin temperature during the main session. There were no significant changes for either group.

6.4 Respiration and EEG

There were no significant differences between groups for alpha, theta and beta power during B1, M or B2. However, during B2, both groups saw a decrease in theta power, while beta power tended to decrease for the control group only.

The decrease in theta was similar to other research on focused attention abdominal breathing [151], [153], however, there were also differences in the results of this study to those of previous research efforts [127], [158]. No meaningful conclusions can be drawn from the decrease in theta power as similar results were seen for both the control and intervention groups.

The Cz EEG probe lies above the primary motor cortex and primary somatosensory cortex, with a decrease in beta power from B1 to B2 suggesting that the control group became less physically alert over time.

The above small differences in EEG results suggest two possibilities. Either slow paced breathing does not impact EEG power, or that one session of slow paced breathing may not be enough to cause a significant shift in EEG results to properly evaluate whether slow paced breathing impacts EEG power.

6.5 Hypothesis revisited

This section revisits the hypotheses that were tested in this study in terms of whether or not the null hypothesis can be accepted or rejected. A summary of the main reasons for

accepting/ rejecting each null hypothesis is also listed. It should, however, be noted that these hypotheses are valid for a single session of slow paced breathing only, and would need to be tested against multiple sessions.

H1: Slow paced breathing initiates resonance in the baroreflex system, and therefore increases heart rate variability. **(Null hypothesis can be rejected)**

- HRV total power increased during the main session for the intervention group but not for the control group.
- HRV total power was significantly higher for the intervention group compared to the control group during the main session.

H2: Slow paced breathing increases relaxation and therefore increases efferent vagal flow represented by increases high frequency HRV. **(The null hypothesis failed to be rejected)**

- No significant differences were found for either group for HRV-HF during any session.
- There was no correlation between respiration frequency and HRV-HF.

H3: Slow paced breathing increases relaxation, which results in less peripheral blood vessel constriction and higher peripheral blood flow, and therefore increases finger temperature. **(The null hypothesis failed to be rejected)**

- No significant difference was found in skin temperature delta for either group during the main session.
- Strangely, the control group (and not the intervention group's) skin temperature increased during the main session, signifying an increase in relaxation.

H4: Slow paced breathing increases relaxation, which diminishes the sweating response, and therefore decreases skin conductance. **(The null hypothesis failed to be rejected)**

- No significant difference for skin conductance delta for either group during the main session.

- There was no significant difference for skin conductance for the intervention group, however skin conductance increased significantly for the control group during B2, signifying an increase in anxiety during B2.

H5: Slow paced breathing increases relaxation and therefore theta and alpha power increase, while beta power decreases. **(The null hypothesis failed to be rejected)**

- For both groups, theta power tended to decrease during B2, while beta power tended to decrease during B2 for the control group only.

6.6 Research limitations and scope for future research

An important limitation to this research is that the impact of only one paced breathing session was investigated. It is possible that EEG results may change significantly if slow paced breathing is practised over multiple sessions and/or a longer period of time per session. It is thus suggested that future research efforts should take this into consideration in order to test the above hypotheses of paced breathing techniques on EEG and physiology.

Another constraint to this study was the small sample size through a limited number of participants. This implies that there may be a greater level of uncertainty in the results described in this study making it difficult to distinguish between a real effect and random variation, and hence caution must be applied when interpreting the results. The results in this study should therefore be considered as correlational, and not causal. It is thus recommended that future research efforts are to focus on a larger sample size in order to strengthen the statistical significance of the project.

There was a high degree of contradiction in the results obtained in this study that may be a function of the individual emotional states of participants as well as the small sample size. The participants in this research project were students with varying degrees of stress in their lives. The majority of these students probably have relatively lower levels of stress, in comparison to people in a working environment with greater amounts of responsibility.

The impact of slow paced breathing would be easier to quantify on participants with high stress levels rather than participants that are already relaxed. Alternatively, it would be interesting to compare groups of stressed and relaxed people.

In addition to the above, the design of the current research was cross-sectional, which means that the data were gathered at one specific point in time only. No pre- and post-event testing was used, and nor were longitudinal processes evaluated. Cross-sectional studies have an inherent temporal limitation, and in the case of this research, where it is possible that the participants emotional and stress states may fluctuate over time or in response to external life circumstances, this limitation should be kept in mind.

Women were not included in this study as women have different EEG [197] and cortisol [198] responses to stress, and therefore also have different physical responses to breathing at different rates. The results of this study are thus invalid for woman and it is recommended that the study be repeated for women and compared to the results of men in order to verify previous research [197], [198].

Based on the experience acquired during this project, the following list provides further research direction possibilities:

- Investigating the impact of more than one biofeedback session and/or longer experimental sessions on EEG and physiology;
- As only slow paced breathing was investigated in this study, it would be interesting to compare the impacts of different types of breathing on EEG and physiology;
- Finding and using a participants individual resonant breathing frequency,
- The use of more EEG probes (and Loreta for determine source localization), MRI or PET to determine the location of the brain that is activated by slow paced breathing;
- Investigation into the impact of breathing and emotional response on BVP amplitude and shape [170]; and
- Investigate the influence of slow paced breathing on different stress levels and in different environments.

6.7 Conclusion

This project aims to investigate HRV, skin conductance, skin temperature and EEG patterns in response to breathing techniques. Given that stress impacts people's health and performance negatively, this research demonstrated that slow paced breathing strongly increases HRV. Therefore, it is plausible that with more frequent practise, basal HRV would increase, and with it benefits such as a reduction in anxiety and increase in performance. Slow paced breathing biofeedback shows promise of a simple, cheap, measurable and effective method to reduce the impact of stress on physiology.

An interesting idea that came from this research was to evaluate the rhythm and speed of the stomach moving in and out during respiration. This was done by finding the percentage power in the low frequency band which indicates initial promise as a good indicator for HRV. This could be used as an alternative to ECG and BVP with advantages of being robust against movement artifact, more robust for people with cold hands (and therefore lack of blood flow and poor signal quality), cheap, simple and inconspicuous to wear.

Even though some of the main advantages of WT (such as 3D representation of signals, superior computational speed and the ability to better detect small changes in EEG [193] than the FFT) were not used in this project, the WT still proved an effective method to evaluate EEG signals.

6.8 Summary

Chapter 6 has presented a discussion and conclusion on the results that are shown in Chapter 5. In summary, slow paced breathing does have an impact on HRV but a limited impact on physiology as indicated by no significant changes in skin conductance and temperature. In terms of the effects of paced breathing on EEG, no meaningful significant conclusions can be drawn and thus future research possibilities are also discussed to address the limitations of this research project.

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Appendix A: Ethics approval letter

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor, Tel +27 (0)11-717-1252
Medical School Secretariat: Medical School Room 10M07, 10th Floor, Tel +27 (0)11-717-2700
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18 November 2013

Mr Brett Klette
School of Electrical and Information Engineering
University of Johannesburg
Sent by email to: bklette@uj.ac.za

Dear Mr Klette

Protocol no: M130611
Protocol Title: Investigation of Electroencephalography and Heart Rate Variability during Spontaneous verses Paced Breathing using Signal Processing Techniques
Principal Investigator: Mr Brett Klette
Protocol Amendment

The letter serves to confirm that the HREC (Medical) chairman has approved the following amendments made on the abovementioned protocol as detailed in your letter dated 22 October 2013:

- Male students will be recruited from the Psychology and Engineering departments from the University of Johannesburg and University of Witwatersrand

Thank you for keeping us informed and updated.

Yours Sincerely,

Ms Zanele Ndlovu
Administrative Officer
Human Research Ethics Committee (Medical)

Appendix B: Questionnaires

Participant questionnaire PARTICIPANT NUMBER: _____

INSTRUCTIONS 1: PLEASE ANSWER THE FOLLOWING QUESTIONS BY CROSSING (x) THE RELEVANT BLOCK OR WRITING DOWN YOUR ANSWER IN THE SPACE PROVIDED.

1 Gender

Male	
Female	

2 Age (in complete years)

INSTRUCTIONS 2: TO WHAT EXTENT DO YOU AGREE WITH EACH OF THE FOLLOWING STATEMENTS? PLEASE INDICATE YOUR ANSWER USING THE FOLLOWING 5-POINT SCALE. PLEASE ANSWER THE FOLLOWING QUESTIONS BY CROSSING (x) THE RELEVANT BLOCK OR WRITING DOWN YOUR ANSWER IN THE SPACE PROVIDED.

1. = never / less than once per month
2. = twice a month
3. = once per week
4. = three times per week
5. = every day

1 I take part in exercise

1	2	3	4	5

Type of exercise: _____

2 I take part in breathing exercises

1	2	3	4	5

Type of breathing exercises: _____

3 I take part in meditation practises?

1	2	3	4	5

Type of meditation: _____

INSTRUCTIONS 3: PLEASE PLACE A VERTICAL MARK ON THE LINE BELOW TO INDICATE YOUR EXPERIENCE:

1 During the first three minute baseline recording, I felt…

Awake Sleepy



2 During the twelve minute main recording, I felt…

Awake Sleepy



3 During the last three minute baseline recording, I felt…

Awake Sleepy



4 During the first three minute baseline recording, my mental activity was…

Quiet Busy



5 During the twelve minute main recording, my mental activity was …

Quiet Busy



6 During the last three minute baseline recording, my mental activity was …

Quiet Busy



INSTRUCTIONS 4: GENERAL FEEDBACK

Do you have any other comments about your experience eg. Feelings of dizziness / alertness / boredom etc?

_____

_____

_____

_____

Do you have any other feedback / comments about this experiment?

_____

_____

_____

_____

SR313

HOW DO YOU FEEL RIGHT NOW? PLEASE CHECK ALL THE ITEMS USING THIS KEY.

RIGHT NOW, I FEEL THIS

①	②	③	④	⑤	⑥
Not at All	A Little	. . Moderately . .		A Lot	Maximum

①	②	③	④	⑤	⑥	1. My mind is SILENT and calm (I am not thinking about anything).
①	②	③	④	⑤	⑥	2. My muscles feel TIGHT and TENSE (clenched fist or jaws; furrowed brow).
①	②	③	④	⑤	⑥	3. I feel AT PEACE.
①	②	③	④	⑤	⑥	4. I feel DROWSY and SLEEPY.
①	②	③	④	⑤	⑥	5. Things seem AMAZING, AWESOME, and EXTRAORDINARY.
①	②	③	④	⑤	⑥	6. Right now I recognize the wisdom of sometimes ACCEPTING things as they are.
①	②	③	④	⑤	⑥	7. My muscles are SO RELAXED that they feel LIMP.
①	②	③	④	⑤	⑥	8. I am HAPPY.
①	②	③	④	⑤	⑥	9. I am WORRYING
①	②	③	④	⑤	⑥	10. I feel AT EASE.
①	②	③	④	⑤	⑥	11. I feel DISTANT and FAR AWAY from my cares and concerns.
①	②	③	④	⑤	⑥	12. I feel ENERGIZED, CONFIDENT, and STRENGTHENED.
①	②	③	④	⑤	⑥	13. I am DOZING OFF or NAPPING.
①	②	③	④	⑤	⑥	14. I feel THANKFUL.
①	②	③	④	⑤	⑥	15. I feel like I am living fully and SIMPLY in the PRESENT, not distracted by past or future concerns.
①	②	③	④	⑤	⑥	16. Things seem TIMELESS, BOUNDLESS, or INFINITE
①	②	③	④	⑤	⑥	17. I feel IRRITATED or ANGRY.
①	②	③	④	⑤	⑥	18. I feel JOYFUL.
①	②	③	④	⑤	⑥	19. I feel SAD, DEPRESSED, or BLUE.
①	②	③	④	⑤	⑥	20. I feel AWARE, FOCUSED, and CLEAR.
①	②	③	④	⑤	⑥	21. My hands, arms, or legs are SO RELAXED that they feel WARM and HEAVY.
①	②	③	④	⑤	⑥	22. I feel INNOCENT and CHILDLIKE.
①	②	③	④	⑤	⑥	23. My BREATHING is NERVOUS and UNEVEN (Or shallow and hurried).
①	②	③	④	⑤	⑥	24. I feel LOVING.
①	②	③	④	⑤	⑥	25. Things seem FRESH and NEW, as if I am seeing them for the first time.
①	②	③	④	⑤	⑥	26. I feel INDIFFERENT and DETACHED from my cares and concerns.
①	②	③	④	⑤	⑥	27. I feel PRAYERFUL or REVERENT.
①	②	③	④	⑤	⑥	28. I feel PHYSICAL DISCOMFORT or PAIN (backaches, headaches, fatigue)
①	②	③	④	⑤	⑥	29. My mind is QUIET and STILL.
①	②	③	④	⑤	⑥	30. I feel ANXIOUS.
①	②	③	④	⑤	⑥	31. I sense the DEEP MYSTERY of things beyond my understanding.
①	②	③	④	⑤	⑥	32. I feel RESTED and REFRESHED
①	②	③	④	⑤	⑥	33. I feel CAREFREE.
①	②	③	④	⑤	⑥	34. TROUBLESOME THOUGHTS are going through my mind.
①	②	③	④	⑤	⑥	35. My body is PHYSICALLY RELAXED.
①	②	③	④	⑤	⑥	36. Presently I feel there's no need to try to change things that simply can't be changed.
①	②	③	④	⑤	⑥	37. I feel fully focused and ABSORBED in what I am doing
①	②	③	④	⑤	⑥	38. I feel OPTIMISTIC, HOPEFUL, or TRUSTING that I can rely on someone or something.

Your age: _____ Gender: ☐ M ☐ F

• 2003, Jonathan C. Smith, Ph.D

SRSI 3d

Below are feelings people sometimes have.

HOW OFTEN HAVE YOU FELT THIS WAY?

1. My mind was SILENT and calm (I wasn't thinking about anything).

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

2. My muscles felt TIGHT and TENSE (clenched fists or jaws; furrowed brow).

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

3. I felt AT PEACE.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

4. I felt DROWSY and SLEEPY before taking a nap or settling for sleep.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

5. Things seemed AMAZING, AWESOME, and EXTRAORDINARY.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

6. I recognized the wisdom of sometimes ACCEPTING things as they are.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

7. My muscles were SO RELAXED that they felt LIMP.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

8. I was HAPPY.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

9. I WORRIED

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

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10. I felt AT BASE.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

11. I felt DISTANT and FAR AWAY from my cares and concerns.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

12. I felt ENERGIZED, CONFIDENT, and STRENGTHENED.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

13. I DOZED OFF or took a NAP when I wanted to.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

14. I had feelings of THANKFULNESS.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

15. I felt like I was living fully and SIMPLY in the PRESENT, not distracted by past or future concerns.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

16. Things seemed TIMELESS, BOUNDLESS, or INFINITE

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

17. I felt IRRITATED or ANGRY.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

18. I felt JOYFUL.

☐ A LOT, more than ONCE A DAY
☐ ONCE A DAY
☐ About once EVERY OTHER DAY

☐ About ONCE A WEEK
☐ About ONCE A MONTH (less than once a week)
☐ RARELY or NEVER, less than once a month

PLEASE CONTINUE ON OTHER SIDE!

18. I felt SAD, DEPRESSED, or BLUE.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

19. I felt AWARE, FOCUSED, and CLEAR.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

20. My hands, arms, or legs were SO RELAXED that they felt WARM and HEAVY.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

21. I felt INNOCENT and CHILDLIKE.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

22. My BREATHING was NERVOUS and UNEVEN (Or shallow and hurried).

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

23. I felt LOVING.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

24. Things seemed FRESH and NEW, like seeing them for the first time.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

25. I felt INDIFFERENT and DETACHED from my cares and concerns.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

26. I felt PRAYERFUL or REVERENT.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

27. I felt PHYSICAL DISCOMFORT or PAIN (backaches, headaches, fatigue)

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

28. My mind was QUIET and STILL.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

29. I felt ANXIOUS.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

30. I sensed the DEEP MYSTERY of things beyond my understanding.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

31. I felt RESTED and REFRESHED

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

32. I felt CAREFREE.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

33. TROUBLESOME THOUGHTS were going through my mind.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

34. My body was PHYSICALLY RELAXED.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

35. I felt there's no need to try to change things that simply can't be changed.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

36. I felt fully focused and ABSORBED in what I was doing.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month

37. I felt OPTIMISTIC, HOPEFUL, or TRUSTING that I could rely on someone or something.

- ☐ A LOT, more than ONCE A DAY
- ☐ ONCE A DAY
- ☐ About once EVERY OTHER DAY
- ☐ About ONCE A WEEK
- ☐ About ONCE A MONTH (less than once a week)
- ☐ RARELY or NEVER, less than once a month