Investigating the mechanisms of neural-visceral coupling for therapeutic applications

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Abstract

Anxiety and mood disorders are commonly characterised by emotion regulation difficulties. Research suggests meditation practice improves emotion reactivity and well-being by modifying attention regulation. Most meditation practices involve directing attention towards internal sensations, such as the breath. It is thought that attending to internal signals re-calibrates executive function processes, such as how attention is allocated and the number of cognitive resources required to process such information. Studies have shown meditation modifies neural activity in brain regions associated with executive function and enhances relaxed states, indicated by an increase in autonomic vagal tone. However, despite meditation originating several thousand years ago, the interaction between these neural and physiological mechanisms and how they relate to therapeutic effects is yet to be understood. This thesis examines the mechanisms of neural-visceral integration during breath-focused meditation (Su-soku) to facilitate psychopathological knowledge and the development of future therapeutic interventions.

To investigate the effects of meditation, 80 healthy participants, across two data sets, engaged in either Su-soku meditation or observed a relaxing woodland scene. Based on previous studies, neural and autonomic activity was recorded using electroencephalography (EEG) and electrocardiography

(ECG), respectively. Studies in this thesis primarily focus on whether there are significant differences in cerebral and autonomic activity between the Su-soku meditation and woodland scene conditions and the relationship between these measures. A secondary aim was to investigate the therapeutic effects of these interventions. As a result, cognitive function and mood were examined using a cognitive flanker task and mood state questionnaire before and after assigned intervention conditions.

Frontal-Midline (FM) oscillatory power in theta, alpha and beta frequencies were significantly modulated from baseline and across Su-soku blocks, which suggests breath-focused meditation induces an altered attentive state. Furthermore, meditation moderately enhanced the vagal tone compared to the relaxing woodland scene. This thesis also demonstrates for the first time that sustained attention towards the breath, via Su-soku meditation, promotes a tendency for FM-oscillatory power to peak during exhalation. According to previous literature, autonomic phases, such as the respiratory and cardiac cycles, have been associated with changes in attention regulation. As a result, finding a relationship between neural oscillations and the respiratory cycle may reflect neural modulation, via cardio-respiratory synchrony mechanisms, to facilitate the processing of internal signals. Exploratory work suggested synchronised cardiac-respiratory signals, referred to as resonance breathing, may enhance the effects of meditation, such as the neural processing of cardiac signals and improving mood.

While further research is warranted, this thesis provides novel insights into the role of neural-visceral interactions during breath-focused meditation and highlights potential future therapeutic applications of such work.

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Contents

List of Tables

List of Figures

Abbreviations

5HT Serotonin

ACC Anterior Cingulate Cortex
Ach Acetylcholine
AI Anterior Insular
ANOVA Analysis Of Variance
AR Autoregressive modelling

BF Basal Forebrain
BOLD Blood-Oxygen-Level-Dependent Imaging

CAN Central Autonomic Network
CNS Central Nervous System
CSI Cardiac Sympathetic Index
CVI Cardiac Vagal Index

DAN Dorsal Attention Network
DMN Default Mode Network
dmPFC Dorsal-medial Prefrontal Cortex

ECG Electrocardiogram
EEG Electroencephalogram
EMG Electromyography
EOG Electrooculography
ERP Event Related Potentials

FFT Fast Fourier Transform

FM Frontal-Midline

fMRI Functional Magnetic Resonance Imaging

GABA γ-Aminobutyric Acid

HEPS Heartbeat Evoked Potentials

HR Heart Rate

HRV Heart Rate Variability

IBI Inter-Beat Intervals

ICA Independent Compontent Analysis

IIR Infinite impulse response

LC Locus Coeruleus

MMN Mismatch Negativity

MPFC Medial Prefrontal Cortex

MRI Magnetic Resonance Imaging

NE Norepinephrine

NTS Nucleus Tractus Solitarius

OB Olfactory bulb

PAG The Periaqueductal Gray

PB Paced breathing

PET Positron Emission Tomography

PFC Prefrontal cortex

PGi Nucleus Paragigantocellularis

pNN50 Percentage difference between RR intervals

PSA Power spectral amplitude

PTSD Post-traumatic stress disorder

rCBF Regional Cerebral Blood Flow
RMSSD Root mean square of successive differences
RSA Respiratory Sinus Arrhythmia

SCR Skin Conductance Response
SDRR Standard deviation in time between heartbeats
SN Salience Network
SWR Sharp-Wave ripples

tVNS Transcutaneous vagus nerve stimulation

vlPFC Ventro-lateral Prefrontal Cortex
vmPFC Ventro-medial Prefrontal Cortex

Chapter 1

Introduction

This thesis investigates the interaction of neural-visceral signals to advance etiological knowledge and therapeutic interventions for mental health.

In Chapter 1, I introduce several core concepts that provide the theoretical framework for the thesis. I begin by providing a short contextual overview, discussing the brain and behaviour in physicalism and predictive modelling (Section 1.1). Following this, the Bayesian predictive coding model is introduced, and its relation to mental illness is explored (Section 1.2). I then outline the importance of central-autonomic interactions for cognition and describe a core theory for this thesis, The Neural-Visceral Integration model (Section 1.3). These previous sections are then furthered by discussing the applications and limitations of these models in the context of meditation therapies (Section 1.4). This is followed by an outline for the direction of the thesis and a COVID-19 adjustments statement (Sections 1.5 and 1.6).

1.1 Overview

Subjective experiences are considered an emergent property from the sum of complex information integration via interactions between myriads of elements within the human system (Tononi, 2004). The transient nature of neural events enables the system to continuously associate and dissociate local and distinct neural network clusters. This behaviour can be expressed as cyclical variations in electrical potential across frequency spectra and between brain regions. Continuous synchronisation of oscillations across the brain formulates a coherent picture of an experience, referred to as neural meta-stability (Fingelkurts & Fingelkurts, 2017). This is proposed to occur through adjusting internal model predictions via properties underpinning the coordination of neuronal nodes (Barrett, Quigley, & Hamilton, 2016; Chanes & Barrett, 2016; Clark, 2013; Feldman & Friston, 2010). Dynamic fluctuating communication within this multi-matrix likely changes how one perceives and experiences life.

However, the malleability of neural networks creates the feasibility of inaccurate propagation within the system (Ainley, Apps, Fotopoulou, & Tsakiris, 2016; Tsakiris & Critchley, 2016). Chronic disturbances to the integration of neural signals likely influence the connectivity profile throughout the brain by altering transient brain states. Consequently, this may lead to the emergence of mental illness (Farb et al., 2015; Sterzer et al., 2018).

Meditative practices are commonly advocated for enhancing mental health and are becoming increasingly global with the advent of meditation technologies, such as mobile phone applications and smart watches (Choi & Ishii, 2020; Gál, Ștefan, & Cristea, 2021; Rubia, 2009; Tang, Hölzel, & Posner, 2015). Meditation practices often involve individuals directing their attention towards internal or external states while maintaining a stable posture and breathing pattern. Studies suggest that meditation can alter neural activity and subsequently their response to internal and external stimuli (Cahn & Polich, 2006; Tang et al., 2009).

Several theories suggest that the perception of bodily states and changes in the body may influence neural connections and, consequently, how one processes and regulates emotion (Pezzulo, 2014; Seth, Suzuki, & Critchley, 2012). Given this bidirectional relationship, there is a possibility that the therapeutic outcomes of meditation could be associated with these effects (Friston, 2012; Seth, 2014). Smith, Badcock, and Friston (2021) furthers this proposition by discussing the clinical applications of predictive processing models. They highlight several insights that associate visceral dysregulation with bodily perception precision estimates, thus, emphasising the model's promising therapeutic potential.

1.2 Theories of the mind

1.2.1 Predictive coding

To process and interpret chaotic existence, the brain must be adaptive in its processing and able to construct internal generative models of the world that provide flexibility for estimating future encounters or experiences. Thus, it is likely that how one comes to experience the world is formulated by the building blocks of ever-going change, or as stated by Friston (2009) “Perception is an inevitable consequence of active exchange with the environment” (p. 293).

Since the basic idea proposed by Helmholtz (1860), that the brain tries to infer causes of sensations, there has been a greater emphasis on hierarchical Bayesian inference (Friston, 2009). Predictive coding implies that a coherent picture of the world is built from estimations and begins with hierarchical top-down communication of ‘prior’ predictions of incoming sensory input. These priors are continuously updated by bottom-up prediction errors, which are the difference between top-down predictions and bottom-up sensory input (Feldman & Friston, 2010). By minimising the sum differences between predictions and encountered sensory sensations, the energy equilibrium of the system can be preserved and thus resist disorder (Friston, 2010).

Predictive learning occurs when the internal model becomes updated. The extent of learning depends on the process of active inference; this is where the prior expectation is selected based on the connection weight of each prior. Weighting is determined by the certainty or precision of attributed evidence (Chekroud, 2015; Hernández-Lobato, Es, Miguel Hernández-Lobato, Shah, & Adams, 2016; Robinson, Overstreet, Charney, Vytal, & Grillon, 2013). For instance, if hypothesis A is true, p(B—A) is the probability of sensory data B given A. In the usual formulation of Bayes' theorem, p (A—B) is the probability that A given B is true, and p(B—A) is the probability that B given A is true. Bayes' theorem gives the formula for calculating p(A—B) given p(B—A), p(A) and p(B). An individual's beliefs based on past experiences or knowledge are reflected in the precision value denoted in this model. This implies that evidence attributed to beliefs varies over time, signifying a vulnerability to modulation and potential plasticity of networks (Barrett & Simmons, 2015; Friston, 2012). A change in the biasing of predictions likely results in an altered internal model and subsequently an altered perception of the world (Clark, 2013; Sterzer et al., 2018).

Active inference argues prediction errors are minimised by updating beliefs or actively changing the environment for sensory inputs to conform to expectations. Interoceptive self-inference theories argue visceral rhythms are closely aligned with expected precision due to the close coupling with neural gain. In turn, adjusting neural gain across the cortex assists in regulating neural populations by enhancing the signal-to-noise of task-specific regions. Allen, Varga, and Heck (2022) proposes these interactions are likely core to performance improvements found in goal-orientated cognition and target detection tasks. Allen also suggests that, unlike other forms of visceral signals, respiratory active inference enables an individual to directly control visceral dynamics and thereby actively partake in optimising sensory precision by adjusting when and how they breathe. Such a proposition highlights the possibility that neural dynamics can be adjusted accordingly via respiratory rhythms.

1.2.2 Neural dynamics

Communication in the brain is encompassed by a chaotic "buzz" of unfolding spatio-temporal dynamics, locally and across the brain. Prediction errors are fed forward by high-frequency oscillations developed in local circuits of pyramidal cells and interneurons, whilst predictions are passed down, with a low-frequency signal originating from deeper pyramidal cells in infragranular layers (Bastos et al., 2012; Chanes & Barrett, 2016; Friston, Bastos, Pinotsis, & Litvak, 2015). Bastos et al. (2012) presented evidence that neural connections in deep layers send backpropagating signals to lower areas, suggesting that low-frequency signals could be seen as communicating top-down "predictions". They also claim that fast frequencies in superficial layers communicate prediction errors (see Figure 1.1).

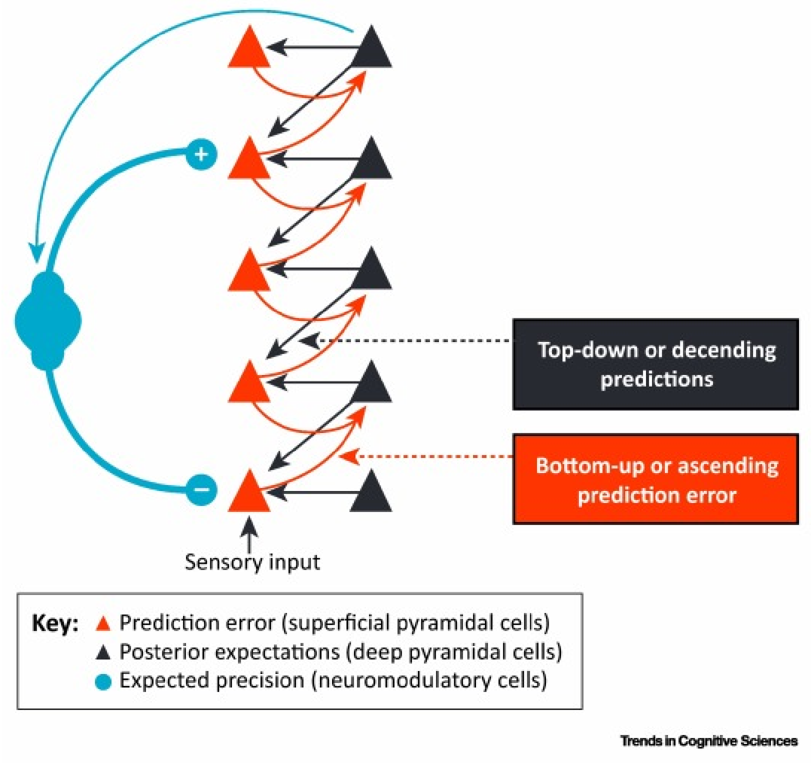


Figure 1.1: Visualising a simple cortical hierarchy with ascending prediction errors and descending predictions (Badcock et al., 2017).

Such runways are proposed to be reflected in Electroencephalogram (EEG) signals, whereby oscillatory activity is generated from synchronous post-synaptic potentials from groups of neurones. The frequency of this signal dictates the recruitment of global or local brain regions (Chanes & Barrett, 2016). High-frequency bands, such as "gamma" (35 Hz upwards), "beta" (13-35Hz) and "alpha" (8-13Hz), are associated with recruiting local regions, whereas the opposite is true for lower bands like "theta" (4-8Hz) and "delta" (1-4Hz).

Precision weighting could be equated to the synaptic strength of neuronal connections, which are modulated by monoaminergic and cholinergic neurotransmitters, such as Serotonin (5HT), Norepinephrine (NE) and Acetylcholine (Ach), respectively. For instance, cholinergic inputs to the medial prefrontal neurones have been found to increase the firing rate of neurones in this area during sustained attention tasks (Gillespie, Brzozowski, & Mitchell, 2018). This synchronous firing and magnitude of individual current dipoles influence the amplitude of EEG oscillations (Nunez, 2006; Whittingstall & Logothetis, 2009). Excitatory neurotransmitters, such as Ach, upregulate activity, whereas inhibitory neurotransmitters downregulate. The combination of amplitude and frequency patterns directly corresponds with the arousal state. Low-voltage fast activity is associated with alertness and is referred to as cortical desynchronization, whereas the opposite is true for drowsiness, which is referred to as synchronization. Neural activity fluctuates between these states, and these changes are often distinguished as a tonic or phasic activity. Short-term events occurring during a particular behavioural state, such as alpha spindles during drowsiness, are called phasic events. In contrast, this activity outside of context, such as at baseline, is referred to as tonic cortical activity. Consequently, this tonic-phasic balance likely underpins how one performs in a task.

Based on Hebbian plasticity, new synapses can be formed, and existing connections can be strengthened by simultaneous activation of neurones (Liggan & Kay, 1999). These consistent, systematic patterns are referred to as meta-stable states (Allefeld, Atmanspacher, & Wackermann, 2009). These states may indicate how one's predictions construct their world and consequently respond to their environment. Supporting this, Fukushima, Betzel, He, Heuvel, and Zuo (2017) found that increasing similarity in node edge weights between white matter fibres and functional connectivity leads to an increase in direct structural connections within active modules. They also mention this phenomenon was prominent in neural networks pertinent to task performance.

1.2.3 Brain networks

Neural oscillations allow for a vast amount of information to be propagated across the brain and form cortical networks based on oscillatory patterns occurring at specific locations in the brain (Sauseng & Klimesch, 2008). These oscillatory patterns enable an organism to adapt and regulate behaviour according to their ever-changing environment. The ability to situationally adapt has been labelled as executive function, which describes a group of cognitive abilities that enable an individual to re-orient behaviour and thoughts (Baddeley, 1998). Attention is one of the cognitive processes, along with working memory, response inhibition and decision-making, thought to comprise executive function. Attention can be defined as a cognitive state focusing on a selection of available information. For an individual to perform a task or respond to stimuli, the allocation of both attention and cognitive effort is required. Several networks have been associated with attention and cognitive processes, namely, the Default Mode Network (DMN), Dorsal Attention Network (DAN) and Salience Network (SN).

The DMN has been associated with mind-wandering and self-referential processing and consists of the following main regions: posterior cingulate cortex and medial prefrontal cortex (Fox, Spreng, Ellamil, Andrews-Hanna, & Christoff, 2015). Neural activity, as indicated by blood oxygen level-dependent signals (BOLD), appears to be greater in these regions during experimental “resting” periods or “baseline” in comparison to goal-directed behaviour (Raichle et al., 2001). It is also activated during internally directed cognitions, such as recalling memories or imagination (Murphy et al., 2018). Moreover, the inability to suppress the DMN during executive function tasks has been associated with psychiatric disorders defined by cognitive impairment, such as major depressive disorder (Bartova et al., 2015). Some research suggests meditative practice can improve the connectivity of the DMN, which in turn could strengthen the ability to regulate ruminating thoughts that could contribute to the development of mood disorders (Jang et al., 2011).

In contrast, the DAN tends to be engaged during goal-oriented attention and consists of distributed brain regions, including the intra-parietal sulcus, frontal eye field and bilateral middle temporal gyrus (Corbetta, Patel, & Shulman, 2008). DMN and DAN are thought to interact competitively, whereby activation of the DAN leads to the DMN becoming less active during externally oriented tasks (Clare, Uddin, Biswal, Castellanos, & Milham, 2008; Di & Biswal, 2014; Fox et al., 2005). It is thought that the anti-correlational nature of these networks helps provide an optimal allocation of mental resources and, consequently, task performance (Owens et al., 2020). Studies have also found meditation to enhance connectivity within the DAN and between the DMN and DAN, which may suggest meditation improves the ability to switch between networks and consequently regulate mental resources (Zhang et al., 2021).

This process of switching between the DMN and DAN is thought to be coordinated by the SN (Menon & Uddin, 2010; Seeley, 2019), which includes the anterior cingulate, insula and ventral anterior insular cortices. The SN plays a crucial role in the bottom-up detection of internal and external salient events. In particular, the insula serves as an integral hub for salience detection and top-down regulation (Menon & Uddin, 2010). These functions are critical for processes, such as emotion regulation and cognitive control, as they require an individual to reappraise their current state in response to the environment (Bressler & Menon, 2010; Seeley et al., 2007).

Emotion regulation relates to how we try to influence our emotions when we have them and how we experience and express them (Gross, 2014). Emotions can affect cognitive processes, either positively or negatively, depending on the situation and desired outcome. Therefore, regulating emotions, either by anticipating an emotional response or responding after an emotional reaction, is critical for exhibiting favourable behaviour. Individuals with anxiety and depression experience difficulties regulating emotions, which has been associated with SN dysfunction. Low-fidelity bottom-up signals resulted in overactive top-down brain activity and exaggerated arousal (Paulus & Stein, 2010). Studies have also shown the strength of connectivity between regions in these networks, such as the MPFC and insula, is also predictive of depression severity (Kaiser, Andrews-Hanna, Wager, & Pizzagalli, 2015). In sum, our ability to attend to the environment and how we process information is determined by optimising the interactions between networks in our meta-stable state.

Studies examining changes in connectivity patterns can provide insight into the relationships between critical regions within and between specific networks. Specifically, effective connectivity methods can be used to test direct causal influences between neural systems and thus reveal how networks are organised regarding feedforward and feedback connections (Friston, 1994, 2011). Understanding connectivity is critical for evaluating predictive coding theories, as such proposes backward connections convey predictions and forward connections deliver prediction errors to higher cortical regions (Friston, 2010). Moreover, investigating differences in hierarchical patterns may be informative for understanding causal processes that maintain normal mental states and disruptions that lead to psychopathology.

1.2.4 Connectivity and mental illness

Much evidence indicates that connectivity disturbances play a role in mental illness (Sheline et al., 2009). There appear to be key nodes within several brain networks associated with depression and anxiety. For instance, decreased amygdala-precuneus and amygdala-hippocampus connectivity has been associated with an increased risk of depression (Rzepa & McCabe, 2016). This may be due to a disruption in connectivity, leading to a decreased ability to suppress and regulate negative memories. Similarly, reductions in functional connectivity between regions in the SN and DMN have been reported in patients with generalised anxiety disorder. These abnormal dynamics may also contribute to pathological tendencies, such as uncontrollable worry, as key structures in these networks, like the medial prefrontal cortex, have been associated with inhibition in response to fear (Xiong, Guo, & Shi, 2020). However, while there is strong evidence for dysfunctional connectivity underpinning problems with emotion regulation, and one could theorise mental illness arises through inaccurate mental models, it is unclear how this process occurs.

Some authors propose that mental illnesses arise from noisy bottom-up signals leading to excessive reliance on top-down predictions (Seth, 2014). This may therefore result in the misclassification of data. Examples of this over-reliance on predictions can be seen in visual illusions, such as Kanizsa triangles (see Figure 1.2), whereby the brain fills in missing information or perceives a particular shape/animal when presented with uncertain sensory information (Sterzer et al., 2018).

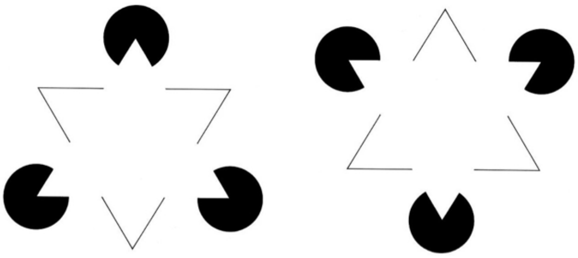


Figure 1.2: Example of Kanizsa triangles (Raz, 2017)

Others propose mental illness is the consequence of dysregulation of processes governing reward mechanisms and attention to reward-associated stimuli (Maier & Seligman, 2016; Seligman, 1975). Such models are analogous to early theories of conditional learning, whereby attention biases are value-driven and, therefore, sensitive to reward predictions. These theories argue that mental illnesses arise from top-down reward processing deficits, leading to under-confidence in one's predictions. Evidence for impairments to reward and punishment can be noted in observed behavioural deficits that characterise these conditions, such as learned helplessness and hyposensitive response to rewards (Eshel & Roiser, 2010; Malberg & Duman, 2003).

Yet, it is difficult to see how these concepts alone can be used to explain emotion deficits (anhedonia) and persistent physical symptoms that accompany mental illness (Henningsen et al., 2018; Treadway & Zald, 2013). Furthermore, sensory information encompasses more than exteroceptive perception (e.g. vision and touch). Interoceptive signals (internal body states, such as heart rate) may be as important, possibly more so, for the mechanisms governing attention and perception (Clark, Watson, & Friston, 2018). Mental health conditions are characterised by a range of systematic disturbances that result in neurocognitive and behavioural deficits. This suggests an interdisciplinary approach may be more suitable for explaining the manifestation and maintenance of these conditions (Critchley, Eccles, & Garfinkel, 2013).

Studies suggest several core regions across brain networks related to perception and the embodiment of emotion, including the amygdala, insula, anterior cingulate and brainstem (Critchley et al., 2005). Interestingly, these regions have also been associated with treatment effects and remission for depression (Whitton et al., 2019). Based on the implication of ACC and insula in generating autonomic changes and awareness of emotional stimuli, these findings may reflect changes to afferent information processing and, therefore, the experience of emotional states (Critchley et al., 2005; Critchley, Wiens, Rotshtein, Öhman, & Dolan, 2004). Badcock et al. (2017) proposed that depressive disorders arise from erroneous interoceptive predictions about the body's physiological state, leading to dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and a cascade of metabolic issues (Barrett et al., 2016; Stephan et al., 2016). Moreover, Badcock et al. (2017) suggests observable symptoms of depression, such as negative affect and fatigue, relate to the body's attempt to minimise prediction error by reducing energy expenditure (Seth & Friston, 2016). This adaptive response increases the precision of bottom-up prediction errors while reducing the certainty of top-down predictions. Ultimately, this results in an inability to evaluate prior beliefs (leading to negative cognitive biases (Beck, 1967; Beck, Epstein, & Harrison, 1983)) and rumination of interoceptive and affiliative concerns.

In summary, this section outlined the framework of predictive coding and discussed its relation to brain function, behaviour and mental illness. In the following section, I will explore several theories and research that considers how neural and interoceptive signals integrate to serve attentional control.

1.3 Mind-body connection

Since the 19th century, the role of peripheral physiology concerning cognition has been an ongoing debate. Initial views proposed that physiological responses precede cognition and emotion, and therefore perception is a consequence of somatovisceral feedback (James, 1884; Lange, 1922). But more recently, there is a growing body of evidence supporting the notion of dynamic and flexible interactions, whereby cognition is thought to generate efferent (top-down) influences on the viscera. In turn, this is shaped by afferent (bottom-up) information from the periphery (Tallon-Baudry, Campana, Park, & Babo-Rebelo, 2018).

1.3.1 The autonomic nervous system

A complex interaction of many brainstem and cortical areas accomplishes the integration of the autonomic nervous system (ANS). Consequently, for this thesis, the relevant pathways will be discussed in detail (refer to Shields and Robert (1993) for an in-depth review of the ANS). The peripheral activity of the ANS is split into two main divisions, the sympathetic and parasympathetic systems, and hierarchically coordinated by neuronal networks in the forebrain, hypothalamus, midbrain and medulla (Langley, 1898; Loewy & Spyer, 1990). Briefly, the sympathetic system facilitates a "fight or flight" response by promoting increased blood flow by signalling an acceleration of the heart via a preganglionic pathway through the paravertebral ganglia and activating vasodilation of vascular muscles via the adrenal medulla. In contrast, the parasympathetic system promotes "rest and digest" functions by regulating vagal tone via the vagus or 10th cranial nerve.

Axons of the vagus nerve emerge from or converge onto the medulla oblongata of the brainstem; this is where the vagal nucleus is located. Vagal efferent signals from the vagal nuclei in the brain stem regulate supradiaphragmatic organs (e.g. heart and lungs), thereby adjusting physiological arousal (Hsu et al., 2012; Karemaker, 2017; Porges, 2009). There are three components of the vagal nuclei involved in the vagal complex: the dorsal motor nucleus of the vagus, the nucleus ambiguus, and the solitary nucleus.

The Polyvagal Theory (Porges, 1995) proposes the vagal pathway can be summarised by two subsystems, the ventral vagal complex and dorsal vagal complex. The ventral vagal complex arises from the nucleus ambiguous and mediates interactions between CNS and ANS by visceral efferent (motor) and afferent (sensory) pathways that slow the heart. As a consequence of these interactions, behaviour and affective responses are modulated according to social demands. While a phylogenetically more primitive system, the dorsal vagal complex mediates the defensive “freeze” response to threat and includes the vagus’s solitary tract and dorsal motor nucleus.

As the vagus nerve is a mixed nerve containing afferent and efferent fibres, it is involved in transmitting information along ascending and descending pathways of the parasympathetic system. This interchange forms a complex neuronal circuity involved in the modulation and feedback of the baroreflex. This mechanism maintains blood pressure by interacting with the central and autonomic nervous systems. Changes in blood pressure within the arterial lumen are detected and feedback to the medulla via ascending afferent fibres of baroreceptors located in the intrathoracic vessels and heart. Sensory afferent fibres from the baroreceptors travelling along branches of the vagus and glossopharygeal nerves project to the Nucleus Tractus Solitarius (NTS).

The NTS serves as a primary site for afferent impulses, including chemoreceptors, pulmonary afferents and cardio-afferent projections (Loewy & Spyer, 1990). Visceral afferent information received by the NTS drives ascending noradrenergic projections to the Nucleus Paragigantocellularis (PGi) in the rostral ventral medulla and forebrain regions, such as the amygdala and the cortex. Through mostly glutamatergic projections, the PGi communicates with the Locus Coeruleus (LC) in the rostral pons' posterior area. In turn, the LC sends noradrenergic projections to the Basal Forebrain (BF), corticopetal cholinergic system and forebrain regions, as the NTS (Aston-Jones, Rajkowski, Kubiak, Valentino, & Shipley, 1996; Papadopoulos & Parnavelas, 1991; Semba, Reiner, McGeer, & Fibiger, 1988). Cholinergic projections from the BF modulate pyramidal cell activity to facilitate sensory information processing within the cortex, while noradrenergic LC activity stimulates the release of norepinephrine to meditate arousal in sensory cortices (Villano et al., 2017).

Evidence suggests the NTS may be a primary afferent-efferent integration hub, as the NTS also receives descending input from cortical areas, such as the hypothalamus and forebrain, which may modulate sensory afferent processing (Andresen & Kunze, 1994). Through the descending pathways, the central nervous system can regulate visceral afferent signals by modulating excitation of barosensitive neurones via γ-Aminobutyric Acid (GABA)-meditated inhibition elicited by the forebrain to the NTS (Coote & Macleod, 1974; McWilliam & Shepheard, 1988; Pagani et al., 1986). As a result of sympatho-inhibition, baroreflex sensitivity is adjusted, leading to changes in the regulation of blood pressure, through the afferent neuronal firing of baroreceptors.

1.3.2 Baroreflex function and resonance

The arterial baroreflex attempts to stabilise arterial pressure by regulating sympathetic nerve activity using two sub-systems: the peripheral and neural arc. Changes in arterial pressure, such as through respiration, affect the sympathetic nerve activity through the neural arc. This involves neural projections along vagal fibres towards midbrain synapses onto the nucleus of the solitary tract. The solitary tract compromises a white bundle of nerve fibres that innervate the solitary nucleus (SN), including the vagus nerves, which convey sensory information about the state of the body to the central nervous system. Information received at the SN from nerve fibres is subsequently projected to many brain regions, including the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala, as well as nuclei of the brain stem, such as the locus coeruleus (LC), which is involved in the regulation of blood pressure. Activity at the LC bidirectionally connects to higher cortical structures through the ventral-medial prefrontal cortex Ventro-medial Prefrontal Cortex (vmPFC) (Guyenet, Koshiya, Huangfu, Verberne, & Riley, 1993; Oyamada, Ballantyne, Mückenhoff, & Scheid, 1998). This region of the brain, in addition to neighbouring structures such as the insula, is thought to play an important role in cardiovascular modulation and emotional response (Hänsel & von Känel, 2008).

The complexity of this reflex arc is notable by the numerous connections and rich interactions involved in autonomic regulation. As a result, the baroreceptor reflex has received much attention and, more recently, is a clinical target for mental health treatments (Duschek, Werner, & del Paso, 2013). Biofeedback therapies attempt to modulate the baroreflex function by maximising respiratory sinus arrhythmia (which relates to HR oscillations according to breathing frequency) by matching the breathing rate with the natural frequency of the baroreceptor reflex (approximately 0.1 Hz). When these frequencies match, these two oscillations reinforce each other (i.e. resonate) (Vaschillo, Lehrer, Rishe, & Konstantinov, 2002). Evidence has shown that amplification of the vagal baroreflex response promotes neuroplasticity and improves physiological health, emotion regulation and cognitive control (Eddie, Price, Bates, & Buckman, 2021; Leyro, Buckman, & Bates, 2019; Thayer, Hansen, Saus-Rose, & Johnsen, 2009).

1.3.3 Relation to cognition

Over 50 years ago, Lacey and Lacey (1970) first identified cardiac cycle time effects, whereby earlier stimulus presentations shortened the cycle and reaction times, and late stimulus presentations lengthened the following cardiac beat-to-beat interval and slowed reaction time. Lacey interpreted these observations as an “afferent feedback hypothesis”. This theory proposes higher cortical centres respond to environmental demands by inducing vagal, autonomic changes in heart rate. In contrast, the heart provides afferent feedback to the brain by decelerating or accelerating depending on task demands. In sum, the effects of autonomic function on cognition can be described as bidirectional: cortical systems adjust in response to afferent feedback, and cognitive demands modulate baroreceptor function (Duschek, Muckenthaler, Werner, & Reyes del Paso, 2009; Reyes Del Paso, González, & Hernández, 2004; Rudas et al., 1999; Yasumasu, del Paso, Takahara, & Nakashima, 2006).

Baroreflex inhibition has been positively associated with and predicts cognitive performance in attention-based tasks (Duschek et al., 2009). Baroreceptor-elicited cardiac deceleration has also been associated with improvements in a visual attention task (Reyes Del Paso et al., 2004). These task-sensitive findings suggest that baroreceptor function, and as a proxy metabolic activity, varies to meet cognitive-attentional demands (Duschek et al., 2009). This interaction suggests that attention, and by consequence, cognitive processing, is influenced by the baroreflex function.

Through noradrenergic and cholinergic routes to forebrain regions, ascending visceral information can modulate thalamocortical rhythms relevant to cognitive demands and consequently influence attention, vigilance and memory (Berntson, Sarter, & Cacioppo, 2003; Drachman, 1977; McCormick, 1989). Pharmacological studies in rats have shown that manipulation of adrenergic function within the NTS, with epinephrine, up-regulates norepinephrine release in the amygdala and enhances memory performance in an inhibitory avoidance task (Clayton & Williams, 2000). Similar findings have been reported in humans after vagal stimulation, which supports the notion of visceral influences shaping cognition (Clark, Naritoku, Smith, Browning, & Jensen, 1999).

Changes to the body's internal physiological state are communicated to forebrain regions to allow for adjustments based on actual or anticipated demand. This ability to detect internal state changes is referred to as interoception. This process can be indexed by Heartbeat Evoked Potentials (HEPS), which reflects temporal changes in neural activity related to the cortical processing of the heartbeat. It has been suggested that interoception may underpin some atypical cognitive performance and attentional bias in mood disorders. HEPs in EEG recordings have been described to be altered by breathing and attenuated by negative emotional stimuli (Garfinkel et al., 2016). HEPs have also been found to be a marker of altered bodily awareness in depressed patients (MacKinnon, Gevirtz, McCraty, & Brown, 2013). Furthermore, decreased interoceptive involvement of the insular cortex detected by fMRI was related to depression and somatic symptom severity in major depressive disorder (Avery et al., 2014). Considering these research findings, further understanding of neural-visceral interactions may highlight key areas for therapeutic applications in mood disorders.

1.3.4 Neural-visceral integration

In recognising changes in baroreceptor function that originate from efferent Central Nervous System (CNS) activity, these findings supply evidence for active inference theory (Barrett & Simmons, 2015). Predictions are acted on by the descending pathway to modulate vagal function, whereas afferent feedback guides efferent control by providing information on prediction error. When an individual cannot generate an appropriate response to the environment, Porges (2009) suggests this is due to the inability to regulate autonomic response.

Thayer and Lane (2000) proposed in their neurovisceral integration model that the regulation of the autonomic nervous system arises from an overarching structural network, referred to as the Central Autonomic Network (CAN). This network interacts with related functional networks, such as the DMN and DAN, to coordinate cardiac, affective, attentional and behavioural responses (Azzalini, Rebollo, & Tallon-Baudry, 2019; Schulkin, Thompson, & Rosen, 2003).

The CAN consists of multiple hierarchical levels from the brainstem to forebrain cortical regions, including but not limited to the insula, cingulate cortex, Medial Prefrontal Cortex (MPFC) and amygdala-hippocampal complex (Valenza et al., 2019). These forebrain regions are defined by their dedicated functions regulating cognition and behaviour. The insula and cingulate cortex are core hubs for integrating multimodal information. The former focuses on linking internal and external information to produce mind-body awareness. The latter is concerned with execution and evaluation processes (Craig, 2002; Critchley et al., 2004; Devinsky, Morrell, & Vogt, 1995; Nguyen, Breakspear, Hu, & Guo, 2016; Pollatos, Gramann, & Schandry, 2007).

The Anterior Cingulate Cortex (ACC), a subdivision of the cingulate cortex, is thought to maintain a current state by reducing conflict with competing states. During decision-making, the ACC increases in activation, and this is often accompanied by activation in the MPFC (Greene & Paxton, 2009; Moll et al., 2006). More recent work suggests the MPFC forms and stores schemas that map context onto actions (Miller & Cohen, 2001). Retrieval of these schemas relies on accessing memory through the nucleus reuniens of the midline thalamus to the hippocampus. The hippocampus is closely connected to the amygdala, where their interrelationship connects memory representation and associated emotional autonomic response (Critchley et al., 2005; Phelps et al., 2001; Williams et al., 2001).

In the context of psychiatric disorders, patients often exhibit prefrontal hypoactivity and differential autonomic responses compared to healthy controls. This has been associated with poor affective information processing and regulation (Balconi & Vanutelli, 2017; Mathews, 1990; Papousek & Schulter, 2001; Thayer, Friedman, & Borkovec, 1996; Thayer & Lane, 2000).

From these studies, we can infer that affective and cognitive processing difficulties could be underlined by dysfunctional neural-visceral mechanisms, which interfere with the ability to update predictive models and produce context-appropriate responses accurately. While the precise order of mechanistic events for the emergence of psychiatric disorder may be unclear, the neural-visceral integration model provides a basis for understanding how afferent and efferent signals result in variability in observable behaviour.

This section outlined several physiological systems relevant to studying neural-visceral mechanisms, including the baroreflex, vagal pathways and central autonomic network. In addition, I discussed how these systems relate to facets of cognition, such as emotion regulation and attention, as well as how this relates to the understanding of specific psychiatric disorders like depression and anxiety. The subsequent section examines how therapies, with a particular focus on meditation, could alter these systems and consequently lead to therapeutic benefits.

1.4 Therapeutic action

Many therapeutics have been used over the years to treat affective and cognitive disturbances that define psychiatric disorders. These approaches are often preferred or used in combination with pharmacological treatments due to concerns with side effects, medication interactions, and adherence.

Meditation has received much attention over the past two decades. Its popularity may be related to promising accumulative empirical support for its therapeutic effects and ease in accessibility, which can improve engagement with treatment (Goldberg et al., 2018). Formal meditation practice likely originated from the Buddha's teachings over 2600 years ago. His teachings emphasised meditative concentration as one of the three teachings of the spiritual path that result in awakening or enlightenment. In modern times, influential Western figures, such as Kabat-Zinn (2003), who founded mindfulness-based stress reduction, brought the benefits of meditative practice to the attention of the public and scientific communities. Meditation requires an individual to focus their attention on an internal or external stimulus, for example, counting inhalations during a defined period, or practice developing thoughts of compassion through loving-kindness meditation (De Benedittis, 2015; Rubia, 2009).

Individuals who engage in meditation report emotional and cognitive benefits associated with the practice, such as positive mood, emotional stability, improved concentration, self-monitoring and cognitive interference control (Kyeong, Kim, Kim, Kim, & Kim, 2017; Moore & Malinowski, 2009; Zeidan, Johnson, Diamond, David, & Goolkasian, 2010). Scientific literature suggests that meditation, even after brief training sessions, can aid mental well-being and cognitive performance (Lane, Seskevich, & Pieper, 2007;

Tang et al., 2007; Zeidan et al., 2010). However, the precise physiological basis of the cardio-respiratory-neuronal interactions during meditation is still unclear. Current evidence indicates the benefits of meditation are likely products of integration between the central and peripheral nervous system (Berntson, Cacioppo, & Quigley, 1993; Critchley & Garfinkel, 2018; Zelano et al., 2016).

According to the neurovisceral integration model, sustained focus on the breath could have a bottom-up inference on the brain via the vagal afferent pathway (Thayer & Lane, 2000). During respiration, intrathoracic pressure varies in response to the movement of the diaphragm and intercostal muscles during inhalation and exhalation. This pressure variation helps maintain gaseous exchange within the lungs, which is necessary for obtaining oxygen for cell function. In addition, intrathoracic pressure influences the hemodynamic response by creating oscillating patterns in systolic blood pressure detected by arterial baroreceptors (Del Negro, Funk, & Feldman, 2018).

Baroreceptors in the heart's chambers initiate the baroreflex mechanism in response to changes in arterial blood pressure and central neural-autonomic pathways. Due to Respiratory Sinus Arrhythmia (RSA), which relates to the phasic effects of respiration influencing baroreflex activity, the time between successive heartbeats, also known as inter-beat intervals, shortens during inspiration and lengthens during expiration (Berntson et al., 1993; Freeman, 2006; García-González, Vaáquez-Seisdedos, & Pallàs-Areny, 2000). The variation in the inter-beat interval is referred to as Heart Rate Variability (HRV). To briefly summarise, the changes in respiratory rate influence RSA frequency, which results in changes to HRV amplitude (see Russo, Santarelli, and O'Rourke (2017), for further reading on physiology).

Some evidence suggests that modulating the autonomic function may be involved in the underlying mechanisms of meditation. Short-term meditation appears to up-regulate parasympathetic activity as indicated by increased HF HRV, compared to a relaxed condition (Tang et al., 2009). Furthermore, consistent practice of slow breathing can result in a long-term shift of autonomic balance toward the parasympathetic state (Pal, Velkumary, & Madanmohan, 2004). Altering cardiac afferent activity has also been shown to improve the interoceptive representation of the insular cortex and reduce depression symptomology (Pinter et al., 2019). Mediators often rated interoceptive performance as superior and difficulty of the heartbeat detection task as easier, despite task performance being no different from nonmeditators across several sessions and respiratory modulation conditions (Khalsa et al., 2008). Ultimately, these studies suggest changes to autonomic activity via meditation can alter the output of the CAN. As a result, this could be a potential explanation for the beneficial effects of meditative practice on cognition and attention (Dooley, 2009; Heck et al., 2017; Rubia, 2009).

To advance therapeutic interventions, it is essential that we gain a solid understanding of how meditative practice delivers these outcomes. This knowledge may then shine a light on how psychiatric disorders emerge and how we could enhance the therapeutic effects of meditation.

1.4.1 Meditation and interoceptive awareness

Generally, meditation can be described as the act of remaining calm while engaging in contemplation, reflection, or awareness for a period of time. As part of religious training, meditation is one of the core practices for purifying the mind and attaining spiritual enlightenment. Most common forms of meditation, such as mindfulness, tend to focus on the observation mode of meditation, which encourages sustained attention towards predominant experiences in the present moment, such as respiration or bodily sensations. Sustained attention to internal signals up-regulates one's awareness of the autonomic state. This is referred to as interoceptive awareness.

The process of heightening one's awareness of internal signals may be relevant to the underlying mechanisms of emotional processing for psychiatric conditions. Pollatos et al. (2007) found a positive association between interoceptive awareness and the intensity of emotional experiences. Furthermore, improved bodily sensations during sadness have been associated with lower levels of depression (Farb et al., 2010). This indicates that interoceptive awareness mechanisms may be relevant for understanding emotional processing in mental illness (Salamone et al., 2021).

Increasing momentary awareness of internal sensations during and after meditation has been associated with increased connectivity in structures belonging to the central autonomic CAN and attentional networks DAN, such as the anterior cingulate cortex and anterior insula. In addition, there is decreasing activation in the DMN structures, such as the medial prefrontal cortex and the amygdala (Brewer et al., 2011; Farb et al., 2007). Changes to these networks are likely key to producing the positive behavioural consequences of meditation, as the extent of connectivity change has been strongly associated with changes in executive function, working memory and perception of negative emotional experiences (Jha, Krompinger, & Baime, 2007; Lutz, Greischar, Perlman, & Davidson, 2009; Zeidan et al., 2010).

The recruitment of brain regions occurs by changing neural oscillatory frequencies, where local recruitment is associated with the amplitude of low-frequency bands, such as theta and delta, and distant for high-frequency bands, like gamma, beta and alpha frequencies. Several studies have shown that meditative practice up-regulates frontal-midline oscillatory activity in a range of frequency bands, alpha and beta, but especially in the theta frequency (Cahn & Polich, 2006; Hölzel et al., 2007). Midfrontal theta reflects the neural mechanism for coordinating cognitive processes involved in top-down control (Cavanagh & Frank, 2014). These changes in amplitude have been source-localised to a key CAN structure, the ACC, which has been shown to increase in activity during affective and attentional states, such as emotion processing and sustained attention (Asada, Fukuda, Tsunoda, Yamaguchi, & Tonoike, 1999; Deiber et al., 2007; Gevins & Smith, 2000; Rachbauer, Labar, Doppelmayr, & Klimesch, 2002; Sauseng, Hoppe, Klimesch, Gerloff, & Hummel, 2007). This suggests the benefits of meditation may be centralised in this brain region.

In addition to enabling cognitive function, evidence suggests the ACC is also involved in regulating autonomic activity (Critchley et al., 2005; Gentil, Eskandar, Marci, Evans, & Dougherty, 2009). Therefore, it is unsurprising that meditative practice facilitates widespread autonomic changes, such as decreased heart rate and skin conductance (Hölzel et al., 2011; Kubota et al., 2001; Tang et al., 2009). Tang et al. (2009) demonstrated greater activity in the ACC, insula, and striatum is possible within five days of meditation. This suggests that modulation of neural-visceral pathways via meditation may be achievable within a brief period.

Cardio-respiratory synchronisation could explain short-term meditation outcomes, whereby breathing cycles and heartbeats occur in the same relative phase for prolonged periods (Bartsch, Schumann, Kantelhardt, Penzel, & Ivanov, 2012). Recent work has indicated a link between cardio-respiratory synchronisation and sustained attention. Mental tasks that require directing attention outside the self appear to lower the magnitude and duration of cardio-respiratory synchronisation epochs and RSA (Mortola, Marghescu, Siegrist-Johnstone, & Matthes, 2020; Zhang, Yu, & Xie, 2010). RSA refers to the synchrony between the heart rate and respiration variation, by which the cardiac R-R interval is shortened during inspiration and prolonged during expiration. Mortola et al. (2020) reported a linear correlation between cardio-respiratory synchronisation and RSA and observed individuals with the lowest drops in cardio-respiratory synchronisation from baseline had insignificant changes in RSA.

These findings indicate that cardio-respiratory interactions could be utilised as a biomarker for central-autonomic stability. Such inference is supported by Zhang et al. (2010), where they demonstrate a decrease in cardio-respiratory synchronisation during mental tasks was negatively correlated with frontal EEG theta power. Extrapolating this to Mortola et al. (2020) 's study, individuals with the lowest drops in cardio-respiratory synchronisation would likely exhibit slight variation from baseline in theta power during the task. While further research is necessary, this suggests that maintenance of high cardio-respiratory synchronisation may facilitate stability and optimisation of the neural-visceral system, as expressed by the insignificant changes in RSA and inferred theta power.

Secondly, cardio-respiratory synchronisation appears to vary according to cognitive demands. Incidentally, studies have shown that meditation significantly increases the length and number of synchronous cardio-respiratory epochs (Cysarz & Büssing, 2005; Wu & Lo, 2010). However, heartbeat and respiration tended to be de-synchronisation during spontaneous meditative breathing, which may suggest maintaining a specific breathing frequency is vital for cardio-respiratory synchronisation during meditation (Cysarz & Büssing, 2005).

1.4.2 Limitations and theorization

While many theories could be proposed for how neural-visceral mechanisms influence cognitive and emotional processing, how meditation might alter cognitive or emotional processes is much more speculative. This is partially due to the complexity of interactions involved in the central autonomic network and differences in experimental design between studies, such as manipulating breathing frequency, that make it difficult to determine the mechanisms of action for interoceptive awareness (Azzalini et al., 2019; Critchley & Garfinkel, 2018).

Based on the current evidence, this thesis argues that meditation induces a state of interoceptive awareness, and this adjusts priors through descending pathways, as signalled by frontal-theta activity in the ACC. Through efferent pathways, increased activation of the ACC leads to enhanced attenuation towards autonomic signals, thereby up-regulating cardio-respiratory synchronization. As a result of cardiac and respiratory interactions, autonomic indices are altered to maintain a homeostatic state. These changes are detected by baroreceptors and communicated back to neural control systems via afferent feedback, eliciting influence on attention and emotional information processing. A detailed thesis outline is provided in the following section.

1.5 Direction of the thesis

In this chapter, I outlined predictive coding theory as a theoretical basis for biological systems and identified there appears to be a connection between neural-physiological signals, which influences cognition and emotion. In addition, I discussed evidence supporting the neural-visceral integration model and how this work can be applied in treating psychiatric disorders.

However, there is a limited mechanistic understanding of neural-visceral integration and how interventions, such as meditation, modulate this. Therefore, advancing our knowledge of neural-visceral interactions could provide insights into the mechanisms of these treatments and potentially open a window into developing new personalised interventions for mood disorders.

This thesis aims to investigate the mechanisms of neural-visceral integration during meditation, to facilitate an understanding of emotional processing for mental disorders and the development of future treatments. This is with the overall goal of exploiting the knowledge of the mechanisms of neural-visceral integration for developing personalised treatments.

The thesis has been organised into the following chapters:
Chapter 2 Literature review on neural-visceral coupling and emotion
Chapter 3 Methodology used in this thesis
Chapter 4 Preliminary analysis of breath-focused meditation (Su-soku)
Chapter 5 Neural-visceral coupling during respiration
Chapter 6 The effects of Su-soku on cognition and mood-state
Chapter 7 Exploring the effects of resonance breathing
Chapter 8 General discussion

1.6 COVID-19 statement

The original plan for this thesis was to develop virtual human therapies based on the findings explored in earlier chapters. However, this was not possible due to delays caused by the COVID-19 pandemic. As a result, this thesis was restructured to focus on the therapeutic effects of meditation, and a study protocol has been developed from this work (see Appendix D).

Egede, J.O., Price, D., Krishnan, D.B., Jaiswal, S., Elliott, N., Morriss, R., Trigo, M.J.G., Nixon, N., Liddle, P., Greenhalgh, C. and Valstar, M. (2021). Design and Evaluation of Virtual Human Mediated Tasks for Assessment of Depression and Anxiety. *In Proceedings of the 21st ACM International Conference on Intelligent Virtual Agents*, 52-59. `https://doi.org/10.1145/3472306.3478361`

Chapter 2

Neural-visceral coupling and emotion

Chapter 1 alluded to the notion that the emergence and maintenance of psychiatric illness may be associated with dysfunctional neural-visceral mechanisms. One of the key characteristics of many mental health disorders is the inability to regulate and process emotions. Therefore, examining the relationship between neural-visceral coupling and emotion may provide fruitful insights for developing therapeutic interventions that modulate these mechanisms.

This chapter presents a literature review investigating the current literature on neural-visceral coupling in the context of emotion processing. This review provides an original contribution to knowledge by identifying key concepts developed over the last 20 years and gaps in the literature that can be addressed in this thesis. Findings from 62 papers are summarised, and three core arguments for investigating neural-visceral mechanisms to advance therapeutic interventions were developed.

2.1 Background

Since the inception of James' theory of emotion, a large body of empirical research has been conducted to investigate the relationship between emotional experiences and different bodily states, notably, specific patterns of autonomic arousal. Experiments conducted by Yerkes, Dodson, et al. (1908) suggested that an optimal level of arousal is required for optimal task performance, whereby the optimal level of arousal is lower the more complex the task. Hebb (1955) 's subsequent adaptation of this law proposed that learning and arousal fit a similar inverted-U function, whereby too little or too much "arousal" learning is not optimal for task performance. Arguably, this Hebbian version of the Yerkes-Dodson law foreshadowed later theorisation of brain-emotion interactions.

In 1967, Lacey's discovery of cardiac cycle time effects led to the first speculation that cardiac variation modulates cognitive function (Lacey, Appley, & Trumbull, 1967). Following these observations, Lacey and Lacey (1970) 's intake/rejection hypothesis proposed that autonomic changes lower sensory thresholds during attention. The rejection aspect of this theory relates to the need for one to inhibit or "reject" incoming sensory information to facilitate mental imagery. Shortly following this, Porges (1972, 1973) observed higher resting levels of cardiac vagal tone, indexed by heart rate variability, were associated with better reaction time performance and improved attentional capacity. Porges, Doussard-Roosevelt, and Maiti (1994) further extended the concept of central-autonomic interaction by relating the development of the vagal system to social/emotional development. Under this framework, social responses to our environment are proposed to be mediated either by vagal input or vagal withdrawal through the components of the limbic system. Autonomic activity effectively tunes the organism, enabling appropriate perception and evaluation for response selection. Therefore, this framework proposes that an inability to regulate autonomic response leads to an inability to respond according to environmental demands (Porges, 1995).

Recent theories have focused on modelling the underlying mechanisms of autonomic regulation and its interaction with the central nervous system. Thayer and Lane (2000) highlight, in the neurovisceral integration model, that autonomic regulation likely arises from an overarching structural mechanism enabling adaptive responses towards situational demands. They suggest that the central autonomic network (CAN) and related functional networks within the brain coordinate cardiac, affective, attentional, and behavioural responses. This process occurs via a complex feedforward-feedback mechanism, whereby direct and indirect pathways, along preganglionic and parasympathetic neurones, modulate output from the CAN and influence the heart rate time series via the sino-atrial node, or vice versa. Despite many recent iterations of the neuro-visceral integration model, there is a consensus that central-autonomic interactions are modulators, rather than direct causes, for how emotion and attention influence information processing during the appraisal of stimuli (Azzalini et al., 2019; Berntson et al., 2003; Damasio & Carvalho, 2013; Schulkin et al., 2003).

Approximately 130 years have passed since James' theory of emotion, and the "embodied" literature continues to expand its applications and therapeutic potential for psychiatric disorders. Several lines of research have reported impairments in autonomic function for psychiatric conditions, but this is particularly pertinent for depression and anxiety disorders (Berntson & Cacioppo, 2004; Herzog, D'Andrea, DePierro, & Khedari, 2018; Koenig, Kemp, Beauchaine, Thayer, & Kaess, 2016; McEwen, Nasca, & Gray, 2016).

In a meta-analysis, participants with depression were found to have lower HRV than healthy control participants and depression severity was negatively correlated with HRV. Furthermore, these effects were not found to be confounded by most common antidepressant medications, such as serotonin reuptake inhibitors, mirtazapine and nefazodone (Kemp, Gray, Felmingham, Brown, & Gatt, 2010). Garfinkel et al. (2016) observed that metacognitive awareness of cardiac signals was associated with reduced anxiety symptoms, whereas poor respiratory accuracy was associated with heightened anxiety. These differences may be related to extent of control an individual has over the internal body sensation, and consequently this may influence the ability to reduce prediction errors. Respiratory accuracy refers to the ability to detect resistive loads, which while respiratory rhythm is more likely to vary compared to cardiac sensations, there is much greater capacity for control of breathing than cardiac regulation. Therapies that attempt to modify respiratory awareness, such as mindfulness meditation, have been shown to improve symptoms of anxiety and lead to changes in frontolimbic areas that have been associated with emotion regulation (i.e amygdala and prefrontal cortex) (McEwen et al., 2016). This suggests optimizing interoceptive awareness, via neural-visceral interactions, may impact how one processes and responds to emotion. Therefore, understanding these mechanisms is particularly pertinent for enhancing knowledge on emotional processing in psychiatric disorders and advancing therapies for these disorders.

This scoping review aims to highlight recent developments in neural-visceral interactions for emotion-based information processing. Specific physiological signatures, representing the interplay between neural and autonomic states, are expected to be related to distinct emotional states. Outcomes from this review will help understand the role of neural-visceral interactions during emotion processing.

2.2 Methodology

2.2.1 Search strategy

The literature search was conducted using PubMed, Web of Science and Google Scholar electronic databases with the criteria “neural” AND “visceral” OR “autonomic” AND “emotion” OR “affective regulation”. This was limited to article title, abstract and keywords, with the dates from 2000 to 2019. This date range starts from 2000, the same year the neurovisceral integration model was proposed by Thayer and Lane (2000).

2.2.2 Study criteria

Studies were only included if they reported both neuroimaging and autonomic measures and investigated emotional processing by elicitation or induction of emotional state. Studies were also required to be journal articles and written in English. This review was conducted according to the framework provided by Hagen-Zanker and Mallett (2013).

Inclusion criteria: **(1)** Neuroimaging methods, such as Functional Magnetic Resonance Imaging (fMRI), EEG, Positron Emission Tomography (PET), or Magnetic Resonance Imaging (MRI); **(2)** Methods of measuring autonomic activity, such as galvanic skin response or heart rate variability; **(3)** Eliciting emotional response, either experimentally or by interventions; **(4)** Human studies only.
Exclusion criteria: **(1)** Studies with psychiatric patients without healthy control groups; **(2)** Studies focusing on physical health conditions; **(3)** Journals without impact factor > 1.99.

While it is recognised that exclusion criterion (1) rules out clinical trials using a standard treatment group design, i.e. randomising participants of interest into an experimental group vs a control placebo group, this design does not fit the purposes of this review. This review explores the effects of neural-visceral interactions on emotion processing for both psychiatric and healthy populations rather than focusing on treatment effects. As a result, it would be tricky to interpret the differences between these populations in studies that do not include a healthy control group.

2.2.3 Data collection

The following variables were extracted from each paper: **(1)** experimental protocol; **(2)** methods for assessing neural activity; **(3)** methods for assessing autonomic activity; **(4)** sample features (clinical or non-clinical); **(5)** number of participants; **(6)** emotion-induction methods and **(7)** outcomes for measures used.

Themes were generated based on the similarities of extracted variables between papers and were subsequently developed into three discussion points for future research to consider.

2.3 Results

2.3.1 Search results

After removing duplicate citations and carefully selecting papers based on primary criteria, 230 potentially relevant articles were identified. Following an abstract review, 48 papers were excluded, and from the full-text review of 182 studies, 120 were excluded. Thus, 62 papers were included in this review (see Figure 2.1).

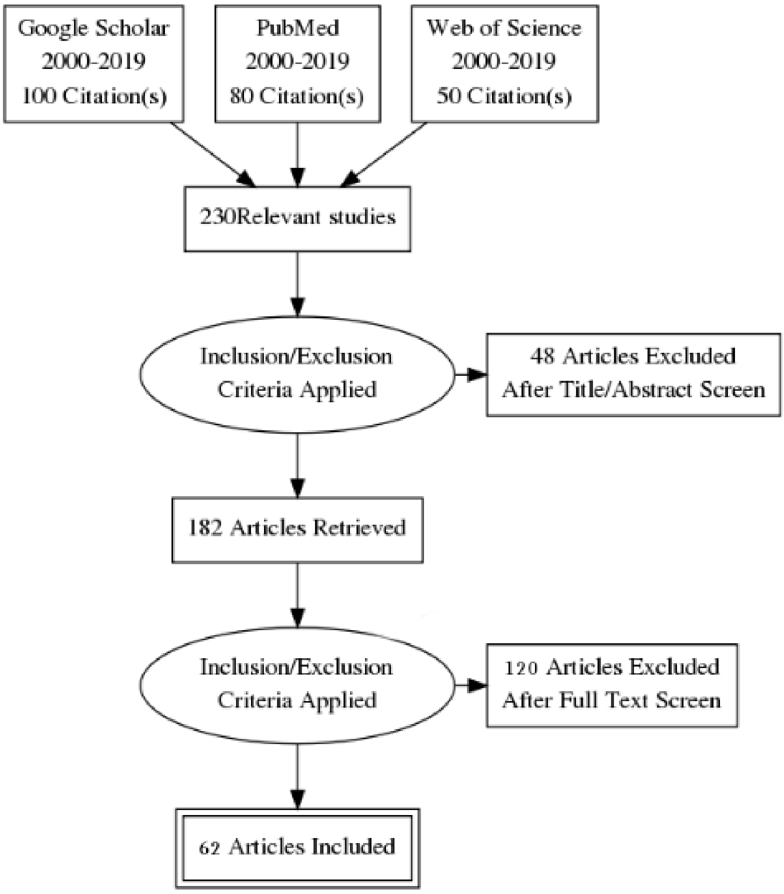


Figure 2.1: PRISMA diagram

Out of a total of 1897 participants, 55 papers focused on healthy participants (n = 1740), and seven papers included participants with a psychiatric disorder (n = 157).

Within the clinical population, the following disorders were reported in these studies: phobia (n = 39), depression (n = 29), post-traumatic stress disorder (n = 75), and obsessive-compulsive disorder (n = 14).

A range of neuroimaging and neurostimulation techniques were employed across the studies (see Figure 2.2): functional magnetic resonance imaging (n = 1120), positron emission tomography (n = 58), electroencephalography (n = 480), single-photon emission computed tomography (n = 78), functional near-infrared spectroscopy (n = 47), transcranial direct current stimulation (n = 21) and transcranial magnetic stimulation (n = 20). Despite most studies employing fMRI to study neural-visceral interactions in the context of emotion processing, this thesis utilises EEG for the purpose of focusing on temporal changes to the neural-viscera.

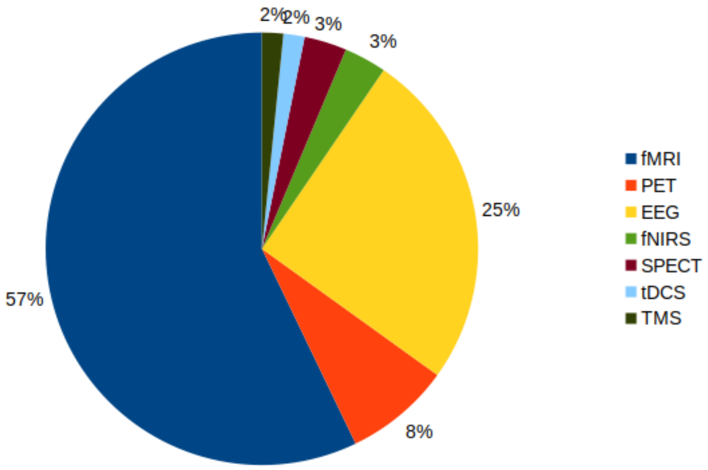


Figure 2.2: Percentage of neuroimaging and neurostimulation techniques employed across included studies

Autonomic activity was quantified by a variety of approaches (see Figure 2.3): pupillometry (n = 228), galvanic skin response (n = 813), temperature change (n = 32) and cardiac measures, such as blood pressure, heart rate and heart rate variability (n = 1262). Based on these findings, autonomic activity is indexed by cardiac autonomic function in this thesis.

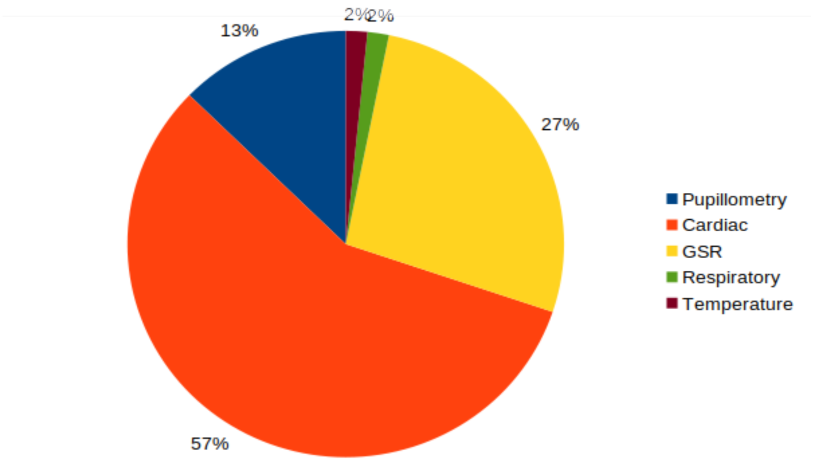


Figure 2.3: Percentage of autonomic activity techniques employed across included studies

Various methods were used across studies to induce emotion (see Figure 2.4): meditation (n = 147), video clips (n = 166), images (n = 627), music (n = 41), biofeedback (n = 17), scripts (n = 66), experimental such as the Stroop task (n = 547) and tactile stimulation (n = 120). As a secondary aim of this thesis is to extend therapeutic interventions, meditation was chosen to induce differential states in participants.

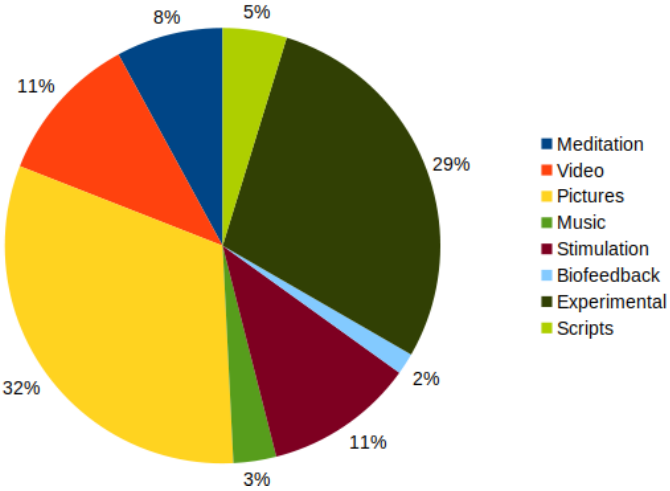


Figure 2.4: Percentage of emotion induction methods employed across included studies

In summary, the key studies suggest neural and peripheral autonomic signals simultaneously react in response to emotion and specific brain areas, such as the anterior insula and anterior cingulate cortex, are associated with peripheral autonomic response and emotional states. Several studies also suggest differences in neural-visceral responsivity for patients with mood and anxiety disorders during negative affect tasks, compared to healthy controls (see Appendix A for key studies table).

2.3.2 Data review

After reviewing the extensive literature, findings were summarised into themes, and then three arguments were developed for exploring neural-visceral interactions for emotion-based therapies.

The first argument supports the proposition that neural-visceral mechanisms are important for understanding emotional processing. Most of the studies reported in this argument utilise emotional transference, where an emotional state is induced via the presentation of emotional stimuli.
In the second argument, interoception is explored and proposed as a core component of neural-visceral interactions and emotional experience.
And finally, the third argument suggests the potential links between therapeutic interventions and central-autonomic mechanisms; by drawing on studies investigating emotion processing in psychiatric populations.

2.4 Discussion

2.4.1 Neural-visceral mechanisms are important for emotion

As predicted, emotional processing can be defined by specific physiological responses. For example, Cuthbert, Schupp, Bradley, Birbaumer, and Lang (2000) investigated the effects of emotional engagement, indexed by autonomic arousal, on scalp-recorded event-related potentials and found a larger delayed positive amplitude peak 200-300ms after picture onset for affective stimuli compared to neutral. They also found this difference was accentuated by affective pictures that prompted increased autonomic response. While these findings suggest psycho-physiological signature could be used to identify emotional states, it is difficult to accurately infer autonomic response from subjective arousal ratings. Many confounding factors could influence arousal ratings, such as attention, subject bias and past experiences. As a result, subjective arousal would likely not directly correlate with a physiological response.

Fortunately, most studies included in this review employed objective methods for assessing autonomic state alongside brain imaging techniques. Lane, Reiman, Ahern, and Thayer (2001) found emotional valence induced by film content and autobiographical emotion recall generates a similar response in high-frequency HRV and Regional Cerebral Blood Flow (rCBF) in the medial prefrontal, left posterior orbitofrontal and Anterior Insular (AI) activity. Although there were several methodological limitations of this research, such as a small sample size, only female participants, and a lack of ecological validity, this work suggests regardless of the emotional induction method employed, specific emotional states appear to generate consistent physiological responses. Given the potential clinical implications this could have for mental health, the following section focuses on studies investigating specific psycho-physiological signatures for negative emotions.

Psychophysiological signatures of negative emotion

While sympathetic arousal can provide beneficial effects, depending on the demands of social interaction, negative emotional states are generally characterised by enhanced sympathetic activity and pronounced activity in the right AI and ACC. The studies included in this review extend this further by implying physiological signatures for specific negative states.

Miskovic and Schmidt (2010); Schirmer and Escoffier (2010) suggest that anger enhances sympathetic activity, as noted by heart rate acceleration and a decrease in cardiac vagal tone when exposed to threatening facial expressions and tones. Moreover, in contrast to neutral and happy stimuli, they observed anger was also associated with specific neural activity. Schirmer and Escoffier (2010) investigated whether pre-attentive change detection, indexed by EEG Mismatch Negativity (MMN), varies with anxiety and sympathetic activation using an auditory oddball paradigm. They found that MMN was larger for angry deviant tones compared to neutral deviants, and this activity was lateralised over the right frontal lobe. In addition, autonomic response, indexed by a change in heart rate, correlated with increasing MMN amplitudes. While these findings further the argument for emotion-specific physiological signatures, it is challenging to infer this interaction is a necessary function. Miskovic and Schmidt (2010) reported the association between right frontal asymmetry and cardiac vagal tone predicted only 37 per cent of the variability in attentional vigilance for angry faces. This is unexpected as if neural-visceral response was required for preparing the body for mobilisation; one would predict this relationship would explain more of the variability in vigilance. One explanation could be that internal perceptual models moderate the neural-visceral connection and modulate vigilance.

Differences in neural-visceral response may relate to the perceived ability to form an actionable response, a process referred to as approach-withdrawal behaviour in the wider literature. For instance, adverse conditions heighten amygdala activation and facilitate mobilisation by enhancing the sympathetic response, as indicated by increased pupil dilation and Skin Conductance Response (SCR) (de Voogd, Fernandez, & Hermans, 2016). However, it is tricky to generalise findings to clinical populations. Participants were exposed to a conditioning paradigm involving shocks, making them explicitly aware and more likely to demonstrate overt behaviours, such as approach or withdrawal. In contrast, traumatic events in clinical populations may trigger pathological hyper-arousal, which could lead to inaction as opposed to mobilisation (Rabellino et al., 2017).

While predicting behaviour based on neural-visceral interactions is challenging, studies suggest similar interactions for more covert paradigms. For example, images eliciting feelings of disgust were rated more intensely during systole, a cardiac phase augmented by sympathetic activity. This was associated with increased activity in the Prefrontal cortex (PFC) and The Periaqueductal Gray (PAG) (Gray et al., 2012). Disgust requires processing and judging stimuli, which corresponds with increased activity of higher cognitive structures, such as the PFC and sympathetic activity. These findings show that physiological changes influence emotional judgment, which neural systems may partially mediate. Such interpretation can be further supported as individuals with higher baseline parasympathetic tone were associated with activity in the bilateral cuneus and parahippocampus, potentially indicating a more efficient regulatory response; pending reproducible findings in men (O'Connor, Gundel, McRae, & Lane, 2007).

Variations in physiological response can also be noted when comparing similar emotional states. Hofmann et al. (2005) reported increased Heart Rate (HR), SCR and lower RSA for worry. In contrast, anticipation induced lower autonomic activity and appeared to facilitate cognitive processing by up-regulating alpha power in the left cortex. Whilst these findings support the argument for differentiating emotions with neural-visceral responses, the lack of management over order effects likely significantly confounds the results. All participants were exposed to the worry block, followed by the anticipation block; thus, the lower autonomic activity observed in the anticipation block may result from a combined effect. Despite the questionable reliability of the anticipation findings, this study highlights the importance of concurrently measuring both central and autonomic activity to derive specific biomarker patterns and understand underlying mechanisms of emotion regulation.

Similar work has been conducted comparing the neural-visceral response to fear and threat. Fear can be described as a strong, uncontrollable, unpleasant emotion caused by actual or perceived danger. Feared stimuli generate right-dominated hemispheric activity with increased Blood-Oxygen-Level-Dependent Imaging (BOLD) in the amygdala, prefrontal, somatosensory, insula and dorsomedial prefrontal cortices. Vagal autonomic activity lowers and responds in a phasic pattern, correlating with amygdala activation (Ruiz-Padial & Ibáñez-Molina, 2018; Williams et al., 2004; Yang et al., 2007). Meanwhile, a threat could be defined as an expression of intent to injure or punish another. During the labelling of threatening images, Ventro-lateral Prefrontal Cortex (vlPFC) activity and SCR increased (Tupak et al., 2014). However, future studies should aim to reproduce these findings in males, to assess whether there are gender differences in response to fear and threatening stimuli.

Overall, there appears to be a central-autonomic pattern for emotions that are typically associated with response or approach and withdrawal or avoidance. For instance, extreme fear can lead to inaction and hence a reduced vagal autonomic activity mediated by the dorsal motor nucleus (Porges, 2009). Whereas observing indirect threats may compel an individual to act, corresponding to a beneficial heightened sympathetic response. These central-autonomic signatures underpinning emotion may also be universal across age (Mather et al., 2017).

While this evidence may be interpreted as similarities in physiological responses due to specific emotional states; sometimes it can be difficult to differentiate a particular state by relying solely on neural and physiological responses. For instance, activities that similarly influence autonomic response, such as mental stress and exercise, have been associated with similar neural activity in the cerebellar vermis, brain stem and right ACC (Critchley, Corfield, Chandler, Mathias, & Dolan, 2000). It might be that high levels of mental stress and intense physical exercise produce high levels of sympathetic arousal necessary to support the fight/flight response. Still, more subtle regulation of autonomic balance is required during everyday social interaction. For instance, stressful situations induced by task difficulty and degrading performance have been associated with increased sympathetic response, indexed by increased pupil diameter, HR and lateral PFC activity. On the other hand, during stressful situations involving threat, pupil response lowered alongside greater HR and PFC activity

(Mandrick, Peysakhovich, Remy, Lepron, & Causse, 2016). These subtle changes in physiological responses to emotion further the argument, supported by many psycho-physiologists, that autonomic response is not bipolar and that context is an important factor to consider when evaluating change in physiological responses with emotion (Porges & Furman, 2011).

Trait/state effects on emotional processing

Individual differences play an important role in understanding the mechanisms of emotional processing. These individual differences refer to either an individual's current state or physiological trait status. The responsivity of autonomic activity can be utilised as one indicator of the current state and, as a result, can be used to deduce potential effects on emotional processing (Makovac et al., 2015; Park, Moon, Kim, & Lee, 2012; Urry, van Reekum, Johnstone, & Davidson, 2009; Weissman, Guyer, Ferrer, Robins, & Hastings, 2018; Yoshihara et al., 2016). Valenza et al. (2016) suggests an individual's autonomic state during either positive or negative emotional induction can predict theta oscillatory power in the PFC. However, whether these findings result from image preference is uncertain, as self-assessment for emotion induction images was not considered.

Another factor worth considering is determining how much a priori traits can influence emotional processing. For instance, emotional priming can influence response to cognitive decisions. During negative emotional affect, individuals with a high trait of anger experience increased blood pressure, enhanced dorsal pons, visual cortex and parietal activity. PAG, occipital and parietal cortices were also linearly correlated with blood pressure increase (Garfinkel et al., 2016). While it is expected that anger increases sympathetic drive, the experimenters did not examine whether emotional priming successfully induced anger. It is more likely that word priming exposed participants to the perception of anger rather than becoming angry. However, successful emotion induction is a general challenge for the study of emotion, especially for emotion induction experiments that attempt to induce emotion via artificial means. Studies with greater ecological validity, such as O'Connor et al. (2007), suggest autonomic baseline can influence emotional processing, which supports the findings reported by Garfinkel et al. (2016).

Trait neural activity is another aspect to consider, as individuals who express greater EEG asymmetry towards the left frontal cortex are associated with enhanced emotional regulation, as indexed by eye-blink startle magnitudes under the threat of shock (Goodman, Rietschel, Lo, Costanzo, & Hatfield, 2013). However, it is questionable whether eye-blinking equates to the complexity of emotion regulation processes. Eye-blinking is typically used as an autonomic response to a threat as a survival mechanism. Therefore, even if eye blinks were associated with regulation, this may be limited to the response to the threat rather than regulating all emotional states. Despite this, more evidence can be found for the effect of trait neural activity on emotional response. During rest, mid-brain and insula activity predicted functional reactivity during fear conditioning, while the dorsal ACC was found to predict SCR and mid-brain function during fear. This suggests that prior physiological states are important for emotion regulation (Linnman, Zeidan, Pitman, & Milad, 2012).

Physiological responses towards emotion may also be context-dependent, especially in social situations involving in-group and out-group biases. Azevedo et al. (2013) reported participants exhibited more significant bilateral anterior insula activity and pupil dilation when observing people in pain of a similar race compared to a different race or control violet race models. Furthermore, increased implicit racial bias predicted increased left anterior insula activity for own race. Interestingly, the only indicators of race used in this study were morphology and hand colour, which means different outcomes may have occurred if facial expressions were included. These studies highlight that the trait effects should be carefully considered when inducing emotion and utilising physiological signatures for identifying emotion.

Furthermore, physiological status can be manipulated and, in turn, influence individual emotional processing. For example, applying neural stimulation can generate different effects. Makovac et al. (2018) examined whether emotional information differs depending on the location of carotid afferent stimulation. They found right carotid baroreceptor stimulation enhances left insula activity more than left stimulation and produced increased intensity rating of fear and neutral faces. And HRV predicted attenuation of right stimulation of amygdala BOLD response during fear processing.

However, several limitations to this study should be noted. The subjective rating scales were likely difficult for participants to use due to the large-scale range (0 to 100) and, as a result, were probably unreliable and inaccurate. SCR would have been a preferable measure. Finally, while the amygdala has been implicated in many emotion studies, a whole brain analysis may have revealed additional activation changes in complementary areas for emotion regulation, such as the prefrontal cortex.

Psychiatric disorders

Given the evidence for trait/state effects on the neuro-visceral mechanism for emotional processing, it is unsurprising to note altered emotional processing associated with psychiatric disorders, as neuro-visceral differences typically characterise these conditions. Interestingly, the studies revealed some similarities between different disorders. For instance, Post-traumatic stress disorder (PTSD) and depression appear to have altered frontal and amygdala activity during emotional processing. These similarities could account for some overlapping symptomatology between affective disorders, such as apathy, low mood and lack of concentration.

Specific neural-visceral processes have also been reported for affective disorders. Major depressive disorder has been characterised by inefficient recruitment of brain regions associated with autonomic response during the response to emotional content (Lane et al., 2013; Papousek & Schulter, 2001). These studies suggest depression is associated with altered physiological connectivity during emotional appraisal, especially stimuli with negative valence. Yet it is unclear whether this association is due to an attentional bias towards negative stimuli or just a general lack of focus/engagement with stimuli employed in these studies. Furthermore, limited clinical implications can be drawn from Papousek and Schulter (2001), as sub-sections of a personality test were used to assess depression. There are at least several clinically validated depression inventories that could have been employed, such as the Hamilton Depression Rating Scale (Lane et al., 2013) or Beck's Depression Inventory. Overall, it is likely the neural differences associated with depression signify an inefficiency in regulating emotion; however, further work is necessary to understand the origin of this dysfunction (Balconi & Vanutelli, 2017).

With regards to PTSD, low parasympathetic drive has been associated with reduced activity in the vmPFC, amygdala, insula (regions which might be regarded as components of the central autonomic network) and also with motor regions (Rabellino et al., 2017; Thome et al., 2017). Although these physiological differences for PTSD may provide some explanatory leverage for PTSD symptoms, such as heightened levels of reactivity towards environmental stimuli and negative emotional affect, the limitations of these studies should be considered. Studies recommend using RMSSD to index vagal function, as high frequency HRV can be confounded by respiration (Shaffer & Ginsberg, 2017). While Thome et al. (2017) acknowledged the potential influence of current depression, there were several individuals with other comorbidities in addition to PTSD, which influences the ability to characterise PTSD with a psycho-physiological profile. Furthermore, there were more males in the PTSD group and more females in the healthy group, which could have influenced the resting HRV and brain connectivity measures.

Anxiety disorders have also been associated with insula differences correlated with increased sympathetic drive and dorsal ACC activity (Davidson, Marshall, Tomarken, & Henriques, 2000; Drabant et al., 2011; Schaefer, Larson, Davidson, & Coan, 2014). This neuro-visceral pattern may explain for reported heightened awareness of bodily sensations associated with anxiety symptoms. However, there are also notable increases in activity in the Dorsal-medial Prefrontal Cortex (dmPFC) and vmPFC for individuals experiencing anxiety (Wager et al., 2009). Future work should aim to understand how these additional brain regions contribute to anxiety symptoms and explore the potential effects of gender. In summary, there is substantial evidence supporting the role of central-autonomic interaction in the ability to process and regulate emotions. For healthy individuals, this may take the form of valence-specific interactions, while in affective disorders, there is substantial evidence for impaired coordination of CNS and ANS activity. This impairment may relate to these conditions' differences in emotional processing and resulting symptomatology. Further understanding of CNS-ANS interactions may provide new insight into these conditions and potentially new treatment developments.

2.4.2 Therapeutic interventions may adjust central-autonomic mechanisms

Physiological response to therapeutic interventions generally provides comparable results for positive emotional states, such as up-regulation of left lateralised frontal brain activity, amygdala, insula, and ACC. In addition, these changes are accompanied by reductions in anxiety and depression scores. The interventions included in this review will be discussed in the following order: stimulation, meditation, acupuncture, and biofeedback.

Neural function can be manipulated by stimulating the brain with small micro-electrical currents. This is applied to alter neuronal firing within a given region and, as a result, manipulate expressed behaviour. Stimulating the brain also appears to manipulate autonomic responsivity towards emotional content (Yasui et al., 2010). For instance, applying low frequency transcranial magnetic stimulation at the left frontal cortex appears to slow heart rate in response to negative stimuli and increase heart rate in the presence of neutral stimuli (Berger, Domes, Balschat, Thome, & Hoppner, 2017). While larger studies with equal gender distributions are required to verify such findings, alteration of the autonomic system in response to emotion processing can also be found for other neuronal stimulation techniques.

Applying anodal transcranial direct current stimulation during down-regulation of negative content leads to decreased SCR and autonomic arousal, whilst up-regulation of emotion leads to increased arousal and SCR (Feeser, Prehn, Kazzer, Mungee, & Bajbouj, 2014). In addition to up-regulation and down-regulation, this study also included a maintenance condition whereby participants were required to maintain an emotional state without modifying it using reappraisal. This is similar to meditative practice, where acceptance of emotions and situations are typically encouraged rather than active reappraisal. Interestingly, they found no significant difference between andol stimulation and sham for the maintenance condition, suggesting a difference in the neural pathways for appraisal compared to emotional acceptance. This suggests there is likely more than one beneficial psycho-physiological state for facilitating mental health.

Autonomic stimulation studies further support the inter-relation connection between the central and autonomic nervous system and therefore widen the potential for manipulating emotional processing. Transcutaneous vagus nerve stimulation (tVNS) is a non-invasive method of stimulating the vagus nerve by applying an electrical current through the left ear to target the auricular branch of the vagus nerve via cutaneous afferent fibres connecting to the transverse cervical nerve (Bermejo et al., 2017). Left auditory canal stimulation with transcutaneous vagus nerve stimulation has been reported to decrease BOLD response in the amygdala, hippocampus, parahippocampus, middle and superior gyrus and subsequently increase activity in the insula, precentral gyrus, and thalamus (Kraus et al., 2007). The potential to manipulate neural function and consequently alter emotional processing with autonomic stimulatory methods provides promise for expanding therapeutic interventions; however, larger studies are required to verify these effects before such research can be translated into clinical practice.

Surprisingly, the effects on neuro-visceral integration can vary based on meditation practice style. For example, during compassion meditation, HR and BOLD in the dorsal ACC were positively correlated; in contrast, mindfulness meditation lowered HR and activity increased in the ventral ACC (Lutz et al., 2009; Tang et al., 2009). Although it is difficult to extrapolate these findings due to small sample sizes and mostly male participants, the differences between mediation styles could be related to the variation in attentive focus. Kyeong et al. (2017) found that focusing on gratitude, in comparison to resentment, significantly lowered HR, and this was correlated with temporostriatal functional connectivity. Following these interventions, functional connectivity analysis revealed changes in the resting-state DMN were distinguishable from those at baseline. After the gratitude intervention, there was a significant positive correlation between the emotional network resting-state functional connectivity and behavioural scales. While these findings suggest physiological response and intervention outcomes depend on the focus of attention, the autonomic state was indexed by HR in this study, which respiratory effects can confound.

Despite this, meditative styles with similar attentional focus, such as zen and mindfulness, have been found to enhance frontal theta power, which was positively correlated with high-frequency HRV (Takahashi, Murata, Hamada, & Omori, 2005; Tang et al., 2009). Although the sample size may limit the generalisability of these findings, we could infer that how the mind wanders/focuses during meditation may be as important as the mechanical aspects of meditating for modulating neuro-visceral interactions. Expertise in meditation also seems to influence physiological outcomes by enhancing the neurovisceral effects (Lutz, Brefczynski-Lewis, Johnstone, & Davidson, 2008; Lutz et al., 2009).

Acupuncture is a therapy originating from Eastern healthcare practices involving inserting small needles into the upper skin dermis. Often treatment is used for relieving pain and muscle injury and occasionally for treating emotional disorders. The general notion is that the needle insertion into the pressure point reduces the inflammatory response associated with discomfort. Evidence suggests acupuncture influences autonomic function by upregulating parasympathetic drive and increasing EEG power for all frequency bands. Such effects were also related to the subjective sensations reported (Sakai et al., 2007). However, more research is required to validate such claims. Several drawbacks of this study, such as sample size, needle size, and age of participants, limit inferences that can be made to develop clinical practice. Furthermore, large confidence intervals for EEG power across all frequency bands reduce the reliability of these findings.

Biofeedback is another therapy commonly implemented for mental health treatment, which involves presenting users feedback on their internal physiological state, such as heart rate. One study suggested biofeedback increases brain activation in central, cortical, and subcortical autonomic regions during relaxation. Meanwhile, during interoceptive representation, activity is upregulated in the anterior cingulate, amygdala and insula. Additionally, it was reported that variation in the responsivity of the amygdala and insula reflected interaction accuracy and sensitivity to biofeedback feedback (Critchley, Melmed, Featherstone, Mathias, & Dolan, 2002). One major caveat of this study relates to the reproducibility of this work. Participants employed their techniques for upregulating and down-regulating their biofeedback signals, and given that recall for individual regulation strategies was poor, it is difficult to understand how these participants successfully manipulated the biofeedback; consequently, it may be difficult to reproduce the same neural activation in a future study.

2.4.3 Interoception is relevant for emotional experiences

Interoception, the awareness of one's autonomic state, appears to play a key role in emotional processing. On a neural level, this is particularly evident by similar physiological substrates that are active during emotion and autonomic awareness, namely, the insula, amygdala, dACC and frontal regions and responsivity of the autonomic nervous system (Kleint, Wittchen, & Lueken, 2015). Interoceptive awareness and intensity of emotional experiences appear to be positively correlated (Pollatos et al., 2007). However, further work is required to define high and low interoceptive awareness, rather than relying on arbitrary cutoffs for group assignment and disregarding large standard deviations.

Literature on interoception emphasizes the role of the insula in autonomic responses, emotional experience and improved well-being (Kleint et al., 2015; Zaki, Davis, & Ochsner, 2012). While it could be argued these findings are limited to the effects of interoception in women, these observations are consistent with evidence suggesting the insula is a hub processing the balance between interoceptive and exteroceptive information sources (Nguyen et al., 2016). On this basis, neural activity associated with bodily awareness may play a key role in the processing of autonomic arousal and, as a result, one's experience of emotion.

Observed behaviours expressed in psychiatric conditions may therefore be associated with dysfunctional activity between interoceptive and exteroceptive components, leading to altered emotional processing. This notion is mainly supported by Pollatos, Kirsch, and Schandry (2005), which reports that those with high accuracy in heartbeat counting experience heightened levels of autonomic response compared to poor heartbeat counters. In addition, heartbeat awareness accuracy positively correlates with P300 amplitude and autonomic arousal state, with no reported differences for emotional valence. Despite contextual differences in emotions, neuro-visceral circuitry for interoceptive awareness and emotional processing appears to be interrelated, thus, providing further evidence for the relevance of interoceptive awareness for emotional processing. This leads one to question the potential flexibility of these mechanisms by attempting to alter bodily awareness to manipulate one's experience of emotion.

2.5 Summary

This review provides an original contribution to knowledge by summarising the past 20 years of research on neuro-visceral integration during emotional processing. Three core areas for further investigation were identified and used to inform the direction of this thesis.

Overall, the studies in this review suggested that more clarity is required to ascertain a mechanistic understanding of the state/trait interrelation in terms of neural-visceral coupling. Based on the assumption of neural plasticity, it is assumed that state changes can ultimately lead to adaptive brain traits; however, systematic trait change is unclear. Furthermore, an individual's awareness of their internal state appears to modify such interactions. While this indicates possible therapeutic applications, there were a minimal number of studies in this review which examined concurrent neural-visceral interactions, interoception and emotion processing. Further understanding this area is critical for developing future interventions for affective disorders, whereby trait mechanisms are likely to be altered, and interventions facilitate state change. As a result, Chapters 4 to 7 focus on investigating the effects of a breath-focused intervention on neural-visceral coupling. Chapter 5 investigates neuro-cardio-respiratory interactions during breath-focused meditation by examining neural-visceral interactions according to the respiratory cycle. Chapter 6 explores the effects of this intervention on cognitive performance, mood and efferent interoceptive processing, as indexed by heartbeat-evoked potentials. Chapter 7 explores the impact of modulating autonomic function, via resonance breathing, on neural-visceral coupling.

In conclusion, this review highlights the importance of studying neuro-visceral interactions for emotional processing and suggests that an individual's awareness of state may be at least partially responsible for variability in emotional response and, consequently, state of mental well-being.
The following chapter describes the primary methods utilised in this thesis for measuring neural and visceral activity and cognitive function. Chapters 4 to 7 encompass experimental investigations examining neural-visceral coupling in the context of conscious breath control.

Chapter 3

Methodology

This chapter describes and discusses techniques applied in this thesis to investigate neuro-visceral integration. The advantages and disadvantages of techniques will be discussed, as well as solutions to resolve issues associated with these methods. Study-specific methods are described in the relevant methods sections in Chapters 4, 5, 6 and 7.

3.1 EEG: Electroencephalogram

3.1.1 Physiological basis

Information is exchanged across the brain by electrical communication between neurons. At a cellular level, this leads to the release and uptake of neurotransmitters between a pre-synaptic membrane and post-synaptic receptor (see Figure 3.1). Neurotransmitters are released into the synaptic cleft once the minimum voltage threshold of the pre-synaptic neuron is crossed, known as cell depolarisation (Osborne, 1985). The neuron's membrane potential rapidly rises and falls in response to a nerve impulse or action potential from a neighbouring neuron. This transference and processing of information via action potentials and neuronal firing are referred to as neuronal spiking or spikes. Excitatory impulses, termed excitatory post-synaptic potentials, increase the likelihood of the next cell firing, while inhibitory post-synaptic potentials make cell firing less likely by lowering the membrane potential.

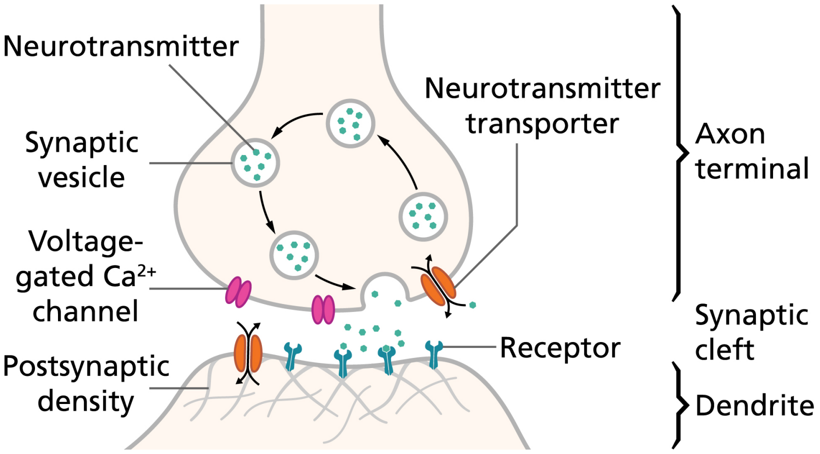


Figure 3.1: Visualisation of neurotransmitters being released across the synaptic cleft from the pre-synaptic membrane to a post-synaptic receptor of a neighbouring neurone. Image adapted from Splettstoesser (2008)

Neuronal firing occurs in a rhythmic pattern leading to the generation of microscopic oscillations, referred to as local field potentials. The synchronized firing of large numbers of neurones can result in macroscopic oscillations, which can be observed in an electroencephalogram (EEG). EEG non-invasively measures the sum of neuronal excitation and inhibition, collectively referred to as neuronal current sources, using an array of scalp electrodes. Dr Hans Berger, a German physiologist, was the first to use EEG to study the human brain in 1929 (Roche & Dockree, 2011). Ever since EEGs have been used extensively worldwide as a clinical diagnostic and research tool for investigating the neural processes underlying cognition and behaviour.

3.1.2 Recording with EEG

Brain activity is recorded using scalp electrode arrays arranged in standardised locations over the main anatomical structures, such as the frontal, temporal or parietal lobes. The most commonly utilised index of standardised locations is the international index 10-20 placement system (Jasper, 1958) (see Figure 3.2). Scalp caps are initially measured relative to the participant's head size to facilitate signal recording accuracy and amplitude strength. Electrodes are attached to the scalp cap worn by the participant and contact with the scalp is enhanced using electroconductive gel.

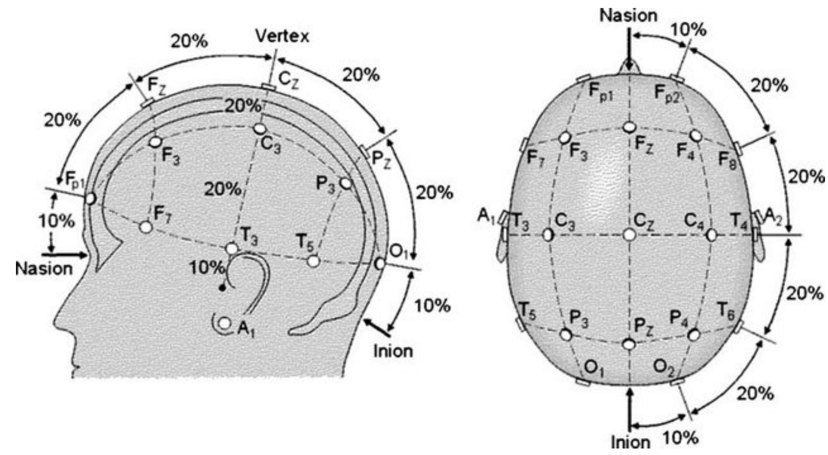


Figure 3.2: International 10-20 system of EEG channels adapted from Sharbrough et al. (1991)

Raw EEG signals are amplified as part of the recording process and are commonly annotated with the occurrence of specific event markers, using voltage triggers during an EEG procedure. For instance, the Su-soku meditation task utilises event markers to represent the onset of the breathing circle stimulus. Pairing EEG signals with markers are beneficial, as the data can be used to infer information relating to the brain at an approximal cortical location with high temporal precision.

3.1.3 Inverse problem

While EEG is useful in measuring neural activity, as it is non-invasive and easy to place electrodes and relatively inexpensive compared to alternative neuroimaging methods, these advantages come at a cost. Due to the pyramidal arrangement of neurones in the cerebral cortex, the neuronal activity recorded with the EEG can be generated from infinite directions. And as a result of volume conduction, the activity from a single source can be captured in many channels. The issue of inferring the origins of neuronal activity from the scalp is referred to as the inverse problem.

Source reconstruction techniques attempt to resolve the inverse problem, whereby the origin is inferred by applying assumptions of brain sources. The single dipole model assumes that the electrical field is created by a point source of the equivalent current dipole. Alternative models, such as the minimum norm solution, estimate the source origin by accounting for multiple current dipoles across the brain (Thakor & Tong, 2004).

Volume conduction effects could be reduced by ensuring efficient electrode placement and increasing the electrode array density. Subsequently, the signal-to-noise ratio can be enhanced by selecting a cluster of neighbouring electrodes before analysis, as performed in this thesis (Oken, 1986).

3.1.4 Pre-processing EEG

As EEG is sensitive to recording brain signals between 5-300μV, this also means that EEG is highly sensitive to outside environmental frequencies that fall within this range (Thakor & Tong, 2004). Therefore, to increase the likelihood of finding true brain signals or, in other words, increase the signal-to-noise ratio, pre-processing techniques must be applied to the data.

Raw EEG is filtered to reduce or separate the noise from the signal of interest. Filters check the signal structure in the time domain and frequency response, known as its Fourier transform. Filters characterise the signal depending on its impulse response type. Infinite impulse response (IIR) filters have internal feedback and continue to respond indefinitely, meaning there is a broad transition between frequencies. IIR filters are commonly used for EEG, as they tend to be more computationally efficient and therefore accessible to users who do not possess higher-end computer systems (see Smith, 2007, for a more mathematical description of digital filtering). For these reasons IIR filters were selected for pre-processing EEG data.

EEG data is typically bandpass filtered to remove slow frequencies of less than 0.1 Hz and frequencies above 40 or 50 Hz. Notch filtering is also applied to remove power line noise in the 50 Hz range. Data should also be baseline corrected to improve signal-to-noise ratio by calculating the average point from baseline and subtracting this from each point in the waveform. The data should be re-referenced to compute the average reference point by taking the average across electrode sites and subtracting this value from each site.

Some EEG artefacts need to be manually assessed to ensure data quality. For instance, ocular artefacts can affect frontal activity, such as eye movements and blinks. These artefacts can be identified by their wave formation, as noted by box shape and sharp wave peaks respectfully (see Figure 3.3).

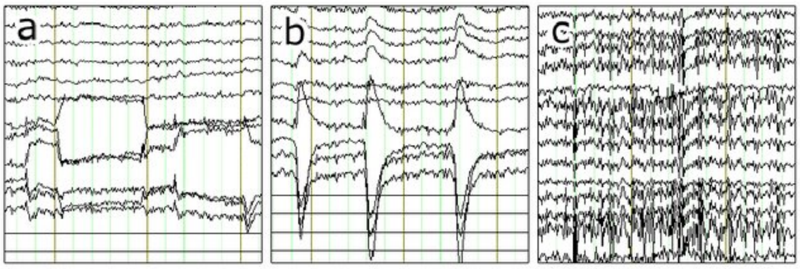


Figure 3.3: Example of physiological artifacts in EEG: a) eye movement, b) eye blink, c) muscle tension. Image adapted from Plass-Oude Bos (2012)

Mathematical algorithms utilise the ocular activity recorded by the electrooculogram to aid the detection and correction of artefacts across trials. Electrooculography (EOG) readings are taken from the additional electrodes placed horizontally and vertically relative to the location of muscles surrounding the eye. The Gratton and Coles algorithm initially estimates correction from EOG and EEG during the experiment and separately estimates correction factors for blinks and eye movement. Finally, after removal, the segments are estimated based on the degree signals are propagated at EOG electrodes (Gratton, Coles, & Donchin, 1983).

An alternative approach to correct ocular artefacts is Independent Compontent Analysis (ICA). ICA reduces data into a small number of patterns, referred to as independent components, to separate noise from brain sources. However, whilst ICA is a statistically valid procedure, there is some subjectivity whereby the user must decide which data to label as an artefact or a brain activity of interest. Therefore, accurately applying ICA requires an adequate level of expertise and, perhaps more importantly, good quality data, large datasets, and suitable computing power to run the ICA (Di Flumeri, Aricò, Borghini, Colosimo, & Babiloni, 2016; Mennes, Wouters, Vanrumste, Lagae, & Stiers, 2010). Based on these reasons, Gratton and Coles was utilised for initial analysis of EEG data and subsequent chapters used an ICA approach for data cleaning.

Muscle movement is more challenging to detect as spatial topographies are not well defined. There has been no standardised approach to resolving muscular artefacts, referred to as Electromyography (EMG) correction methods (Urigüen & Garcia-Zapirain, 2015). Several papers argue whether ICA is effective in removing muscle artefact from data; however, as aforementioned, this approach suffers from concerns relating to data reliability and validity (Muthukumaraswamy, 2013). EMG presents a wide spectral distribution; therefore, reducing noise relating to muscular artefacts may only be possible. Artefact removal algorithms allow the user to specify criteria for marking bad segments in the data, including segments relating to a muscular artefact. The user can subsequently inspect these markers to verify that artefacts have been correctly identified. As a result, muscular artefacts are more likely to be random and short in duration, and only a small number of segments will be dropped during pre-processing. Furthermore, setting an artefact rejection criterion can potentially improve the reproducibility of findings. Suppose an entire channel is very noisy, which could be caused by incorrect electrode placement, head movement or insufficient conductive gel. In that case, these channels can be replaced with mathematically corrected data using topographical interpolation. This estimates the EEG signal for the bad channel by using a combination of values measured from several channels. By utilising this function, channels are not dropped, and therefore signal-to-noise is maintained.

After data cleaning, various approaches can be taken to analyse electrophysiological data due to the richness of the data obtained. The techniques used in this thesis will now be explored in further detail: event-related potentials and spectral analysis.

3.1.5 Event related potentials

Event Related Potentials (ERP) describe peaks and troughs of the electrical activity waveform that are locked to experimental events in terms of time or phase. Trials of experiment blocks are repeated to allow ERPS to be averaged over trials to increase the signal/noise ratio for non-phase locked activity (Dawson, 1954). As ERPs reflect a change in electrical activity in response to a specific occurrence of a cognitive, motor, or perceptual event, they are informative for indicating waveforms associated with cognition during a task (see Figure 3.4).

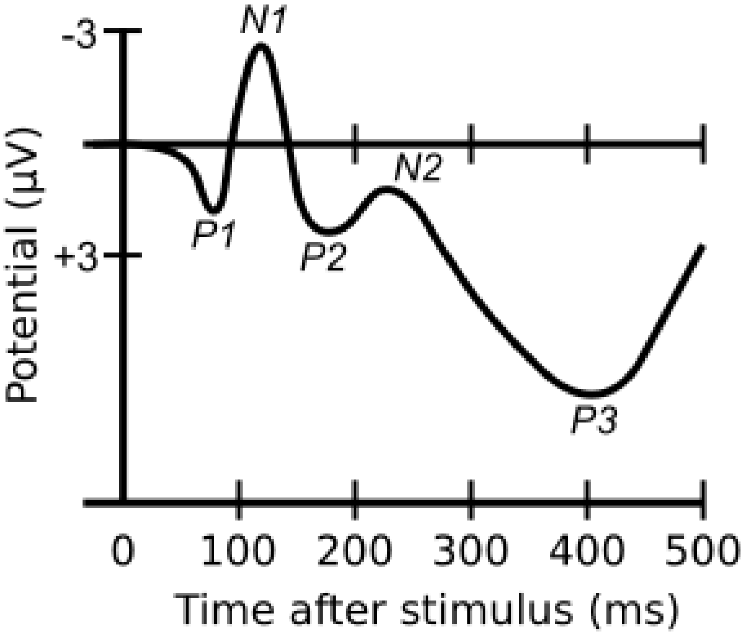


Figure 3.4: Simple graph showing potential and latency differences between event related potential components, adapted from Riera (2014).

The ERP method has identified common topographical locations and time windows during specific tasks. For example, negative deflection at 200ms from stimulus onset, located at the frontal-central location (N200), has been associated with response conflict when presented with incongruent information (Li et al., 2014). This deflection is thought to reflect cognitive control over one's behaviour in response to the presentation of distracting flanking stimuli. Emotionally stimuli also appear to enhance early posterior negativity and late positive potential, compared to neutral stimuli (Abid, Middlebrooks, Rawls, & Lamm, 2021; Kissler, Winkler, & Junghofer, 2009).

Positive deflections associated with stimulus onset are thought to reflect attention resource allocation, which suggests that emotional stimuli require additional resources for information processing. Interestingly, Pollatos et al. (2007) discovered that individuals with high interoceptive awareness exhibited larger positive deflections 300 ms after stimulus onset in response to emotional stimuli. These results suggest ERPs can provide insights into mechanisms governing attention and interacts with internal body awareness, which has high relevance for understanding conditions with aberrant emotion regulation and autonomic function, such as mood and anxiety disorders (Burwell, Malone, & Iacono, 2016). Consequently, this thesis includes analysis of ERPs in the context of understanding the effects of meditation on cognitive control and attention.

3.1.6 Spectral EEG

Spectral EEG refers to decomposing brain rhythms into different frequency bands, for instance, Delta (0.5–4 Hz), Theta (4–8 Hz), Alpha (8–12 Hz), Beta (12–30 Hz), and Gamma (>40 Hz). Neural frequencies have been associated with various cognitive functions; for instance, deep sleep is associated with increased power in the delta frequency band.

Fast Fourier Transform (FFT) can estimate power spectral density by breaking down the signal into constituent sinusoids of different frequencies and determining their relative strengths (Ramirez, 1975). However, switching from the time domain to the frequency domain limits an FFT analysis by the time window selected. Fourier decomposition identifies the local minimum and maximum stationary points in a given signal window to derive a spectral curve. Consequently, FFT assumes the signal varies slowly, which can lead to issues with resolution and spectral leakage, referred to as aliasing effects. Leakage is where the signal is distributed or smeared across many frequencies due to FFT analysing a short slice of a signal. Spectral leakage visually is presented as bumps in the spectrogram rather than a single sharp peak.

Aliasing effects can be reduced by using a tapering function, such as the Gabor filter, hanning window or multitaper method (Thakor & Tong, 2004). Windowing improves frequency response by reducing the amplitude of the time domain signal, which is translated into smoothing the signal in the frequency domain (Scholl, 2016). As an extension of the Fourier transform, wavelet transform analysis includes time-offset weights and the sum of sine/cosine waves. This means that wavelet transform can be informative for research questions that wish to understand how power in the frequency domain changes over the time domain (see Luck, 2014, for a more comprehensive review of EEG).

Changes to EEG spectral amplitude have been associated with cognition, emotion, and psychopathology (Cavanagh & Frank, 2014; Jin, Kim, Kim, Hyun, & Lee, 2017; MacKinnon et al., 2013). Task-related differences in spectral power have been noted for emotional valence processing in depressed patients and studies have shown alpha and theta EEG spectral bands can be used to identify depression when differentiating between depressed patients and healthy controls (Koller-Schlaud, 2017; Mahato, 2020). Clinical trials suggest that increasing internal awareness through meditation can significantly improve symptoms and enhance alpha and theta power (Kang, Sponheim, & Lim, 2022). Based on these findings, EEG spectral power was chosen to be examined in this thesis for the purpose of understanding the effects of meditation therapies on neural activity.

3.1.7 Heartbeat evoked potentials

HEPS describe the electroencephalographic activity that occurs synchronously with the heartbeat, which can provide insight into afferent mechanisms from the heart to the brain (see Figure 3.5).

EEG activity is typically segmented relative to the ECG signal, approximately 200 to 600ms after the R-wave, and then averaged into an evoked potential (in microvolts). The ECG signal is also utilised for the removal of cardioballistic artifacts using an artifact subtraction method, whereby detected R peaks are subtracted from the original EEG signal using a sliding-window approach (Allen, Polizzi, Karkow, Fish, & Lemieux, 1998). Changes in HEP amplitude are proposed to reflect either facilitation or reduction in afferent influence (MacKinnon et al., 2013).

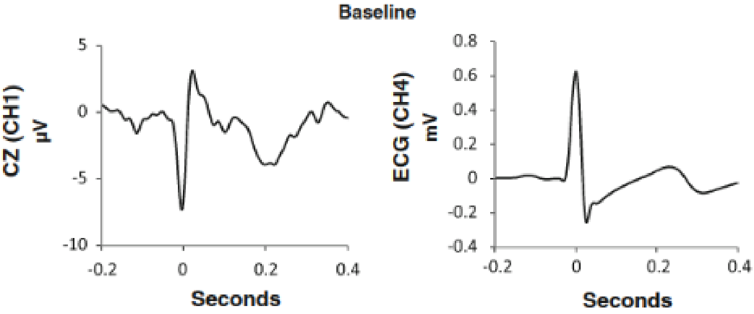


Figure 3.5: Heartbeat evoked potential recorded at the Cz EEG channel during baseline, compared to the ECG signal. Adapted from MacKinnon et al. (2013)

HEP amplitude appears sensitive to attention orientation, especially interoceptive compared to exteroceptive attention and has been previously associated with meditative practice (Kang, Sponheim, & Lim, 2020; Petzschner et al., 2019). However, HEPs are thought to indirectly index interoceptive awareness, as HEP amplitude reflects only the cortical processing of the heartbeat. Several studies have shown a lack of reliable association between HEP amplitude and bodily awareness (Verdonk et al., 2021). As a result, the current thesis investigates HEP amplitude as an exploratory analysis, and instead focuses on understanding interactions between spectral EEG amplitude and autonomic measures.

3.2 Autonomic measures

ANS activity can be inferred from heart rate changes, so that concurrent ECG measurement can be a useful adjunct to EEG measurements. In this thesis, heart rate variability has been utilised for measuring autonomic function, and the respiratory rate has been used as a subsidy measure for interpretation purposes. Consequently, data collection and analysis of these measures have been described below.

3.2.1 Recording with ECG

The heart's activity can be derived from electrocardiograms (ECG) which measure the voltage difference over time between electrodes placed across the body, such as on the left and right wrists. An ECG trace graphically describes the electrical activity in the heart initiated by the sinoatrial node (see Figure 3.6). The initial P wave represents the depolarization of the left and right atrium, which causes the blood within the heart to move from the atrium chambers into the ventricles. A short delay follows this to allow the ventricles to fill adequately. At this point, indicated as the QRS complex, the electrical impulse is received and subsequently conducted from the bundle of His across Purkinje fibres causing the ventricles to contract.

PR interval denotes the time from the beginning of arterial depolarization (P wave) to the start of the QRS complex. It is thought to reflect AV conduction. The ST segment encompasses the time between the end of the QRS complex and the beginning of the T wave, reflecting ventricular repolarization.

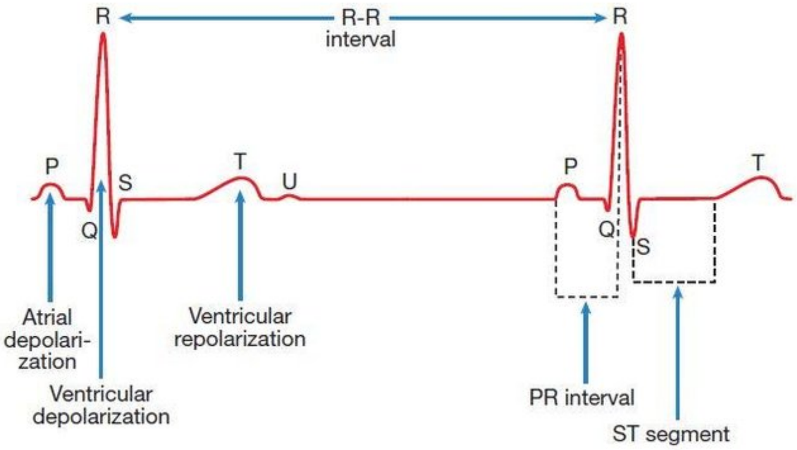


Figure 3.6: A “typical” ECG trace adapted from Belgacem et al. (2012)

The variation in the distance between successive R waves, referred to as heart rate variability (HRV), is an indicator of autonomic function. Sympathetic activity tends to increase heart rate and decrease high-frequency heart rate variability, whereas parasympathetic decreases heart rate and increases high-frequency heart rate variability (Berntson et al., 1997). The sampling frequency of ECG is recommended between 250 to 500 Hz (Malik et al., 1996).

3.2.2 Pre-processing ECG

The analysis of ECG is usually performed offline and must be initially pre-processed before applying analysis techniques. Initially, the data should be cleaned of artefacts typically caused by motion or cardiac fluctuations. In this thesis, artefacts were identified by visual inspection and corrected by applying interpolation; this replaces the data point by estimating its value based on several other points within the same dataset.

3.2.3 Heart rate variability analysis

Various methods have been developed for analysing heart rate variability. According to the Task Force of Electrophysiology, no single measure of HRV has been deemed as a "gold standard" for measuring autonomic state (Malik et al., 1996). Several papers suggest the most appropriate measure depends on the research question and population sample (Allen, Chambers, & Towers, 2007; Hnatkova, Copie, Staunton, & Malik, 1995; Laborde, Mosley, & Thayer, 2017).

Time-domain methods

The time-domain analysis consists of straightforward statistical approaches to analysing heart rate variability over time. Time-domain HRV parameters include: computing the Standard deviation in time between heartbeats (SDRR), Percentage difference between RR intervals (pNN50), and Root mean square of successive differences (RMSSD). It should be noted that RMSSD is identical to the short-term non-linear measure, SD1, which is the standard deviation (hence SD) of the distance of each point from the y = x axis (SD1) and specifies the ellipse's width (Ciccone et al., 2017).

As this thesis is primarily concerned with vagal-mediated changes in HRV, RMSSD was selected as the preferred time-domain measure (Shaffer & Ginsberg, 2017).

Frequency-domain methods

Heart rate variability can be split into low and high-frequency components, corresponding to the function of the autonomic system. High frequency is noted between 0.15 to 0.4Hz and is mostly related to the parasympathetic nervous activity. Power values of HF are derived from the relationship between the respiratory frequency and the cardiac system, referred to as respiratory sinus arrhythmia (RSA). Low frequency ranges from 0.04 to 0.15Hz and reflects both sympathetic and parasympathetic activity (Berntson et al., 1997). LF/HF ratio is an indicator of sympathovagal balance.

The signal series can be divided into separate sinusoidal components of different amplitude, frequency and phase to derive frequency components. This can be performed using either a fast Fourier transform (FFT) algorithm, as previously described for EEG analysis, or Autoregressive modelling (AR). AR uses existing data points to predict future values within the same data series, resulting in the overall spectrum being decomposed into single spectral components without apriori assumptions. As a result, AR is beneficial for analysing short-segmented data, and as a result this was applied to analysis in this thesis.

Non-linear methods

Time-domain and frequency-domain approaches employ linear algorithms to estimate HRV parameters across various time points. However, cardiac data is typically highly transient and non-linear over time; therefore, linear approaches are unlikely to fully encompass the structure or complexity of the data time series. Alternatively, non-linear methods, such as the Poincaré plot, can be applied. Based on previous literature investigating the neurovisceral mechanisms and pharmacological support, this thesis reports the non-linear values derived from Poincaré plots (Kubota et al., 2001; Toichi, Sugiura, Murai, & Sengoku, 1997).

The Poincaré plot (also known as a Lorenz plot) graphically represents the correlation between RR intervals by plotting each RR interval against the following RR interval (see Figure 3.7). This shows how likely it is that an RR interval will be followed by one of similar duration, as well as the range of RR duration in the time series. The spread of these values forms an ellipsoidal shape, which describes the heart rate time series for the given data. Along the ellipsoid, starting at the point of origin, is the length referred to as SD2, which is thought to reflect long-term variability. In the transverse direction is SD1, which is thought to reflect short-term variability. SD1/SD2 is the ratio of these measures.

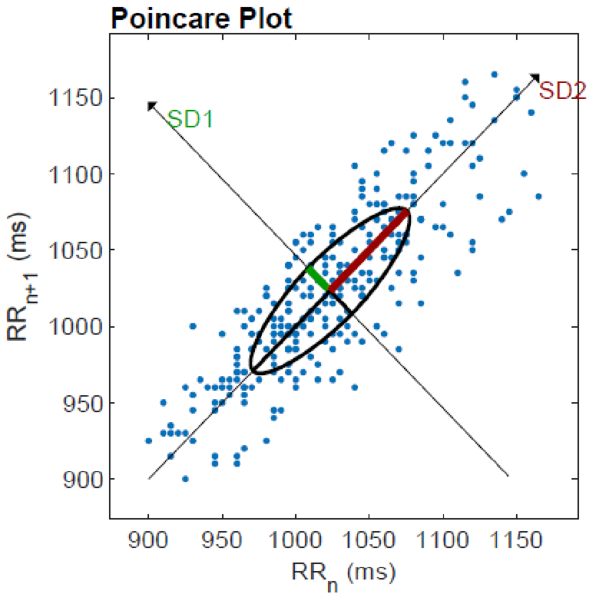


Figure 3.7: Example of a Poincaré plot using Kubios HRV software

Toichi et al. (1997) proposed an algorithmic extension of the Poincaré plot to provide a direct estimate of autonomic function. In pharmacological studies, Toichi et al. (1997) showed that $\log_{10}(SD2 \times SD1)$ is sensitive to cardiac vagal function as noted by the decrease in $L \times T$ under conditions elicited by atropine. Output from this equation is referred to as the Cardiac Vagal Index (CVI). In contrast, SD2/SD1 ratio did not significantly change in response to atropine, but a decrease was noted in conditions elicited with propranolol. As a result of this drug interaction, SD2/SD1 ratio is referred to as the Cardiac Sympathetic Index (CSI).

Based on the pharmacological evidence produced by Toichi et al. (1997) and previous literature Kubota et al. (2001), CVI and CSI measures were employed to examine autonomic function. However, the combined product of SD1 and SD2 is likely noisier than SD1 alone, as slow changes influence the autonomic state over the entire observation period. For this reason, I utilise both non-linear and time-domain measures in this thesis.

3.2.4 Respiration

Due to the strong association between respiration and autonomic activity, the effects of respiration should be considered to determine whether the neurovisceral effects of an experimental condition are confounded by the effects of respiration (Bernardi, Porta, Gabutti, Spicuzza, & Sleight, 2001).

Respiratory rate should ideally be measured using dedicated equipment to ensure the accuracy of results, such as using a respiratory belt around the rib cage and abdomen or mouthpieces (Seppänen, Alho, & Seppänen, 2013). However, if such equipment is unavailable such as in the experiments reported in this thesis, respiratory rate can be derived from high frequency HRV (Churpek, Snyder, Twu, & Edelson, 2018; Cysarz et al., 2018). For analyses involving the respiratory cycle in this thesis, there was a breathing pacer and the rate of the pacer was taken as the breathing rate, and that RSA peak frequency was used to check compliance with the pacer.

3.3 Su-soku meditation

Su-soku meditation attempts to bring the participant into a state of relaxation by requesting the subject to focus on counting their inhalations and exhalations. This form of meditation could be described as focused attention meditation, as attention is sustained on a chosen object, i.e. the breath (Lutz et al., 2008). Other forms of focused attention meditation styles include transcendental meditation, yoga meditation and meditative prayer.

Meditative practice is thought to help calm the mind and body, as indicated by a reduction in breathing rate and heart rate and improved performance on attentional tasks (Dooley, 2009; Kubota et al., 2001; Lutz et al., 2008; Tang et al., 2007). However, participants who are naïve to meditation practice may struggle to maintain task engagement and consequently may not experience all the benefits from the practice. Therefore it is important to consider supplying a point of focus during meditative techniques (Kubose, 1976; Rowe, Shepstone, Carnelley, Cavanagh, & Millings, 2016). In the studies reported in this thesis, participants are represented with an expanding and contracting circle to aid with attentional focus and avoid sudden onset stimulus at each breath intake which might evoke an EEG response.

Chapter 4

Preliminary analysis of Su-soku meditation

This thesis aims to advance therapeutic treatments for mental health by extending the current knowledge on neural-visceral mechanisms. Chapter 2 highlighted that an individual's baseline physiological state might determine the nature of the response to therapeutic interventions.

Therefore, this chapter presents a preliminary analysis, examining the effects of Su-soku meditation from baseline on neural oscillations and autonomic indices. The results from this study will provide insights into the therapeutic applications of neural-visceral mechanisms by extending the current knowledge of baseline state and thereby providing an original contribution to knowledge. In turn, these findings will inform the direction of later chapters.

4.1 Introduction

Scientific literature suggests that meditation, even after brief training sessions, can aid mental well-being and cognitive performance (Lane et al., 2007; Tang et al., 2007; Zeidan et al., 2010). However, the precise physiological mechanisms involved in this process is still unclear. Current evidence indicates that the benefits of meditation are likely products of integration between the central and peripheral nervous system (Berntson et al., 1993; Critchley & Garfinkel, 2018; Zelano et al., 2016).

Based on physiology, one could speculate that low-frequency activity is predominantly involved in the central-autonomic interaction. Support for such postulation can be noted in in-vivo animal-based research on olfactory sense. In this line of research, they refer to the effects of respiration on the brain as “respiratory-entrained brain rhythms” and report that respiratory rhythms facilitate inter-regional neural communication; in particular, theta frequency is involved in promoting cross-frequency coupling (Biskamp, Bartos, & Sauer, 2017; Tort et al., 2018).

Furthermore, meditation studies that concurrently record EEG and report oscillatory spectral analysis to provide similar support for the importance of low-frequency oscillations. One of the most notable findings is that an increase in theta power at the frontal-midline is correlated with meditative experience and high-frequency HRV (Kasamatsu & Hirai, 1966; Tang et al., 2009). Kubota et al. (2001) reported a transient increase in FM theta activity during Su-soku meditation, which involved maintaining a consistent breathing rate and was associated with increased high-frequency HRV. They reported a close link between brain theta oscillatory activity and cardiac autonomic function during breath-focused meditation (Su-soku).

However, the validity of these findings could be undermined by the preselection of participants with frontal-midline theta before conducting the final analysis. In addition, as with most meditation studies, the small sample size in this study likely artificially inflated observed effects.

In a similar study with a larger sample size, Tyrell (2016) reported significantly greater frontal theta power during Su-soku meditation, compared to a relaxing control condition. However, it is difficult to ascertain the validity of these findings due to unequal task durations of Su-soku and control conditions. As a result, a longer duration of meditation may provide a greater opportunity to enter the attentive state (Aftanas & Golocheikine, 2001). Therefore, this study could have been underpinned by a greater likelihood of enhanced frontal theta within the Su-soku condition.

Despite this apprehension, a recent systematic review, which included 187 studies on meditation and EEG, suggested research findings in alpha and theta modulation are robust and are strong neural correlates of meditative activity (Grau et al., 2020). They also note topographical differences and evidence for meditation modulating high-frequency bands, such as beta and gamma. However, these findings are less consistent, which may relate to the focus of meditative practice.

Faber et al. (2017) found undirected Transcendental meditation increased coverage and occurrence of a large-scale microstate, referred to as the default mode network (DMN), compared to transcendent periods and baseline. Frontal-theta activity has been found to negatively correlate with brain regions within the DMN (e.g posterior cingulate cortex and medial frontal regions) (Scheeringa et al., 2008). This highlights the importance of investigating topographical effects of oscillatory activity and broad frequency bands, as variations may allude to differences in neural network activation and, subsequently, effects on cognition.

In terms of explaining the effects of Su-soku meditation, there is some debate as to whether sustained, controlled breathing alone, as practised in Su-soku meditation, can be enough to produce the baroreflex effects. Previous literature highlights that respiratory rate may influence theta power, which likely relates to the hemodynamic interaction effects explored earlier (Busek & Kemlink, 2005). Regular controlled breathing is quoted as “insufficient to produce a decrease in blood pressure” and therefore does not involve the baroreflex mechanism (Joseph et al., 2005). Other studies suggest that controlled breathing may even inhibit parasympathetic nerve activity (Sasaki & Maruyama, 2014). They note differential cardiac effects between controlled breathing at 0.25 Hz and 0.10 Hz. This suggests that the baroreflex effects of Su-soku breathing may be influenced by an individual’s optimal slow breathing rate when there is the most resonance between cardiac and respiratory patterns, as exhibited by RSA amplitude.

Further investigation to confirm the physiological mechanisms of meditative practice has significant clinical implications. Breathing exercises and meditation are commonly prescribed for anxiety and affective disorders, and therapeutic benefits have been noted for improving mood and cognitive processing (Del Negro et al., 2018; Rubia, 2009). Given the current evidence basis suggests that neuro-visceral integration may influence cognition and subsequently lead to improvements in mental health, research in this area has the potential to create new insights into the future treatment for psychiatric disorders. This clinical potential is further compounded by the increasing technological advancement in modern society, enabling increased flexibility and availability of well-being therapies. Consequently, understanding the mechanisms underlying the interaction between respiration, autonomic and central nervous systems is even more critical to uncover due to the potential of future mental health treatments to impact a wider population of people via technology.

This chapter aims to assess the neuro-visceral effects of Su-soku meditation and further investigate how mechanisms governing the central-peripheral system alter during a sustained breathing task.

4.1.1 Research questions and hypotheses

In order to ascertain a foundational understanding of the neuro-visceral effects of Su-soku meditation, this study will primarily focus on measuring the changes in theta oscillatory power and sympathetic and parasympathetic index. As an initial exploratory study, alpha and beta frequency bands have also been included to aid the interpretation of theta power.

Research questions

RQ1. Does Su-soku meditation significantly enhance frontal-midline theta power, compared to a relaxed baseline condition?

RQ2. Is there a topographical difference between theta, beta and alpha oscillatory frequencies during Su-soku meditation?

RQ3. Does change in oscillatory power correlate with change in autonomic function?

Hypotheses

H1. Su-soku meditation will increase frontal-midline theta oscillatory power from baseline.

H2. Frontal-midline theta oscillatory power will continue to increase across Su-soku meditation blocks.

H3. Theta power will be greater in frontal regions, alpha will be predominant in posterior electrodes and beta power will be noted in frontal-central regions.

H4. Changes in autonomic function will correlate with oscillatory power changes.

4.2 Methods

4.2.1 Study design

This study presents a preliminary analysis investigating the changes in neural and autonomic activity in Su-soku meditation from baseline in healthy participants. A pre-collected dataset was utilised to test the research questions set out in the current study. This dataset was initially acquired to explore brain oscillations and cardiac autonomic measures in healthy participants and patients with schizophrenia during both Su-soku meditation and a cognitively demanding task in a cross-over design. A relaxing woodland condition provided a baseline estimate.

4.2.2 Participants

In the original dataset, 46 participants were recruited from the surrounding Nottinghamshire area using advertisement flyers, and three patients with schizophrenia were explicitly recruited from health services. All participants were naïve meditators with little to no experience with meditation. Participants received compensation for volunteering their time for the study. Patients were recruited in the original data set for developing potential therapeutic treatments.

As the current study focuses on understanding neural-visceral interactions, data corresponding to these three patients were removed to create a modified dataset containing healthy participants. In addition, three participants were removed based on noisy EKG data. As a result, forty healthy participants (19 males, 21 females, M age = 25, SD age = 6.10) are included in the current study.

Healthy participants were defined by meeting the following eligibility criteria: i) No personal history of psychotic, medical or neurological illness, ii) No first-degree relative with a history of psychotic illness, iii) Excluded participants with drug abuse (according to the DSM-IV criteria, American Psychiatric Association, 2013) within the last three months or any illicit drug use 48 hours before the EEG session, and iv) No substantial change to nicotine or caffeine use before the EEG session.

G*power estimated a sample size of 36 participants for a repeated measures ANOVA testing oscillatory power across four time periods would provide 95% power to detect a moderate effect ($f = 0.50$) with a significance level of $p<0.05$ (Faul, Erdfelder, Lang, & Buchner, 2007). Additional participants were recruited in the original study to account for the possibility of missing or incomplete data. For a two-tailed bi-variate correlation, assessing the relationship between oscillatory power and autonomic function, G*power estimated a sample size of 29 participants would provide 80% power to detect a large effect (ρH1=0.5) with a significance level of $p<0.05$, while a sample size of 84 provides 80% power to detect a moderate effect (ρH1=0.3) with a significance level of $p<0.05$. All other tests reported in this study are treated as exploratory; therefore, the interpretation of these findings should be treated with caution.

4.2.3 Procedure

As the current study focuses on addressing questions related to brain oscillations and autonomic activity during Su-soku meditation, only the data obtained from the Su-soku meditation task and the woodland block preceding the meditation task were extracted from the original dataset. This preceding block will be utilised directly as the participant's baseline state before Su-soku. For facilitating study replicability, the design and procedure utilised in the original collection of the data are described below.

The study was initially designed to compare the effect of two interventions: Su-soku meditation and the demanding time estimation task (the "Bugs" task), on subsequent brain states. Neural activity was assessed by recording EEG during two probe conditions: an auditory oddball task and a relaxing woodland scene. Participants were randomly assigned to either Task group A or Task group B. Task group A (n = 20) performed Su-soku first, whilst Task group B (n = 20) completed the bugs task first. However, all participants experienced both interventions (Su-soku and "Bugs" task) in an order randomised between participants. A mixed group design controls order effects on attention while accounting for individual variations within participant groups.

Su-soku was performed in three blocks lasting five minutes, with an interval of approximately two minutes between blocks. The two probe conditions were administered before the first intervention to provide a baseline estimate of brain state between the two interventions to assess brain changes induced by the first intervention and after the second intervention to assess brain state after completing both interventions. The timeline of the probes and interventions is shown in Table 4.1. EEG activity was measured throughout the entire series of activities. For this thesis, EEG was recorded during the bugs, and oddball tasks were not analysed. The questions of interest regarding brain state during Su-soku were addressed by analysing EEG recorded during each of the three Su-soku blocks. EEG recorded during the woodland scene immediately preceding the Su-soku blocks was treated as a baseline, as both groups experienced the same preceding task (oddball task). Therefore any changes in the attentive state should be similar between groups. Because prior engagement in the "Bugs" task might influence activity during the Su-soku blocks, assignment to either Task group A or Task group B was treated as a variable of interest.

The breathing rate for each participant was recorded before the commencement of the tasks while they were comfortable and relaxed; this set the pace of the expanding and contracting circle during Su-soku meditation. In addition, all participants were provided with practice sessions of all the tasks before EEG recording. In some cases, the assigned breathing rate was adjusted after practising Su-soku to ensure the participant was comfortable, not hypo/hyperventilating and the sequence of tasks was not interrupted by practice versions. The assigned breathing rate was tailored on an individual subject basis to ensure participants were comfortable and relaxed during Su-soku.

Task Group A	**Task Group B**
Oddball task	Oddball task
Eyes open woodland scene	Eyes open woodland scene
Su-soku breathing meditation	"Bugs" game
Oddball task	Oddball task
Eyes open woodland scene	Eyes open woodland scene
"Bugs" game	Su-soku breathing meditation
Oddball task	Oddball task
Eyes open woodland scene	Eyes open woodland scene

Table 4.1: Task order by group condition

Initially, all participants completed an auditory oddball Go/No go task consisting of low tones (non-targets) and high tones (targets). The targets occurred less frequently than the non-targets. Participants were instructed to respond to the targets by either responding or inhibiting button press actions upon hearing the non-targets (data not reported in this thesis).

Secondly, all participants entered a resting state facilitated by viewing a looped video of a relaxing natural woodland scene. The participants were presented with a fixation cross, which they were required to maintain their gaze upon for two minutes while participants listened to a soundtrack of quiet woodland noises. The looped audio track included sounds of running water and chirping birds. This task occurred in three non-consecutive blocks (Woodland1, Woodland2, and Woodland3) and was designed to induce relaxation; it required nothing other than for participants to observe the screen.

The breathing task consisted of three consecutive sections of Su-soku meditation (Block1, Block2, and Block3); the participants completed Su-soku at different stages in the procedure depending on their task order group. Participants were instructed to count their breaths over a five-minute duration for each breathing section and then inform the researcher of how many breaths were counted. The background soundtrack of woodland noises was kept consistent between the Su-soku and woodland conditions.

The “Bugs” task was an anticipatory reaction task; if the participant timed their reaction correctly after hearing the stimulus (a clicking sound), they would be successful in squashing a bug that was about to appear on the screen. There were five parts to this task that involved differing times between the clicking sound and the bug’s appearance (data not reported in this thesis). Overall, the experiment took participants roughly 60 minutes to complete.

4.2.4 Electrophysiological recordings

A 128-channel BioSemi Active Two system (Amsterdam, Netherlands) was used to detect electrical signals on the scalp at a sampling rate of 512Hz. Scalp electrodes were applied using the standard 10-20 international placement, with the reference electrode on ear lobes to align the electrode sites according to the scalp surface. Horizontal eye movements were monitored using electrodes located laterally to the outer canthus of the left and right eyes. Eye blinks and vertical movements were monitored by an electrode below the right eye and scalp electrode FP2. An electrode was applied to each wrist to measure the participant's electrocardiographic (ECG) trace.

4.2.5 EEG analysis

EEG data were processed offline using Brain Vision Analyzer software (Brain Vision Analyzer, Brain Products GmbH, Gilching, Germany).

Pre-processing

Initially, raw EEG data was referenced to ear lobe channels, then passed through Butterworth Zero Phase filters with a low cut-off of 0.5 Hz and high cut-off of 45 Hz to reduce spectral leakage and aliasing effects. A notch filter of 50 Hz was also applied to minimise powerline noise. The data was down-sampled to reduce the load on available computing power from 510 Hz to 250 Hz, then segmented into the conditions of interest: three blocks of Su-soku meditation and the first woodland block. Data were then manually inspected for noisy channels, and missing data were subsequently estimated by topographical interpolation, using spline interpolation. Condition blocks were then split into equal segments of 10 seconds.

Ocular movements were corrected using the automated Gratton and Coles algorithm method (Gratton et al., 1983). Finally, artefacts within segments were identified using a semi-automatic approach using the following criteria: Allowing for maximal voltage of 50μV/ms, a maximal allowed difference of values in intervals of 200μV, the minimal allowed amplitude of -200μV, the maximal allowed amplitude of 200μV and lowest allowed activity in intervals at 0.5μV.

Frontal-midline activity was defined as the average oscillatory power across Fz, FCz, FC1 and FC2 electrodes (see Figure 4.1). By using a pre-defined region of interest, statistical power loss for a dense electrode array is kept to a minimum (Oken, 1986).

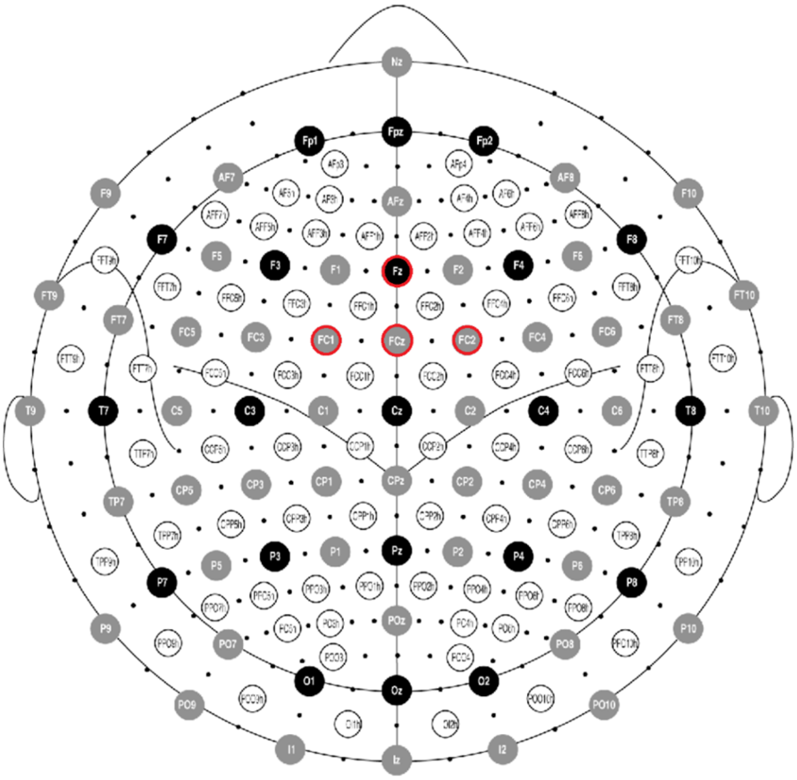


Figure 4.1: 10-20 topographical map presenting the averaged frontal-midline electrodes used in this thesis highlighted in red.

Average spectral power

Absolute power (μV^2) for baseline and the three blocks of Su-soku was calculated using fast-Fourier transform with a resolution of 1Hz, 10 percent Hanning window length, full spectrum, normalized segments and variance correction (see Figure 4.2). The data was averaged across the epochs for individual participants and extracted at the FM-electrodes in the following frequency bands: theta: 4-7 Hz, alpha: 8-12 Hz, beta: 12.5–20 Hz.

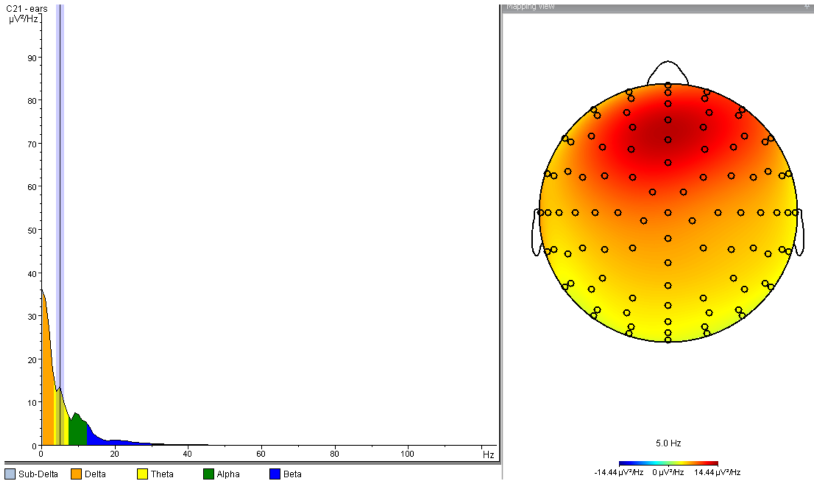


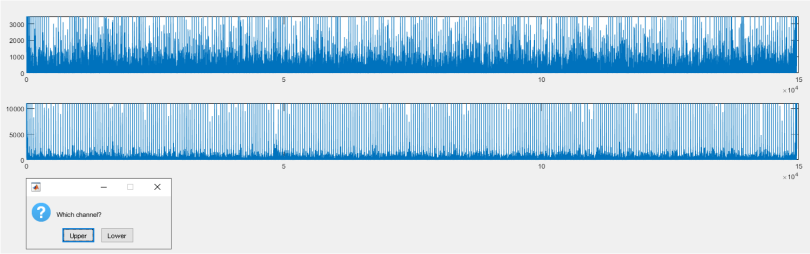
Figure 4.2: Average FFT time-frequency plot for an individual subject at electrode Fz

4.2.6 HRV analysis

ECG was recorded at a sampling rate of 512Hz using external wrist electrodes (BioSemi, Netherlands). The two ECG channels for each participant, block and marker files were exported from Brain Vision Analyzer (BVA), corresponding to the three blocks of Su-soku meditation and baseline condition. Marker files contained time stamps for each event marker and sampling rate information.

ECG pre-processing

The ECG data was cleaned and R peak were identified using an in-house MATLAB script. This script provided a GUI for the user to manually select a high and low cut-off threshold (see Figures 4.3 and 4.4). A high cut-off was defined as removing IBIs that were too long to be a single beat, and a low cut-off was set for where IBIs were too short to be a single beat.



Which channel?

Figure 4.3: The user is presented with the two ECG channels and is asked which channel they wish to use. The lower is chosen in this case, as it has the least amount of noise.

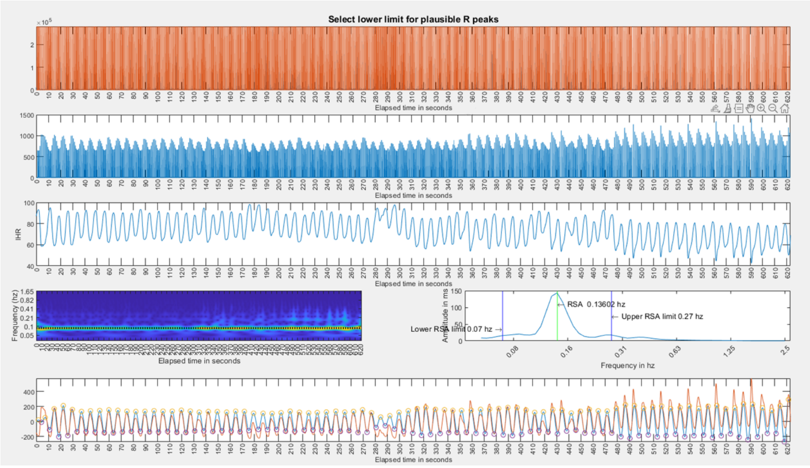


Figure 4.4: Final graphical output from the in-house MATLAB script

After implausible IBIs were removed and verified by the user, a spline interpolation algorithm (pchip) was used to interpolate missing IBI values to give a time series with the same sampling rate as the original ECG. The resampling rate was 360 samples per breathing cycle, regardless of the original sampling rate. This allows for the time course to be preserved after deleting artifactual IBIs by interpolation across missing values. Then, to verify this interpolated correction, the user is presented with the new IBI time series as instantaneous HR values, which are easier to understand intuitively.

A wavelet decomposition of interpolated IBIs was applied to create a time-frequency spectrogram and spectrum (median values at each frequency). This allows Power spectral amplitude (PSA) peak to be visualised and the amplitude at that peak (in ms) to be calculated. This information was then used to generate a range of summary parameters, such as HR and RMSSD, for each Su-soku meditation block. The assigned breathing rate from the S1 intervals was marked as a red line, where we expect an RSA peak to occur if they are breathing according to the assigned rate. A Pearson's Correlation Coefficient analysis was used to determine the relationship between participant respiratory rate and the peak of high-frequency heart rate variability to confirm participants' breathing according to the prescribed respiratory rate. The results suggested a significant positive correlation between prescribed respiratory rate and RSA, $r(31) = 0.94$, $p = <0.005$. These results indicate that most participants were breathing at their prescribed rate.

Data cleaning and estimate of HRV using in-house software were compared with HRV measures generated by ARTiiFACT 2.0 Kaufmann, Sütterlin, Schulz, and Vögele (2011) and Kubios HRV (version 2.2, University of Eastern Finland, Finland) as reported in Appendix B. This comparison indicated that the in-house software achieved a greater signal-to-noise ratio.

Non-linear HRV analysis

An in-house MATLAB script produced a Poincaré plot for individual participants for each condition to derive nonlinear measures. This plots IBIs against previous IBIs to assess the correlation between consecutive intervals (see Figure 4.5). The shape of the ellipse along the line of identity is essential for deriving nonlinear measures. The width of the ellipse measures dispersion perpendicular to the line of identity, which describes the short-term variability in HRV, referred to as SD1. On the other hand, the length of the ellipse, measured by the dispersion along the line of identity, reflects the long-term variability in HRV, known as SD2.

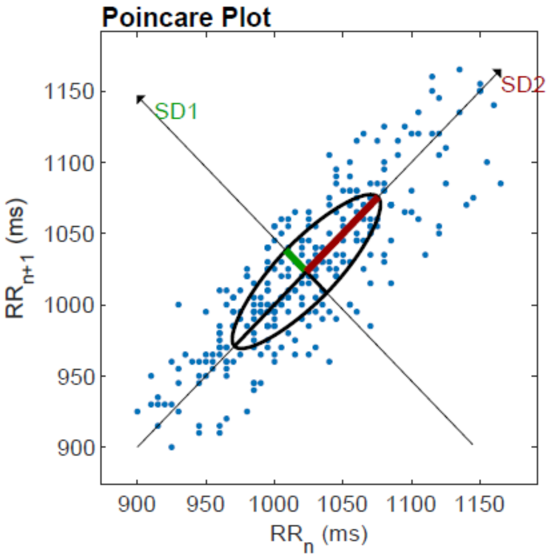


Figure 4.5: Poincaré plot for subject 1

SD1 and SD2 measures were extracted to ascertain autonomic state using Toichi's cardiac sympathetic index (CSI) and cardiac vagal index (CVI) (Toichi et al., 1997). The average autonomic state during Su-soku meditation was calculated by taking the mean CSI and CVI across the three blocks of Su-soku. Likewise, oscillatory power was the average of the three Su-soku meditation blocks.

CSI: The cardiosympathetic index is calculated as follows:

$$CSI = \frac{SD2}{SD1}. \tag{4.1}$$

CVI: The cardiovagal index is calculated as follows:

$$CVI = log[SD2 \times SD1] \tag{4.2}$$

4.2.7 Statistical analysis

All statistical analysis was performed using IBM SPSS 22. Assumptions of the general linear model were initially assessed visually using histograms of the residuals and further verified numerically using normality, homogeneity, skewness and kurtosis testing. Skewness and kurtosis values exceeding -1 to +1 were considered violations of normal distribution (Blanca, Alarcón, Arnau, Bono, & Bendayan, 2017). Suggested deviations from normality were further investigated with quantile-quantile plots and Cook's distance values.

Any Cook's distance values above 0.5 would be considered an outlier and subsequently removed and replaced with linear interpolation. Fortunately, none of the Cook's distance values lay above the criterion, despite some variables showing high kurtosis >1. Outlier detection sensitivity for Cooks distance has been reported as very high; therefore, this approach was chosen to be an appropriate method for objectively identifying outliers to complement the interpretation of QQ-plots (Cousineau & Chartier, 2010; Senthamarai & Manoj, 2015). An alpha level of p = <0.05 was used for all analyses.

4.3 Results

4.3.1 Does FM-theta power increase across time during Su-soku meditation?

Kubota et al. (2001) reported a peak in theta power at Fz during Su-soku meditation, but they pre-selected participants with the highest theta power. Therefore, this initial analysis was performed to confirm the effects of Su-soku meditation without pre-selecting participants based on theta power.

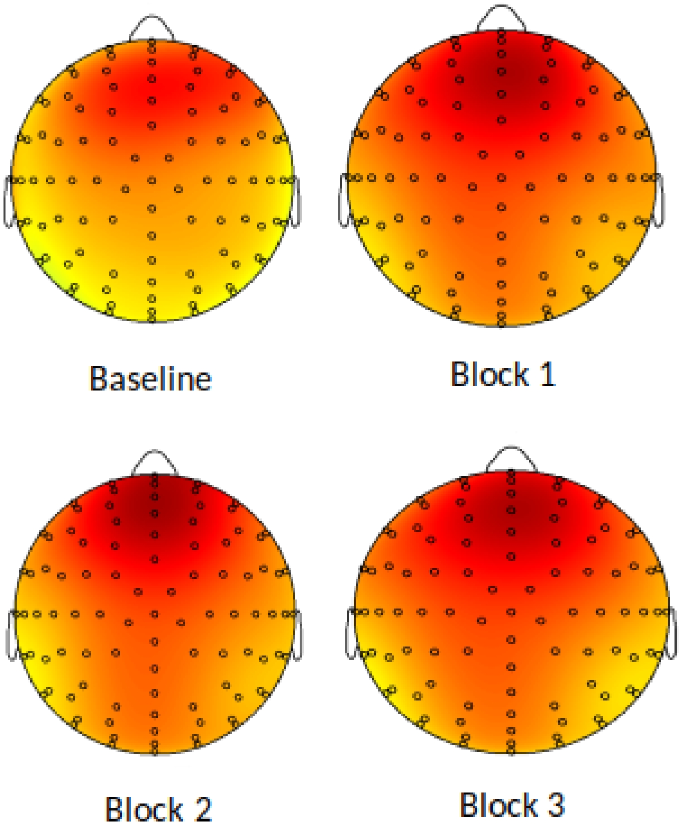


Figure 4.6: Topographical maps of theta power from baseline and Su-soku

Figure 4.6 indicates that frontal-midline theta power appears to increase from baseline and across subsequent blocks of Su-soku meditation. To confirm this effect, a repeated measure 4x4 Analysis Of Variance (ANOVA) was conducted to assess the effects of Su-soku meditation on frontal-midline theta power. Two within-subject factors of time and electrode location were included in this analysis. Time was split into four levels corresponding to baseline and three consecutive blocks of Su-soku meditation. The electrode location was divided into four levels of frontal electrodes corresponding to Fz, FCz, FC1, and FC2. Group allocation (Group A or B) was included as a between-groups variable to assess the effects of task order between groups. Degrees of freedom have been corrected where appropriate, based on epsilon generated from Mauchly's test of Sphericity.

There was a significant main effect of time on FM-theta power, $F(1.74, 66.0) = 4.49$, $p = <0.01$, $\eta_p^2 = 0.106$, but no significant interaction between time and group, $F(1.74, 66.0) = 0.043$, $p = 0.94$, $\eta_p^2 = 0.001$. I conducted a Helmert series of contrasts to test whether the main effect of time indicated a significant effect of the Su-soku task. This revealed that there was significantly more theta power during Su-soku than baseline, $F(1, 38) = 4.78$, $p = 0.035$, $\eta_p^2 = 0.112$, as well as significantly more theta power during the second Su-soku block than the first, $F\ (1, 38) = 4.86$, $p = 0.034$, $\eta_p^2 = 0.113$. There was no significant difference found between block 2 and block 3 for theta power, $F\ (1, 38) = 2.78$, $p = 0.10$, $\eta_p^2 = 0.068$.

There was also a significant main effect of electrode, $F(1.59, 60.4) = 11.30$ $p = <0.001$, $\eta_p^2 = 0.23$, but no significant interaction between electrode and group allocation, $F(1.59, 60.4) = 0.23$, $p = 0.75$, $\eta_p^2 = 0.006$. A Helmert contrast indicated that power at Fz was significantly greater than the more posterior electrodes, $F(1, 38) = 14.38$, $p = <0.001$, $\eta_p^2 = 0.274$; significantly greater at FCz than at the two lateral frontocentral electrodes, $F(1,\ 38) = 0.924$, $p = 0.343$, $\eta_p^2 = 0.024$, and that there was no difference in power between the two lateral electrodes, $F(1,\ 38) = 3.83$, $p = 0.058$, $\eta_p^2 = 0.092$. This supports the inference that theta power increase induced by Su-soku was in the frontal midline (Fz) theta source (see Figure 4.7).

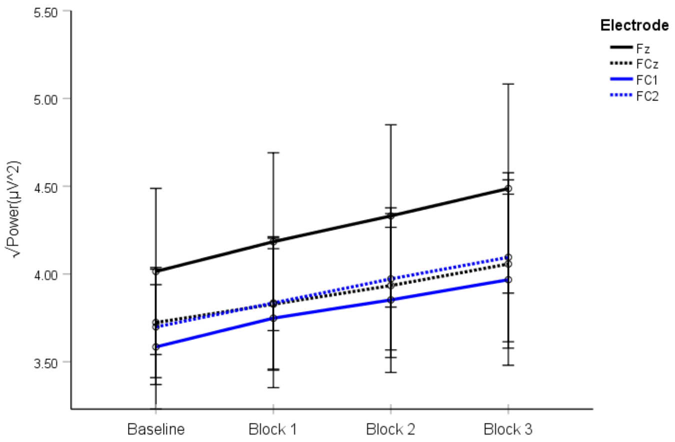


Figure 4.7: FM-theta power during baseline and Su-soku with 95% confidence intervals

There was no significant interaction between time and electrode, $F(\ 2.86,\ 108.6) = 1.03$, $p = 0.38$, $\eta_p^2 = 0.03$ and no significant interaction between time, electrode and group allocation, $F(2.86,\ 108.6) = 0.49$, $p = 0.68$, $\eta_p^2 = 0.013$. This suggests that the time course of variation of Su-soku power was not significantly different between electrodes, as there was an absence of a significant interaction between time and electrode. The between-subject effect of group was insignificant, $F(1,\ 38) = 0.97$, $p = 0.33$, $\eta_p^2 = 0.025$, which means that the effects of Su-soku on theta power were unlikely influenced by task order (see Figure 4.8).

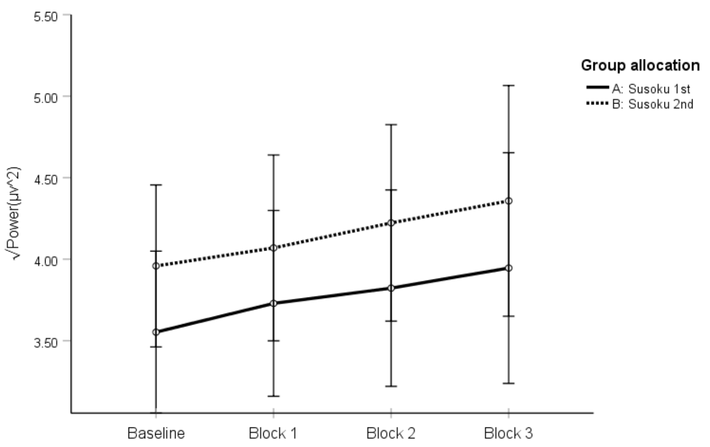


Figure 4.8: FM-theta power for group allocation during baseline and Su-soku with 95% confidence intervals

This analysis has demonstrated that frontal theta increases from baseline to Su-soku and across Su-soku blocks, with a prominent peak in the frontal midline (Fz). However, it isn't easy to interpret an increase in theta power without inspecting higher frequency oscillatory power. Drowsiness causes an increase in low frequencies, such as theta, and a decrease in high frequencies, like alpha and beta power. To aid interpretation of the theta results from Su-soku, a further repeated measures 4x4 analysis of variance was conducted to assess change in frontal-midline alpha and beta power (using frontal-midline electrodes: Fz, FCz, FC1, and FC2).

4.3.2 Does FM-alpha and FM-beta oscillatory power change during Su-soku meditation?

FM-Alpha Power

There was a significant main effect of time on FM-alpha power, $F(1.99, 75.61) = 23.11$, $p = <0.001$, $\eta_p^2 = 0.378$, but no significant interaction between time and group, $F(1.99, 75.61) = 0.46$, $p = 0.63$, $\eta_p^2 = 0.012$. A

Helmert contrast suggested that there was significantly more alpha power during Su-soku than baseline, $F(1,\ 38) = 33.59$, $p = <0.001$, as well as significantly more alpha power in subsequent Su-soku blocks, block 1 vs block 2, $F(1,\ 38) = 10.44$, $p = 0.003$, block 2 vs block 3, $F(1,\ 38) = 5.95$, $p = 0.020$.

There was also a significant main effect of electrode, $F(2.55,\ 96.82) = 10.97$, $p = <0.001$, $\eta_p^2 = 0.224$, but no significant interaction between electrode and group allocation, $F(2.55,\ 96.83) = 0.35$, $p = 0.75$, $\eta_p^2 = 0.009$. A Helmert contrast indicated that power at Fz was significantly greater than the more posterior electrodes, $F(1,\ 38) = 17.82$, $p = <0.001$, while there was no difference in power between FCz and the two lateral electrodes, $F(1,\ 38) = 2.14$, $p = 0.152$. There was a significant difference in alpha power between the two lateral electrodes, with greater alpha power in the right electrode (FC2) compared to the left (FC1), $F(1,\ 38) = 12.60$, $p = 0.001$.

There was a significant interaction between time and electrode, $F(3.67,\ 139.41) = 3.50$, $p =<0.01$, $\eta_p^2 = 0.084$, but no significant interaction between time, electrode and group allocation, $F(3.67,\ 139.41) = 1.68$, $p = 0.16$, $\eta_p^2 = 0.043$. Alpha power significantly increased across Su-soku blocks 1, 2 and 3 for FCz, Level 2 vs later (time) x level 2 vs later (electrode), $F(1,\ 38) = 4.60$, $p = 0.04$, Level 3 vs level 4 (time) x level 2 vs later (electrode), $F(1,\ 38) = 4.77$, $p = 0.04$. However, alpha power was significantly greater across Su-soku blocks for the two lateral electrodes, Level 2 vs later (time) x level 3 vs level 4 (electrode), $F(1,\ 38) = 14.96$, $p = <0.001$, Level 3 vs level 4 (time) x level 3 vs level 4 (electrode), $F(1,\ 38) = 8.11$, $p = 0.007$ (see Table 4.2).

Time	**Electrode**	$\boldsymbol{F(1,\ 38) =}$	$\boldsymbol{p =}$
Baseline vs Su-soku	Fz vs others	1.19	0.28
	FCz vs others	0.38	0.54
	FC1 vs FC2	1.74	0.19
Block 1 vs Block 2	Fz vs others	0.008	0.92
	FCz vs others	4.60	0.04
	FC1 vs FC2	14.96	0.001
Block 2 vs Block 3	Fz vs others	2.63	0.11
	FCz vs others	4.77	0.04
	FC1 vs FC2	8.11	0.007

Table 4.2: Within-subject contrasts for interaction between time and electrode

And finally, there were no significant effects found for group, $F(1,\ 38) = 1.90$, $p = 0.18$, $\eta_p^2 = 0.048$, meaning that the findings reported here were not influenced by task order differences between groups (see Figure 4.9). These results suggest that Su-soku may increase attention, as opposed to enhancing drowsiness, as there was a prominent peak in alpha power at Fz induced by Su-soku. There is also a suggested lateralization effect, as lateral alpha power was significantly greater for the right electrode compared to the left between block 2 and block 3 (see Figure 4.10).

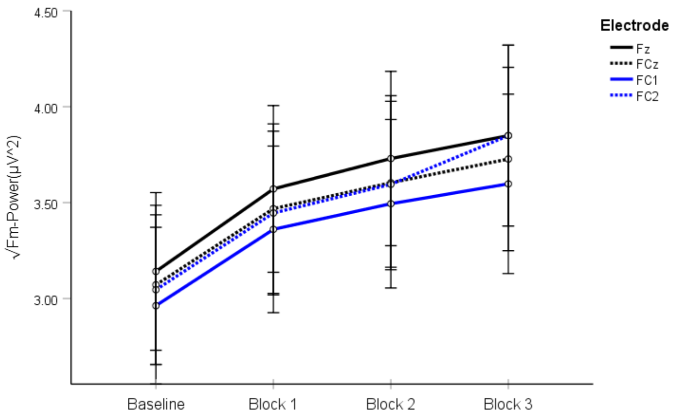


Figure 4.9: Alpha power during baseline and Su-soku by electrode with 95% confidence intervals

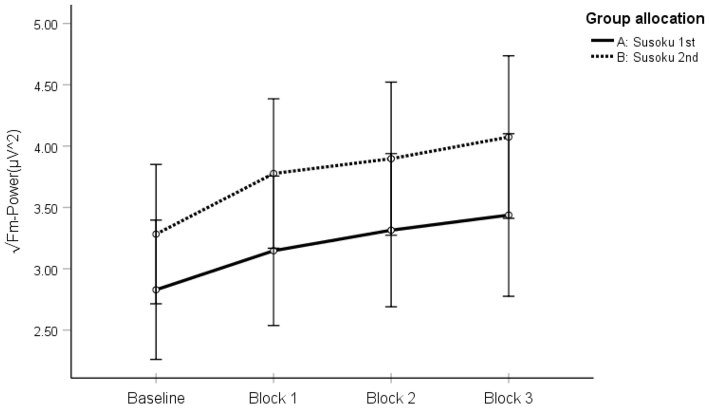


Figure 4.10: Alpha power during baseline and Su-soku by group allocation with 95% confidence intervals

FM-Beta Power

There was a significant main effect of time on FM-beta power, $F(1.87, 71.2) = 11.01$, $p = <0.001$, $\eta_p^2 = 0.23$, but no significant interaction between time and group, $F(1.87, 71.2) = 0.85$, $p = 0.47$, $\eta_p^2 = 0.022$. A Helmert contrast suggested that there was significantly more beta power during Su-soku than baseline, Level 1 vs later: $F(1, 38) = 18.26$, $p = <0.001$, $\eta_p^2 = 0.33$. But there was no significant difference in beta power across the Su-soku blocks, Level 2 vs later, $F(1,\ 38) = 3.811$, $p = 0.058$, $\eta_p^2 = 0.091$, Level 3 vs level 4, $F(1,\ 38) = 0.027$, $p = 0.87$, $\eta_p^2 = 0.001$. However, unlike theta and alpha power, there was no significant main effect of electrode, $F(3,\ 114) = 2.61$, $p = 0.06$, $\eta_p^2 = 0.064$. And there was no significant interaction between electrode and group allocation, $F(3,\ 114) = 0.46$, $p = 0.71$, $\eta_p^2 = 0.012$. While there was a significant effect for group, $F(1,\ 38) = 4.84$, $p = 0.034$, there was no significant interactions between time, electrode and group allocation, $F(4.51,\ 171.59) = 0.81$, $p = 0.53$, $\eta_p^2 =0.021$. There was also no significant interaction between time and electrode, $F(4.51,\ 171.59) = 2.00$, $p = 0.088$, $\eta_p^2 = 0.050$. This means that while beta power was different between groups, this effect did not influence the effects found for time and electrode (see Figure 4.11).

These results indicate that there was a constant increase in generalized beta power from baseline and across Su-soku blocks, and beta power was significantly greater in the first Su-soku block compared to baseline (see Figure 4.12).

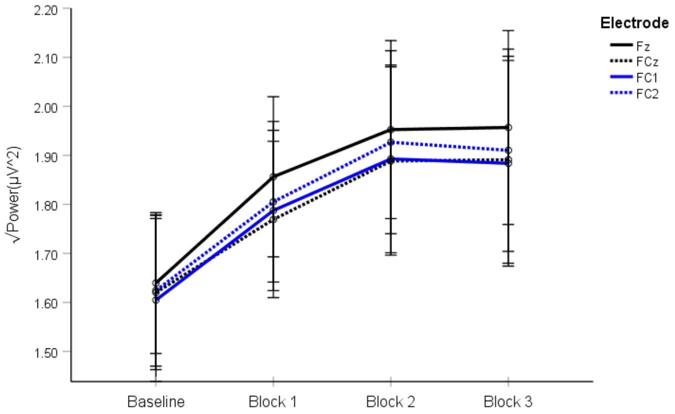


Figure 4.11: Beta power during Su-soku and baseline by electrode with 95% confidence intervals

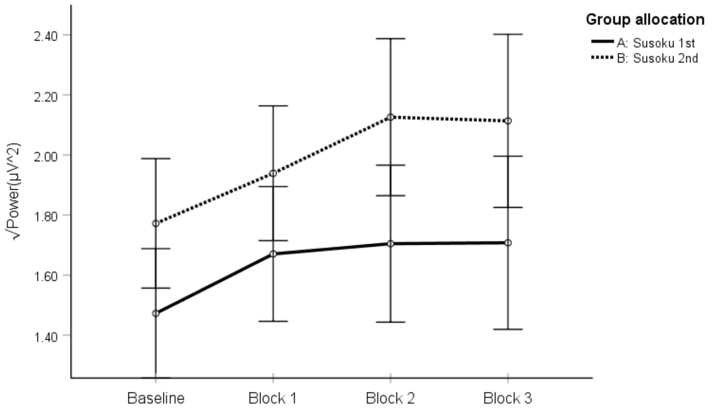


Figure 4.12: Beta power during Su-soku and baseline by group allocation with 95% confidence intervals

So far, Su-soku appears to increase frontal-midline theta, beta and alpha frequencies, compared to the baseline condition. This supports the notion that Su-soku is promoting an attentive state in the participants, and subsequently, this extends the initial findings reported by Kubota et al. (2001).

However, the relationships between frequencies may also be informative for confirming the effects reported here. Theta/beta ratio is considered a marker for executive control processes and has been found to increase during mind wandering compared to focused attention (van Son et al., 2019). On this basis, the frontal-midline theta-beta ratio was investigated.

4.3.3 FM-theta/beta ratio

There was a significant main effect of time on theta-beta power, $F(3,\ 114) = 5.46$, $p = 0.002$, $\eta_p^2 = 0.126$, but no significant interaction between time and group, $F(3,\ 114) = 0.25$, $p = 0.47$, $\eta_p^2 = 0.022$. A Helmert contrast suggested that there was significantly less theta-beta power during Su-soku than baseline, $F(1,\ 38) = 11.39$, $p = 0.002$, but there was no significant difference in theta-beta power across the three Su-soku blocks, block 1 vs later, $F(1,\ 38) = 0.0020$, $p = 0.65$, block 2 vs block3, $F(1,\ 38) = 0.14$, $p = 0.28$.

There was also a significant main effect of electrode, $F(1.21,\ 45.99) = 7.53$, $p =$ <0.001, $\eta_p^2 = 0.165$, but no interaction between electrode and group allocation, $F(1.21,\ 45.99) = 0.24$, $p = 0.67$, η_p^2= 0.006. The follow up Helmert contrast revealed there was significantly more theta-beta power in Fz compared to FCz, $F(1,\ 38) = 7.93$, $p = 0.008$, and significantly greater theta-beta power in FCz compared to the two lateral electrodes, $F(1,\ 38) = 0.063$, $p = 0.013$. However, there was no significant difference in theta-beta power between the two lateral electrodes (FC1 and FC2), $F(1,\ 38) = 0.028$, $p = 0.096$.

There were no significant effects of group, $F(1,\ 38) = 1.03$, $p = 0.32$, η_p^2= 0.027, nor any significant interaction between time, electrode and group allocation, $F(3.62,\ 137.9) = 0.778$, $p = 0.78$, $\eta_p^2 = 0.011$. There was also no significant interaction between time and electrode, $F(3.63,\ 137.9) = 0.57$, $p = 0.82$, $\eta_p^2 = 0.015$. This confirms that the effects found for time and electrode were not influenced by task order between groups (see Figure 4.13). This decline in theta-beta power from baseline and subsequent sustained effect across time supports the concept that Su-soku is promoting a focused, attentive state, as opposed to mind-wandering. The difference in theta-beta power between electrodes at baseline suggests theta-beta power may originate from a more frontal source, which makes sense given that more frontal brain regions, such as the prefrontal cortex, are considered to operate executive functions (see Figure 4.14).

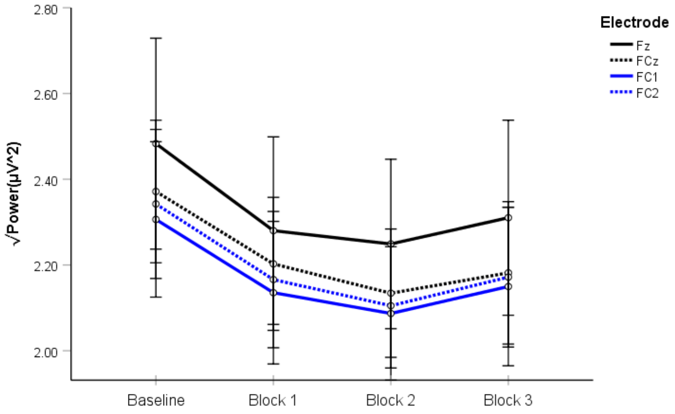


Figure 4.13: Theta/beta ratio from baseline and across Su-soku by electrode with 95% confidence intervals

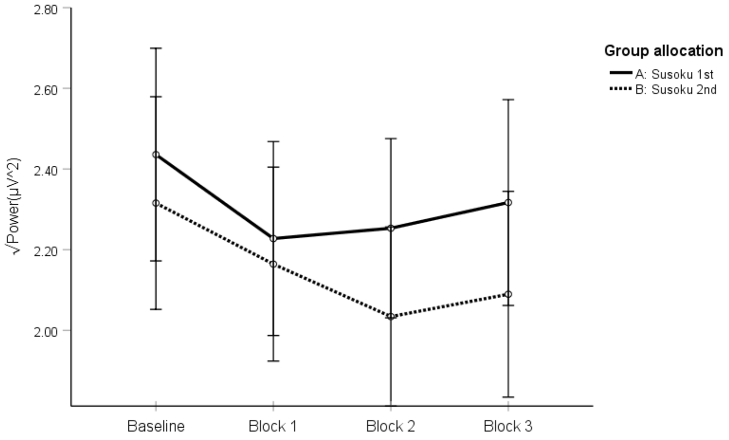


Figure 4.14: Theta/beta ratio from baseline and across Su-soku by group allocation with 95% confidence intervals

This analysis of the theta-beta ratio suggests that Su-soku may enhance top-down attentional control. However, the alpha analysis reported in Section 4.3.2 indicated that FM-alpha increases across Su-soku, which may imply low levels of vigilance, also referred to as tonic alertness or sustained attention. Within the same analysis, a potential lateralization effect was reported, which may indicate that alpha power could increase across Su-soku in other electrode locations. Greater levels of posterior alpha than frontal is a sign of vigilance (Hegerl et al., 2008). Therefore, depending on where alpha is located, this may indicate either drowsiness or alertness. To determine whether there is a reduction in vigilance from baseline to Su-soku and across Su-soku blocks, a repeated measures ANOVA was conducted to evaluate change in Pz/Fz alpha ratio at four levels of time.

4.3.4 Posterior/frontal electrode alpha ratio

There was no significant main effect of time, $F(1.80,\ 68.4) = 1.08$, $p = 0.34$, $\eta_p^2 = 0.028$, and no significant interaction between time and group allocation , $F(1.80,\ 68.4) = 0.35$, $p = 0.68$, $\eta_p^2 = 0.009$. There was also no significant effect of group, $F(1,\ 38) = 2.03$, $p = 0.16$, $\eta_p^2 = 0.051$ (see Figure 4.15).

These results suggest there is no reduction in vigilance from baseline to Su-soku and across the Su-soku blocks, and from visually inspecting mean values, there appears to be greater posterior alpha power than frontal alpha. Although these values may not be significantly different, an additional statistical test is not necessary as the ratio analysis already confirms vigilance is maintained (see Figure 4.16).

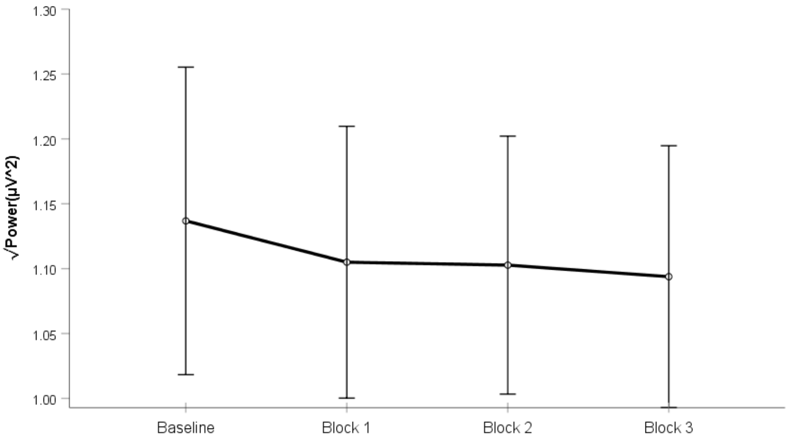


Figure 4.15: Change in Pz/Fz alpha ratio from baseline and across Su-soku blocks

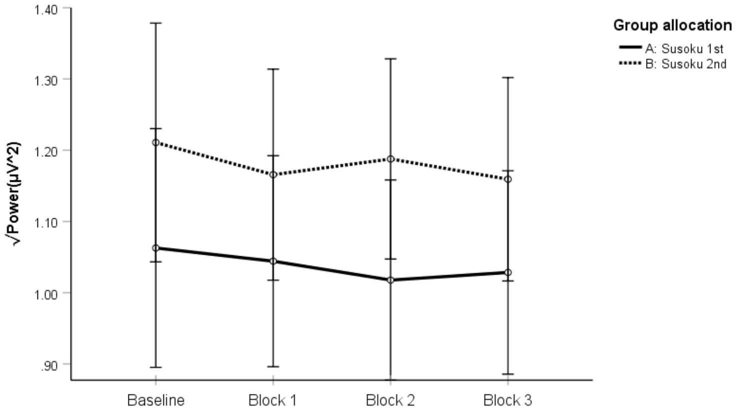


Figure 4.16: Change in Pz/Fz alpha ratio by group allocation across Susoku blocks

These initial results confirm and extend Kubota et al. (2001)'s frontal-midline findings. Kubota et al. (2001) also reported that theta increases in the midline region at the Cz electrode. Therefore, it would be informative to examine further how frontal signals relate to central and posterior sites. Based on previous literature, it is predicted that there will be topographical differences between alpha, beta and theta power (Grau et al., 2020).

4.3.5 Is there topographical variation in oscillatory power across time during Su-soku meditation?

To investigate the variation in oscillatory power from the front to the back of the brain, a separate mid-line analysis was performed using Fz, FCz, Cz and Pz. Three repeated measures 4x4 ANOVA was conducted for alpha, theta and beta frequencies. Time was measured at four levels: baseline, block 1, block 2 and block 3, and electrode: Fz, FCz, Cz, and Pz.

Theta Power

There was a significant main effect of time, $F(1.\ 52,\ 57.9) = 3.83$, p= 0.04, η_p^2= 0.092, and there was no significant interaction between time and group, $F(1.52,\ 57.9) = 0.34$, $p = 0.65$, η_p^2= 0.009. This suggests that the average theta power across the four electrodes was significantly greater at one of the time points. A Helmert contrast revealed that theta was significantly greater in first Su-soku block compared with the subsequent two Su-soku blocks, $F(1,\ 38) = 4.57$, $p = 0.04$, $\eta_p^2 = 0.11$. There was no significant difference in theta power between baseline and Su-soku block 1, $F(1,\ 38) = 3.69$, $p = 0.06$, $\eta_p^2 = 0.089$, and no significant difference between Su-soku block 2 and block 3, $F(\ 1,\ 38) = 2.89$, $p = 0.097$, $\eta_p^2 = 0.071$.

Also, there was a significant main effect of electrode, $F(1.72,\ 65.5) = 24$, $p = <0.001$, $\eta_p^2 = 0.38$, but there was no significant interaction between electrode and group allocation, $F(1.72,\ 65.5) = 0.78$, $p = 0.45$, $\eta_p^2 = 1.34$. This suggests that theta power varies at different electrode locations. A Helmert contrast indicated that Fz had significantly greater theta power than more posterior electrodes, $F(\ 1,\ 38) = 25.98$, $p = <0.001$, $\eta_p^2 = 0.40$, FCz had significantly greater theta power than central (Cz) and posterior sites (Pz), $F(1,\ 38) = 45.76$, $p = <0.001$, $\eta_p^2 = 0.55$. However, there was no significant difference in theta power between central (Cz) and posterior sites (Pz), $F(1,\ 38) = 2.02$, $p = 0.16$, $\eta_p^2 = 0.050$. These results suggest that theta power is more frontally concentrated than generated in the midline or posterior sites (see Figure 4.17).

There was no significant interaction between time and electrode, $F(1.70,\ 64.8) = 1.30$, $p = 0.28$, η_p^2= 0.033, no significant interaction between time, electrode and group, $F(1.71,\ 64.8) = 1.15$, $p = 0.32$, $\eta_p^2 = 0.029$, and no significant between-subject effect of group, $F(1, 38) = 2.06$, $p = 0.16$, $\eta_p^2 = 0.051$. This indicates that the effects found for electrodes do not alter based on task order (see Figure 4.18).

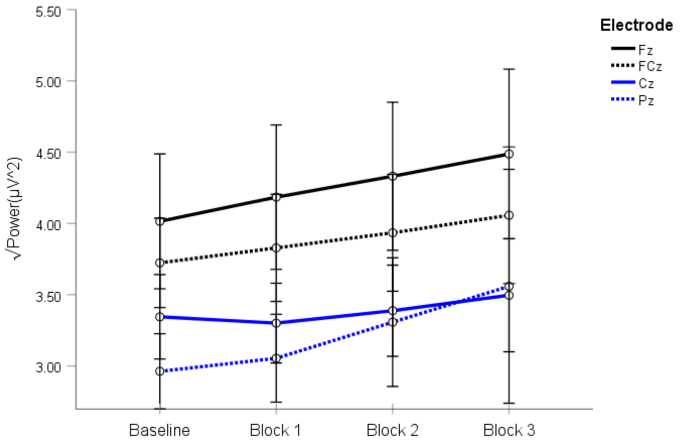


Figure 4.17: Midline theta analysis across time by electrodes

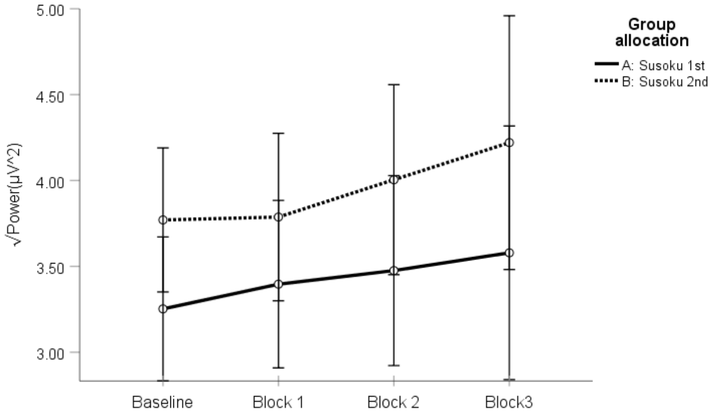


Figure 4.18: Midline-theta across time by group

Alpha Power

There was a significant main effect for time, $F(1.85, 70.6) = 16.74$, $p = <0.001$, $\eta_p^2 = 0.31$, and no significant interaction between time and group, $F(1.86, 70.6) = 0.45$, $p = 0.62$, $\eta_p^2 = 0.012$. A Helmert contrast revealed that the average alpha power across frontal, central and posterior electrodes was significantly greater at block 1 Su-soku compared to baseline, $F(1, 38) = 23.8$, $p = <0.001$, $\eta_p^2 = 0.38$, then at block 2 compared to block 1, $F(1, 38) = 7.01$, $p = 0.01$, $\eta_p^2 = 0.16$, and at block 3 compared to block 2, $F(1, 38) = 0.76$, $p = 0.04$, $\eta_p^2 = 0.11$. This suggests that alpha power generally increases across the brain from baseline to Su-soku and during Su-soku.

However, there was also a significant main effect for electrode, $F(1.36, 51.6) = 8.43$, $p = <0.001$, $\eta_p^2 = 0.18$, and no significant interaction between electrode and group, $F(1.36, 51.6) = 3.36$, $p = 0.06$, $\eta_p^2 = 0.081$. The Helmert contrast indicated there was not a significant difference in alpha power between Fz and FCz, $F(1, 38) = 0.54$, $p = 0.47$, $\eta_p^2 = 0.014$, however there was significantly greater alpha power at FCz compared to Cz, $F(1, 38) = 4.71$, $p = 0.04$, $\eta_p^2 = 0.11$, and the greatest amount of alpha power was recorded at Pz, $F(1, 38) = 16.42$, $p = <0.001$, $\eta_p^2 = 0.30$. These results suggest that although there was a significant average increase in alpha power across all the included electrodes across time, there was, however, a difference in the amount of power at each electrode. Alpha power appears to be greatest posteriorly rather than frontally generated (see Figure 4.19).

There was no significant interaction between time and electrode, $F(3.77, 143) = 1.80$, $p = 0.13$, $\eta_p^2 = 0.14$, and there was no significant interaction between time, electrode and group, $F(3.77, 143) = 1.90$, $p = 0.12$, $\eta_p^2 = 0.05$. There was no significant between-subject effect for group, $F(1, 38) = 2.33$, $p = 0.14$, $\eta_p^2 = 0.06$. These findings highlight that the effects found for time was not influenced by specific electrodes, nor was the power difference at electrodes influenced by time. In addition, task order had no effect on the findings reported (see Figure 4.20).

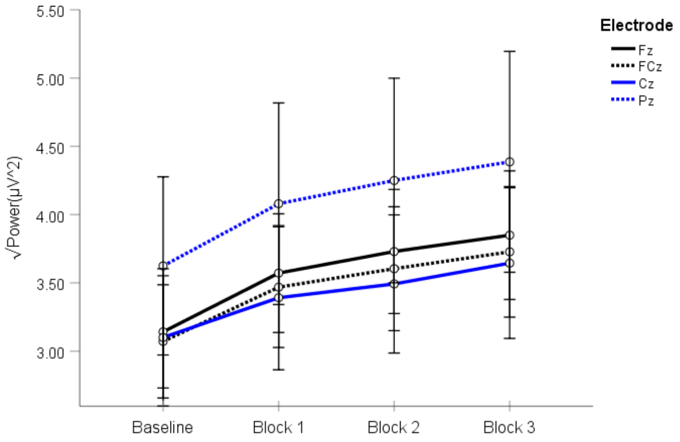


Figure 4.19: Change in alpha power across time by electrode

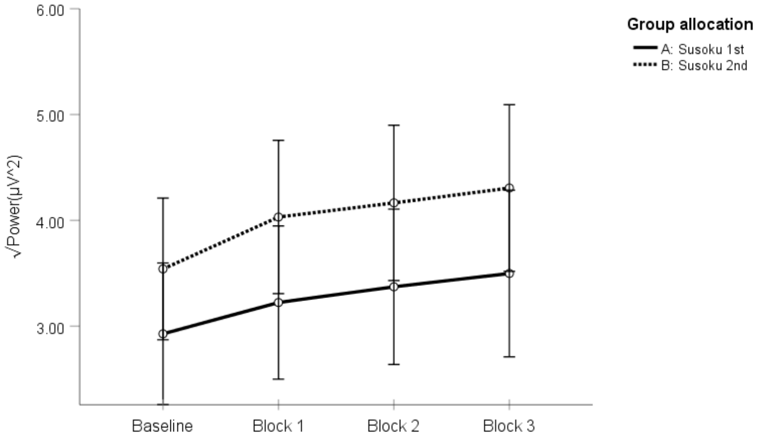


Figure 4.20: Change in alpha power across time by group

Beta Power

There was a significant main effect for time, $F(1.67,\ 63.5) = 10.47$, p =<0.001, $\eta_p^2 = 0.22$, and there was no significant interaction between time and group, $F(1.67, 63.5) = 1.20$, $p = 0.30$, $\eta_p^2 = 0.031$. A Helmert contrast revealed the average beta power over the included electrodes was significantly greater at Su-soku block 1 compared to baseline, $F(1, 38) = 17.51$, p = <0.001, $\eta_p^2 = 0.32$. Beta power significantly increased from block 1 to block 2, $F(1,\ 38) = 5.17$, $p = 0.03$, $\eta_p^2 = 0.12$, but beta power was not significantly different between block 2 and block 3, $F(1,\ 38) = 0.76$, $p = 0.39$, $\eta_p^2 = 0.020$. These findings show that general beta power across the brain increased from baseline to Su-soku and during the first two blocks.

There was a significant main effect for electrode, $F(1.46,\ 55.35) = 8.09$, $p = <0.001$, $\eta_p^2 = 0.18$, and there was no significant interaction between electrode and group, $F(1.46,\ 55.35) = 0.40$, $p = 0.61$, $\eta_p^2 = 0.010$. Further investigation using a Helmert contrast indicated that Fz had significantly greater beta power than the more posterior electrodes, $F(1,\ 38) = 15.96$, $p = <0.001$, $\eta_p^2 = 0.29$, and FCz had significantly greater beta power than Cz and Pz, $F(1,\ 38) = 11.26$, $p = 0.002$, $\eta_p^2 = 0.223$. However, there was no significant difference in beta power between Cz and Pz, $F(1,\ 38) = 2.42$, $p = 0.13$, $\eta_p^2 = 0.009$. These results indicate that beta power was stronger at more frontal sources than central or posterior sites (see Figure 4.21).

There was no significant interaction between time and electrode, $F(3.16,\ 119.9) = 2.19$, $p = 0.09$, $\eta_p^2 = 0.054$, and there was no significant interaction between time, electrode and group, $F(3.16,\ 119.9) = 1.32$, $p = 0.27$, $\eta_p^2 = 0.034$. There was no significant effect of group, $F(1,\ 38) = 5.24$, $p = 0.03$, $\eta_p^2 = 0.12$. Fortunately, the lack of significance found for these interactions highlights the prior results were not influenced by any specific time point, electrode location or task order (see Figure 4.22).

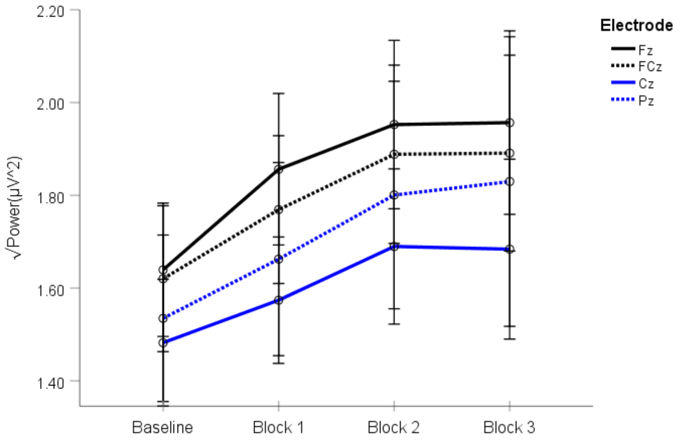


Figure 4.21: Beta power over time by electrode

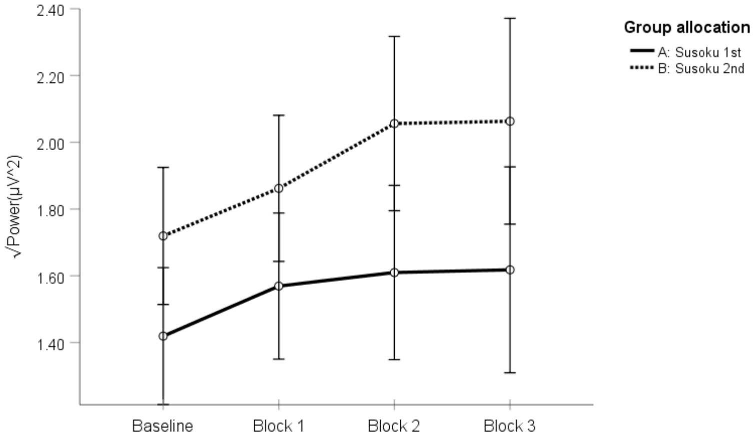


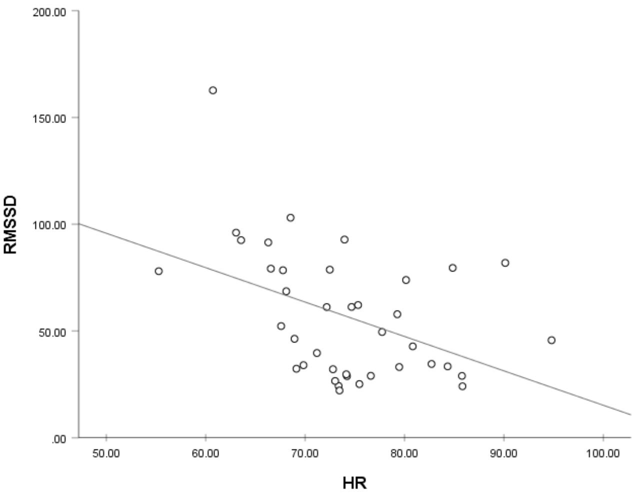
Figure 4.22: Beta power over time by group

4.3.6 Does Su-soku meditation influence autonomic state?

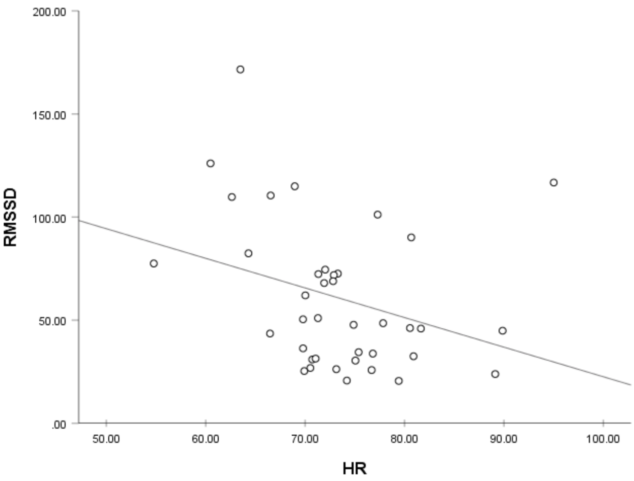
To initially assess whether Su-soku influenced autonomic state, I examined whether there was a relationship between heart rate and autonomic measures during Su-soku using a Pearson's correlation analysis.

There was a statistically significant negative correlation between RMSSD and HR until Su-soku block 3, block 1 $r(39)$ = -0.44, p = 0.006, block 2 $r(39)$ = -0.31, p = 0.04, block 3 $r(39)$ = -0.20, p = 0.2 (see Figure 4.23). Similarly, CVI and HR were also found to be significantly negatively correlated, block 1 $r(39)$ = -0.41, p = 0.008, block 2 $r(39)$ = -0.37, p = 0.02, block 3 $r(39)$ = -0.27, p = 0.10 (see Figure 4.24). However, only a moderate positive relationship was revealed between CSI and HR, block 1 $r(39)$ = 0.20, p =0.21, block 2 $r(39)$= 0.049, p = 0.78, block 3 $r(39)$ = 0.25, p = 0.13. RSA amplitude and heart rate were also significantly negatively correlated, block 1 $r(39)$ = -0.38, p =.02, block 2 $r(39)$ = -0.34, p = .03. , block 3 $r(39)$ = -0.34, p =.03. This means that individuals with a faster heart rate tended to exhibit a lower RSA amplitude (see Figure 4.25). These results are unsurprising based on the literature given that greater sympathetic drive would be associated with a faster heart rate and, therefore, lower RMSSD, CVI and RSA amplitude and greater CSI.

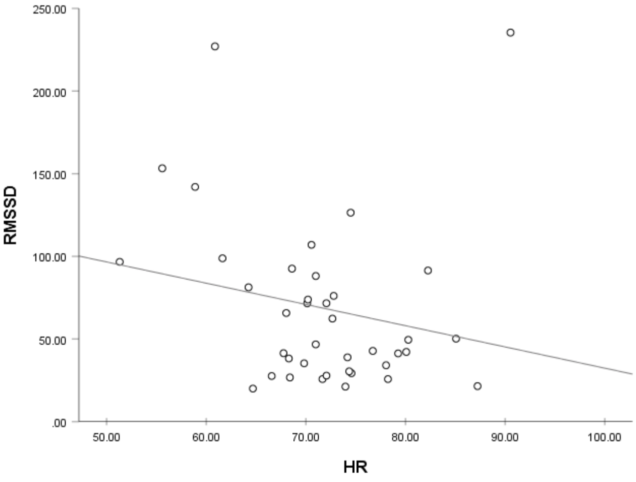
Respiratory rate and heart rate were moderately correlated, although the relationship was not statistically significant, $r(39)$ = 0.23, p = 0.15. This lack of statistical significance may be associated with unequal samples for respiratory rate groups, as the assigned respiratory rate was determined by the participant's resting rate rather than a standardised fixed rate.



(a) Block 1

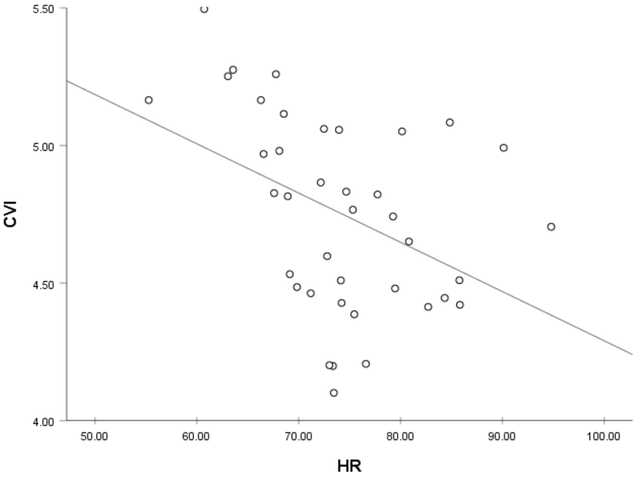


(b) Block 2

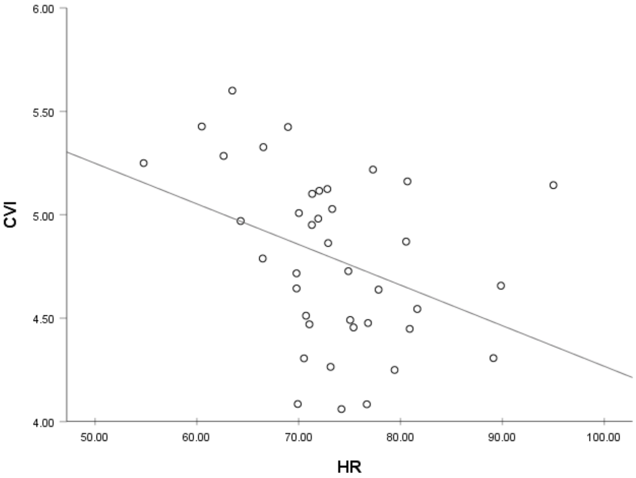


(c) Block 3

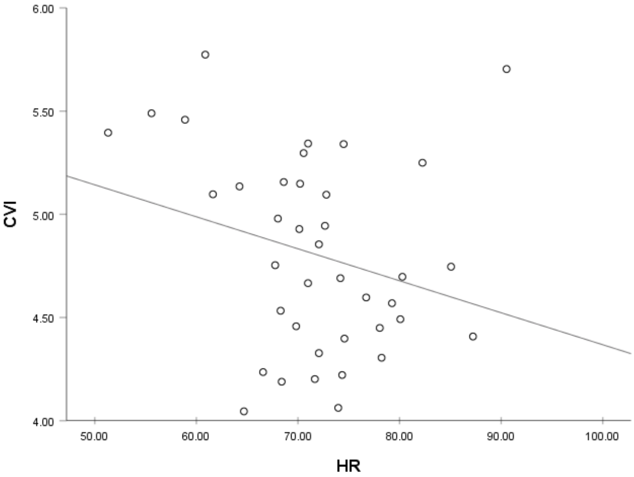
Figure 4.23: Significant negative relationship between RMSSD and heart rate across Su-soku blocks



(a) Block 1

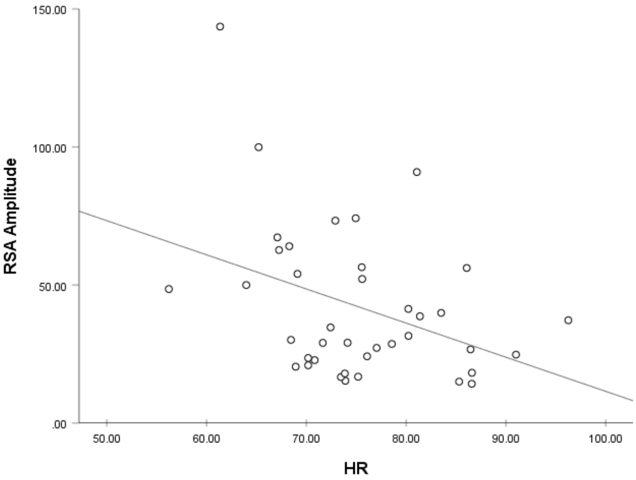


(b) Block 2

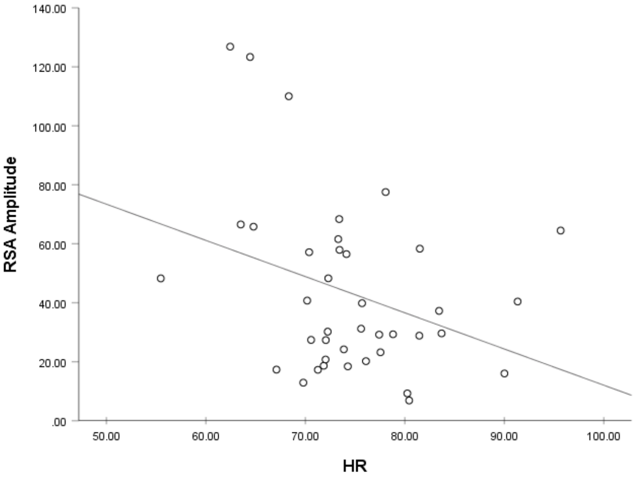


(c) Block 3

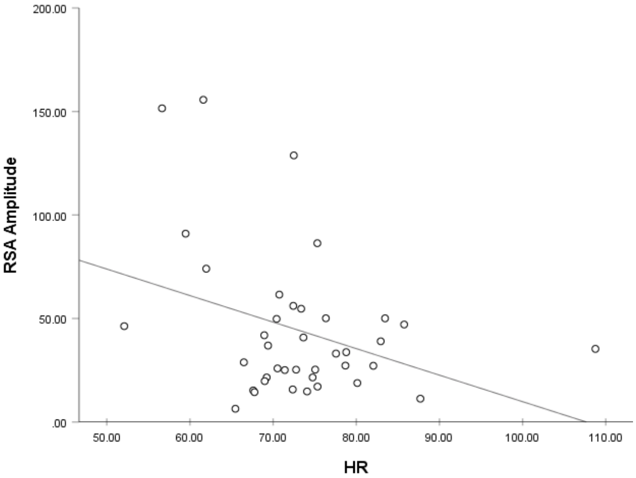
Figure 4.24: Significant negative relationship between CVI and heart rate across Su-soku blocks



(a) Block 1



(b) Block 2



(c) Block 3

Figure 4.25: Significant negative relationship between RSA amplitude and HR across Su-soku blocks

However, the best measure of cardiac vagal activity remains a topic of debate. Toichi et al. (1997)'s pharmacological research on the autonomic system proposed CVI would be the most appropriate measure of cardiac vagal activity. On the other hand, based on a comprehensive review of many studies, Shaffer and Ginsberg (2017) states that RMSSD is the most widely used measure to estimate the vagal meditated change in HRV. RMSSD is identical to SD1, which is utilised in the computation of CVI. RMSSD is also affected less by respiration than RSA; therefore, it could be considered a less noisy measure of vagal tone.

Based on previous research and my initial findings, I investigated whether there is a significant change in RMSSD across meditation blocks. However, since Kubota et al. (2001) employed CVI to test the relationship between theta oscillations and vagal effects, I will perform a secondary analysis using CVI, in addition to the primary analysis using RMSSD, to test Kubota's finding.

A repeated measure three-way ANOVA revealed that meditation block had a significant effect on RMSSD, $F(1.44,\ 53.2) = 4.86$, $p = 0.02$, $\eta_p^2 = 0.12$. And there was no interaction effects between block and group allocation, $F(1.44,\ 53.2) = 0.10$, $p = 0.84$, $\eta_p^2 = 0.003$. Follow-up Helmert contrasts revealed a significant increase in RMSSD from block 1 to block 2, $F(1,\ 37) = 5.53$, $p = 0.02$, $\eta_p^2 = 0.13$, and between block 2 and block 3, $F(1,\ 37) = 4.20$, $p = 0.05$, $\eta_p^2 = 0.10$. There was no significant between-subject effect of group allocation, $F(1,\ 37) = 0.28$, $p = 0.60$, $\eta_p^2 = 0.008$. These results suggest that Su-soku meditation significantly increased vagal tone (see Figure 4.26).

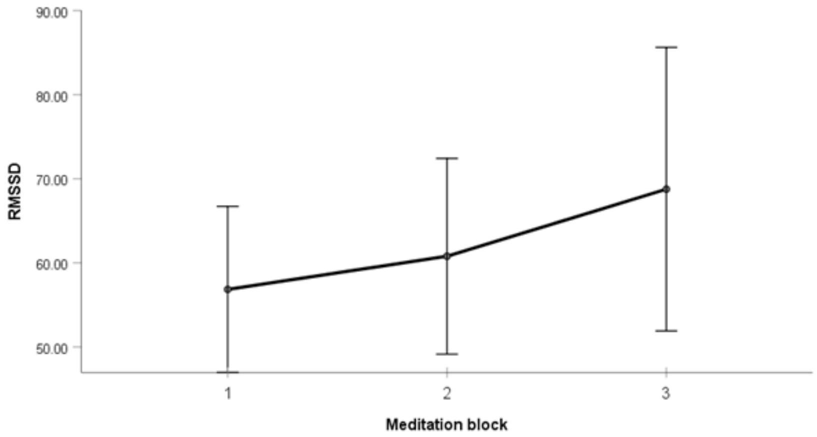


Figure 4.26: RMSSD change across meditation blocks

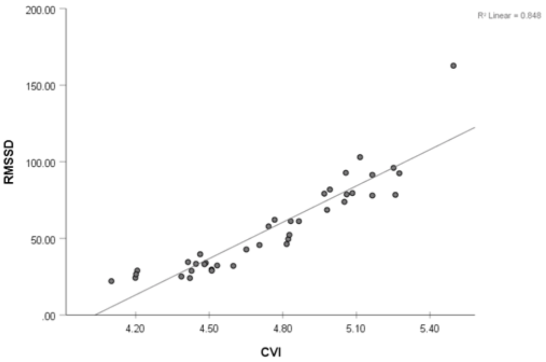
4.3.7 Does change in FM-theta correlate with change in autonomic function?

Following these findings, I investigated whether there was a similar underlying mechanism governing the increase in theta power and vagal tone by conducting a repeated measure multivariate analysis of variance with a within-subject factor of time at three levels of meditation block (block 1, block 2, and block 3) and two measures of theta and RMSSD. Theta power reflects the average power across the four frontal-midline electrodes (Fz, FCz, FC1, and FC2). It is expected that if theta power and vagal tone are associated with the same underlying mechanism, then they should be similarly affected by Su-soku meditation.

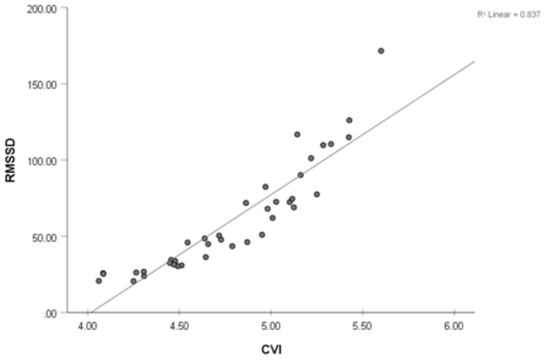
There was a significant main effect for time, $F(4,\ 144) = 3.36$, $p = 0.012$, $\eta_p^2 = 0.085$, but no interaction between time and group allocation, $F(4,\ 114) = 0.095$, $p = 0.94$, $\eta_p^2 = 0.003$. Uni-variate tests showed that time significantly affected theta power, $F(1.77,\ 63.68) = 3.74$, $p = 0.03$, $\eta_p^2 = 0.094$, and RMSSD, $F\ (1.44,\ 63.68) = 4.47$, $p = 0.03$, $\eta_p^2 = 0.11$.

Follow up Helmert contrasts revealed that theta power significantly increases from block 1 (M = 3.93, SD = 1.28) to block 2 (M = 4.06, SD = 1.35), $F = 4.71$, $p = 0.04$, $\eta_p^2 = 0.12$. However, there was no significant difference in theta power between blocks 2 and 3 (M =4.18, SD=1.59), $F = 2.62$, $p = 0.15$, $\eta_p^2 = 0.06$. RMSSD was also found to significantly increase from block 1 (M = 56.21, SD = 30.39) to block 2 (M = 60.28, SD = 35.85), $F = 5.20$, $p = 0.03$, $\eta_p^2 = 0.13$. And there was no significant difference in RMSSD between blocks 2 and 3 (M = 67.91, SD = 51.91), $F = 3.73$, $p = 0.06$, $\eta_p^2 = 0.09$. These results suggest that Su-soku meditation similarly influences the vagal tone and frontal oscillatory activity in the theta frequency.

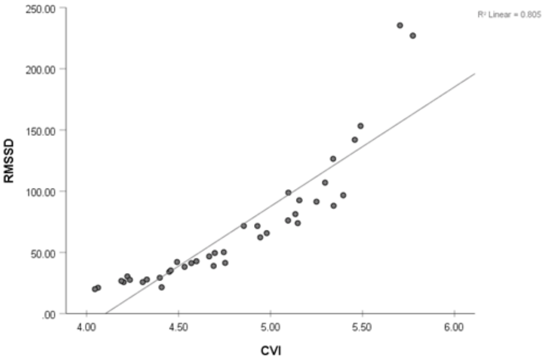
Kubota et al. (2001) reported increasing FM-theta during Su-soku meditation positively correlated with cardiac vagal index. Given the recent analysis suggesting that Su-soku meditation increases RMSSD, I wanted to explore the relationship between RMSSD and cardiac vagal index visually. As RMSSD is primarily used to estimate vagal tone, I suspect these measures to be tightly correlated (see Figure 4.27).



(a) Block 1



(b) Block 2



(c) Block 3

Figure 4.27: Relationship between RMSSD and CVI during Su-soku

As predicted, because of the logarithmic relationship between CVI and RMSSD, these scatter plots exhibit a curvilinear relationship between these variables. Based on this verification and previous work by Kubota et al. (2001), I investigated whether a change in theta power may be associated with a change in CVI and CSI during meditation. Based on previous RMSSD findings and literature, I expect Su-soku meditation to significantly influence theta power, CVI and CSI. I further investigated the association between vagal tone and theta power changes by repeating the above analysis with CVI and CSI.

Interestingly, the analysis revealed no significant main effects for time, $F(6, 142) = 2.05$, $p = 0.06$, $\eta_p^2 = 0.081$ and there was no significant interaction between time and group allocation, $F(6, 142) = 0.11$, $p = 0.99$, $\eta_p^2 = 0.005$. These results suggest that Su-soku does not significantly influence CVI and CSI (see Figures 4.28 to 4.30).

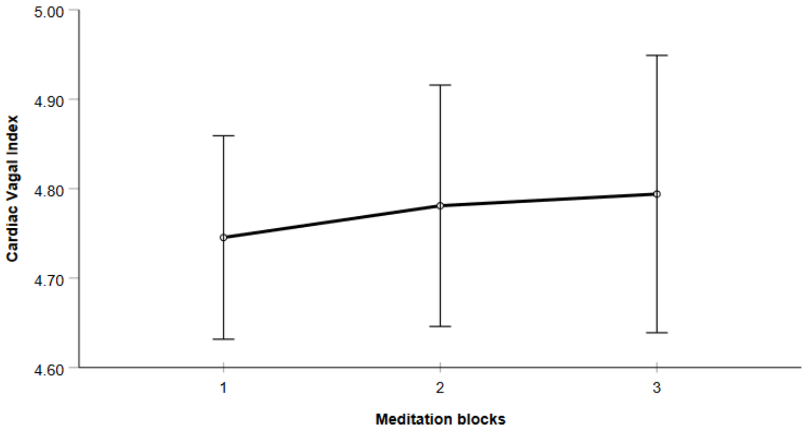


Figure 4.28: Change in cardiac vagal index during Su-soku

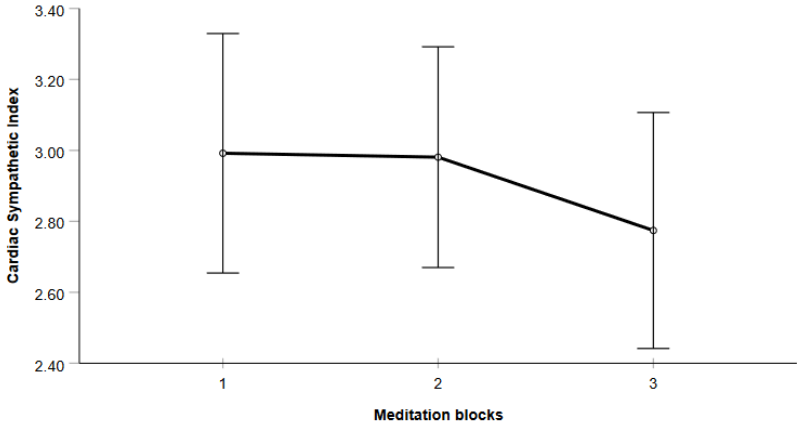


Figure 4.29: Change in cardiac sympathetic index during Su-soku

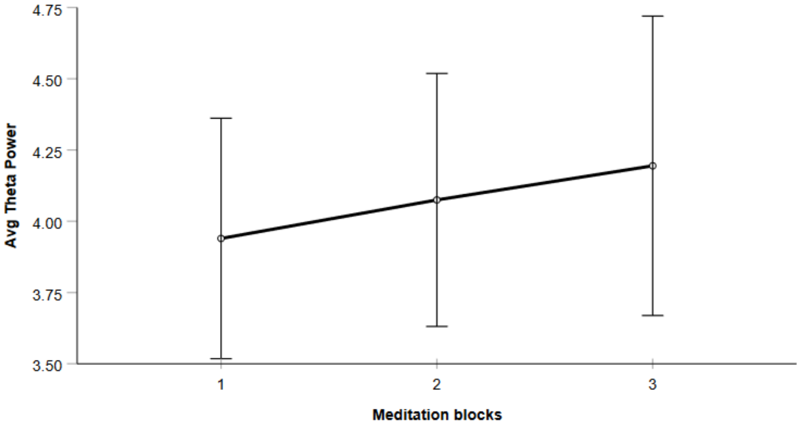


Figure 4.30: Average frontal theta power from baseline and across Su-soku

RMSSD is a less noisy metric of vagal tone than CVI, as the combined product of CVI is influenced by slow changes in the autonomic state occurring over the entire observation period. Therefore, the insignificant results reported here may be related to physiological noise in these vagal metrics. As mentioned in Chapter 3, the most appropriate measure for the autonomic state depends on the population and research question. As this thesis is primarily interested in vagally meditated changes during meditation, the RMSSD analysis likely provides the most reliable estimate of autonomic changes during Su-soku (Shaffer & Ginsberg, 2017).

Based on the previous analyses, I examined whether there was a relationship between theta power and autonomic activity across Su-soku blocks. While RMSSD and theta power appear to increase during Su-soku, as indicated by the ANOVA analysis, the Pearson's correlation revealed a moderate but statistically insignificant relationship between RMSSD and theta power, block 1 $r(39) = 0.19$, $p = 0.23$, block 2 $r(39) = 0.22$, $p = 0.17$, block 3 $r(39) = 0.26$, $p = 0.12$. Similarly, heart rate and theta had a moderate but not statistically significant correlation, block 1 $r(39) = 0.17$, $p = 0.30$, block 2 $r(39) = 0.14$, $p = 0.37$, block 3 $r(39) = 0.20$, $p = 0.21$.

RSA amplitude and theta were correlated, but this was not statistically significant, block 1 $r(39) = 0.11$, $p = 0.50$, block 2 $r(39) = 0.12$, $p = 0.46$, block 3 $r(39) = 0.061$, $p = 0.71$. Pearson's correlation revealed respiratory rate and theta power to be negatively correlated, although this was also not statistically significant, $r(39) =$ -0.50, $p = 0.76$.

In sum, these findings suggest that while vagal tone and oscillatory power appear to be influenced by Su-soku meditation in the current study, they do not appear to have a significant linear correlation.

4.4 Discussion

4.4.1 Main findings

This study examined whether Su-soku meditation significantly affects the central-autonomic network by assessing frontal-midline oscillatory power and autonomic activity changes. The results confirmed the prior hypotheses that Su-soku meditation increases FM-theta power from the baseline and across subsequent Su-soku blocks. This effect was concentrated in frontal and central locations rather than posteriorly, indicating prefrontal cortex involvement. Further exploratory analyses, including higher frequency bands and additional electrode locations, indicated that Su-soku induces a more focused, attentive state. Compared to baseline, the theta-beta ratio declined, and alpha power was significantly greater at posterior sites, suggesting Su-soku promotes vigilance rather than drowsiness. I predicted frontal-midline theta power would be associated with vagal tone because baroreceptors are sensitive to a sustained breathing rate, subsequently leading to an alteration within the central-autonomic network. Initially, I examined the relationship between HR and HRV measures across Su-soku sessions to determine the effects of Su-soku on the autonomic state. Most of these correlations suggested a significant negative correlation, which supports existing literature on sympathetic-parasympathetic balance reflected in heart rate. A follow up ANOVA suggested vagal tone (indexed by RMSSD) significantly increased during Su-soku meditation. When investigating the association between oscillatory power and autonomic function, RMSSD and frontal-midline theta power exhibited a significant increase from meditation blocks 1 to 2, suggesting that similar underlying mechanisms may govern central-autonomic changes during Su-soku. Based on the findings of Kubota et al. (2001), I also examined the relationship between CVI, CSI and theta power during Su-soku. While this study could not confirm previous findings, this was likely due to differences in experimental design between the studies and the physiological noise of CSI/CVI autonomic measures. I also did not find any significant correlations between theta power, RSA amplitude, HR and respiratory rate.

Despite the unclear relationship between neural and autonomic signals, the findings for theta and beta power suggest, in contrast to the relaxing woodland scene, Su-soku induces a reliance on top-down processing. These results are typical of sustained attention tasks; however, a change in beta power is inconsistent with meditation studies (van Son et al., 2019; Yoshida et al., 2020). According to a recent literature review, most meditation studies suggest modulations in alpha and theta frequencies are strong neuronal correlates of meditative activity, irrespective of meditation style and expertise (Grau et al., 2020). Some studies also report spectral modulations in higher frequency bands, such as beta and gamma (Braboszcz, Cahn, Levy, Fernandez, & Delorme, 2017; Chandra, Sharma, Sharma, Jha, & Mittal, 2017). However, the direction of effect on spectral power is still unclear. This is unexpected, as studies suggest meditation improves cognitive control, yet there appears to be mixed evidence for meditation modulating high-frequency bands associated with cognitive control (Del Negro et al., 2018; Grau et al., 2020; Rubia, 2009; Spitzer & Haegens, 2017; Tang et al., 2015). There could be many reasons for this. One possibility is that meditative practice is required to modulate the amplitude of high-frequency bands (Braboszcz et al., 2017; Cahn, Delorme, & Polich, 2012). Several studies indicate that low frequencies, such as theta, can modulate the amplitude of faster oscillations. The phase of slow oscillations can couple with the rhythm of nasal respiration, and phase-amplitude plots reveal that beta amplitude couples to a preferred phase of the respiratory cycle (Herrero, Khuvis, Yeagle, Cerf, & Mehta, 2018; Lockmann, Laplagne, & Tort, 2018; Zelano et al., 2016). Based on this evidence, the effects of Su-soku meditation on the central-autonomic system may initially delineate from the phase synchrony between slow oscillations and respiration.

According to the predictive coding framework, respiratory-driven oscillatory dynamics influence the process of active inference (Friston, 2010; Friston, FitzGerald, Rigoli, Schwartenbeck, & Pezzulo, 2017). As the breathing rate is maintained, the uncertainty between future states is reduced, thus minimising the usual free energy expenditure for predicting future states. More simply, a consistent breathing state optimises the trade-off between information gain and expected utility. Consequently, physiological resources can be reallocated, leading to an increase in frontal-midline low-frequency oscillatory power to facilitate cognitive control (Cavanagh & Frank, 2014; Jensen & Mazaheri, 2010). By attending to the internal state and maintaining a constant breathing rate, the free energy principle proposes this leads to an increase in top-down neural activity, favouring precision weighting towards incoming sensations. This is especially relevant for the role of alpha power, as this frequency band has been specifically considered as a top-down control mechanism for inhibiting conflicting information (Jensen & Mazaheri, 2010). On the other hand, frontal-midline theta power has previously been correlated with prediction error, and response-locked frontal theta has been linearly related to uncertainty during reinforcement learning task (Cavanagh, Zambrano-Vazquez, & Allen, 2012).

Generally, this concept of meditation influencing mechanisms associated with attentional control has been a long-standing theme within meditative literature for several years (Lutz et al., 2008; Lutz, Mattout, & Pagnoni, 2019). On this basis, I speculate that: directing internal attention towards the respiratory rhythm attenuates the phase synchrony between low-frequency oscillations and respiratory rhythm, which increases frontal-midline beta power leading to a decrease in arousal state via the inhibition of noradrenaline at the locus coeruleus. By maintaining a constant respiratory rate, frontal-midline low-frequency oscillatory power increases and, as a result, facilitates the cognitive benefits found in meditation. However, even if this mechanistic speculation holds true, numerous unanswered questions remain. For instance, what is the optimal state of phase-synchrony, and how can it be achieved? Some studies suggest that synchrony should occur dynamically, based on behaviour, state and task-dependant (Kocsis, Pittman-Polletta, & Roy, 2017). Therefore, the amount of synchrony may be highly contextual and individualistic.

4.4.2 Limitations

However, there are limitations of the current study to be noted. A potential limitation of the study was the allocation of participants to task-order dependent groups, as the original study designed the experiment to allow a cross-over comparison of the effects of Su-soku with an arousing task (the "Bugs" task, not analysed in this thesis). Consequently, the current study utilised the resting period immediately before Su-soku to provide a consistent baseline for estimating the effects of Su-soku irrespective of the task order. Considering group allocation had no significant effect on oscillatory power between baseline and Su-soku, nor the rate of change over the three Su-soku blocks, this indicates task order had minimal effect on the results reported in this chapter. Earlier, I suggested that a change in beta power during Su-soku could indicate a reliance on top-down processing. However, it is still being determined whether this is solely related to meditation practice due to inconsistent findings on beta power in wider meditation literature. In the current study, participants were required to count their breaths during Su-soku and report this to the experimenter at the end of the session to verify their assigned breathing. Therefore, a confounding variable could be that some participants struggled with breath counting during Su-soku, which may lead to the more attentional or cognitive effort. This may be one explanation for why the results indicate heavy reliance on top-down processes, as opposed to just the effects of Su-soku meditation alone.

Due to the lack of a respiratory belt, the current study could not directly verify the breathing rate during data acquisition. However, there is no reason to think that a belt would have allowed a more accurate prescription of a comfortable breathing rate. Kubota et al. (2001) had used a fixed rate of 17 breaths/min for males and 18 breaths/min for females. During protocol development, it was discovered that some participants found that 17 breaths/min was uncomfortably fast. Therefore, an upper limit of 17 was elected, allowing participants to breathe more slowly if a slower rate felt comfortable. This was determined before data acquisition by asking each participant to count breaths while breathing comfortably for 1 minute after placement of the electrode cap. Following this, participants engaged in practice sessions whereby the circle's rate of expansion and contraction depended on the participant's reported breathing rate. For participants who reported 17 or more breaths per minute, this rate was fixed at 17. All participants reported feeling comfortable at the prescribed breathing rate. Participants appeared to be breathing at the prescribed rate, as indicated by the significant positive correlation between the prescribed breathing rate and respiratory sinus arrhythmia.

Kubota et al. (2001) selected participants and trials based on observable FM-theta power in Su-soku. As a result, only 12 out of 25 participants were included in their analysis, increasing the potential of a type 1 error. In contrast, regardless of theta induction, all eligible participants were examined in the current study. While this reduces potential bias in the analysis, increased variability requires a larger sample size to detect an effect. Based on the initial power calculation, this correlation analysis was likely underpowered. However, in a preliminary analysis that achieved adequate power, Su-soku meditation similarly influenced the vagal tone and frontal oscillatory activity in the theta frequency, which suggests neural-autonomic interaction warrants further investigation.

4.4.3 Implications and conclusions

Despite these minor limitations, this study provides preliminary evidence that the cardio-respiratory-neuronal interaction during Su-soku meditation is worth further investigation. The results presented in this chapter support previous studies that propose Su-soku meditation up-regulates frontal-midline theta power and vagal tone. In addition, this occurred irrespective of meditation practice and successful theta induction. Regarding the exploratory work, including additional frequency bands and topographical locations, Su-soku meditation appears to facilitate an attentive, vigilant state compared to a relaxing baseline condition. Furthermore, Su-soku-induced changes in beta amplitude may be related to respiratory-driven oscillatory dynamics as a consequence of maintaining attention towards the breath. To further investigate the potential underlying mechanisms of this process, the next chapter examines how oscillatory power and vagal tone changes according to the respiratory cycle during Su-soku meditation.

Chapter 5

Neural-visceral coupling during respiration

Recent studies propose optimal neural-visceral coupling may depend on respiratory-driven oscillatory dynamics (Herrero et al., 2018; Lockmann et al., 2018; Zelano et al., 2016). In Chapter 4, I suggested that cardio-neural-respiratory interactions may explain the unexpected findings between oscillatory power and autonomic indices. Phase synchrony between respiration and low-frequency oscillations may trigger respiratory-driven oscillatory modulation of high frequencies, which are involved in coordinating attention resources.

As an extension of Chapter 4, this chapter investigates how oscillatory power and autonomic activity are modulated by respiration during Su-soku. This work provides an original contribution to knowledge by being one of the first meditation studies to apply circular statistics to examine neural-respiratory interactions. Findings from this chapter will inform the research direction and interpretation of later chapters that investigate the effects of Su-soku on cognition, mood and interoception (Chapter 6).

5.1 Introduction

Meditation practices that involve attending to the breath, such as the Su-soku meditation, tend to influence oscillatory power in several frequencies at the fronto-midline, which are associated with executive function and attention. Chapter 4 confirmed these effects by noting increased frontal-midline oscillatory power in alpha, beta and theta frequencies, with increasing effects across the Su-soku experimental blocks. However, contrary to predictions, oscillatory power across Su-soku blocks was not correlated with autonomic changes. These unexpected findings may be related to the differences in attentional context between Su-soku and woodland conditions, whereby the former focused on internal and the latter on external signals. Overall, the results presented in Chapter 4 suggest that attending to sensory information may modulate the deployment of attentional resources towards respiration.

Theoretical accounts suggest that attending to particular inputs leads to system bias towards these inputs and consequently up-regulates their effects (Beck, 1967; Beck et al., 1983; Friston, 2009). Therefore, if we wish to understand the effects of increasing internal sensory awareness, also known as interoception, we must first consider the effects of the sensory signals and, in the context of this thesis, the effects of respiration on neuronal oscillations.

Understanding the mechanism of neuronal-respiratory entrainment has come a long way since researching the brains of hedgehogs (Adrian, 1942). Zelano et al. (2016) studied the effects of nasal respiration on human limbic oscillations by recording intracranial EEG on seven epilepsy patients during respiration. They found that local field potentials in the pyriform cortex can be phase-entrained by breathing, with increasing oscillatory power in delta, theta and beta during the inspiratory phase of breathing. Inspiration also entrained theta power in the amygdala and hippocampus regions. They also noted beta power was modulated by theta phase and theta-gamma coupling within the pyriform cortex during nasal breathing. As beta is typically associated with attentional control, they suggested that the respiratory-entrainment of theta and its subsequent influence on beta power suggests that respiration, specifically at the inspiratory phase, helps to organise spatio-temporal excitability.

Several studies suggest that respiration and low-frequency oscillations interact by phase-phase coupling. Melnychuk et al. (2018) reported that during meditation, the respiratory phase couples with frontal theta activity near the location of the anterior cingulate cortex. Mechanistically, this association is likely related to the chemo-sensitivity of locus coeruleus neurones and its connection to the ACC.
When attending to respiration, Herrero et al. (2018) found respiration-locked oscillations in alpha and beta frequencies increased, particularly in the anterior cingulate cortex. And the amplitude of these oscillations was modulated by the oscillatory phase of the lower frequency oscillations. In participants that were more aware of their breath, the ACC did not show a high degree of respiration-locked oscillations at rest, but this iEEG-breath coherence increased during breath-awareness. They also found that brain areas involved in interoception, such as the insula, had stronger respiration-locked oscillations when participants correctly tracked their breath (Craig, 2004, 2009). The finding of increased hippocampal coherence during breath tracking is consistent with that structure's involvement in the task, which required remembering a count (Šveljo, Koprivšek, Lučić, Prvulović, & Ćulić, 2010). This research suggests that directing at-

tention towards respiration modulates core regions involved in monitoring physiological processes and executive function. And for individuals with heightened interoceptive awareness, these respiratory-neural effects are further pronounced. This indicates that enhancing awareness of the internal world strengthens the frontoparietal attentional system, and as a result, the system becomes more adaptive to task demands.

Given the support for low-frequency oscillations (e.g. theta) driving high-frequency oscillatory activity (alpha, beta), there may well be a systematic pattern of oscillatory activity during the respiratory cycle, which corresponds to the underlying regulation of the frontoparietal system. Animal studies have shown promising cases for this possibility. Rojas-Líbano and Kay (2008) found that gamma oscillations always occurred around the transition point between inhalation and exhalation; meanwhile, beta oscillations appeared between early exhalation and the end of inhalation.
While Liu, McAfee, and Heck (2017) and colleagues showed local field potential Sharp-Wave ripples (SWR) characteristics in the hippocampus were found to be modulated by the respiratory cycle. They found an increased probability of SWR during expiration, and this effect was eliminated when the Olfactory bulb (OB) activity was inhibited. The OB region is crucial for relaying sensory information like smell to higher cortical brain regions such as the hippocampus. Therefore, the evidence that inhibiting OB activity eliminated SWR during exhalation suggests that the respiratory phase plays a crucial role in processing incoming sensory information.

However, while this animal work encourages the idea that the respiratory phase is important in respiratory-entrained oscillations, it is challenging to assess the effects of attending to the breath or interoceptive awareness in animals. Theoretically, we could imagine that the respiratory phase pattern exerted by respiratory-entrained oscillations would enhance internal awareness, as the minimal energy would be required to adjust priors in the presence of precise prediction errors (Friston, 2009; Tsakiris & Critchley, 2016).

Therefore, the current study aims to further investigate the effects of the respiratory phase on neuronal oscillatory power during a breath-focused meditative task. It is thought that the results from this work will help to inform the mechanistic underpinnings of respiratory interoceptive awareness.

5.1.1 Research questions and hypotheses

Research questions

RQ1. Is there a difference in oscillatory power across the respiratory cycle?

RQ2. Is there a difference in respiratory phase angle between frequencies?

RQ3. Does oscillatory power and respiratory phase direction change across meditation blocks?

RQ4. Do interbeat-intervals significantly change across the respiratory cycle, in terms of amplitude and phase angle?

RQ5. Is there a relationship between neural phase angle and RSA amplitude?

Hypotheses

H1. Oscillatory power will be up-regulated during inhalation, compared to exhalation.

H2. Alpha and beta oscillations are likely to appear at the transition points between inhalation to exhalation/exhalation to inhalation. Theta is more likely to be located at inhalation.

H3. Oscillatory power is likely to become stronger and oscillations are likely to cluster towards a mean direction across meditation blocks.

H4. Heart rate will increase during inhalation.

H5. There will be a relationship between the optimal neural phase angle and increased RSA amplitude.

5.2 Methods

The current study utilised the same data set as Chapter 4. Therefore, see Chapter 4 for information regarding data collection, experimental design, participants, experimental procedure, electrophysiological data collection and pre-processing.

5.2.1 EEG analysis

EEG data was processed offline using Brain Vision Analyzer software (BrainVision Analyzer, Brain Products GmbH, Gilching, Germany).

Spectral amplitude

A continuous wavelet transform was applied to the data using the Morlet complex wavelet function between 2 to 30 Hz frequencies, using 29 linear frequency steps. Wavelet normalization was set to instantaneous amplitude with a Morlet parameter of 5. The output values of this analysis were the real values of the spectral amplitude (μV).

Data was subsequently segmented relative to the expansion of the Su-soku circle (intended to signal the onset of inspiration) with a segment size of 6000 ms. Bad intervals were skipped, and overlapping segments were allowed. Spectral amplitude was averaged across epochs. Subsequently, amplitude data was extracted from Fz for each Su-soku meditation block, given its proximity to the ACC. Event markers relating to the stimulus onset were extracted.

5.2.2 Data preparation

The successive difference between stimulus markers and median breath duration was calculated using custom Python scripts. This value was then converted to seconds by dividing by 1000 and multiplying by the sampling rate of 512. This final value represents the number of samples to keep per subject, based on median breath duration.

A subsequent Python script was used to trim the number of samples per subject based on the individual's median breath duration and to average over the frequency bands of interest (Theta 4-8 Hz, Alpha 10-12 Hz and Beta 14-20 Hz). All files were then downsampled to 1000 samples to ensure data samples for each subject were the same length with minimal data loss, regardless of respiratory rate. The index of the sample number, representing the respiratory cycle phase, was translated into radians, whereby the 1000th sample is 2π and the 500th sample is π. The value at the sample number then reflected the radius from the circle's centre. From this information, the complex number for the 1000 samples for each subject was calculated, enabling one to deduce the average complex number, phase angle and average radius per subject. This information enables the investigation of the average respiratory phase at which EEG oscillatory power had peak amplitude. This procedure was repeated for frequency bands of interest and across experimental blocks. Group averages were then calculated for experimental blocks.

Several polar plots for experimental blocks and frequencies were produced to visualize the data before proceeding to statistical analysis. Participants were plotted using their individual average radius and phase values and group averages based on block.

The following libraries were utilised in the custom Python scripts: Pandas (McKinney et al., 2010), Numpy (Harris et al., 2020), Plotly (Plotly Technologies, 2015), Scipy (Virtanen et al., 2020) and the Circular R package (Agostinelli & Lund, 2017). See Appendix C for an example of the Python script used in this study.

5.2.3 Circular statistics

A preliminary analysis of variation of oscillatory power in equally spaced time intervals in individual participants indicated that oscillatory power varies systematically across the respiratory cycle. However, when respiratory rates differ between participants, brain signals quantified in a time bin occurring at a particular time after the onset of the breathing cycle represent brain activity at a different phase of the respiratory cycle in different participants. This confounds the interpretation of average values across participants within a particular time bin. Therefore, I employed circular statistics based on averages across participants at corresponding phases of the respiratory cycle.

At the time of writing, no existing Python functions could directly compute the circular statistics required for this research. As the data was already in Python, I imported R functions using the r2py package.

Initially, the data was plotted using a p-p plot against a von Mises distribution, the circular equivalent of a normal distribution (see Figure 5.1). This step was used to identify outliers in the data and compare them to a normal distribution. All data appeared to fit the linear line reasonably, indicating that the data is normally distributed.

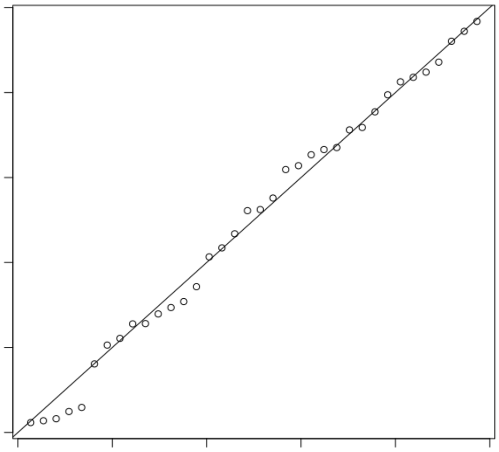


Figure 5.1: Example of a P-P plot with alpha, Fz, block 3 data plotted against the von Mises distribution, made using the circular R package.

A Rayleigh test was then employed to examine whether the oscillatory phase angle, relative to the respiratory cycle, significantly changes across meditation blocks. If the value is greater than the critical value ($p = <0.05$), then the null hypothesis for uniformity will be rejected. In contrast, if the p-value is less than 0.05 we can assume the data is not uniform, and there is a tendency for the oscillatory activity to be concentrated at a particular point during the respiratory cycle.

5.2.4 ECG analysis

An in-house MATLAB script was used to plot the interpolated IBIs from Chapter 4 around the respiratory cycle, using a sampling rate of 360 samples per cycle. The time stamps defined phases of the respiratory cycle for event markers extracted from BVA. Histograms were generated for each participant of the mean phase angle for each cycle to check for bimodality.

5.2.5 Statistical analysis

Linear statistical analysis was performed using IBM SPSS 22, and circular statistics were calculated using libraries/toolboxes in Python and MATLAB, such as the circstat2012a toolbox (Berens, 2009) to estimate the mean, median, and standard deviation of phase angle around the cycle and the probability that the distribution is not a von Mises distribution. An Alpha level of p = <0.05 was used for all analyses.

Assumptions of the general linear model were initially assessed visually using histograms of the residuals and further verified numerically using normality, homogeneity, skewness and kurtosis testing. Skewness and kurtosis values exceeding -1 to +1 were considered violations of normal distribution (Blanca et al., 2017). Deviations from normality were further investigated with quantile-quantile plots and Cook's distance values.

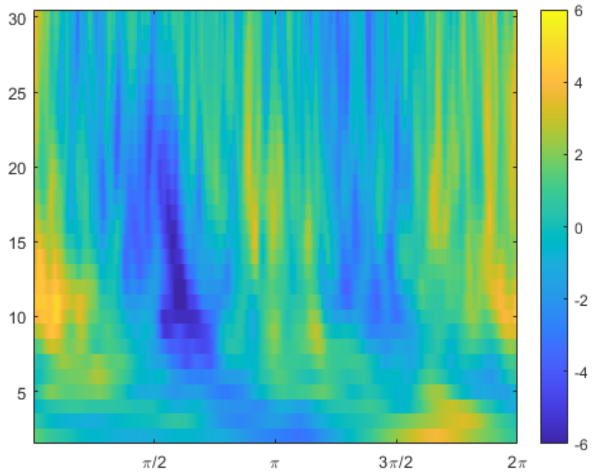
Any observations above 0.5 would be considered an outlier and subsequently removed and replaced with linear interpolation. Fortunately, none of the Cook's distance values lay above the criterion, despite some variables showing high kurtosis >1. Outlier detection sensitivity for Cook's distance has been reported as very high; therefore, this approach was chosen to be an appropriate method for objectively identifying outliers to complement the interpretation of QQ-plots (Cousineau & Chartier, 2010; Senthamarai & Manoj, 2015).

5.3 Results

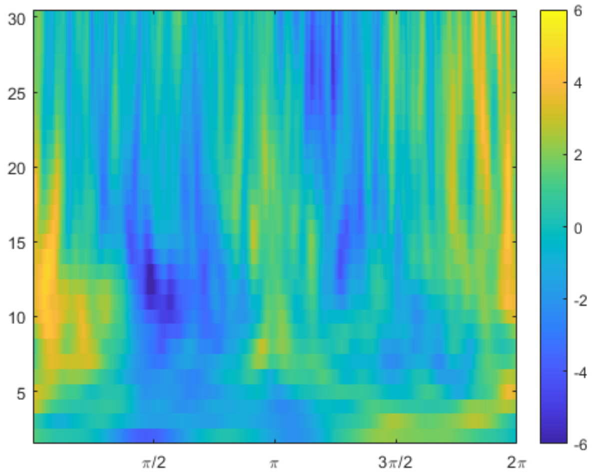
5.3.1 Oscillatory power across the respiratory cycle

To understand the overall picture of oscillatory power across the breathing cycle, I explored the data by dividing the respiratory cycle for each participant into 360 “bins” representing respiratory phase, regardless of respiratory rate, and plotted a time-frequency spectrum of oscillatory brain amplitude for each meditation block (see Figure 5.2).

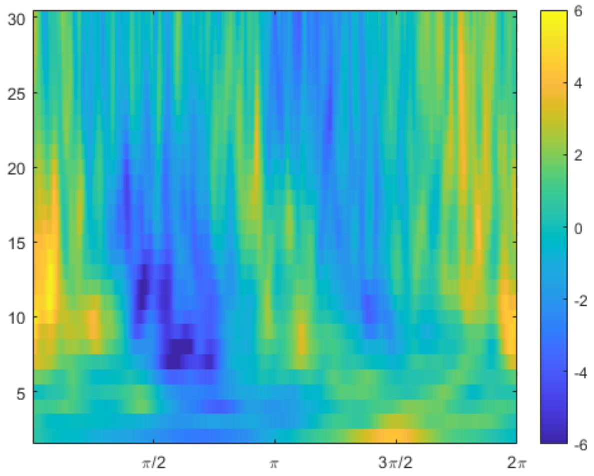
Respiratory phase is represented on the horizontal axis, oscillatory frequency on the vertical axis, and colour represents the oscillatory amplitude. The colour axis represents the t value across all participants from a one-sample t-test against a null of zero, i.e. the extent to which the values deviate from zero. This means that bright blue or bright orange areas are consistent across participants (large t-values) and so likely to reflect processes common to all participants. Oscillatory amplitude is expressed as z scores, i.e. as standard deviations from the person’s mean, where the mean is defined as zero, so blue (negative) areas mean that participants had consistently less oscillatory power than their average in that part of the respiratory cycle, and orange areas mean they had consistently more. The most prominent effects are for alpha and low beta (frequency is marked in Hz on the vertical axis).



(a) Block 1



(b) Block 2



(c) Block 3

Figure 5.2: Time-frequency spectrum plots

Interim discussion

These time-frequency plots suggest that oscillatory power changes across the respiratory cycle. However, with linear statistics, it difficult to interpret whether there is an average tendency for oscillatory power to peak at the start of inhalation or the end of exhalation. Furthermore, it would be helpful to know whether there is a quantitative change in respiratory phase angle across meditation blocks.

In the next section, I further explore oscillatory power variance during respiration using circular statistics.

5.3.2 Maximal oscillatory power via respiratory phase

This analysis furthers findings from Section 5.3.1, as I explore where the maximal oscillatory amplitude is occurring with respect to the respiratory cycle using circular statistics. The primary question here is to investigate whether there is a tendency for oscillatory power to peak at a particular phase of the respiratory cycle. I predict that maximal oscillatory power would exhibit an increasing trend to be located at a specific respiratory phase angle during sustained meditative practice to facilitate optimal function. Therefore, I expect to see a significant difference in phase angle when comparing blocks.

Initially, I explored polar histograms to examine respiratory phase angle direction for oscillatory power for alpha, theta and beta frequencies across meditation blocks. These phase angles were then further analysed with summary circular statistics: mean direction or average phase angle in relation to the respiratory cycle, mean resultant length or average amplitude, circular variance, circular standard deviation and the Rayleigh test statistics for each Su-soku block and frequencies of interest (alpha, theta and beta).

The Rayleigh test evaluates whether the data is drawn from a uniform distribution or one that is concentrated around one or more preferred directions. The null hypothesis of such test assumes a symmetric unimodal data distribution, referred to as a von Mises distribution, described as a circular equivalent of the normal distribution. The Rayleigh test is often used in circular statistics and recommended when a unimodal department for uniformity is expected (Landler, Ruxton, & Malkemper, 2019). A significant Rayleigh's statistic, indicated by *, suggests a non-uniform von-Mises distribution.

Alpha

According to Figure 5.3 and Table 5.1, maximal alpha power appears to exhibit an increasing tendency to lie at the end of the exhalation phase across meditation blocks. This suggests that maximal alpha power just before inhalation may be optimal for processing respiratory signals.

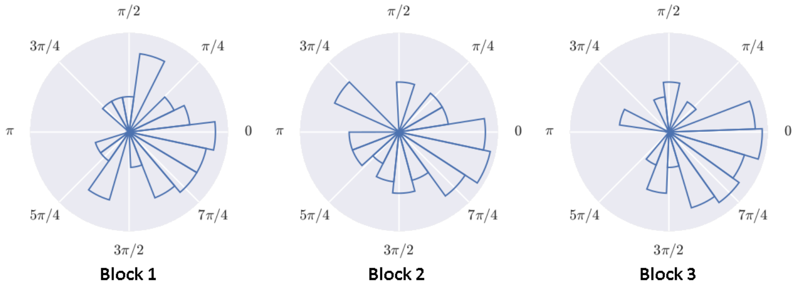


Figure 5.3: Polar histogram of alpha power variation across respiratory phases during meditation

Parameter	Block 1	Block 2	Block 3
Mean direction	-0.30	-0.49	-0.18
Mean resultant length	0.13	0.12	0.11
Circular variance	0.87	0.88	0.89
Circular SD	2.16	2.21	2.21
Rayleighs test statistic	0.42	0.40	0.57
p value	0.001*	0.003*	0*

Table 5.1: Alpha frequency circular statistics for respiratory phase angle of the oscillatory power

Theta

As with alpha power, Figure 5.4 and Table 5.2 suggest a greater tendency for maximal theta power to lie at the end of the exhalation phase across meditation blocks. According to the Rayleigh statistic in Table 5.2, theta power at block one exhibited a uniform von Mises distribution (normal distribution). This complements the polar histogram for Block 1, which suggests no particular phase angle direction for theta power. Block two exhibits a deviation from uniformity, with a tendency towards the end of exhalation or the start of inhalation. Block three was also identified to deviate from uniformity, but there is a clear direction towards the exhalation phase. This change from uniformity to non-uniformity may reflect a shift in attention resources according to the respiratory rhythm.

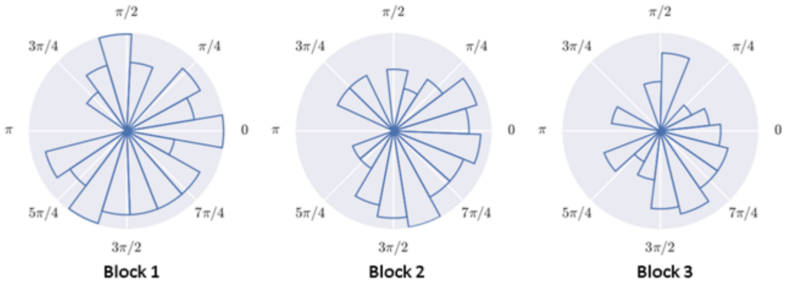


Figure 5.4: Polar histogram of theta power variation across respiratory phases during meditation

Parameter	Block 1	Block 2	Block 3
Mean direction	-0.36	-0.18	-0.33
Mean resultant length	0.08	0.10	0.10
Circular variance	0.92	0.90	0.89
Circular SD	2.36	2.25	2.27
Rayleighs test statistic	0.22	0.31	0.35
p value	0.17	0.03*	0.010*

Table 5.2: Theta frequency: circular statistics for respiratory phase angle of the oscillatory power

Beta

Similarly to alpha and theta power, Figure 5.5 and Table 5.3 suggest maximal beta power exhibits an increasing tendency towards the end of exhalation across meditation blocks. According to the circular standard deviation, beta oscillatory power appears to be the most variable during respiration and across meditation blocks. This may correspond to individual differences in the ability to control breathing to match the external stimulus and one's awareness of their breath.

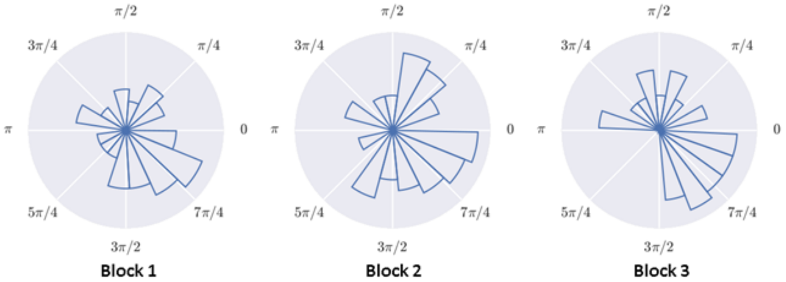


Figure 5.5: Polar histogram of beta power variation across respiratory phases during meditation

Parameter	**Block 1**	**Block 2**	**Block 3**
Mean direction	-0.14	-0.16	0.15
Mean resultant length	0.06	0.06	0.05
Circular variance	0.94	0.95	0.95
Circular SD	2.47	2.50	2.48
Rayleighs test statistic	0.31	0.34	0.41
p value	0.03*	0.01*	0.001*

Table 5.3: Beta frequency: circular statistics for respiratory phase angle of the oscillatory power

Interim discussion

These results complement findings from Section 5.3.1, as I initially found that oscillatory power appeared to peak at the start and end of respiration. The findings of Section 5.3.2 suggest that alpha, beta and theta power appear to become less uniformly distributed according to the respiratory phase across meditation blocks, as indicated by the change in Rayleigh's statistic. Oscillatory power for an alpha, theta and beta tend to be concentrated just before inhalation. In addition, Section 5.3.2 suggests that beta activity is susceptible to repeated, sustained attention tasks, as most participants' phase angles shifted across all three meditation blocks towards a direction. The polar histogram graphs also suggest maximal theta appears to occur before beta, followed by alpha during a sustained breath task.

In the following section, I investigate whether autonomic activity significantly changes across the respiratory cycle in terms of amplitude and phase angle.

5.3.3 Cardiac vagal variation during meditation

This analysis aims to assess whether autonomic activity varies across the respiratory cycle. I expect inter-beat intervals (IBIs) to be concentrated at the inhalation phase due to cardiorespiratory interactions, which cause the heart rate to speed up during inhalation and decrease during exhalation. However, it is unclear what effect sustained meditation would have on IBIs respiratory phase angle. I postulate that, across meditation blocks, IBIs would become increasingly concentrated towards a particular direction as more participants can modulate their IBIs in relation to the breath with practice. For a similar reason, I would expect RSA amplitude to increase across meditation blocks.

The Rayleigh test revealed phase angle to be statistically significant compared to a von Mises distribution. Furthermore, the visualisations suggest a tendency for phase angle to be concentrated during the inhalation phase of respiration (Figure 5.6).

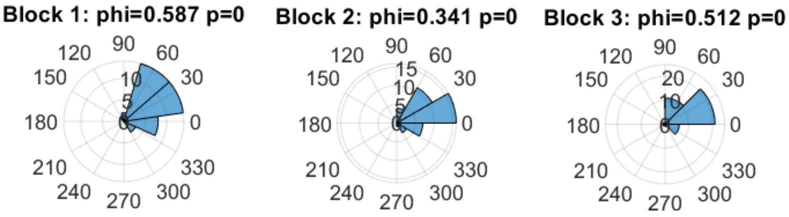


Figure 5.6: Circular plots for IBIs across meditation blocks

Parameter	Block 1	Block 2	Block 3
Mean angle (SD)	0.45 (0.75)	0.33 (0.79)	0.26 (0.82)
Median angle	0.68	0.49	0.40
Mean heart rate (SD)	75.58 (8.23)	74.78 (7.73)	73.22 (9.33)
Median heart rate	74.53	73.99	72.45
Mean RSA amplitude (SD)	42.19 (27.13)	43.37 (29.33)	44.32 (35.15)
Median RSA amplitude	33.10	34.21	34.52

Table 5.4: Group autonomic activity data

A repeated measures analysis of variance was used to calculate whether there is a significant change in mean IBI phase angle and RSA amplitude across meditation blocks. RSA amplitude corresponds to the HRV peak in the HRV power spectrum.

This analysis revealed no significant effect of block on amplitude, $F(2, 82) = 0.58$, $p = .6$, $\eta_p^2 = 0.014$ (see Figure 5.7). And no significant effect of block on phase angle, $F(1.43, 58.7) = 1.79$, $p = .18$, $\eta_p^2 = 0.042$ (see Figure 5.8).

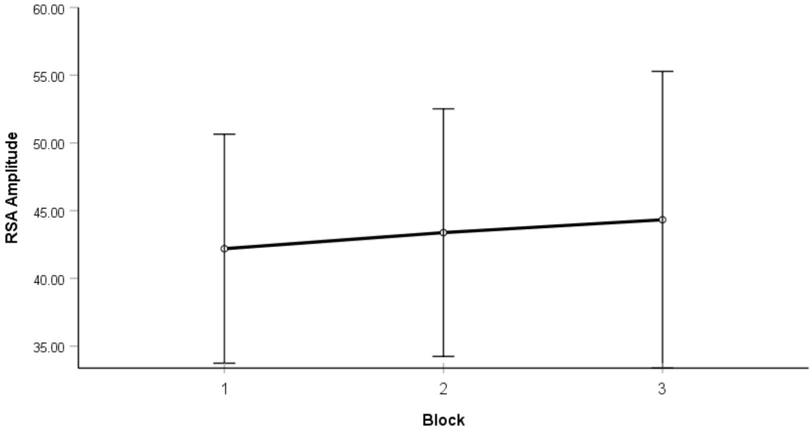


Figure 5.7: RSA amplitude across meditation blocks

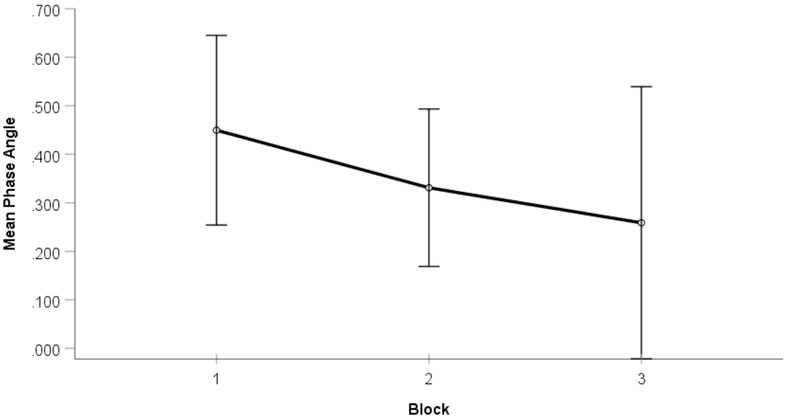


Figure 5.8: Mean phase angle across meditation blocks

Interim discussion

Despite the change across blocks not being significant, these visualisations suggest that Inter-Beat Intervals (IBI) phase angle appears to become more concentrated towards the start of inhalation. And this directional effect is emphasised with the significant Rayleigh's Test. However, RSA amplitude does not significantly change across meditation blocks.

In the following section, I investigate whether oscillatory changes are associated with autonomic changes during respiration.

5.3.4 Neural-cardiac relationship via respiratory phase

Oscillatory power varies across the respiratory cycle and tends to be concentrated before inhalation. In addition, IBIs tend to be focused at the start of inhalation. Several studies suggest that vagal tone predicts cognitive function, especially attention and working memory. Surprisingly, Chapter 4 found little evidence for a relationship between heart rate variability and oscillatory amplitude during Su-soku meditation. But the most recent findings suggest maximal oscillatory power varies in respiratory phase angle across meditation blocks. This temporal change in oscillatory power in relation to the respiratory cycle may reflect a difference in the attentive state during a breath task.

Here I assess whether RSA amplitude predicts respiratory phase angle shifts in oscillatory power during Su-soku meditation using multivariate regression, the predictor variable being RSA amplitude and dependent variables being the respiratory phase angle differences for oscillatory power between the three meditation blocks. This analysis is repeated for alpha, beta and theta, respectively.

Alpha

The findings from the regression analysis suggest that RSA amplitude significantly predicts respiratory phase angle shifts for alpha power from Block 2 to Block 3. However, large confidence intervals for this effect also suggest this finding may be spurious (see Table 5.5).

Parameter	B1 to B2	B2 to B3	B3 to B1
$t(38) =$	1.39	-2.07	1.04
$p =$	0.17	0.04*	0.31
95% CI	[-6.58 to 35.70]	[-45.98 to -0.52]	[-8.26 to 25.69]
η_p^2	0.05	0.1	0.03

Table 5.5: Multivariate regression results for respiratory phase angle shifts for Fz Alpha across meditation blocks. B1, B2 and B3 refer to blocks 1, 2 and 3 respectively.

Therefore, to further investigate this effect, I plotted a simple scatter plot (see Figure 5.9). These visualisations suggest that my previous concern was likely correct. There is not a straightforward linear correlation between respiratory phase angle shifts for alpha power and HRV amplitude, as most values are centred at 0 with no clear indication of whether this angle is associated with increased or decreased vagal tone.

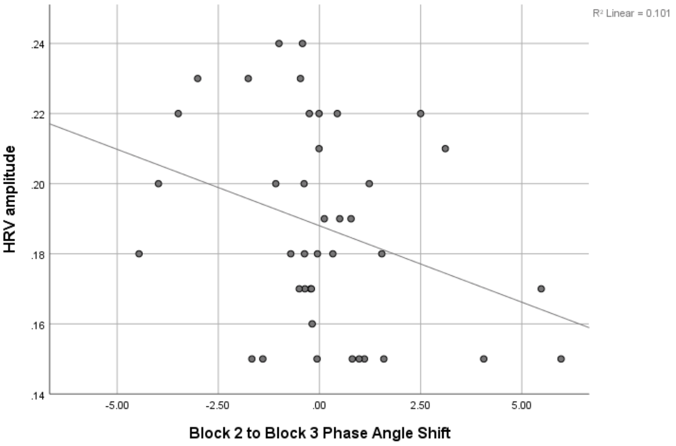


Figure 5.9: Scatter plot for relationship between HRV amplitude and respiratory phase shift for alpha power from block 2 to block 3.

Beta

HRV amplitude significantly predicted respiratory phase angle shifts for beta from Block 1 to Block 2 (see Table 5.6). As with the respiratory phase angle shifts for alpha power, confidence intervals are substantial, which requires further inspection by scatter plot (Figure 5.10).

Parameter	**B1 to B2**	**B2 to B3**	**B3 to B1**
$t(38) =$	-2.05	1.81	-0.51
$p =$	0.04	0.08	0.61
95% CI	[-31.78 to -0.19]	[-1.64 to 28.60]	[-15.89 to 20.85]
η_p^2	0.09	0.08	0

Table 5.6: Multivariate regression results for respiratory phase angle shifts for Fz beta across meditation blocks. B1, B2 and B3 refer to blocks 1, 2 and 3 respectively.

Unlike alpha, there does appear to be a weak trend between HRV amplitude and respiratory phase angle shift. Participants with a positive phase angle shift appear to have lower HRV amplitude. This is interesting as I identified in 5.3.2 that from Block 1 to Block 2, there is a generally positive phase angle shift and a significant positive phase angle shift between Block 3 to Block 1. There may be an optimal respiratory phase angle for beta, especially as the highest HRV amplitudes can be seen for participants centred just before the 0 phase.

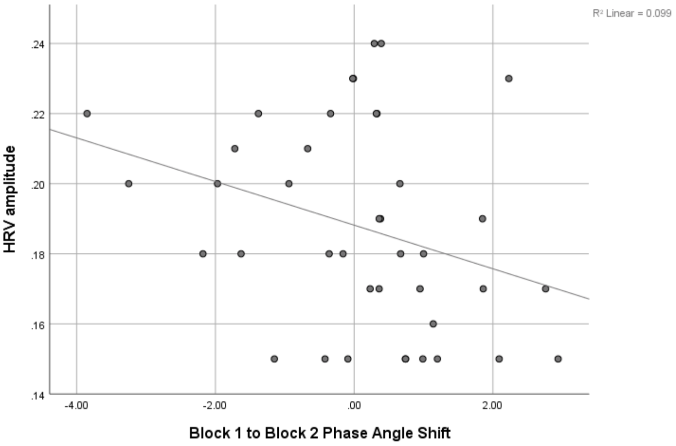


Figure 5.10: Scatter plot for relationship between HRV amplitude and respiratory phase shift for beta from block 1 to block 2.

Theta

HRV did not significantly predict respiratory phase angle shifts for theta (see Table 5.7). While one could interpret this finding as the result of lower power, it is more likely that the effect size for theta is smaller than beta and alpha.

Parameter	**B1 to B2**	**B2 to B3**	**B3 to B1**
$t(38) =$	1.12	-0.2	-0.79
$p =$	0.31	0.84	0.43
95% CI	[-9.82 to 29.70]	[-21.31 to 17.41]	[-28.28 to 12.30]
η_p^2	0.03	0	0.02

Table 5.7: Multivariate regression results for respiratory phase angle shifts for Fz theta across meditation blocks. B1, B2 and B3 refer to blocks 1, 2 and 3 respectively.

5.4 Discussion

5.4.1 Main findings

The purpose of this chapter was to expand on previous findings by investigating three different aspects of how neural-visceral signals change across respiration during Su-soku meditation: (1) oscillatory amplitude will be up-regulated during inhalation; (2) there will be an increasing tendency across meditation blocks for this amplitude to peak during the respiratory transition; (3) this oscillatory tendency will be coupled with changes in the viscera during respiration.

Initial exploratory work indicated that oscillatory power fluctuates across the respiratory cycle, with greater oscillatory power during inhalation than exhalation at theta, alpha and beta frequencies. On further investigation using circular statistics, there was a significant tendency for oscillatory power in theta, beta and alpha frequencies to peak at the end of exhalation rather than during inhalation. Across meditation blocks, beta activity became significantly less uniform/more concentrated towards the exhalation to the inhalation transition point, whereas alpha and theta activity moved significantly towards $-\pi/2$. Also, on average, theta occurred before the alpha phase angle.

Subsequent analysis investigated whether these changes in oscillatory activity were related to autonomic changes during respiration. Interbeat-intervals (IBI) phase angle tended to be concentrated at the inhalation phase, which supports the cardiac-respiratory hypothesis that heart rate increases during inspiration. However, there were no significant changes in IBI phase angle and amplitude across meditation blocks. This was an unexpected finding, as it was predicted that if Su-soku meditation influences neural-visceral interactions, then there should be relative changes in IBI as found in oscillatory power across meditation blocks.

The relationship between central and autonomic neural activity during respiration was examined to clarify these findings further. Participants with greater RSA amplitudes tended to have beta and alpha phase angles shift closer to the exhale to inhale transition point, and that RSA predicted this respiratory phase angle shift. This suggests that the cardiac-respiratory synchrony, as indicated by RSA amplitude, may facilitate the cognitive processing of visceral signals. Previous work has demonstrated that short-term interoceptive processing can enhance perceptual processes (Garfinkel et al., 2014; Park, Correia, Ducorps, & Tallon-Baudry, 2014; Zelano et al., 2016).

Longstanding evidence supports respiratory effects on cognition, specifically attention (Winkler, 1898). Earlier work has shown that attention fluctuates in phases with the respiratory cycle, and specific phases either enhance or detriment cognitive processing (Lehmann, 1894). While more recent work has reported that the frontal theta-beta ratio fluctuates in correspondence to the respiratory phase, suggesting that the respiratory phase plays a core role in attentional shifts (Melnychuk, Robertson, Plini, & Dockree, 2021). As the current study involved focused breathing, these findings likely reflect a combination effect of both interoceptive attention and respiration. And due to the lack of a comparable control condition, the exact contributions of these effects are difficult to determine (Flexman, Demaree, & Simpson, 1974; Grassmann, Vlemincx, Von Leupoldt, Mittelstädt, & Van den Bergh, 2016).

On the other hand, differences in respiratory frequency between participants were controlled, and research has shown that the amplitude of RSA is not affected by respiration frequency (Denver, Reed, & Porges, 2007; Giardino, Glenny, Borson, & Chan, 2003). Therefore, any respiratory effects in the current study are likely due to paced breathing rather than differences in respiration frequency between participants. But it is worth mentioning that the potential effects of respiration could have been further explored if additional physiological parameters were measured in the original study, such as maximal oxygen uptake (also known as VO_2 max) and tidal volume.

A few studies report that paced breathing, as opposed to spontaneous breathing, can influence cognition; which suggests that attending to internal signals via paced breathing could be the primary contributor to the effects reported in the current study (Li, Park, & Borg, 2012; Zautra, Fasman, Davis, & Arthur, 2010). Predictive coding theory states that a living organism attempts to minimise prediction errors by adjusting internal world priors (Friston, 2009). In the current study, oscillatory power across meditation blocks increased and became increasingly concentrated towards a particular respiratory phase direction. These changes may signify oscillatory optimisation towards visceral signals, thereby increasing signal gain and minimising prediction errors (Farb, Segal, & Anderson, 2013).
Furthermore, there were differences in the respiratory phase angles between frequency bands: frontal theta peak occurred before beta, which was then followed by the alpha peak. As prefrontal regions are proposed to exert control over perceptual processing, phase angle differences between frequencies may indicate the process of attention control.

Up-regulated theta amplitude during exhalation may relate to its association with facilitating cognitive processing. Theta is thought to promote the integration of thalamocortical inputs and detect conflict between current and intended behaviours by a rhythmic sampling of the environment when prior information is available. As a result, attentional performance often varies as a function of theta, whereby frontal-midline theta is associated with increased cognitive control over short time scales and reduced attention during prolonged cognitive engagements (Helfrich et al., 2018). Low-frequency rhythms, such as theta, entail long periods of low excitability, which would make it increasingly challenging to detect random stimuli (Schroeder & Lakatos, 2009). Therefore, suppressing theta during inhalation may benefit sensory selection and, as a result, the processing of visceral information.

Regarding beta, this frequency is typically measured alongside theta to indicate attentional control balance, whereby theta acts as a parameter for bottom-up processing and beta for top-down attentional processing (Spitzer & Haegens, 2017). In distracted states, high levels of theta and low levels of the beta have been observed, which suggests beta plays an executive function role (Braboszcz, 2011). Moreover, functional connectivity studies, such as van Son et al. (2019), report that a lower theta/beta ratio, and thereby high beta, correlates with greater connectivity between brain regions associated with executive control, such as the ACC and dorsolateral PFC, during focused attention. A spike in theta amplitude followed by beta could indicate an attentional switch from sensory input to integrating inputs with existing information. Such interpretation is in line with computational models of beta generation, which suggest beta is generated through concurrent input of feedforward inputs via the granular layer, and feedback driven by higher-order cortical regions (Kop et al., 2011).

In contrast, alpha is typically associated with the role of inhibition; therefore, one interpretation of alpha respiratory phase angle occurring last could be that alpha acts as a gating mechanism to filter task-irrelevant processes before the subject's next inhalation (Jones et al., 2010; Kerr, Sacchet, Lazar, Moore, & Jones, 2013; Klimesch, 2012; Klimesch, Sauseng, & Hanslmayr, 2007; Worden, Foxe, Wang, & Simpson, 2000). Melnychuk et al. (2021) also found alpha amplitude to increase when theta/beta ratio was lowest, which further supports the notion of an arousal-related component fluctuating with the respiratory cycle. The circular analysis in the current study extends existing literature, as the findings indicate that not only does alpha amplitude increase during meditation, there is a potential respiratory-phase preference enhancing across meditation blocks. This finding may allude to oscillatory changes found in long-term meditation, where alpha modulation is associated with enhanced prefrontal attentional control (Davidson, 2004; Farb et al., 2010, 2007). When the alpha peak occurs in relation to respiration could be a potentially helpful indicator for training attentional control.

5.4.2 Limitations

Although the current study may provide helpful insights into the mechanisms of neural-visceral interactions during paced breathing, its limitations should also be noted.

As aforementioned, the study design utilised in this data set was less than ideal for comparing differences between "treatment" conditions due to the interleaving cognitive tasks in a cross-over design. There were also differences in engagement duration between the relaxing meditative states. During Su-soku, participants were interrupted every 5 minutes to inform the researcher of how many breaths they had counted; in comparison, participants were uninterrupted during the woodland condition. These differences may have impacted the ability of participants to engage in the task and therefore affected their attentive state. An appropriate, equal-length control condition would have helped verify the "treatment" effects of Su-soku on attention.

While the breathing rate was verified by matching the prescribed breathing rate to RSA frequency, it would have been helpful to explore whether or not participants provided accurate reports on breath counting to investigate attention and interoceptive awareness. Furthermore, additional measures of respiration, interoception and cognition may have been informative in untangling the effects of interoceptive attention from the effects of respiration.

5.4.3 Implications

Despite these limitations, several implications can be drawn from the current study. To my knowledge, this study is one of the first to apply circular analysis to explore the effects of paced breathing on central-autonomic activity across the respiratory cycle. As a result, this novel approach opens up potential new avenues for studying electrophysiological effects on breath-focused meditation. This work suggests that not only does attention oscillate in phase with the breath, but there may be an optimal phase angle for each frequency associated with cardio-respiratory synchrony.

This finding furthers the therapeutic possibilities of targeted breath training for mental health in treatment and clinical monitoring. Generally, existing literature already supports the therapeutic benefits of breath control for improving quality of life and mood; however, there has been minimal research into quantitatively tracking the effectiveness of breath training (Chiesa & Serretti, 2010; Grossman, Niemann, Schmidt, & Walach, 2004).

As some neural changes take years of regular meditative practice before fruition, most beginners are unlikely to experience the full benefits of practice (Engen, Bernhardt, Skottnik, Ricard, & Singer, 2018; Kral et al., 2018). As a consequence, some individuals may choose to discontinue meditative practice before receiving any mental health benefits. Therefore, future work should consider the potential of developing tracking meditation systems and possibly utilise the current findings as a starting point. The ability for individuals to track their meditation progress would likely improve clinical adherence and, as a result, potentially the mental state of an individual.

5.4.4 Conclusions

In summary, the findings presented in this chapter suggest that increasing interoceptive awareness via paced breathing alters feedforward-feedback regulation as indicated by oscillatory changes across the respiratory cycle in frequencies associated with the ability to filter and prioritize information flow. The novel application of circular statistics to investigate these effects provides an original contribution to knowledge.

The next chapter aims to confirm and extend current findings by:

1. Repeating previous analyses used in Chapters 4 and 5 on a different data set with a comparable woodland condition.

2. Assessing the influence of meditative practice on cognition and mood.

3. Examining the relationship between interoceptive awareness and central-autonomic changes during meditation.

Chapter 6

Su-soku effects on cognition and emotion

According to predictive coding accounts, as a consequence of minimising prediction errors, attending to internal bodily signals up-regulate their influence on the physiological system. This theory could be plausible, as neural and autonomic rhythms appear biased towards a specific respiratory phase across meditation blocks. In Chapter 5, I argued these results might reflect the effects of increasing interoceptive awareness, and in turn, this may modulate cognitive performance and emotion regulation.

The current chapter confirms and extends previous findings by assessing the differences in oscillatory power and autonomic indices between meditative states and exploring meditation effects on cognitive performance and mood state. In addition, the effects of meditation on interoception are examined by analysing the neural processing of visceral signals indexed by heartbeat-evoked potentials (HEPs). Research investigating the role of HEPs in meditation is relatively sparse; therefore, the findings in this chapter provide an original contribution to knowledge.

6.1 Introduction

Previous chapters assessed the effects of Su-soku meditation on neural-autonomic activity by comparing Su-soku meditation with a baseline period featuring a relaxing woodland scene. Findings in Chapter 4 suggested Su-soku meditation appears to up-regulate attentiveness, inferred by the increase in frontal-midline oscillatory power in theta, alpha and beta frequencies from baseline and across subsequent Su-soku blocks. Furthermore, Su-soku meditation appeared to facilitate a relaxed state as vagal tone increased across meditation blocks (as indexed by RMSSD). While simultaneous up-regulation of theta power and vagal tone suggested a similar mechanism may underpin this effect, there were no significant correlations between changes in theta oscillatory power and cardiac vagal tone. I concluded from these findings that, compared to baseline, Su-soku appears to facilitate an attentive, vigilant state, and the relationship between oscillatory power and vagal tone may be mediated by respiration.

This proposal was further investigated in Chapter 5 by assessing how oscillatory activity alters across the respiratory cycle during Su-soku meditation. These findings indicated that Su-soku might heighten attention towards the transition period of exhalation to inhalation, as across Su-soku blocks, there was an increasing mean direction for theta, alpha and beta phase angles to be located towards the end of exhalation. Interestingly, the change in oscillatory phase angle and amplitude was not correlated, which suggests independent mechanisms may govern the changes found in amplitude power and when amplitude power peaks in relation to the respiratory cycle. These mechanisms were further probed by assessing the relationship between phase angle change and autonomic state. Respiratory sinus arrhythmia amplitude predicted the beta and alpha phase angle shift. The greater the RSA amplitude, the closer the phase angle was to the exhale to the inhale transition point. These findings indicate that both average amplitudes change and when amplitude peaks occur during the respiratory cycle may play a significant role in the effects of Su-soku meditation.

While previous findings led to several interesting discoveries, the crossover experimental design of the original study was not ideal for comparing the differences between the Su-soku and relaxing woodland baseline conditions. The relaxing woodland condition was originally intended to be used as a rest period rather than to compare treatment effects with Su-soku. In addition, this baseline block was employed before and after a complex cognitive probe task, which may have affected the reliability of the results obtained from the baseline period.

One of the primary aims of the current chapter is to extend my previous work by assessing the differences between a Su-soku meditation condition and a relaxed state condition in a between-subject group design. Furthermore, a simplified cognitive attention task will be utilised to evaluate the effects of Su-soku on cognitive performance. If my previous findings are robust enough, I expect comparable results to Chapters 4 and 5; however, it is uncertain whether these findings will be statistically significant; due to the differences in experimental design. I anticipate that autonomic activity may differ from my previous results, as the continuous block design will enable participants to engage in the meditative tasks for longer. Perhaps this will facilitate the ability to achieve an altered state of consciousness. Based on previous literature, I hypothesise that a change in oscillatory power induced by Su-soku will facilitate cognitive performance. Moreover, this cognitive effect will be significantly greater in Su-soku than in the relaxing woodland condition due to an interaction between interoceptive awareness and neural-visceral signalling (Ainley et al., 2016; Cavanagh & Frank, 2014). However, currently, the exact mechanisms involved in this process are unclear.

Heartbeat evoked potentials (HEPS) are commonly measured to index interoceptive awareness. These can be described as a positive event-related potential at frontocentral electrodes occurring between 200 to 600ms after the R-wave of a heartbeat. Research has shown that directing attention toward internal stimuli up-regulates HEP amplitudes with the most prominent effects at frontal electrodes, and greater interoceptive accuracy has been associated with greater amplitude of HEPs (Petzschner et al., 2019). Studies suggest changes in frontal theta power in heartbeat-evoked brain responses meditate the effects of mindfulness. And these heartbeat-evoked response effects were localised to brain regions commonly associated with attention control, such as the anterior cingulate cortex (ACC), AI, and the lateral prefrontal cortex (Kang et al., 2020). These findings indicate that the interoceptive benefits of meditation may be a key mechanism in improving brain functions for attention control (Coll, Hobson, Bird, & Murphy, 2021).

Several studies propose that brain regions within the salience network contribute individual functions to serve as a “unified model” of sensory experiences (Critchley et al., 2004; Taggart, Critchley, van Duijvendoden, & Lambiase, 2016). The anterior insula and ACC provide a conjoined awareness of the moment and of self, where the AI integrates cortical-sensory information and the ACC re-represents this information for higher cognitive functions, such as selection and preparation for response. They found that increased brain activity, indicated by blood oxygenation response (BOLD), in the right AI predicts a participant’s accuracy in a heartbeat detection task, and AI grey matter volume correlates with interoceptive accuracy and subjective ratings of visceral awareness. They also found that interoceptive accuracy correlates with emotional experience, thus alluding to the notion that interoceptive awareness may be critical in regulating emotional states.

Further support for these proposals can be found in meditation studies. Individuals who regularly engage in meditation tend to have high heartbeat awareness, greater efficiency in controlling responses to conflicts, and are consistently reported to have changes in the salience network (Jha et al., 2007; Jo, Schmidt, Inacker, Markowiak, & Hinterberger, 2016; Kang et al., 2020; Moore & Malinowski, 2009; Tang et al., 2015, 2007; van den Hurk, Giommi, Gielen, Speckens, & Barendregt, 2010). These cognitive benefits can even be noted in short-term meditation, where the meditation group activity in the ACC significantly predicted behavioural performance on cognitive tasks (Kwak et al., 2020). These studies suggest that developing interoceptive awareness via meditative practice may benefit cognition, specifically, executive function.

Executive function is a term used to describe several mental processes that enable us to plan, focus attention, remember instructions, and juggle multiple tasks successfully. The efficiency of attention allocation is a core component of executive function, as how well we process, and consequently act on, information relies on using resource efficiency. Cognitive tasks, such as the Eriksen Flanker task, can be used to evaluate executive function by assessing responses to various stimuli based on reaction time, accuracy and electrophysiological measures.

In terms of understanding the communication in the brain, electrical potentials generated by the brain that are related to specific internal or external events, referred to as event-related potentials (ERPS) can be measured as a function of assessing cognitive function. These ERPS can have distinct waveform signatures in terms of the time of occurrence and polarity, indicating various cognitive processes in the brain. For instance, the N200 component represents a negative deflection after 200ms post-stimulus and is thought to relate to conflict or sensory deviation; in contrast, the P300 component, reflected as a positive deflection in the EEG at about 300–600 ms after stimulus onset, is associated with target sensory selection.

The P300 component is larger for incongruent trials relative to congruent trials (Frühholz, Godde, Finke, & Herrmann, 2011; Kopp, Mattler, Goertz, & Rist, 1996). Meanwhile, experienced meditators express higher amplitudes for P300 components during congruent and incongruent trials (Cahn et al., 2012; Jo et al., 2016). One might predict a decrease in the difference between congruent and incongruent trials. But it appears plausible that improving the ability to focus attention would increase P300 in both incongruent and congruent trials due to more consistent timing of the P300 peak across trials. Studies also report that meditators tend to express larger N200 ERP amplitudes, compared to non-meditators, for conflict trials (Norris, Creem, Hendler, & Kober, 2018; Quaglia et al., 2019).
This difference in ERP components may be associated with changes in global brain activity, as several studies report increases in network efficiency and connectivity of brain regions immediately following brief meditation (Srinivasan & Baijal, 2007; Xue, Tang, & Posner, 2011). Subsequently, improving the efficiency of network communication, and thereby allocation of attentional resources, contributes to the improvement in conflict monitoring and emotion regulation seen in meditation research. This concept can be inferred from stimulation studies, whereby stimulation of the frontal cortex that leads to inhibition of cognitive networks, such as the frontal-temporal anterior insular, has been shown to result in emotional flattening

towards affective stimuli (Mai, Braun, Probst, Kammer, & Pollatos, 2019). Meditation may improve cognitive processes by dampening the effects of emotion on cognitive processing. Evidence for this can be found in a few studies exploring meditation's cognitive effects on participants who exhibit emotion regulation difficulties. For instance, Norris et al. (2018) reported moderately neurotic participants who meditated for 10 minutes before the Flanker task had improved accuracy on incongruent trials and larger N200 amplitudes compared to those who listened to a relaxing control tape. While no differences were reported for individuals with higher levels of neuroticism, these findings suggest that brief meditation can improve the allocation of attentional resources in some novices with a tendency to experience negative emotions.

I propose that the effects of meditation on cognition and emotion can be explained using the predictive coding account. Meditation facilitates cognitive control and emotion regulation by maximising predictive models through active inference. As a result, additional resources can be allocated to higher order processing, which could influence how one perceives their environment (Tsakiris & Critchley, 2016). In sum, our awareness of internal signals may affect how we process our environment. Evidence for this can be seen in interoceptive studies, whereby low interoceptive awareness tends to be associated with reduced ERP amplitudes and cognitive performance and the opposite is often reported for high interoceptive individuals (Ainley et al., 2016; Georgiou, Mai, Fernandez, & Pollatos, 2018; Klawohn, Santopetro, Meyer, & Hajcak, 2020; Pang et al., 2019; Terhaar, Viola, Bär, & Debener, 2012).

In the context of this work, Su-soku meditation is a task that requires individuals to focus on their internal signals via breath counting. I hypothesise that attending to an internal body rhythm up-regulates its influence on the physiological system: respiratory effects on neural and cardiac rhythms. The findings in Chapters 4 and 5 suggest that neural and cardiac rhythms are altered by the respiratory cycle, and the system appears biased towards this state across blocks.

This chapter aims to further this story by confirming previous findings and investigating the influence of neural-cardiac-respiratory modulation on cognitive performance and mood state with a between-subjects design.

6.1.1 Research questions and hypotheses

Research questions

RQ1. Is there a replicable pattern of neural and autonomic activity during Su-soku meditation?

RQ2. Does Su-soku meditation significantly influence cognitive task performance and/or ERPs (P300, N200) during a cognitive control task?

RQ3. Is there a significant difference in mood state after Su-soku meditation, compared to the woodland condition?

RQ4. Is there a difference in heartbeat evoked potential (HEP) amplitude between Su-soku and woodland conditions?

Hypotheses

H1. It is expected the current study will reproduce similar results as found in Chapters 4 and 5.

H2. Compared to the woodland condition, Su-soku will enhance cognitive performance, and this change will be correlated with differences in ERP amplitudes (P300, N200).

H3. Su-soku induces a greater positive change to mood, in comparison to the woodland condition.

H4. HEP amplitude will be greater in the Su-soku condition compared to the woodland.

6.2 Methods

6.2.1 Study design

In order to validate and further existing findings on neural-visceral interactions during meditative states, this independent measures study had several aims:

1. To replicate previous findings, namely, the difference in neural and autonomic activity between meditative states;
2. Investigate how Su-soku meditation influences cognition based on electrophysiological changes in the frontal-midline and how this relates to change in performance during a flanker task, assessed by reaction time and percentage accuracy, and mood state, measured by changes on the abbreviated Profile of Mood States examination (Grove & Prapavessis, 1992);
3. Explore the extent to which differences in neural and autonomic states are related to interoceptive awareness, as indicated by HEPS.

6.2.2 Participants

Forty healthy participants (26 females and 14 males) between the ages of 20 to 42 (M = 25.10, SD = 4.77) were recruited in the original study via self-selection methods, such as poster advertisements across university campuses and social media groups in Nottingham (Baldwin, 2020). All participants identified as naïve meditators at the time of recruitment via self-report, this describes as having little to no previous experience with meditation. Participants were paid a standard inconvenience allowance for participation.

This sample size was selected due to an a priori analysis, which indicated that a sample size of 34 participants was needed to find a medium effect size of oscillatory power across time between group conditions with 80% power (G*Power; Faul, Erdfelder, Lang, and Buchner (2007)); recruitment exceeded this number in accounting for potential attrition or technical error.

Participants were considered healthy and therefore included in the study if they answered no to the following screening questions: i) currently receiving treatment for a psychiatric disorder, ii) personal history of psychotic illness, iii) a first-degree relative with a history of psychotic illness, iv) history of neurological illness or trauma, v) history of drug abuse (according to the DSM-IV criteria, American Psychiatric Association, 2013) within the past three months, vi) use of illicit drugs in the 48 hours (about two days) before the EEG recording, and vii) a recent and substantial change to their nicotine or caffeine intake.

6.2.3 Measures

Profile of Mood States (POMS)

The POMS is a self-report inventory that assesses six dimensions of mood construct: anger, confusion, depression, fatigue, tension, and vigour. In the shortened version, 24 items load onto these six different scales and participants are asked questions which refer to periods, such as “last 24 hours” or “last week”, and respond using a five-point response scale, from 0 (not at all) to 4 (extremely).

6.2.4 Procedures

The experiment was designed to have two sessions of a cognitive attention task, referred to as the flanker task, separated by either Su-soku meditation or a relaxing woodland scene, depending on the participant's group assignment. Participants were randomly assigned to either Su-soku or woodland relaxation group conditions. Resting breathing rate was recorded by breath counting during a trial period prior to the experimental recording. This resting breathing rate was used to set the pace of the breath-pacer stimuli used in the meditation block.

The original study also recorded responses to several other questionnaires, including the "Healthy Heart Questionnaire" (University of Colorado, n.d) and "The Schizotypal Personality Questionnaire – Brief Version" (Raine & Benishay, 1995), which are not relevant for this thesis and therefore not reported here.

Flanker task

The Eriksen Flanker task is a cognitive task which involves focused attention and response inhibition to make accurate judgements within a given period (Eriksen & Eriksen, 1974).

The flanker task consisted of presenting a white arrowhead stimulus surrounded by randomised congruent or in-congruent stimuli, referred to as "flankers", on a black background. In congruent trials, the arrowhead stimuli were "flanked" by arrows pointing in the corresponding direction (<<<<<, >>>>>), whereas in-congruent trials presented flankers pointing in a conflicting direction (<<><<, >><>>). Neutral trials presented an arrowhead flanked by diamond symbols. There were 40 trials per trial type presented in a random order, with a maximum of 241 trials per session.

At the start of the experiment, a fixation cross was displayed in the centre of the screen for 300ms, followed by a 900ms response window. Participants were required to indicate the direction of the central arrowhead by responding on the keyboard; “V” was used to indicate left-facing stimuli, while “M” was used for right-facing stimuli. Participants were instructed to respond as quickly and accurately as possible.

Between the end of one trial and the beginning of the next, an inter-trial interval was jittered between 1700 to 3200ms of each trial, with an average overall trial duration of 2.5 seconds. Participants performed the entire task twice, before and after their meditation session. For both task periods, this consisted of four blocks of 60 trials each; a rest was provided between each block, which varied in length depending on the participant’s preferences. Participants were required to press the space bar to proceed through each block.

After the first flanker session, all participants engaged in a 10-minute meditative session: either Su-soku meditation or observing a relaxing woodland scene. In both conditions, participants were exposed to a continuous looping video with audio of a relaxing woodland forest, which included sounds of running water and chirping birds. However, the Su-soku group was also presented with expanding/contracting circles in the centre of the screen as breath-pacer stimuli. The speed of the circles’ expansion matched each participant’s resting breathing rate. As the circles expanded and contracted, they faded in colour between green, blue and red. Equally, the background rhythmically faded from light to dark blue. Participants were asked to inhale as the circle expanded and exhale as the circle contracted. The Su-soku block lasted one minute in the practice task and ten minutes during the experiment. In order to keep participants focused on the task, participants were also asked to count their breaths during the task silently; one full inhalation and exhalation counted as one breath.

In the woodland condition, a looped video of dense woodland with a moving stream was presented in the centre of the screen. Participants were asked to relax during this task and keep their eyes fixed on a specific screen spot. As with the Su-soku condition, this task lasted one minute during practice and ten minutes during the actual experiment. The background soundtrack and volume were kept consistent between Su-soku and woodland conditions. All stimuli were coded and presented using MATLAB 2014b with the COGENT toolbox (CogentTeam, 2000).

Electrophysiological recording

Electrophysiological data were recorded using a 128-electrode system (Active II System, Biosemi, Amsterdam, Netherlands), and electrodes were placed according to the 10-20 international electrode placement system (Oostenveld & Praamstra, 2001). All electrodes were referenced to the Common Mode Sense (CMS), positioned to the immediate left of the central electrode (Cz). Data were digitised at a sampling rate of 512 Hz.

Additional electrodes were placed as points of reference. Two electrodes were positioned onto the left and right earlobes, as the earlobe is expected to produce minimal muscular artefacts. Two further electrodes were placed on the outer canthi of either eye to record horizontal saccadic movements. Two additional electrodes were placed above and below the left eye to account for vertical ocular movements and blinks. A final two electrodes were positioned on the inner wrist of both the left and right arm to record heart-rate variability.

6.2.5 EEG analysis

EEG data was processed offline using Brain Vision Analyzer software (BVA; Brain Vision Analyzer, Brain Products GmbH, Gilching, Germany).

Data pre-processing

Raw EEG data was initially referenced to ear lobe channels, then passed through Butterworth Zero Phase filters with a low cut-off of 0.5 Hz and high cut-off of 45 Hz. Then the data was manually inspected for noisy channels, and missing data was subsequently estimated by topographical interpolation, using spline interpolation.

Artefacts were corrected using a semi-automatic approach with the following criteria: Maximal rate of voltage change of 50μV/ms, the maximal allowed difference of values in intervals of 200μV, minimal allowed amplitude of -200μV, the maximal allowed amplitude of 200μV and lowest allowed average activity in 200ms of 0.5μV. Ocular movements were corrected using independent components analysis infomax restricted algorithm with 512 steps, using a slope algorithm for blink detection.

The data was segmented into three epochs: 1) Su-soku/woodland condition, 2) Flanker task before, and 3) Flanker after. The meditative epoch (Su-soku or woodland) was then segmented into three equal time bins.

Average spectral power analysis

To verify the oscillatory findings of Chapter 4, the analysis for spectral power was repeated. Absolute power (μV^2) for the three-time segments in both Su-soku and woodland conditions was calculated using fast-Fourier transform with a resolution of 1Hz, 10% Hanning window length, full spectrum, normalized segments, and variance correction. For individual participants, data were averaged across the epochs and extracted at the frontomedial electrodes (Fz, FCz, FC1 left, and FC2 right electrodes) in the following frequency bands: theta: 4-7 Hz, alpha: 8-12 Hz, and beta: 12.5–20 Hz.

Time-frequency analysis

Similarly, the same time-frequency analysis applied in Chapter 5 was used to assess the change in oscillatory power across the respiratory cycle during Su-soku meditation. A continuous wavelet transform was applied to the data using the Morlet complex wavelet function between 2 to 30 Hz frequencies, using 29 linear frequency steps. Wavelet normalization was set to instantaneous amplitude with a Morlet parameter of 5. The output values of this analysis were the real values of the spectral amplitude (μV).

For the Su-soku blocks, the data was subsequently segmented into an epoch size of 6000ms relative to the stimulus onset position; this was when the circles changed from decreasing to increasing in size to cue the start of inhalation. Bad intervals were skipped, and overlapping segments were allowed. Spectral amplitude was averaged across epochs and extracted from Fz. Marker locations for stimulus onset were extracted to calculate the duration of the respiratory cycle in milliseconds.

Event related potential analysis

After the pre-processing stages, EEG flanker data was segmented based on trial type: congruent, neutral and incongruent trials. EEG data epoch lengths of 1000ms were extracted, starting from 200ms before stimulus onset for correct trials and then averaged for each trial type. The 200ms pre-stimulus interval served as the baseline and was used for subsequent baseline correction.

The P300 was quantified as the maximum positive inflection between 350 to 500 ms at electrode site Fz. N200 was defined by the maximum negative deflection evoked 200 to 350ms following stimulus presentation.

Heartbeat evoked potential analysis

An R-wave is defined as the first and greatest upward deflection within an Electrocardiogram (ECG) time course in the line 1 configuration used in this study. This large wave is generated by electrical stimulation passing through the main portion of the ventricular walls to trigger ventricular contraction.

In this study, the R-waves were manually identified within BVA and tagged with markers to represent “R-wave events”. These markers were then subsequently utilised to segment the EEG data, which was time-locked to the R-wave reference marker starting at -100ms to 700ms. As part of the segmentation process, overlapping segments were allowed, and bad intervals were skipped. Segments were baseline-corrected using the period -100 to 0. HEPs are defined as the highest positive peak within the Fz channel in the window 200 to 600ms after the R wave and used an automated peak detected in BVA to detect the peaks and record the latency and amplitude.

6.2.6 Statistical analysis

Statistical analysis was performed using IBM SPSS 22 and Python with imported R libraries “CircStats” and “circular”.

Assumptions were initially assessed visually using histograms of the residuals and further verified numerically using normality, homogeneity, skewness, and kurtosis testing. Skewness and kurtosis values of z-score values exceeding -1 to +1 were considered normal distribution violations. These were further investigated with quantile-quantile plots and Cook’s distance values (Blanca et al., 2017).

Any Cook’s distance values above 0.5 would be considered an outlier and subsequently removed and replaced with linear interpolation. Outlier detection sensitivity for Cook’s distance has been previously reported as very high. Therefore this approach was chosen to be an appropriate method for objectively identifying outliers to complement the interpretation of QQ plots (Cousineau & Chartier, 2010; Senthamarai & Manoj, 2015).

Subject 23 had many missing observations and therefore was excluded from the analysis. Consequently, the total sample size for this analysis was 39. Significant interactions were followed-up with t-tests. Effect sizes for significant results were reported as partial eta squared (η_p^2). All statistical tests were two-tailed with an alpha-level of $p = .05$.

6.3 Results

6.3.1 Oscillatory power

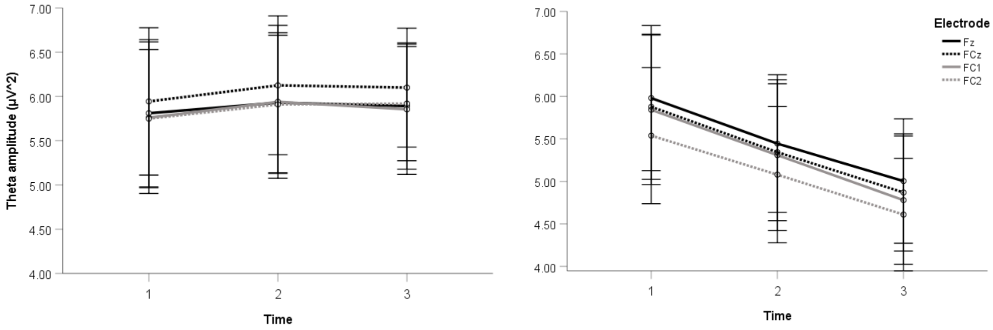
For the first analysis, I checked whether there is a difference in oscillatory power between woodland and Su-soku, between electrodes across time with a 3 x 4 x 2 mixed ANOVA, and within-subject factors of time (three levels) and electrode (four levels, Fz, FCz, FC1 left and FC2 right). Group was a between-subject factor with two levels (woodland or Su-soku).

Theta

There was a significant main effect of time, in which mean theta decreased monotonically across blocks, $F(3,\ 111) = 4.89$, $p = .003$, $\eta_p^2 = 0.116$. However, there was a significant group x time interaction, showing that this effect of time differed between groups, $F(2,\ 74) = 12.89$, $p = .001$, $\eta_p^2 = 0.16$. Follow up one-way ANOVAs showed that both groups had a significant decrease in theta across time, Time 1 vs later: $F\ (1,\ 37) = 20.31$, $p = <.001$, $\eta_p^2 = 0.35$, time 2 vs time 3: $F(1,\ 37) = 4.63$, $p = .04$, $\eta_p^2 = 0.11$. But the decrease was greater in the Su-soku group, $F(1,\ 37) = 10.21$, $p = .003$, $\eta_p^2 = 0.22$.

Theta power significantly differed between electrodes, whereby mean theta was greater for frontal than more posterior electrodes, $F(3,\ 111) = 4.89$, $p = .003$, $\eta_p^2 = 0.116$. However, there was also a significant group x electrode interaction, meaning that the effect of electrode differed between groups, $F(3,\ 111) = 3.07$, $p = .03$, $\eta_p^2 = 0.077$. Follow up one-way ANOVAs confirmed that mean theta was greatest in FCz for the woodland group, whereas mean theta power was greatest in Fz for the Su-soku group, $F(1,\ 37) = 9.58$, $p = .004$, $\eta_p^2 = 0.21$ (see Figure 6.1).

There was no significant interaction between time and electrode, $F(6, 222) = 0.77$, $p = .6$, $\eta_p^2 = 0.020$, between time, electrode and group, $F(6, 222) = 0.55$, $p = .76$, $\eta_p^2 = 0.015$, and no significant between-subject effects of group, $F(1, 37) = 2.08$, $p = .16$, $\eta_p^2 = 0.053$.



(a) Woodland

(b) Su-soku

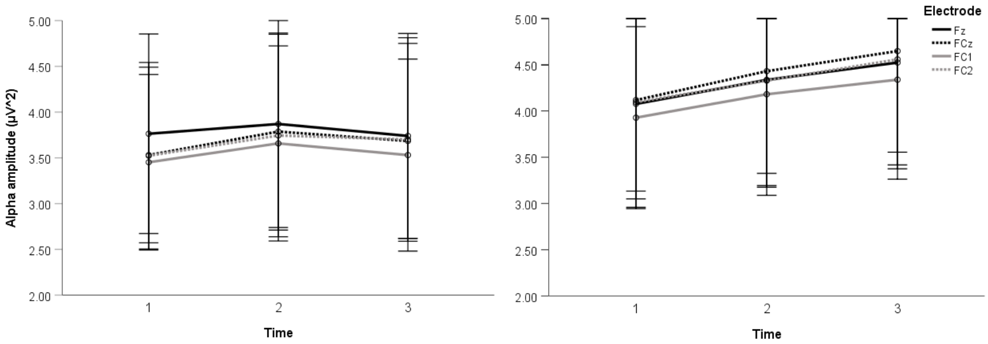
Figure 6.1: Change in theta amplitude across time by electrode for both group conditions with 95% within subject confidence intervals. (a) Shows that for the woodland condition, mean theta power does not significantly vary across time. Meanwhile (b) suggests that mean theta oscillatory power decreases monotonically across time.

Alpha

There was no significant main effect of time, F (1.28, 47.37) = 2.02, $p = .16$, η_p^2= 0.052, which suggests that alpha power did not significantly change during the meditative phase and this effect did not significantly vary between group conditions, F (1.28, 47.37) = 0.20, $p = .71$, $\eta_p^2 =$ 0.005.

Alpha power significantly differed between electrodes, in which mean alpha was greater for frontal than more posterior electrodes, F (2.54, 93.96) = 7.46, p = <.001, η_p^2= 0.17, and there was no significant interaction between electrode and group allocation, F (2.54, 93.96) = 1.30, p = .28, η_p^2= 0.034. This means that alpha oscillatory power was significantly different between electrodes, but this effect was not significantly different between group conditions. Follow up one-way ANOVAs revealed that Fz and FCz had the greatest mean alpha power compared to the other electrodes, F (1, 37) = 14.24, p = .001, η_p^2 = 0.27, and there was no significant difference in mean alpha power between FC1 left and FC2 right, F(1, 37) = 7.46, p = .010, η_p^2 = 0.17. Overall this indicates that in both Su-soku and woodland, mean alpha power increases at a fronto-central source.

There was no significant interaction between block and electrode, F (3.11, 115) = 2.70, p = .05, η_p^2 = 0.068, and there was no significant interaction with group, F(3.10, 115) = 1.03, p = .38, η_p^2= 0.027. This means the differences in oscillatory power between electrodes did not significantly vary over time or between groups. There was no significant between-subject effects of group allocation, F(1, 37) = 0.86, p = .36, η_p^2 = 0.023. (see Figure 6.2).



(a) Woodland (b) Su-soku

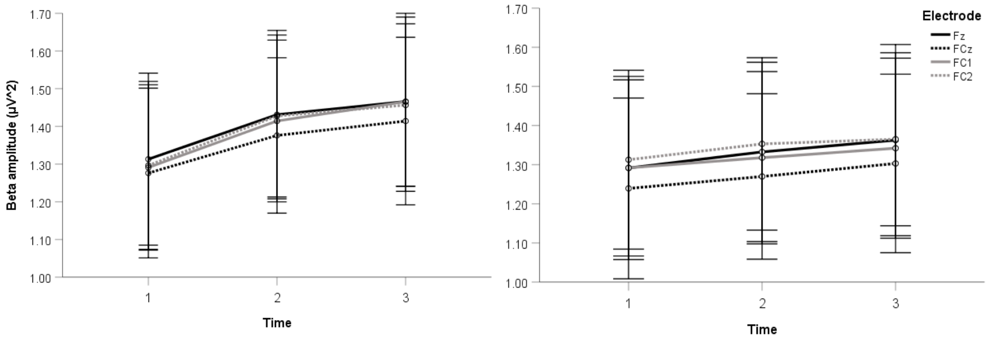
Figure 6.2: Change in mean alpha power across time by electrode for both group conditions with 95% within subject confidence intervals. (a) Shows in the woodland condition there is no significant change in mean alpha power across time. (b) While the Su-soku condition appears to show that mean alpha power increases across time, this effect was not statistically significant.

Beta

There was no significant main effect of time, meaning that, although beta power tended to increase across time, the extent of change was not enough to warrant significance, F (1.44, 53.2) = 3.64, p = .05, $\eta_p^2 = 0.090$. Furthermore, there was no significant difference in beta power across time between group conditions, $F(1.44, 53.2) = 1.01$, p = .37, $\eta_p^2 = 0.027$.

Beta power significantly differed between electrodes, $F(2.04, 75.81) = 3.69$, p = .03, $\eta_p^2 = 0.091$, however, this effect did not vary between group conditions, $F(2.05, 75.81) = 0.34$, p = .78, $\eta_p^2 = 0.004$. Follow up one-way ANOVA revealed that beta power was significantly lower in FCz compared to the other fronto-medial electrodes, $F(1, 37) = 5.66$, p = .023, $\eta_p^2 = 0.13$. While there was no significant difference in beta power between Fz and other FM electrodes, $F(1, 37) = 1.51$, p = .23, η_p^2= 0.04, and between FC1 and FC2, $F(1, 37) = 0.96$, p = .33, $\eta_p^2 = 0.025$ (see Figure 6.3).

There was no significant interaction between block and electrode, $F(2.03, 74.96) = 0.82$, $p = .45$, $\eta_p^2 = 0.022$ and this did not significantly differ between group conditions, $F(2.03, 74.96) = 1.19$, $p = .31$, $\eta_p^2 = 0.031$. There was no significant between subject effects of group allocation, $F(1, 37) = 0.22$, $p = .64$, $\eta_p^2 = 0.006$.



(a) Woodland

(b) Su-soku

Figure 6.3: Mean beta oscillatory power across time by electrode for both group conditions with 95% within subject confidence intervals. (a) Shows that beta power appears to increase across time for the woodland condition. (b) In contrast, beta power appears to moderately increase across time. For both conditions we note here that FCz had significantly lower mean beta power, denoted by the black dotted line.

Theta/beta ratio

Chapter 4 explored whether there is a change in theta/beta ratio during Su-soku in order to clarify interpretations of attention. I reported in this chapter that the theta/beta ratio decreases across Su-soku, which suggests that Su-soku increases attention awareness and reduces mind-wandering states. If my original findings are robust, I expected to find a similar result for the replication analysis.

In the current analysis, theta/beta ratio significantly changed across time, F (1.41, 52) = 8.40, $p = 0.001$, $\eta_p^2 = 0.19$, but this effect did not significantly differ between group conditions, $F(1.41,\ 52) = 0.68$, $p = .001$ (see Figure 6.4). Follow up contrasts revealed theta/beta ratio significantly decreased from time 1, $F(1,\ 37) = 9.32$, $p = .004$, $\eta_p^2 = 0.20$. But there was no significant difference between time 2 and time 3, $F(1,\ 37) = 4.08$, $p =$ .051 (see Figure 6.4).

There was a significant difference in theta/beta ratios between electrodes, $F(2.55,\ 94.3) = 3.72$, $p = 0.02$, $\eta_p^2 = 0.091$, but this effect did not significantly differ between group conditions, $F(2.55,\ 94.3) = 1.23$, $p = .30$. Follow up contrasts suggest FCz had significantly greater theta/beta ratio than posterior electrodes, $F(1,\ 37) = 6.34$, $p = 0.016$, $\eta_p^2 = 0.15$, but there was no significant difference between Fz and other electrodes, F (1, 37) = 0.55, $p = .46$, $\eta_p^2 = 0.015$, and between FC1 and FC2, $F(1,\ 37) = 1.56$, $p =$ 0.22, $\eta_p^2 = 0.040$, power = .23. While theta-beta ratio was greater for FCz, there was a general decline for most electrodes across time (see Figure 6.5).

There was no significant interaction between time and electrode, $F($ 1.30, 47.9) = 0.83, $p = .40$, $\eta_p^2 = 0.022$, and no significant interaction between time electrode and group, $F(1.29,\ 47.9) = 1.25$, $p = .28$, $\eta_p^2 = 0.033$. While there were no significant between-subject effects of group, $F($ 1, 37) = 0.34, $p = .56$, FCz appears to rapidly increase between blocks for the woodland condition, whereas there was only a moderate increase between time two and time three for the Su-soku condition.

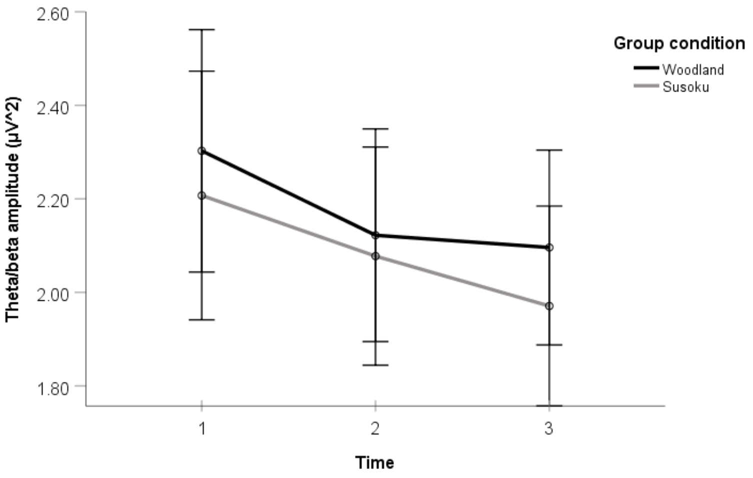


Figure 6.4: Comparing changes between woodland and Su-soku in theta/-beta amplitude across time

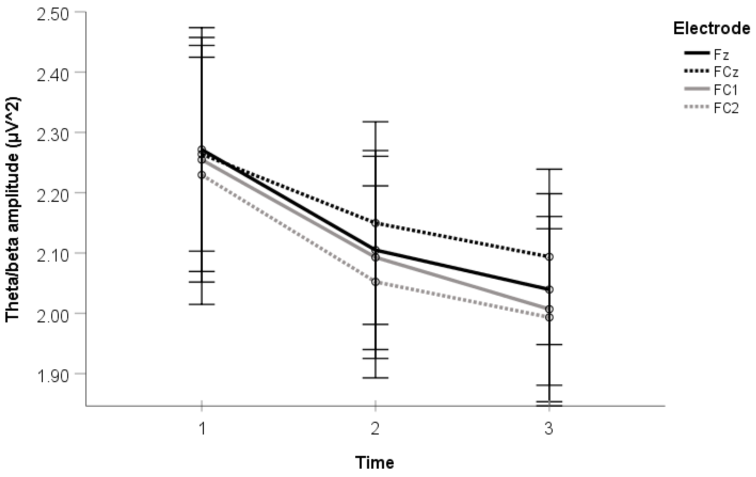


Figure 6.5: Mean theta/beta oscillatory power appears to significantly decrease across time for most electrodes (Fz, FC1 and FC2). FCz appears to somewhat increase between time 2 and time 3, but this was not significantly different from the other FM-electrodes.

6.3.2 Autonomic activity

Chapter 4 explored whether the change in theta power was associated with a change in autonomic activity, indexed by CVI and CSI measures. Su-soku appeared to influence vagal tone, indicated by the up-regulating of mean RMSSD across Su-soku blocks. However, Su-soku did not appear to influence CVI and CSI, nor was there a significant relationship between theta power and autonomic measures.

The findings in this chapter suggested an opposite effect of theta power for Su-soku, compared to Chapter 4, as mean theta power decreases across time. If there is a connection between theta power and vagal tone, I suspected there might also be a difference in autonomic findings compared to Chapter 4. Initially, I assessed whether there was a relationship between HR and autonomic measures using Pearson's correlation analysis.

Autonomic measure	$r(39)$	$p =$
RMSSD	-0.26	0.11
CVI	-0.27	0.10
CSI	0.11	0.49
RSA amplitude	-0.12	0.46
Respiratory rate	-0.09	0.61

Table 6.1: Pearson's correlation analysis results between heart rate and several autonomic indices.

According to my findings, all autonomic indices measured in this study, apart from CSI, appeared to be negatively correlated with heart rate (see Table 6.1). This is expected as a faster heart rate tends to be associated with a greater sympathetic drive, which is the opposite of vagal tone measures such as RMSSD and CVI. However, there were no statistically significant results between heart rate and autonomic indices.

Following this, I examined whether there were statistical differences in autonomic measures between Su-soku and the woodland condition. Assumptions of homogeneity and normality were confirmed by Levene's test and histograms (see Table 6.2).

Autonomic measure	$F(1, 37)$	$p =$
RSA peak frequency	0.010	0.92
RSA amplitude	5.81	0.02*
RMSSD	0.38	0.54
CVI	0.024	0.88
CSI	0.022	0.88

Table 6.2: Levene's test results for autonomic measures between groups

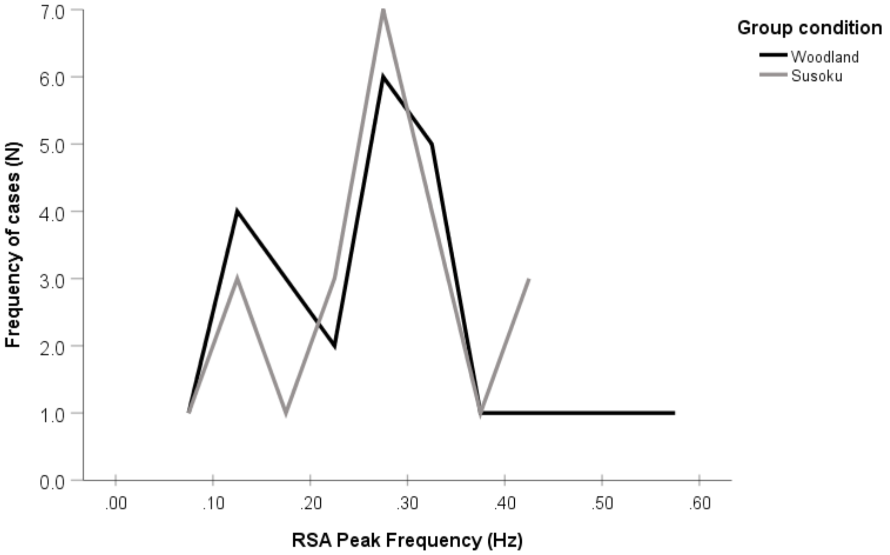
Based on these results, I performed a one-way analysis of variance to calculate the main effects, with an independent variable of group allocation (Su-soku or woodland) and five dependent variables of autonomic measure (RSA peak frequency, RSA amplitude, RMSSD, CVI and CSI). Due to the indication of unequal variances for RSA amplitude, Welch's one-way ANOVA was performed for this parameter. 95% confidence intervals for the fixed ANOVA model are also reported here (see Table 6.3 for results).

Autonomic measure	$F(1, 37)$	$p =$	**CI**
RSA peak frequency	0.30	0.59	[0.22+0.29]
RSA amplitude*	1.58	0.22	[26.89+48.86]
RMSSD	0.42	0.52	[42.56+55.86]
CVI	0.23	0.63	[4.54+4.75]
CSI	0.65	0.43	[2.61+3.28]

Table 6.3: One-way ANOVA results for autonomic measures with 95% confidence intervals (*with Welch's ANOVA values reported for RSA amplitude)

Regarding Table 6.3, no statistically significant differences between woodland and Su-soku conditions were found for any autonomic measures. I produced several graphs to visualise group differences in autonomic measures (see Figures 6.6, 6.7 and 6.8). These findings suggest that the differences in theta power between Su-soku and woodland are unlikely to be related to autonomic change.

In summary, the current study brief meditative states are unlikely to significantly influence the autonomic state, compared to a relaxing woodland scene.



(a) RSA peak Frequency

(b) RSA Amplitude

Figure 6.6: (a) When comparing the differences between woodland and Susoku group conditions there appears to be a large portion of participants in both groups who exhibited an RSA peak frequency around 0.3Hz, and another spike around 0.1Hz range. (b) Mean RSA amplitude appears to be greater for Su-soku than woodland, which is likely due to paced breathing in Su-soku. As RSA depends on breathing rate, a more variable breathing rate, which you would expect in non-paced conditions, will tend to give a broader RSA.

(a) Mean CVI

(b) Mean CSI

Figure 6.7: (a) When comparing the differences in mean CVI between group condition with 95% within subject confidence intervals there is little difference in overall CVI between group conditions. (b) Similarly when comparing the differences in mean CSI between group conditions there is little difference in overall CSI between group conditions.

Figure 6.8: Comparing the differences in mean RMSSD between group conditions, there is little difference in RMSSD between group conditions

6.3.3 FM-theta vs autonomic tone

Despite the lack of group differences, Chapter 4 suggested that change in theta power may be associated with autonomic activity. Given previous analyses indicating a change in frontal-midline theta power in the current dataset, I performed a Pearson's correlation analysis to examine whether there was a relationship between autonomic tone and theta power across the group conditions. This analysis highlighted a moderate correlation between RMSSD and theta power, a small relationship between theta, RSA amplitude and respiratory rate and a negative correlation between theta and heart rate. However, there were no statistically significant correlations (see Table 6.4).

Autonomic measure	$r(39)$	$p =$
RMSSD	0.14	0.39
HR	-0.22	0.18
RSA amplitude	0.09	0.59
Respiratory rate	0.10	0.56

Table 6.4: Pearson's correlation analysis results between frontal-midline theta power and several autonomic indices

Interim discussion

In summary, the results from the Chapter 4 replication analysis were unexpected. Based on previous findings, I hypothesised that, during Su-soku, frontal-midline theta would increase across time, but instead, frontal-midline theta power decreased across time. One explanation could be the differences in task delivery between the experiments.

For the data set used in Chapter 4, referred to as Su-soku 1, the Su-soku blocks were shorter and presented in-between various cognitive blocks. In comparison, for the data used in this chapter, referred to as Su-soku 2, there was only a single uninterrupted session of Su-soku. Therefore, participants could sustain paced breathing for longer compared to Su-soku 1. It could be argued that a continuous session of Su-soku enables participants to become more in tune with their breath. As a result, this reduces the requirement for monitoring resources leading to either a reduction in arousal or enhanced cognitive control during sustained practice of Su-soku. This interpretation is furthered by the instruction differences between Su-soku and woodland. Su-soku meditation requires participants to maintain a regular breathing pace, reinforcing the reduced need for monitoring resources. In contrast, woodland requires participants to observe a constantly changing animated clip that they have no direct control over.

Alpha power was significantly greater in frontal regions (Fz and FCz) compared with central areas, but there were no differences between group conditions. The lack of group differences could be explained by the fact that both tasks require attention. While beta power did not significantly change across time, there is a general increase across blocks. The most interesting finding relates to the differences in electrodes, as previously, no significant differences were reported between electrodes for beta power. Beta power was significantly lower in FCz for both groups compared to the other electrodes.

These findings could be related to the differences in the contrasting "woodland" condition between the data sets. In Su-soku 1, the "woodland" condition was initially utilised as a baseline condition. As a result, there is a disparity in duration between this baseline condition and Su-soku, respectively 2 minutes vs 10 minutes. Whereas, due to the between-subject experimental design in Su-soku 2, the durations were identical between Su-soku and woodland conditions. As a result, the attentional resources required by these tasks are more comparable in the Su-soku 2 data set. But despite this, there were no significant differences in autonomic measures between group conditions, and there was no statistically significant association between theta power and vagal tone.

6.3.4 Oscillatory power by respiratory phase

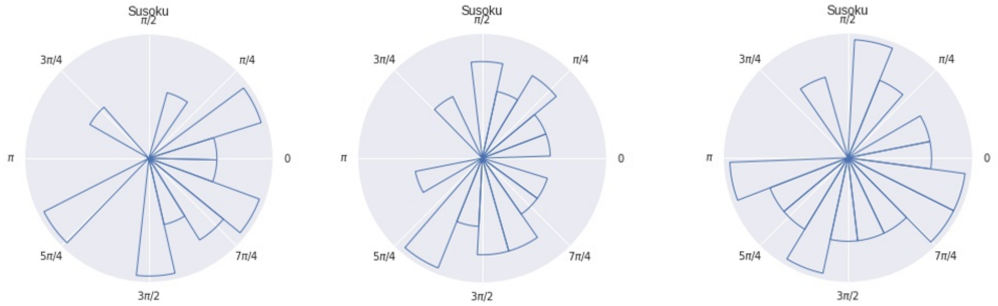
Chapter 5 investigated whether there was a difference in respiratory phase angle between frequency bands during Su-soku. I found average phase angles were significant for all frequency bands and tended to lie in the quarter just before inhalation. As in Chapter 5, Rayleigh's test was performed to assess whether the average phase angles for each subject at each frequency significantly differed from a von Mises distribution (analogous to a normal distribution) for the Su-soku condition. Surprisingly, these summary statistics suggest that only alpha oscillations significantly differed from a von Mises distribution, $p = 0.0017$ (see Table 6.5).

Parameter	**Alpha**	**Theta**	**Beta**
Mean direction	-0.60	-0.41	-0.74
Mean resultant length	0.042	0.036	0.044
Circular variance	0.95	0.96	0.95
Circular Std	2.58	2.67	2.55
Rayleigh test statistic	0.45	0.22	0.21
$p =$	0.0017	0.4	0.42

Table 6.5: Summary statistics for electrode Fz for Su-soku condition

Chapter 5 also investigated how phase angle changes over blocks and found a significant mean direction for theta, beta and alpha oscillatory activity. Beta activity became significantly more concentrated towards the exhalation to the inhalation transition point, whereas alpha and theta activity moved significantly towards $-\pi/2$. And on average, theta occurred before the alpha phase angle. These results suggested an optimal phase angle for sustained attention, as oscillatory power increases across blocks and oscillations become increasingly clustered towards a mean direction. As the current data set was continuously collected, it is challenging to segment the data into blocks and accurately align the data according to the start of inhalation. Therefore, I could only explore how phase angle changes on average across the respiratory cycle during Su-soku (see Figure 6.9).

Similarly to Chapter 5, the oscillatory data collected during Su-soku was assigned a time marker relative to the start of the respiratory cycle, which was then converted into angles. Therefore the polar plots represent the average tendency for neural activity to occur during respiration. From these polar plots, we can observe a general tendency for alpha oscillatory power to occur in the latter quarter before inhalation - similar to the findings reported in Chapter 5. In addition, a more uniform distribution of phase angles can be observed for theta and beta.



(a) Alpha (b) Beta (c) Theta

Figure 6.9: Polar plots representing average phase angles across participants for Su-soku: This visual representation suggests there is only a tendency for alpha to be distributed towards a mean direction, the lower right quadrants.

Respiratory phase angle discussion

In brief, the results reported here differ from those reported in Chapter 5. The previous phase angle analysis suggested a significant mean direction for oscillatory power across all frequency bands. In contrast, the current study only found a significant mean phase direction for an alpha power towards the end of exhalation/start of inhalation. Despite these results, it is difficult to interpret findings for other frequencies due to many participants' mean phase angles in various directions in the polar plot.

6.3.5 Mood state change

A one-way ANOVA was used to assess whether the change in Profile of Mood States significantly differed between group conditions (Su-soku and woodland). The results revealed there was no significant difference in POM score change between Su-soku (M = -1.30 , SD = 13.97, CI [-7.83 - 5.24]) and woodland (M = 2.40, SD = 8.48, CI [-7.84 - 5.24]), F (1, 37) = 1.025, p = .32 (see Figure 6.10).

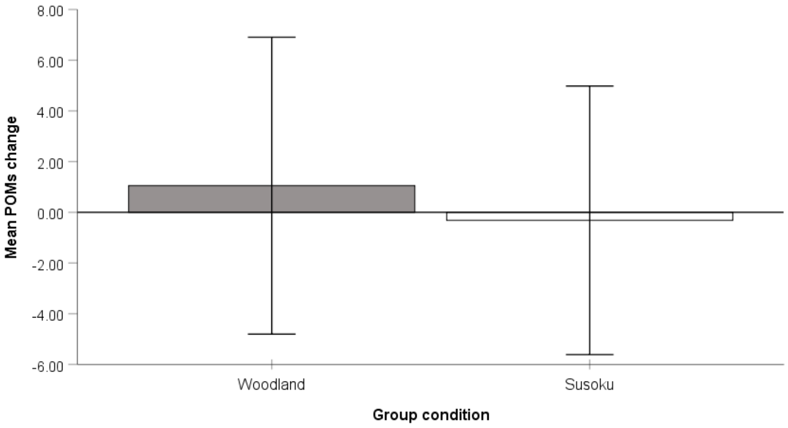
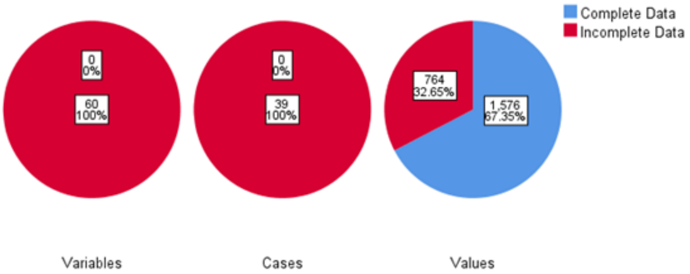


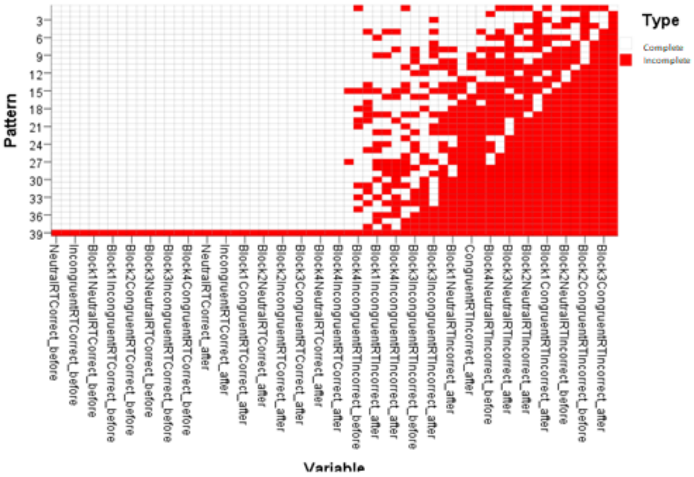
Figure 6.10: Comparing the differences in POM change between woodland and Su-soku group conditions. The overlapping 95% confidence intervals visually demonstrates the lack of statistical difference between group conditions.

6.3.6 Flanker task performance

Initially, the flanker task data was examined to see whether dividing the data into correct and incorrect trials would be statistically feasible. As most participants responded correctly to all trial types, there was an insufficient number of incorrect trials for a meaningful comparison between correct and incorrect trial types (see Figure 6.11). Given this discrepancy between correct and incorrect trials, only correct trials were chosen to be examined in the flanker analysis.



(a) Summary of overall complete data



(b) Data value patterns

Figure 6.11: This graph reflects data values collected by trial type for the Flanker task. From this we can observe the most participants were accurate across all trial types, and as a result there are a minimal number of recorded incorrect trials (shown as incomplete data here).

Reaction time

A three-way mixed ANOVA was performed to test whether group allocation influences reaction time during the flanker task. In this analysis, the dependent variable was reaction time, and the independent variables were: 1) Congruency at three levels (neutral, congruent and incongruent), 2) Block at four levels, and 3) Time at two levels (before and after interventions), and a between-subject factor of group allocation, Su-soku or woodland.

There was a significant main effect of time on RT, $F(1,\ 36) = 9.23$, $p = .004$, $\eta_p^2 = 0.20$. But there was no significant interaction effect with group condition, $F(1,\ 36) = 0.10$, $p = .75$. Follow up contrasts revealed reaction time was significantly slower before the group intervention condition, compared to after, $F(1,\ 36) = 9.24$, $p = .004$, $\eta_p^2 = 0.20$. While participants were faster after their group intervention, there was an absence of block effect on RT; therefore, these findings are unlikely due to just practice across a continuous series of blocks, $F(1.86,\ 66.89) = 0.87$, $p = .46$. There were also significant interaction effects by group condition, $F(1.86,\ 66.89) = 0.12$, $p = .87$. Therefore, it appears that practice, followed by a relaxing break, is associated with decreased reaction time (see Figure 6.12).

There was a significant main effect for congruency on RT, $F(1.31,\ 47.26) = 275.24$, $p = <.001$, $\eta_p^2 = 0.88$. But there were no significant interaction effects by group condition, $F(1.31,\ 47.26) = 0.22$, $p = .70$. As a result, these findings indicate there was no significant effect of group allocation on reaction time during the flanker task, $F(1,\ 36) = 0.083$, $p = .78$, $\eta_p^2 = 0.002$.

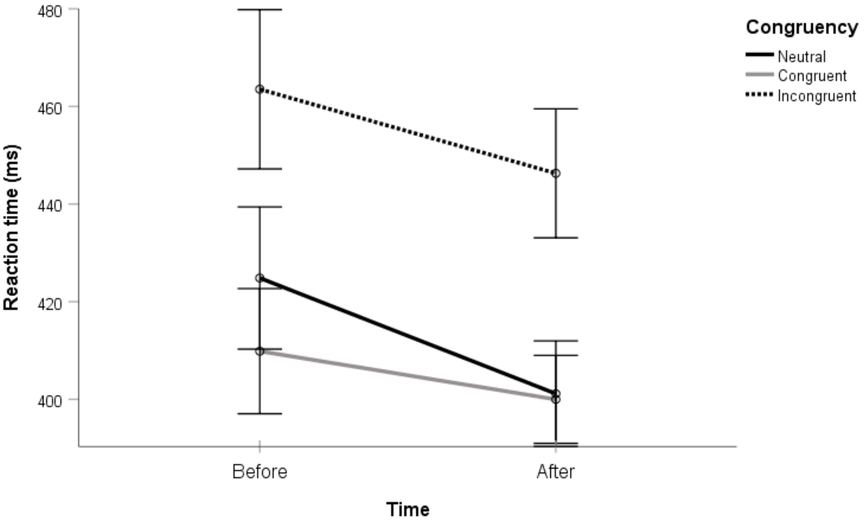


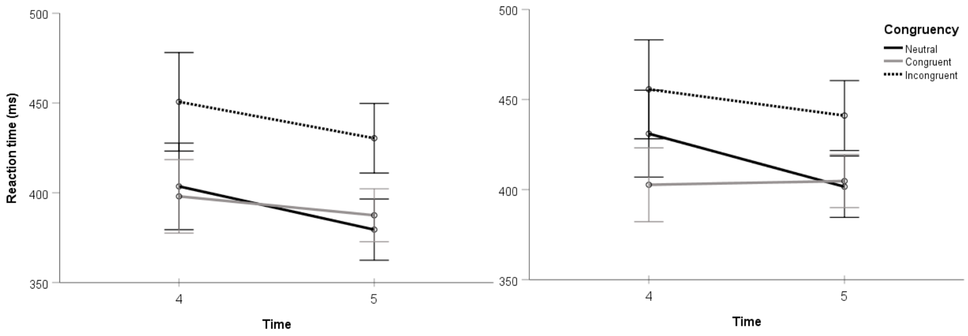
Figure 6.12: Change in reaction time between before and after group conditions by congruency. We can note here that there is a significant decrease in reaction time from before the meditation conditions compared to after.

To further investigate whether there is a difference in consolidation effect between group conditions, a second two-way mixed ANOVA was performed with independent variables of 1) Time at two levels (task block before intervention vs task block after intervention), 2) Congruency at three levels (neutral, congruent and incongruent) with a between-subject factor of group allocation (Su-soku or woodland).

There were no significant differences in reaction time before and after group conditions, $F(1,\ 36) = 4.26$, $p = .05$, $\eta_p^2 = 0.11$. And there were no significant differences between group conditions, $F(1,\ 36) = 0.08$, $p = .78$, $\eta_p^2 = 0.002$. This finding further clarifies previous analysis, where significant effects for time were reported. These results suggest that Su-soku did not differ from woodland; however, we cannot exclude the possibility that a relaxing 'meditative' state promotes consolidation of performance gain (see Figure 6.13).

Similarly to the previous analysis, there was a significant effect of congruency on reaction time, $F(1.733, 62.39) = 81.76$, $p = <.01$, $\eta_p^2 = 0.69$, but no significant interaction between congruency and group condition, $F(1.73, 62.39) = 2.61$, $p = .080$, $\eta_p^2 = 0.07$. (see Figure 6.13). Follow up contrasts revealed a significant difference between neutral and congruent reaction time, $F(1, 36) = 25.72$, $p = <.001$, $\eta_p^2 = 0.42$,and a significant difference between congruent and incongruent reaction time, $F(1, 36) = 139.16$, $p =<.001$, $\eta_p^2 = 0.79$.

There was a significant interaction between time and congruency, $F(1.58, 56.71) = 6.11$, $p = .007$, $\eta_p = 2 = 0.15$. There was not a significant interaction between time, congruency and group condition, $F(1.58, 56.71) = 0.98$, $p = .36$, $\eta_p^2 = 0.026$. There was no significant between-subject factors of group condition, $F(91, 36) = 1.79$, $p = .19$, $\eta_p^2 = 0.047$. As a result of this analysis, it seems that there is not much difference in the consolidation effect between these two conditions.



(a) Woodland (b) Su-soku

Figure 6.13: These line graphs present the change in reaction time between before and after group conditions by congruency type with 95% confidence intervals. Regardless of group allocation, we can note that participants were slower in reacting to incongruent stimuli than congruent and neutral trial types. We can also note that participants significantly improved their reaction time for neutral trial types, whereas there was little change for congruent trials.

Accuracy

A 2x4x3 repeated measures ANOVA was conducted to assess the effects of meditation on performance accuracy at two levels of time (before and after), four levels of time and three levels of congruency (neutral, congruent and incongruent) with a between-subject factor of group condition. For the purposes of comparison, accuracy percentages were first converted into Z-scores. 100% accuracy trials were subtracted by 0.01 to allow for Z-score conversion.

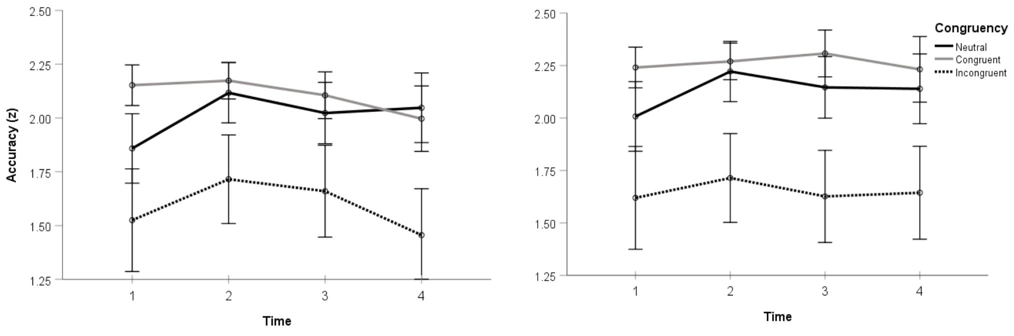
Accuracy did not significantly change between before and after group conditions, $F(1,\ 36) = 1.19$, $p = .28$, $\eta_p^2 = 0.032$, and there was no significant interaction with group condition, $F(1,\ 36) = 0.57$, $p = .46$, $\eta_p^2 = 0.016$.

However, accuracy did significantly change across time, $F(2.43,\ 87.29) = 4.91$, $p = .003$, $\eta_p^2 = 0.12$, and this effect was not significantly different between group conditions, $F(2.43,\ 87.29) = 0.34$, $p = .75$, $\eta_p^2 = 0.009$ (see Figure 6.14). Accuracy also significantly changed depending congruency trial type, $F(1.81,\ 42.53) = 48.68$, $p = <.001$, $\eta_p^2 = 0.58$, and this effect was not significantly different between group conditions, $F(1.18,\ 42.53) = 1.08$, $p = .35$, $\eta_p^2 = 0.029$.

There was a significant interaction between time and block, $F(2.53,\ 91.23) = 10.48$, $p = <.001$, $\eta_p^2 = 0.23$, which means that accuracy was significantly different across blocks between before and after group conditions. But this effect on accuracy does not appear to be sensitive to group condition type, as there was no significant interaction between time, block and group condition, $F(2.53,\ 91.23) = 2.72$, $p = .098$, $\eta_p^2 = 0.023$.

Follow up contrasts revealed that accuracy was highest at block 2 compared to later blocks, $F(1, 36) = 9.56$, $p = .004$, $\eta_p^2 = 0.21$. But there was no significant difference in accuracy at block 1 vs later, $F(1, 36) = 3.93$, $p = .06$, $\eta_p^2 = 0.098$, and between level 3 vs level 4, $F(1, 36) = 2.57$, $p = .12$, $\eta_p^2 = 0.067$. For congruency, neutral trials resulted in significantly lower accuracy than congruent trials, but significantly greater accuracy than incongruent trials, $F(1, 36) = 31.73$, $p = <.001$, $\eta_p^2 = 0.47$. The greatest difference was between incongruent and congruent trials, whereby accuracy was greatest for congruent trials compared to all other trial types, $F(1, 36) = 52.59$, $p = <.001$, $\eta_p^2 = 0.59$.

For the interaction between time and block, accuracy was significantly increased from block1 after group conditions compared to before, $F(1, 36) = 20.90$, $p = <.001$, $\eta_p^2 = 0.37$. But there were no significant differences found between block 2 and block 3, $F(1, 36) = 2.33$, $p = .14$, $\eta_p^2 = 0.061$, and block 3 and block 4, $F(1, 36) = 0.61$, $p = .44$, $\eta_p^2 = 0.017$.
There was no significant between subject effect of group condition, $F(1, 36) = 0.32$, $p = .58$, $\eta_p^2 = 0.009$.



(a) Woodland

(b) Su-soku

Figure 6.14: Accuracy between before and after blocks by congruency with 95% confidence intervals

Flanker results discussion

Encouragingly, these flanker results echo consistent findings in the congruence effect literature, whereby reaction time is slowest for incongruent trials and fastest for congruent trials. These differences are often explained by the additional cognitive resources required to process incongruent/distractor flankers compared to congruent/facilitating flankers.

However, surprisingly findings indicate that reaction time for neutral trials was significantly faster for woodland than for Su-soku. One explanation could be related to a “cognitive overloading” effect. In neutral trials, the distractor stimuli were not relevant to the target, i.e. they were not arrows, which may have been easier to process for woodland participants as they were passively observing a woodland scene rather than counting their paced breathing as required for the Su-soku condition.

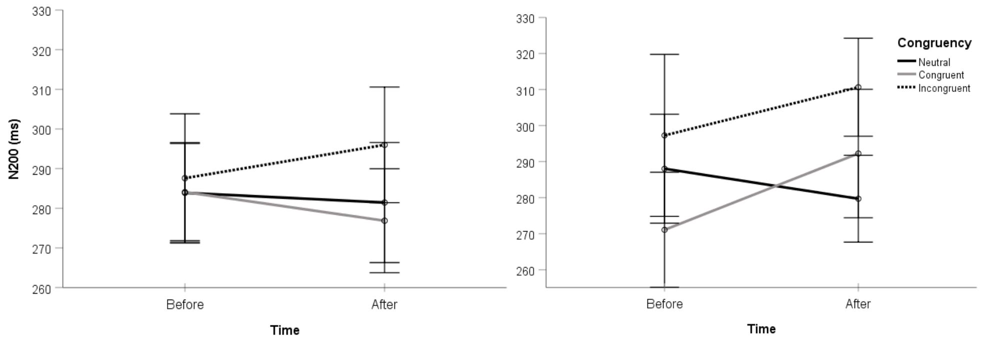
There were no differences between groups for accuracy, but accuracy generally improved over time. A straightforward explanation for this would be “learning effects”, whereby performance improves the longer they are engaged in the task. These findings also suggest that the break from task performance may promote consolidation.

6.3.7 Event related potentials during Flanker

N200 Latency for correct trials

There was a significant effect of congruency on N200 latency, $F(1.46, 53.86) = 10.45$, $p = <.001$, $\eta_p^2 = 0.22$. But there was no significant interaction with group condition, $F(1.46, 53.86) = 1.26$, $p = .28$, $\eta_p^2 =0.033$. There was no significant effect of time on N200 latency, $F(1, 37) = 1.13$, $p = .30$, $\eta_p^2 = 0.030$. And there was no significant interaction with group condition, $F(1, 37) = 1.37$, $p = .25$, $\eta_p^2 = 0.04$. There was a significant interaction between congruency and time, $F(1.76, 65.40) = 4.69$, $p = .02$, $\eta_p^2 = 0.11$, and there was a significant interaction with group condition, $F(1.76, 65.40) = 5.00$, $p = .012$, $\eta_p^2 = 0.12$.

Follow up contrasts revealed that N200 occurred significantly later for in-congruent trials ($M = 303.1$, $SD = 30.29$) than congruent trials ($M = 284.36$, $SD = 33.2$), $F\ (1, 37) = 12.94$, $p = .001$, and neutral trials ($M = 279.71$, $SD = 24.99$) occurred significantly earlier than in-congruent trials, $F(1, 37) = 5.07$, $p = .030$, $\eta_p^2 = 0.12$. There was no significant between subject effect of group condition on latency, $F(1, 37) = 0.40$, $p = .53$, $\eta_p^2 = 0.011$. See Figure 6.15 for a visual representation of these results.



(a) Woodland (b) Su-soku

Figure 6.15: Mean N200 latency between before and after group conditions by congruency type. Both (a) and (b) show the increased latency for incongruent trials compared to other trial types, presented by the black dotted line.

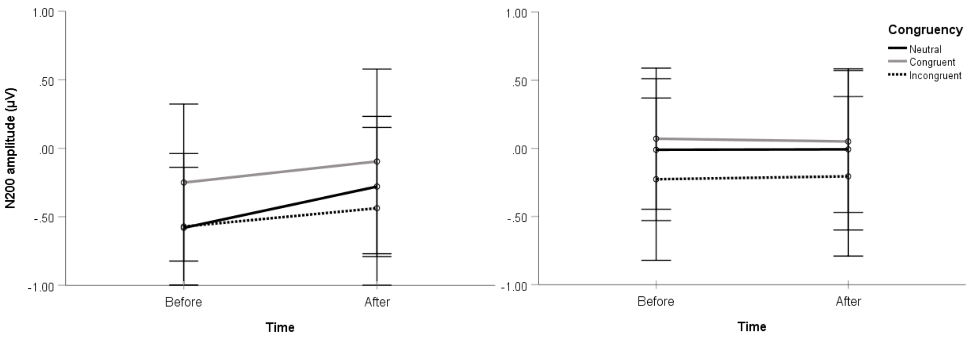
N200 Amplitude

Conducted a 3x2 repeated measures ANOVA to assess whether there is a difference in N200 amplitude based on congruency type (neutral, congruent and incongruent) and time (before and after group condition).

There was a significant effect of congruency on N200 amplitude, $F(1.98, 73.41) = 12.16$, $p = <.001$, $\eta_p^2 = 0.25$, but no significant interaction effects between congruency and group condition, $F(1.98, 73.41) = 1.24$, $p = .30$, $\eta_p^2 = 0.032$ (see Figure 6.16). There was no significant effect of time on N200 amplitude, $F(1, 37) = 0.59$, $p = .45$, $\eta_p^2 = 0.016$, and no significant interaction effects between time and group condition, $F(1, 37) = 0.58$, $p = .45$, $\eta_p^2 = 0.015$.

There was no significant interaction between congruency and time, $F(1.83, 67.73) = 0.50$, $p = .59$, $\eta_p^2 = 0.013$. There was no significant interaction between congruency, time and group condition, $F(1.83, 67.73) = 0.51$, $p = .59$, $\eta_p^2 = 0.014$.

Follow up contrasts revealed the N200 amplitude was significantly more negative during in-congruent (M = -0.32, SD = 1.23) than congruent trials, (M = -0.025, SD = 1.07), $F(1, 37) = 25.99$, $p = <.001$, $\eta_p^2 = 0.41$. However there was no significant difference between neutral (M = -0.14, SD = 1.15) and other congruent trials, $F(1, 37) = 0.044$, $p = .84$, $\eta_p^2 = 0.01$. There was no significant between-subject effects of group condition, $F(1, 37) = 0.84$, $p = .36$, $\eta_p^2 = 0.022$.



(a) Woodland (b) Su-soku

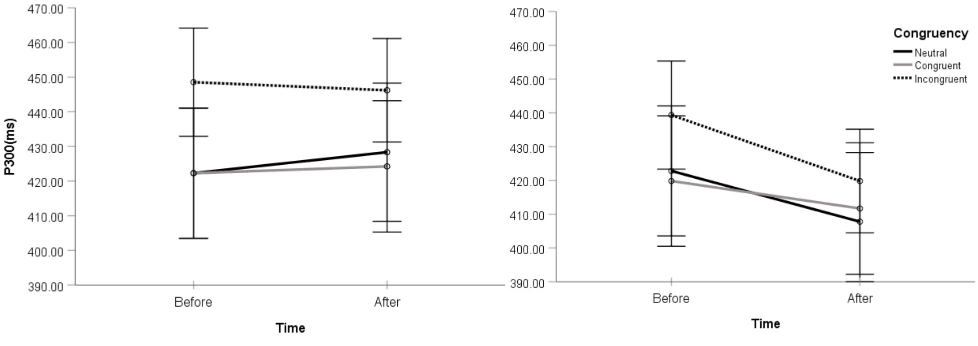
Figure 6.16: Change in mean N200 amplitude before and after group conditions by congruency types with 95% confidence intervals. In both (a) and (b) graphs we can see that incongruent trials produced significantly greater negative N200 amplitude, compared to other trial types.

P300 Latency

To assess the effects of group allocation on P300 latency, I conducted a two-way mixed ANOVA with independent variables of 1) congruency at three levels (neutral, congruent and incongruent), 2) time (before and after group conditions) with a between-subject factor of group allocation (woodland or Su-soku).

There was a significant effect of congruency on P300 latency, $F(1.36, 50.16) = 9.21$, $p = <.001$, $\eta_p^2 = 0.20$, but no significant interaction with group condition, $F(1.36,\ 50.16) = 0.58$, $p = .50$, $\eta_p^2 = 0.02$. Furthermore, there was no significant effect of time on P300 latency, $F(1,\ 37) = 1.75$, $p = .19$, $\eta_p^2 = 0.05$, and no significant interaction with group condition, $F(1,\ 37) = 2.99$, $p = .075$. There was no significant interaction between congruency and time, $F(1.47,\ 54.47) = 0.58$, $p = .51$, $\eta_p^2 = 0.02$. And there was no significant interaction with group condition, $F(1.47,\ 54.47) = 0.26$, $p = .91$, $\eta_p^2 = 0.007$.

Despite finding little evidence for a statistically significant effect of group condition, $F(1,\ 37) = 1.61$, $p = .21$, $\eta_p^2 = 0.042$, Figure 6.17 shows an interesting P300 latency decrease between before and after for all trial types in Su-soku, but not for woodland. While this may indicate an improved processing efficiency attributable to Su-soku, this analysis was adequately powered to detect a small effect (f=0.2) with 80% power and a significant level of p<0.05.



(a) Woodland (b) Su-soku

Figure 6.17: Mean P300 latency between before and after group conditions by congruency type. In both (a) and (b) P300 occurs earlier in congruent and neutral trials, compared to incongruent trial types. In addition, note (b) that P300 appears to occur earlier for all trials after Su-soku - where as there appears to be little change for the woodland condition.

P300 Amplitude

Conducted a 3x2 repeated measures ANOVA to assess whether there is a difference in P300 amplitude based on congruency type (neutral, congruent and incongruent) and time (before and after group condition).

Congruency had a significant effect on P300 amplitude, $F(1.48,\ 54.89) = 4.61$, $p = .01$, $\eta_p^2 = 0.11$, and there was no significant interaction with group condition, $F(1.48,\ 54.89) = 0.43$, $p = .59$, $\eta_p^2 = 0.01$ (see Figure 6.18). Time did not have a significant effect on P300 amplitude, $F(1,\ 37) = 0.056$, $p = .81$, $\eta_p^2 = 0.002$, and there was no significant interaction with group condition, $F(1,\ 37) = 0.02$, $p = .89$, $\eta_p^2 = 0.001$.
There was no significant interaction between time and congruency, $F(1.58,\ 58.49) = 0.06$, $p = .91$, $\eta_p^2 = 0.001$, and no significant interaction with group condition, $F(1.58,\ 58.49) = 0.12$, $p = .89$, $\eta_p^2 = 0.003$.

Follow up contrasts revealed that P300 amplitude was significantly greater for incongruent trial types than congruent, $F(1,\ 37) = 5.54$, $p = .02$, $\eta_p^2 = 0.13$, but there was no significant difference between neutral and congruent, $F(1,\ 37) = 1.79$, $p = .19$, $\eta_p^2 = 0.05$ (see Figure 6.19). There was no significant effect between subject factor of group conditions on P300 amplitude, $F(1,\ 37) = 0.82$, $p = .37$, $\eta_p^2 = 0.02$.

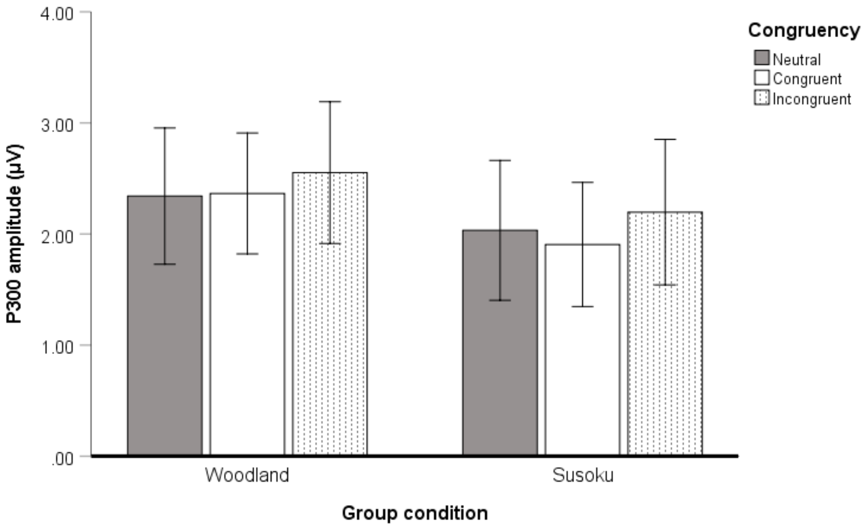
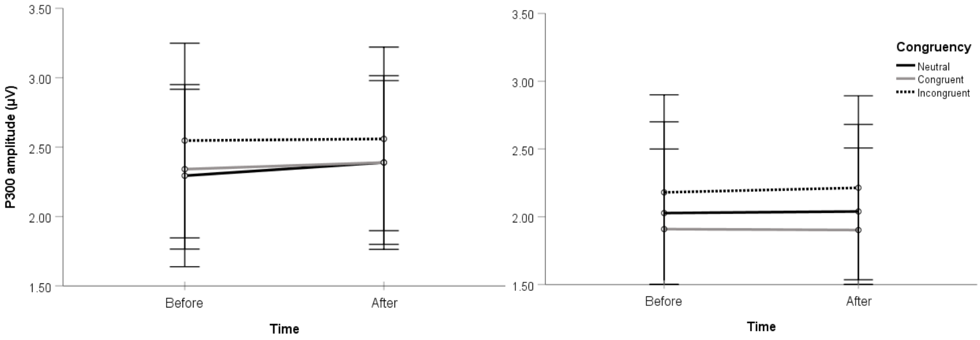


Figure 6.18: Visualising the differences in P300 amplitude between group conditions by congruency type, we can note that incongruent trials generated greater P300 amplitude than other trial types, and there was no significant difference between group conditions.



(a) Woodland

(b) Su-soku

Figure 6.19: Mean P300 amplitude for Su-soku between congruency types across time

ERP discussion

These event-related potential results echoed consistent findings in the congruency literature, whereby the N200 component was significantly larger, as in more negative, and occurred significantly later for incongruent, compared to other congruency trial types. These findings support the idea that the N200 component may reflect the processing of conflict, whereby increased resources are necessary for processing, and this is indicated by a later and enlarged N200 amplitude.

Unexpectedly, the P300 component occurred significantly earlier for neutral trials than in other trial types. This finding could be again explained by the differences in stimuli for the neutral condition than other trial types, as flankers were irrelevant stimuli; this may have been faster to process than deciphering between arrow directions. The finding for P300 amplitude also adds weight to this argument, as P300 amplitude was greatest for incongruent trial types, which suggests that participants may have required more resources to process incongruent trials, as it took participants longer to identify them, as indicated by the P300 latency.

6.3.8 Heartbeat evoked potentials

A simple independent samples T-test was performed to assess the group differences for HEP amplitude between the Su-soku condition and the woodland condition groups.

The result from the test revealed that there is not a statistically significant difference between Su-soku (M = 0.63 , SD = 0.43) and woodland conditions (M = 0.42, SD = 0.39) for HEP amplitude, t (37) = 1.52, p = .14, CI [0.47 to -0.67] (see Figure 6.20).

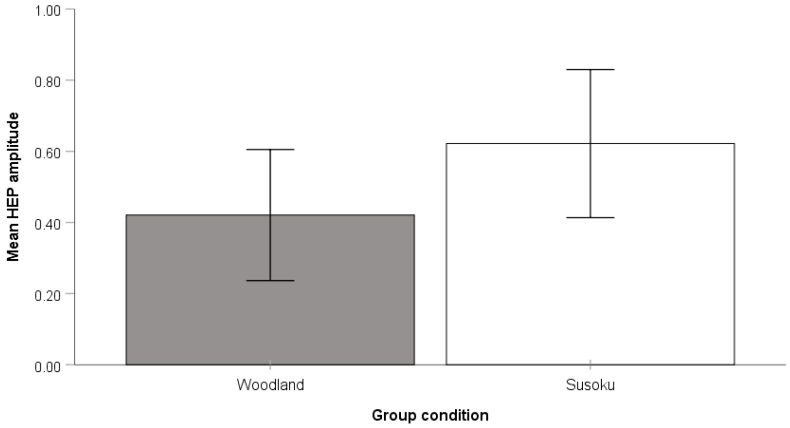


Figure 6.20: Comparing the mean HEP amplitude between woodland and Su-soku group conditions. In addition, while not significant, this graph suggests Su-soku facilitates greater HEP amplitude compared to woodland.

HEP discussion

While there were no significant differences between groups for HEP potentials, it is worth discussing possible reasons why this may have been the case. As heartbeat evoked potentials are thought to reflect the neural processing of vagal afferents, some participants within the woodland condition could have high internal visceral awareness; in contrast, participants in the Su-soku condition were actively improving their internal visceral awareness during the experiment. This potential explanation suggests that future HEP studies should consider controlling or assessing for extraneous factors that could influence HEP amplitude, such as including a heartbeat awareness test.

6.4 Discussion

6.4.1 Main findings

The aim of this chapter was to confirm and extend my previous work in Chapters 4 and 5, to investigate the mechanism of neural-visceral interactions during meditative states. Some of the results from the current study contradicted previous findings on frontal-midline theta power. For instance, rather than an increase in FM-theta, I found FM-theta power significantly decreased across Su-soku meditation blocks compared to the relaxing control condition. Alpha and beta power also increased across Su-soku meditation blocks, as reported in Chapter 4, however this was not statistically significant. Similarly, RMSSD was not significantly different between meditation groups. The circular analysis revealed only alpha oscillations had a significant mean tendency toward the exhalation phase during Su-soku; in contrast, Chapter 5 suggested that theta and beta oscillations also show a phase angle tendency towards the end of exhalation. I confirmed there was no difference in autonomic change between Su-soku and the relaxed control condition, which strengthens the argument that brief Su-soku meditation does not significantly alter autonomic balance; when compared to a relaxed state. Moreover, while a moderate relationship was detected between RMSSD and frontal-midline theta power, this was not statistically significant.

There has been little consensus regarding the effects of meditation on frontal-midline theta amplitude, and as a result, the role of FM-theta in meditation is currently unclear (Kubota et al., 2001; Lagopoulos et al., 2009; Lomas, Ivtzan, & Fu, 2015). Park et al. (2012) also reported theta power decreased during Paced breathing (PB) and alpha power globally increased. However, they also mention PB enhanced the parasympathetic drive, as indicated by increased HRV HF power and decreased low to high-frequency ratio during PB. The difference in these findings compared to the current study could be explained by the variation in practice and experimental time for the meditation task. In Park et al. (2012)'s study, the participants engaged in PB for 30 minutes, which involved 15 minutes of practice and experiment time. In contrast, the current study only required participants to engage in Su-soku for 11 minutes, whereby one minute was dedicated to the practice, and the remaining time was recorded. Based on these differences, Su-soku may up-regulate parasympathetic drive if the practice is effective or if adequate time has been allocated towards engaging with the task.

However, while differences in the meditation task duration could be a feasible explanation for autonomic differences between studies, this does not necessarily explain the discrepancies in theta power across meditation studies. Studies with similar meditation practice durations report differences in theta power (Lagopoulos et al., 2009; Park et al., 2012). In the wider attention literature, theta amplitude increases relative to cognitive control demands. It is therefore proposed to reflect a fundamental coordination mechanism, allowing higher cortical regions, such as the ACC, to integrate and convey task-selective information (Womelsdorf, Johnston, Vinck, & Everling, 2010). On this basis, decreasing theta power suggests that Su-soku meditation requires fewer cognitive demands than the relaxing control condition. Theta power, in this instance, may relate to the repetitive nature of the Su-soku task, which may, over time, require fewer resources due to the predictability of paced breathing.

Disappointingly, there was minimal evidence for Su-soku meditation providing cognitive performance benefits. There were no significant group differences between Su-soku and control tasks; for reaction time and accuracy during the Flanker task for all congruency trial types. Furthermore, I investigated whether there were information processing differences between groups by examining ERP changes (N200 and P300) before and after meditation tasks. And again, there were no significant group differences, which suggests that Su-soku does not significantly influence cognitive performance in a Flanker task compared to a relaxed state in the current study. One interpretation could be that participants in the current study may have required additional meditation sessions before cognitive benefits could be observed. This idea is supported by Cahn and Polich (2006)'s systematic review, where they concluded that meditation-induced oscillatory changes in the ACC and PFC are related to the proficiency of practice. There appears to be a potential trend concerning meditative practice duration. Earlier, I mentioned that the Su-soku duration may have been too short for enhancing parasympathetic activity. Likewise, it is suggested that neural changes in higher cortical regions involved in cognitive control may depend on practice time. Therefore, in the absence of results, we may infer that successful modulation of neural-visceral mechanisms is important for improving cognitive performance.

Following this inference, how would we know that breath-focused meditation has successfully modulated neural-visceral mechanisms? The final analysis explored this question by investigating the relationship between interoceptive awareness and the central-autonomic neural system. Su-soku induced earlier and greater HEP amplitudes compared to the woodland condition, which suggests that brief breath-focused meditation has some influence on the processing of cardio-afferent inputs to the brain (MacKinnon et al., 2013; Petzschner et al., 2019). However, the difference between these group conditions was insignificant, and HEP amplitude did not significantly correlate with oscillatory power and autonomic activity. One could speculate that the lack of significance may relate to the delivery of the Su-soku task. During Su-soku, participants maintained their resting breathing rate, as simulated by an expanding-contracting circle. This means that the breathing rate differed between participants, which may have affected HEP amplitudes.

Previous studies have shown that a slow breathing rate (0.1 Hz) can boost the vagal baroreflex response. When the respiratory rate is controlled, there are no longer significant differences in baroreflex gain (Lehrer et al., 2003; McCraty, Atkinson, Tomasino, & Bradley, 2009). The baroreflex response is responsible for the maintenance of blood pressure change and thus intrinsically linked to the beat-to-beat fluctuations in heart rate, as measured with HRV. MacKinnon et al. (2013) investigated the relationships between HEP amplitudes, HRV and emotional states. They reported HF and LF HRV parameters were consistently associated with increased HEP amplitudes, during resonance frequency breathing (0.1 Hz). They also found that the negative emotion reduced HRV and produced the weakest evoked potentials. This suggests that, in addition to attending to the breath, breathing rate may be a critical component in successfully modulating neural-visceral mechanisms, given that there were no significant between-group differences in HRV parameters, HEP amplitude, and mood state change. Despite a lack of statistical significance, Su-soku produced earlier, and larger HEP amplitudes than woodland, which suggests the mechanistic neural-visceral interaction may begin with the ability to perceive internal signals, and resonance breathing could be one way to facilitate the conscious perception of these signals (Leganes-Fonteneau et al., 2021).

6.4.2 Limitations

A few comments could be made about the potential limitations of this study. One could argue that the findings may be an inaccurate reflection of meditation effects, as the delivery of the meditation task differed from conventional meditation practices. While participants focused on the expanding-contracting circle during Su-soku, they were also asked to count the number of breaths they took. Breath counting would have facilitated sustained attention on the breath, but also it might have distracted some participants from relaxing in the moment; due to perceived pressure on breath accuracy. As a result, this additional requirement may have affected the results. An alternative approach could use sound cues to guide breathing, as this would still fulfil the intended effect on attention but without effects from potential implicit pressure (Park et al., 2012).

There are also issues regarding subjectivity with the utilisation of a mood state questionnaire to evaluate the effects of meditation on emotion. Due to only a brief period between the initial and second completion of the questionnaire, participants may remember their answers which in turn affects the reliability of mood state change results. Instead of using a questionnaire to estimate mood state, one could infer mood state changes by assessing the participant's processing of emotional stimuli, as several studies have found mood state influences how emotions are processed (Chepenik, Cornew, & Farah, 2007; Lawrie, Jackson, & Phillips, 2019; Trilla, Weigand, & Dziobek, 2021). For instance, a positive mood broadens global attention, thereby eliminating reaction time differences between positive and negative words. In comparison, a negative mood narrows attention to negative words and less effective mood-induced facilitation (Sereno, Scott, Yao, Thaden, & O'Donnell, 2015).

6.4.3 Implications

Despite these limitations, there are several implications of this research. I evaluated the validity of my previous work and successfully replicated some of the previous findings on the changes in oscillatory amplitude during meditation. Overall, this work has contributed toward understanding the mechanistic interactions between afferent and efferent pathways. Considering the failure to observe significant effects of Su-soku on HEP, we cannot exclude the possibility that performance enhancement is conditional on achieving neuro-visceral integration. Continued exploration in this area of research will bring clarity to the underlying mechanisms affecting autonomic and emotional function and guide the development of new interventions for psychological and physiological-based disorders.

6.4.4 Conclusions

While the direction of effects is unclear, this study suggests that brief breath-focused meditation can influence fronto-medial neural oscillations in amplitude, respiratory phase angle, and heartbeat evoked potentials. The role of interoception in the central-autonomic nervous system dynamic may be more important than initially realised, and exploring the therapeutic effects of resonance breathing could be fruitful.

When analysing the respiratory frequency of participants, I came across some individuals with a natural breathing rhythm at the resonance frequency (0.1 Hz). Consequently, Chapter 7 explores the neural-visceral components associated with these resonant individuals as case studies. This further clarifies the current hypothesis that mental health disorders could be characterised by interoceptive awareness.

Chapter 7

Exploring the effects of resonance breathing

In sum, the results from Chapters 4, 5 and 6 suggest the effects of Su-soku meditation on neural-visceral coupling may be associated with enhanced interoceptive awareness of respiratory signals. Recent studies indicate that when heart rate variability and respiration occur in-phase, known as resonance breathing, interoceptive prediction errors are reduced through up-regulation of interoceptive neural functioning, which consequently entrains oscillatory rhythms in brain regions associated with emotion regulation (Kang, Sponheim, & Lim, 2021; Leganes-Fonteneau et al., 2021).

This chapter presents a case study report exploring the effects of resonance breathing on neural-visceral coupling. These findings provide a preliminary insight into the effects of resonance breathing on neural-visceral coupling and a novel contribution to knowledge by exploring the neural-respiratory effects of resonance breathing.

7.1 Introduction

Heart rate variability (HRV) describes the variation of inter-beat intervals of the heart and is considered to reflect an organism's capacity to adapt to environmental change (Thayer, Åhs, Fredrikson, Sollers III, & Wager, 2012). The rate of cardiac rhythm varies according to respiration, whereby inspiration is associated with heart rate acceleration (RR-interval decrease) and expiration with a heart rate deceleration (RR-interval increase). This interaction is referred to as respiratory sinus arrhythmia (RSA).

When heart rate variability and inspiration/expiration oscillate in phase with each other, the amplitude of HRV is maximised (Hsieh, Mao, Young, Yeh, & Yeh, 2003; Vaschillo et al., 2002). This peak in amplitude is pronounced at the "resonance frequency" of 0.1 Hz (6 breaths per minute) (Lehrer, 2013). The term "resonance" is used to describe this frequency, as some evidence suggests it induces a coherent, synchronous resonance frequency in various physiological signals (e.g. HR, baroreceptor reflex, blood pressure, and brain perfusion), thus entraining different physiological oscillatory systems (McCraty et al., 2009; Mejía-Mejía, Torres, & Restrepo, 2018).

HRV amplitude is enhanced through the mechanisms of the baroreflex. When respiration and heart rate occur in-phase, there is a 180° phase relationship between heart rate and blood pressure, which triggers the stimulation of baroreceptors to regulate blood pressure (Vaschillo et al., 2002). Regular stimulation of this response has been shown to increase resting baroreflex gain, i.e. strengthen the baroreflex system (Lehrer et al., 2003). In turn, the augmentation of the baroreflex increases the adaptability of the cardiovascular system and enhances afferent vagal communication through reciprocal interconnections intervening in the medulla and cerebral cortices (Russo et al., 2017; Shaffer, McCraty, & Zerr, 2014; Zaccaro et al., 2018).

Afferent vagal communication is measured by the neural response to heartbeats, referred to as heartbeat evoked potentials (MacKinnon et al., 2013). Vagal autonomic stimulation studies suggest that altered frontal heartbeat evoked potentials can be localised to brain regions associated with emotion regulation and mood, including the insula, operculum, somatosensory cortex, and orbital ventromedial prefrontal regions (Poppa et al., 2021). Resonance breathing has been associated with improved emotional well-being, including reduced depression and anxiety symptoms and resilience to stressors (Goessl, Curtiss, & Hofmann, 2017; Mather & Thayer, 2018). These findings suggest that modulation of vagally-meditated HRV may facilitate emotional well-being by entraining brain rhythms in regions relevant to emotional regulation (Babo-Rebelo, Richter, & Tallon-Baudry, 2016; Lehrer et al., 2003; Mather & Thayer, 2018; Steinfurth et al., 2018).

Several EEG studies have reported that resonance breathing induces increased oscillatory power in alpha and theta frequencies, which correlated with changes in heart rate oscillations (Busek & Kemlink, 2005; Hinterberger, Walter, Doliwa, & Loew, 2019; Park et al., 2012). Brain oscillations in these frequencies are proposed to facilitate key cognition functions, such as memory and executive control, and consequently influence emotion regulation (Ertl, Hildebrandt, Ourina, Leicht, & Mulert, 2013). However, the mechanisms underlying vagal afferent modulation's therapeutic effects are unclear (Lehrer, 2013; Zaccaro et al., 2018). In a recent post-traumatic stress disorder clinical trial, mindfulness meditation improved PTSD symptoms and increased alpha, frontal theta oscillatory power, and frontal theta heartbeat-evoked brain responses (Kang et al., 2021). Mediation analysis revealed only frontal theta HEPs were found to underline the therapeutic effects of mindfulness meditation. These findings indicate the strength of visceral-afferent cardiac signalling may predict therapeutic outcomes. Therefore, up-regulation of interoceptive neural functioning via resonance breathing may enhance therapeutic effects.

One of the first reports examining how baroreflex functioning shapes interoceptive awareness suggested resonance breathing may facilitate the conscious perception of interoceptive signals by reducing interoceptive prediction errors through systematic synchronisation (Leganes-Fonteneau et al., 2021). Respiration is known to dynamically tune by performance optimisation of sensory detection and correlate with respiratory sinus arrhythmia and cortical potentials (Hinterberger et al., 2019; Rassi, Dorffner, Gruber, Schabus, & Klimesch, 2019). Kluger, Balestrieri, Busch, and Gross (2021) suggests respiration instigates transient cycles of heightened excitability, as reflected by the modulation of perceptual sensitivity and posterior alpha power during the respiratory cycle. Speculatively, it is likely that the effects of resonance breathing on interoceptive prediction errors would also be visible during the respiratory cycle.

However, there are several unknowns concerning HRV modulation that must be noted. Firstly, HRV modulation also induces hemodynamic changes in the brain, which may be an essential or a primary contributor to facilitating electrical changes in the brain (Pfurtscheller, Daly, Bauernfeind, & Müller-Putz, 2012). If this is the case, we would expect reduced psychological benefits of resonance breathing in individuals with cardiovascular disease due to plaques reducing cerebral perfusion. Therefore, this has important implications for the suitability of resonance breathing. Secondly, 0.1 Hz breathing could lead to hyperventilation, which could have negative consequences for individuals with a diagnosis of anxiety (Szulczewski & Rynkiewicz, 2018). Thirdly, individual resonance frequency seems to differ slightly between individuals in the range of 0.075–0.12 Hz, and therefore the ability to generalise findings may prove difficult (e.g. Vaschillo, Vaschillo, and Lehrer (2006)). Similarly, how resonance breathing is delivered seems to influence effectiveness (Zelano et al., 2016).

In summary, resonance breathing may promote adaptive physiological and emotional functioning via facilitating systematic neural-visceral coherence. As a result, the therapeutic potential of resonance breathing seems promising. During the analysis of previous chapters, several participants were identified with a natural resting breathing rate at the resonance frequency. This unexpected observation provides a unique opportunity to explore the effects of resonance breathing as a case study. As resonance is associated with up-regulated RSA, we might anticipate enhanced parasympathetic activity during expiration leading to enhancement of any cerebral phenomena related to parasympathetic activity. For example, despite the relationship between parasympathetic activity and theta oscillations reported in Chapter 4 was not replicated in Chapter 5, it is plausible that during resonance, there will be an increase in theta power during expiration. Exploring the effects of resonance breathing during respiration provides a novel contribution to this rapidly growing research area and may offer critical preliminary insights into understanding the effects of breathing on mental health.

7.1.1 Research questions and hypotheses

Research questions

RQ1. Does resonance breathing influence emotional regulation, as indicated by mood state?

RQ2. Does resonance breathing enhance vagal-afferent processing, in terms of RSA and HEP amplitudes?

RQ3. Does resonant breathing modify the pattern of variation of brain oscillatory power across the respiratory cycle?

Hypotheses

H1. It is predicted that resonance breathing will enhance mood state.

H2. Resonance breathing is also anticipated to enhance RSA and HEP amplitudes.

H3. Based on previous findings in this thesis and interoception literature, it is predicted that resonance breathing will strengthen perceptual detection of afferent signals which will be reflected as a consistent respiratory phase direction for oscillatory power during respiration.

7.2 Methods

7.2.1 Identifying resonant participants

When processing the cardiac data in Chapter 6, four participants in the Su-soku condition exhibited an RSA peak at the resonance frequency of 0.1 Hz; and their prescribed breathing rate during Su-soku was near 0.1 Hz. It should be noted that the prescribed breathing frequency for Su-soku was self-selected, i.e. these were participants with a naturally low breathing rate at rest. One subject in the woodland condition also exhibited an RSA peak at the resonance frequency. These resonance participants presented a consistent IBI pattern, as indicated by the blue peaks and troughs in Figure 7.1, a clear frequency band, and an RSA peak at 0.1 Hz. This occurrence of resonance may be associated with feeling comfortable at a low breathing rate.

Non-resonant participants are defined as individuals who did not express an RSA peak at the 0.1Hz resonance frequency, regardless of their breathing rate. There was one non-resonant subject with a breathing frequency close to the vicinity of 0.1 Hz, yet they did not exhibit resonance. This case study includes five resonant participants and 33 non-resonant participants.

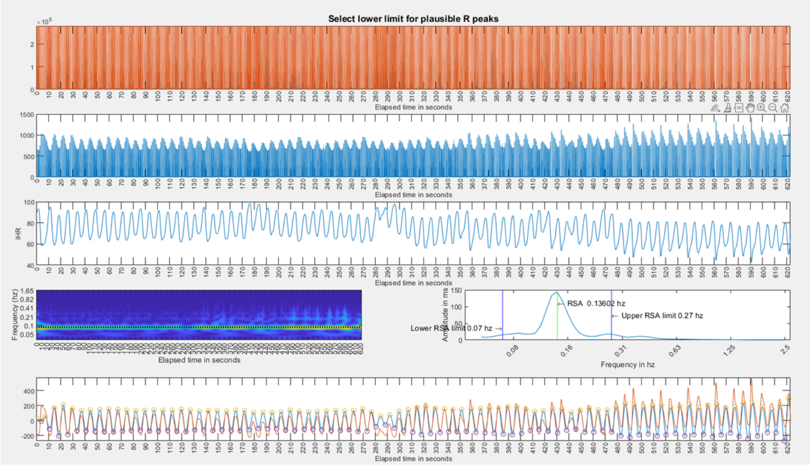


Figure 7.1: Identifying resonant participants

7.2.2 Analysis of resonant cases

Due to the small sample size, estimates of variance are likely to be unreliable. Therefore, instead of a formal statistical analysis of group differences, Z-scores indicating the changes in relevant physiological values will be presented for each participant to illustrate the degree of consistency across participants of any changes and to provide an indication of the degree to which any observed effects might be generalized.

Z-scores were calculated by obtaining the mean and standard deviation for all non-resonant participants' scores for each performance parameter. An individual's score was calculated by subtracting the non-resonant mean from the individual's data point and then dividing it by the standard deviation.

$$z = \frac{x - \mu}{\sigma}$$

The Z-score measures the extent to which a resonant subject deviated from the rest of the sample (non-resonant participants).

7.3 Results

7.3.1 Mood state change

Current evidence suggests that resonance breathing can lead to improvements in emotion regulation. As a result, it may lead to an improved ability to regulate their own emotions and enhanced mood (Goessl et al., 2017; Mather & Thayer, 2018). Therefore, I predicted resonant participants would have a greater positive change in mood state compared to non-resonant participants.

Participant number	POM Change (Z-score)
#5	9 (0.75)
#7	-4 (-0.31)
#31	5 (0.43)
#37	10 (0.84)
#8	3 (0.26)
NR Group mean (SD)	-0.24 (12.14)

Table 7.1: Mood state results by resonance

Based on the Z-scores in Table 7.1, most resonant participants exhibited a positive mood state change after Su-soku or woodland compared to non-resonant participants. Only subject seven appeared to have a negative mood state change. This data was graphically visualised to assess whether these resonant subject values significantly differed from the non-resonant participants (see Figure 7.2).

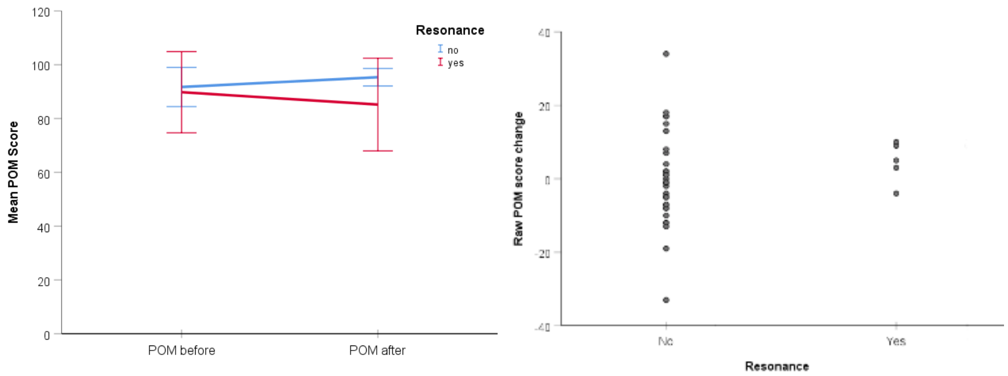


Figure 7.2: Visualising mood state change by resonance group

Figure 7.2 suggests that despite most resonance participants experiencing a positive mood change, these differences lie within the range of non-resonant participants. While this suggests there is not a significant difference between groups, these findings must be interpreted cautiously given the small sample size of participants.

7.3.2 Autonomic function

RSA amplitude

Resonance breathing is thought to up-regulate HRV amplitude via action on the baroreflex function (Lehrer, 2013). To confirm these effects, I compared the differences in RSA amplitude between resonant and non-resonant participants (see Table 7.2 and Figure 7.3).

Participant number	RSA amplitude (Z-score)
#5	166.55 (11.4)
#7	58.52 (2.52)
#31	85.32 (4.66)
#37	143.54 (9.31)
#8	89.86 (5.027)
NR Group mean (SD)	26.82 (12.54)

Table 7.2: RSA amplitude by resonance

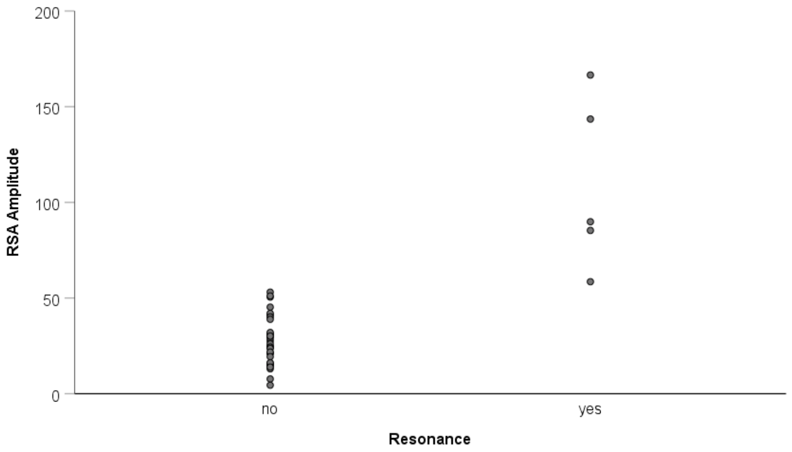


Figure 7.3: RSA amplitude by resonant group

Enhancement of RSA is marked in resonant participants, as expected from the definition of resonance. However, this information alone does not confirm the involvement of the baroreceptor. This is further discussed in Section 7.4.

HEP amplitude

Several studies suggest that resonance breathing enhances interoceptive neural function, as indexed by HEP amplitude (Huang, Gevirtz, Onton, & Criado, 2018; MacKinnon et al., 2013). Therefore, exploring the HEP amplitude differences between resonance groups is of interest.

Participant number	HEP amplitude (Z-score)
#5	0.82 (0.79)
#7	0.48 (0)
#31	0.78 (0.69)
#37	0.89 (0.95)
#8	1.02 (1.25)
NR Group mean (SD)	0.48 (0.43)

Table 7.3: HEP amplitude by resonance

Based on the Z-scores in Table 7.3, it appears that, except for subject 7, resonance breathing enhances HEP amplitude. A scatter plot was produced to explore these effects further.

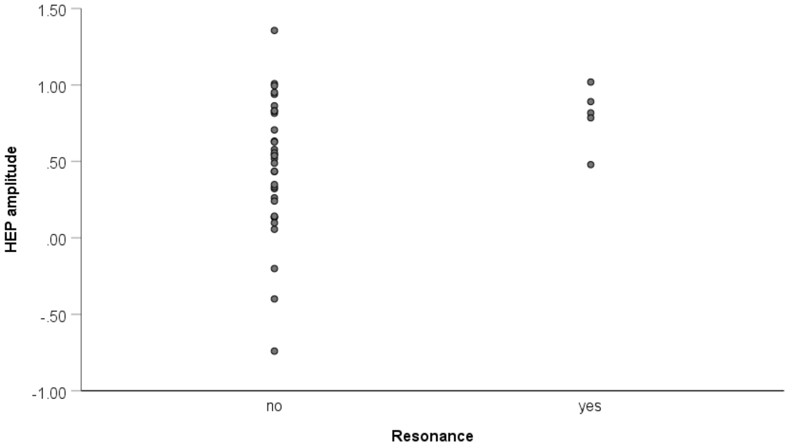


Figure 7.4: HEP amplitude by resonance group

Figure 7.4 suggests that despite the group HEP amplitude mean of resonance breathing being greater than the non-resonance group, the individual subject scores lie within the range of non-resonant participants.

7.3.3 Oscillatory power by respiratory phase

Recent literature proposes that resonance breathing strengthens vagal-afferent signalling by enhancing synchronous neuro-cardio-respiratory activity (Kluger et al., 2021; Leganes-Fonteneau et al., 2021). This enhanced bottom-up signalling reduces prediction error and, as a result, facilitates emotional well-being. The respiratory system is an example whereby dynamic changes occur to optimise cognitive performance, especially the detection of sensory stimuli (Hinterberger et al., 2019; Rassi et al., 2019). As resonance breathing involves the modulation of respiratory rate, we may observe differences in oscillatory power during the respiratory cycle for resonant participants. In this section, I explore the variation in oscillatory amplitude during respiration in resonant and non-resonant groups with polar histograms.

Theta

Figure 7.5 suggests resonant participants tend to express theta amplitude peaks during exhalation. Two resonant participants exhibited a theta amplitude peak at the same phase angle, represented by a larger histogram bar. In contrast, there is no clear theta phase angle pattern shown by non-resonant participants.

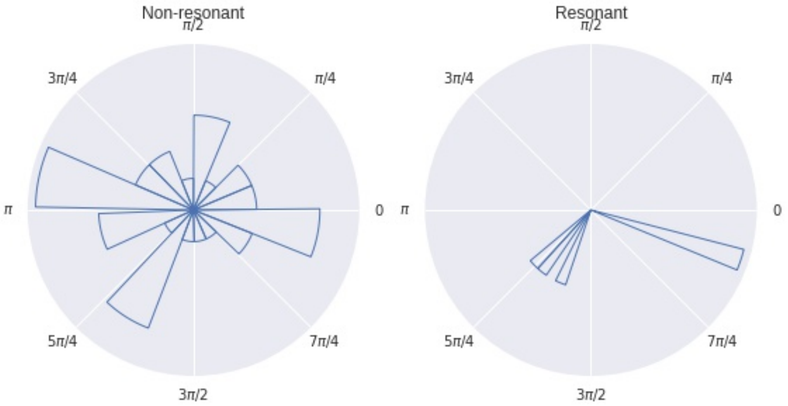


Figure 7.5: Polar histogram of theta amplitude peak across respiration

Alpha

In the alpha frequency, there appears to be no clear pattern dissociating the resonant and non-resonant groups (see Figure 7.6).

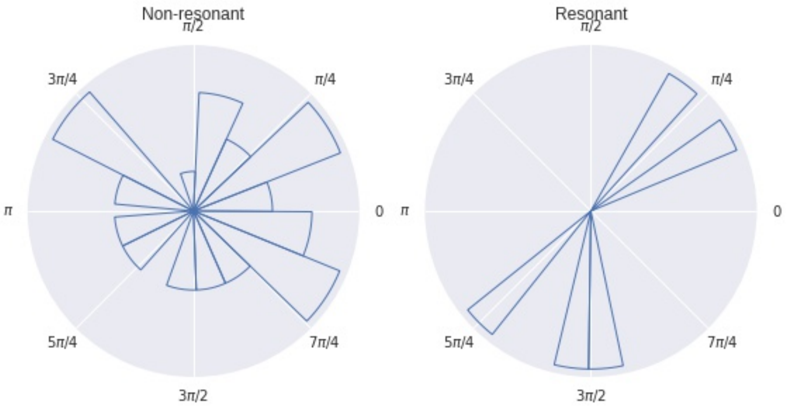


Figure 7.6: Polar histogram of alpha amplitude peak across respiration

Beta

Figure 7.7 suggests beta amplitude peaks tend to occur during exhalation for resonant participants, while there is a tendency towards inhalation for non-resonant participants. This may reflect differences in how resonant participants prepare for incoming afferent signals compared to non-resonant participants.

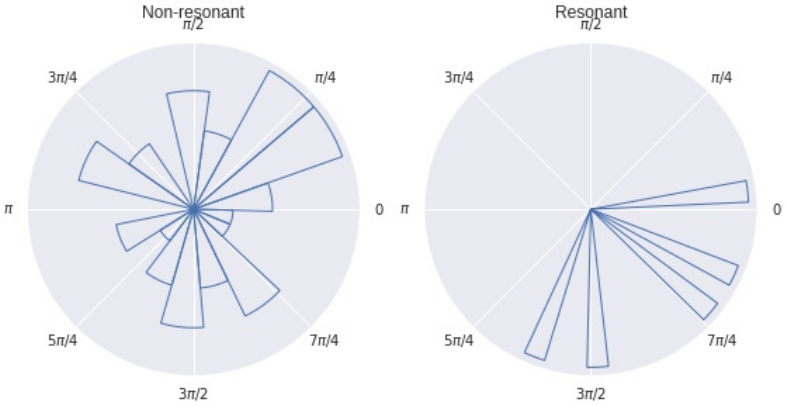


Figure 7.7: Polar histogram of beta amplitude peak across respiration

7.4 Discussion

With the interest of exploring the effects of paced breathing on the neural-visceral system, this chapter investigated differences between participants that displayed resonance, whereby the oscillation frequency between breathing and cardiac rhythms occurred in phase at 0.1Hz, with non-resonant participants. Recent literature predicted that afferent vagal modulation, via resonance breathing, might enhance interoceptive processing and emotional well-being by inducing neural oscillatory entrainment (Hinterberger et al., 2019; Leganes-Fonteneau et al., 2021; Steinfurth et al., 2018).

These preliminary findings showed an improvement of mood and enhancement of HEPs in resonant cases, but these changes lie within the range of non-resonant cases. There are two potential interpretations for this. Firstly, statistical power was insufficient to detect significant differences between resonant and non-resonant participants. Future studies with a larger sample size would be necessary to assess the effect size of resonance breathing. Secondly, several studies have shown significant clinical improvements in depressive symptoms and enhanced HEP strength following daily HRV biofeedback sessions (Lehrer, 2013; MacKinnon et al., 2013; Mather & Thayer, 2018). Therefore, the inability to detect a significant difference between the groups may be related to inadequate practice frequency for the resonance breathing group.

However, the benefits of HRV biofeedback therapy have been associated with augmentation of the baroreflex rhythm through slow diaphragmatic breathing; which is proposed to increase the amplitude of heart rate oscillations (Appelhans & Luecken, 2006; Kemp & Quintana, 2013). While resonant participants appeared to exhibit enhanced RSA amplitude, it is unclear whether the effect of resonance breathing during Su-soku meditation is due to baroreflex modulation via baroreceptor stimulation. Blood pressure can be manipulated by breathing rate, and pressure receptors (“baroreceptors”) detect changes and convey this information to hypothalamic centres, which trigger homeostatic reflexes. However, non-resonant participants with a similar breathing rate as resonant participants did not express an enhanced RSA amplitude. Overall the effects of Su-soku on autonomic activity appear to be unclear as they contradict prior expectations regarding the physiological mechanism of paced breathing. If breathing rate and attention to the breath were critical for increasing vagal tone, we would expect consistent RSA amplitude across participants with similar breathing rates and heightened parasympathetic response during Su-soku. Instead, these findings might suggest the involvement of top-down efferent communication during resonance breathing.

In resonant cases, theta and alpha amplitude peaks clustered towards the exhalation phase. This pattern is particularly noteworthy as this is similar to what was observed in Chapters 5 and 6, whereby respiratory phase angles in alpha, theta, and beta frequencies expressed an increasing tendency to peak during exhalation across Su-soku blocks. This pattern of oscillatory synchrony during exhalation may imply the significance of a temporal relationship. Brain rhythms are thought to transmit information through synchronization (Fingelkurts & Fingelkurts, 2017; Tononi, 2004). As oscillatory frequencies facilitate distinct functions via recruitment of various brain regions, overlapping respiratory phase angles may reflect altered coordination of neural coupling (Chanes & Barrett, 2016; Heck et al., 2017). Incidentally, Rodriguez-Larios, Wong, Lim, and Alaerts (2020) observed harmonic locking of alpha and theta rhythms during meditation and noted a more pronounced effect over central and left temporoparietal electrodes.

Based on the proposed functions of these frequencies and spatial distribution, they suggest these results may reflect integration between memory and executive function. While this thesis did not find direct evidence for Su-soku meditation modulating executive function in the Eriksen Flanker task, the speculative temporal relationship between alpha and theta could suggest early signs of modulation prior to behavioural outcomes.

Brain-respiratory studies have shown neural oscillations at various frequencies can be locked to and modulated by the respiratory phase (Kluger et al., 2021; Zelano et al., 2016). They suggest that fluctuating peripheral-neural coupling may heighten cortical excitability and therefore facilitate the sampling of sensory information. It is interesting that the current study suggests temporal coupling of theta and alpha frequencies during exhalation, as Zelano et al. (2016) reported faster and more accurate sensory identification during inhalation compared to exhalation. These results suggest respiratory phase may provide a specific function for meeting cognitive demands.

Respiration is known to dynamically tune by performance optimization of sensory detection and correlate with respiratory sinus arrhythmia and cortical potentials (Hinterberger et al., 2019; Rassi et al., 2019). Studies employing nonlinear methods have shown phase synchronization between blood pressure and efferent nerve activity and frequency locking of infra-slow oscillations of brain potentials by respiration (Karavaev et al., 2018; Vandenhouten, Lambertz, Langhorst, & Grebe, 2000). Similarly, Karavaev et al. (2018) reported coherence between neural oscillations and respiration is maximal at frequencies close to the frequency of respiration and found no evidence of synchronization between infra-slow oscillations and respiration during spontaneous breathing.

In conclusion, while interpretations should be viewed cautiously, these results appear to reflect similar observations reported in more robust larger-scale studies. As a result, my work provides preliminary evidence that paced breathing may facilitate resonance and influence neural-visceral interactions. Future studies may wish to expand on this work by further investigating the therapeutic significance of the peripheral phase on oscillatory coupling during meditative training.

Chapter 8

Discussion

This thesis investigated neural-visceral coupling during breath-focused meditation (Su-soku) to enhance our understanding of its therapeutic applications for mental health.

The purpose of this chapter is to provide a summarised overview of the findings reported in this thesis, followed by a critical interpretation and reflection on findings from similar studies. Given the available evidence, a revised mechanism of action for the effects of breath-focused meditation will be proposed. Based on this mechanistic model, several new research questions will be formulated and discussed in the final section of this chapter, alongside a suggested study protocol for testing the model's validity.

8.1 Summary of findings

8.1.1 Chapter 4

Preliminary analyses presented in Chapter 4 suggested that Su-soku induces a more focused, attentive state, as indicated by the rise in frontal-midline oscillatory power from baseline and across Su-soku blocks. Based on previous literature, I predicted up-regulation of oscillatory power would be associated with changes to the baroreflex (Kubota et al., 2001; Tang et al., 2009).

FM-theta oscillatory power and RMSSD significantly increased from meditation block 1 to block 2 and was sustained through block 3. These results confirm previous studies findings that Su-soku meditation appears to influence the central and autonomic nervous systems. Moreover, given the association between meditative practice, improved cognition, and mental well-being, these findings may speculatively indicate an optimal state involves optimising interactions between neural-visceral mechanisms (Del Negro et al., 2018; Grau et al., 2020; Rubia, 2009; Tang et al., 2015). Such interpretation can be related to previous attention theory concepts, such as the Yerkes and Dodson law, which states arousal can be described by an inverted u-shape function, whereby until a specific point, there is a positive relationship between arousal and performance (Yerkes et al., 1908). To this end, my findings might signify that neural-visceral coupling can be optimised, which likely has consequential effects on the attentive state.

8.1.2 Chapter 5

These preliminary insights were developed in Chapter 5 by assessing how oscillatory and autonomic activity changes relative to the respiratory cycle. I predicted oscillatory power would cluster towards a mean respiratory phase direction across meditation blocks, and this change would be associated with autonomic activity. Oscillatory power varied across the respiratory cycle, with a general tendency towards the end of exhalation. In contrast, cardiac interbeat intervals tended to concentrate at the start of inhalation, with no significant change across meditation blocks. The concentration of IBIs early in the cycle is equivalent to saying that heart rate was higher earlier in the respiratory cycle and lower later in the cycle. These results could indicate greater parasympathetic influence late in the cycle at the time when the oscillatory amplitudes tend to be greater. As baroceptors play an important role in mediating the parasympathetic effects, this might suggest that greater baroreceptor influence on both the brain and heart occurs late in the respiratory cycle. Furthermore, this difference in respiratory phase angle between physiological signals may explain why a linear association between oscillatory power and autonomic activity could not be detected in Chapter 4.

Changes in oscillatory phase angles relative to the respiratory cycle may reflect oscillatory optimization toward processing visceral signals. As the nucleus tractus solitarius (NTS) receives facilitatory vagal inputs during exhalation, increasing frontal cortical activity during this phase may enhance the interoceptive processing of vagal information (Gazi et al., 2021; Grennan, Balasubramani, Maric, Ramanathan, & Mishra, 2021). Greater RSA amplitude has been associated with improved cognitive control, well-being and parasympathetic state; therefore, I investigated whether RSA amplitude predicts respiratory phase angle shifts of neural oscillations (Porges et al., 1994; Tonhajzerova, Visnovcova, Mestanikova, Jurko, & Mestanik, 2016). However, I could not find a consistent relationship between RSA amplitude and oscillatory phase angle shifts across participants.

8.1.3 Chapter 6

Following this, Chapter 6 suggested the mechanism underpinning the effects of Su-soku may involve interoceptive vagal processing, as heartbeat-evoked potential amplitude increased during Su-soku but not during the relaxing woodland condition. This work also highlighted that meditation duration might influence the effects of Su-soku as FM-theta amplitude decreased across Su-soku blocks. Duration likely affects the ability of an individual to engage in meditation; therefore, differences in FM-theta may reflect meditation depth (Katyal & Goldin, 2021).

Chapter 6 also investigated the effects of Su-soku on flanker task performance and event-related potentials. While these findings confirmed well-established congruency effects, there were no significant differences between Su-soku and relaxing woodland conditions for reaction time, accuracy, N200 latency, N200 amplitude, P300 latency, and P300 amplitude. This suggests that any cognitive performance benefit from Su-soku meditation is no greater than those from a period of quiet relaxation. In the broader context of meditation literature, this finding is not unusual. Meditation studies tend to examine changes in attentional performance after a period of meditation practice (>3 months), and report maintained performance improvements (Lutz et al., 2008; Tang et al., 2015; Zanesco, King, MacLean, & Saron, 2018). Although meditation has been positively associated with cognitive benefits, Su-soku may be a meditation type that requires dedicated practice before such effects are realized (Gill, Renault, Campbell, Rainville, & Khoury, 2020).

8.1.4 Chapter 7

Chapter 7 featured a case study exploring the effects of resonance breathing on neural-visceral coupling. The results from this work suggested that cardio-respiratory synchrony may further enhance the processing of interoceptive vagal signals, as inferred by enhanced oscillatory power during exhalation and HEP amplitude in resonant participants compared to non-resonant participants.

One of the first studies to examine neural-vagal interactions during emotion and resonance breathing found that individuals who experience negative emotions had more chaotic or disordered HRV. In contrast, participants in positive mood states had increased order or resonance in HRV (MacKinnon et al., 2013). They also observed HRV influencing afferent input to the brain, as measured with HEPs and discovered HEP amplitude was particularly enhanced by the resonant condition.

As predicted, resonant participants exhibited greater HEP amplitudes compared to non-resonant participants. Furthermore, resonant participants experienced a significant positive mood change after Su-soku. However, there were few resonant participants in the woodland group to ascertain whether a significant positive mood change would only be observed in Su-soku. There could be a bi-directional relationship between emotion and the viscera; a positive mood state could facilitate HRV resonance. Likewise, we could potentially up-regulate the mood state through the breath via cardio-respiratory-neural pathways. While further research on the effects of resonant breathing would be required to validate these findings, this research suggests resonance breathing may potentially provide a therapeutic effect on mental health via the vagal afferent pathway.

8.1.5 Conclusion

In summary, the findings of this thesis suggest Su-soku meditation induces differential attentional states, as indicated by changes in frontal-midline oscillatory power across meditation sessions. Furthermore, changes in oscillatory power relative to the respiratory cycle, a moderate increase in vagal tone and enhanced neural-visceral effects in participants exhibiting resonant breathing highlights evidence for predictive coding. The theory states that top-down signals are updated from bottom-up prediction errors by adjusting the neural gain to enhance the signal-to-noise ratio. Coordinating activity and detection of salient events is conducted by the anterior cingulate cortex, which is proposed to be a key region generating neural activity at the frontal-midline region. The ACC is part of the SN, which modulates the activity of the DMN and DAN according to task demands.

While there is a lack of consistency between the two datasets utilised in this thesis for theta power and oscillatory power by respiratory phase, both highlighted a decrease in theta/beta ratio, which indicates Su-soku increases attentional awareness and reduces mind wandering. The pilot work presented in Chapter 7 furthers the idea that increasing awareness of visceral signals via resonant breathing may enhance the optimisation of oscillatory power during respiration and the ability to regulate emotion.

Although there was no evidence for Su-soku enhancing cognitive performance or ERPs associated with the Flanker task, this does not rule out the possibility that Su-soku may improve performance in a task involving emotion regulation. As a result, one may infer from the findings of this thesis that Su-soku meditation modulates top-down signals in response to visceral information across sessions and respiration. The novel research questions and approaches in this thesis, such as utilising circular statistics to investigate the effects of respiration on neural-visceral coupling, provide an original contribution to the neural-visceral integration literature and insight into future therapeutic applications of meditation.

Considering the current literature and contributions provided by this thesis, the following section proposes a potential mechanistic pathway for how breath-focused meditation influences neural-visceral coupling and, as a consequence, how this can lead to therapeutic benefits.

8.2 Proposed mechanism for Su-soku

Tuning of the brain is important for attentional control and emotion regulation. The key brain areas thought to coordinate executive function are the prefrontal and anterior cingulate cortex (ACC) (Kanske & Kotz, 2010; Tang et al., 2015). The ACC contributes to cognitive control by detecting conflicts in information processing and signalling when increased top-down control is required (Critchley et al., 2005; Kanske & Kotz, 2010; Kwak et al., 2020). This process of conflict monitoring echoes similar concepts proposed by the Predictive Coding Framework, which states prediction errors depend on incongruency between predictions and actual outcomes and that an organism seeks to minimize these discrepancies to maintain homeostasis (Clark, 2013; Friston, 2010).

Recent work has suggested components of this allostatic process may be related to specific neural oscillatory frequencies; for instance, frontal theta oscillations reflect prediction error/surprise, and parietal alpha and beta oscillations indicate anticipation of conflict (Williams, Ferguson, Hassall, Wright, & Krigolson, 2021). Although Su-soku did not explicitly involve decision-making, modifying a wandering attentive state towards a sustained attentive state involves engaging cognitive control resources. Several studies have shown meditation regulates response-conflict adaption (Chan et al., 2020; Colzato, Sellaro, Samara, & Hommel, 2015). This thesis suggests that Su-soku modulates frontal-midline oscillatory power in theta, alpha and beta frequencies. Therefore, there is a possibility that adaptations to the conflict monitoring process might underpin the effects of Su-soku.

I propose the act of sustained interoceptive attention, as seen in Su-soku meditation, alters weightings associated with attention sampling, which ultimately modifies the ability to detect and respond to conflicts in the environment, such as changes in the visceral state induced by a sustained breathing rate. This argument is further developed in the subsequent subsections.

8.2.1 Attention sampling

Perceptual rhythms may constitute fluctuations in precision weighting, whereby rhythms during unfavourable phases represent moments when precision is insufficient (Ainley et al., 2016; Feldman & Friston, 2010; VanRullen, 2018). According to Fiebelkorn and Kastner (2019) "Rhythmic Theory of Attention", perceptual rhythms are temporally organised to facilitate the resolution of conflicts between sensory and oculomotor functions. As a result, attention reorientation is only possible during certain rhythmic phases. For instance, alpha rhythms tend to be modulated and nested within the theta rhythm (Dugué & VanRullen, 2017; Fiebelkorn & Kastner, 2019; Helfrich et al., 2018; Hong, Sun, Wang, Li, & Tong, 2020). This interaction suggests theta gates reorientation, whereby other frequencies aid cognitive processing through the facilitation or suppression of neural noise. Situations that require adaptive control, such as response conflict, stimulus novelty and behavioural errors, tend to share a common medial-frontal spectral signature in the theta band, which further supports that reorientation may be gated by theta (Cavanagh & Frank, 2014).

However, it is interesting to note that many studies report meditation enhances theta power. Therefore the effects of meditation may be associated with changes in attention reorientation; there has been little work investigating the physiological mechanisms involved in this up-regulation (Aftanas & Golocheikine, 2001; Cahn et al., 2012; Kang et al., 2020; Kubota et al., 2001; Lagopoulos et al., 2009; Lutz et al., 2009; Takahashi et al., 2005; Tyrell, 2016). Therefore, the following sections attempt to explore the potential mechanisms underlying the effects of meditation by referring to the broader neural-visceral literature.

8.2.2 Baroreflex response

Evidence from cardiac and respiratory studies suggests that alterations to the baroreflex function are associated with changes in attention sampling (Azzalini et al., 2019; Duschek, Wörsching, & Reyes del Paso, 2015). While most of this work has focused on the effects of the cardiac cycle phase on attention, a few notable studies attempt to modulate vagal activity during exhalation. These studies are particularly interesting, as one of the key findings in this thesis was that resonance breathing appeared to induce exhalation-locked oscillations. This suggests that the modulation of vagal tone may influence the coordination of temporal cortical rhythms and, consequently, how one samples their environment. The following sections further explore this proposal.

Cardiac Cycle

Peripheral signals from baroreceptors are communicated to the brain through afferent pathways via the vagus nerve to provide information about the timing and intensity of the heartbeat (Cameron, 2009; Taggart et al., 2016). Furthermore, phases of the cardiac cycle have been associated with changes in attention. Galvez-Pol, McConnell, and Kilner (2020) found that participants generated more ocular saccades during the early phase of the cardiac cycle (systole) compared to the later diastole. The systole period is concurrent with sensors on vessels, namely baroreceptors, that fire during the heart's ejection of blood. Baroreceptors activate in response to changes in blood pressure and adjust heart activity accordingly via the negative feedback loop of the baroreflex. The sensitivity of this response modulates as a function of cognitive demand (Garfinkel et al., 2014; Reyes Del Paso et al., 2004).

In their active interference model, Allen, Levy, Parr, and Friston (2019) endorsed the notion that baroreceptors exert an inhibitory influence on the brain, whereby intrinsic noise fluctuations influence overall neural excitability and connectivity. Cyclic changes in heart function are proposed to modulate attenuation of sensory precision, and as a result, baroreceptor feedback becomes less frequent and more predictable over time. This increased predictability provides an opportunity for high-precision sensory sampling and, consequently, reduces prediction error and improves future predictions.

Respiratory Cycle

Similarly to the cardiac cycle, the respiratory cycle phases have been associated with changes in attention regulation (Melnychuk et al., 2018; Zelano et al., 2016). Melnychuk et al. (2018) suggests this may be partially due to synchronization between respiratory and attentional systems via locus coeruleus (LC) coupling. The LC optimizes firing to suit task demands, inferred by CO_2 levels, influencing respiratory phrenic nerve firing. Studies have observed stimulation of the NTS during exhalation increased

HF-HRV, enhanced activation of the locus coeruleus and increased NTS connectivity to the insula and mid-cingulate cortex (Garcia et al., 2017; Sclocco et al., 2019). This suggests that exhalation-gated stimulation of vagal pathways may enhance afferent signalling and cardiovagal modulation.

Recent work has shown daily HRV biofeedback sessions can increase heart rate variability, which in turn plays a causal role in improving emotion regulation. Mather and Thayer (2018) propose this occurs by physiological oscillations stimulating oscillatory activity in shared brain regions involved in emotion regulation. Consequently, this activity strengthens functional connectivity within these regions and benefits emotional well-being. In Chapters 5 and 6, I found consistent evidence for a relationship between frontal alpha oscillations and the respiratory cycle phase. These findings may reflect the stimulatory effects of physiological signalling on neural oscillations. This interpretation is further emphasized by Chapter 7, whereby resonant participants exhibited a tendency for exhalation-locked neural oscillations, increased HF-HRV amplitude and enhanced interoceptive neural processing.

8.2.3 Relevance to meditation therapies

However, unlike Su-soku meditation, mindfulness meditation therapies do not require individuals to maintain a breathing rate at their resonance frequency; instead, individuals are usually encouraged to breathe at a comfortable slow pace and focus on the sensory feedback of this experience. In this regard, interoceptive attention toward the breath may be as significant as the mechanical mechanisms governing these systems.

Herrero et al. (2018) demonstrated that interoceptive attention to the breath increases respiration-locked oscillations, particularly in the anterior cingulate cortex. As participants became more aware of their breath, brain areas involved in interoception, such as the insula, had stronger respiration-locked oscillations. As the ACC is involved in conflict monitoring, these findings suggest the attention benefits of breath-focused meditation may relate to the modulation of respiration-locked oscillations. Although there are differences between Herrero et al. (2018)'s study and the findings presented in this thesis, the overarching similarity is that respiration rhythms organise cortical oscillations in the brain and resonant breathing potentially facilitates these effects. Oscillatory frequencies are thought to characterise various cognitive functions, and the amplitude of high-frequency oscillations, such as gamma and beta, are modulated by the oscillatory phase of lower frequency oscillations (Heck et al., 2017). In Chapter 7, only consistent respiration-locked oscillatory patterns in the beta frequency were noted for resonant participants, which speculatively could suggest that resonance breathing facilitates the effects of meditation by increasing vagal signal predictability (Chapter 7). However, additional studies are necessary to explore these preliminary observations further.

8.3 Limitations

8.3.1 Study design

While the data sets utilised in this study were well-designed for their intended purposes, some aspects were less than ideal for the current investigations. The Su-soku 1 data set, featured in Chapters 4 and 5, accounted for individual baseline measures, which improved the overall power of my studies. However, as woodland was used as a rest period in the original study, its duration differed from Su-soku. Therefore this would have affected the ability to compare conditions accurately. While the Su-soku 2 data set, included in Chapters 6 and 7, did not have this issue, the between-subject design makes it difficult to determine the effects of individual differences. An ideal experimental design would include a baseline period for each condition and examine the effects of meditation with three treatment groups (1) Su-soku meditation, (2) relaxing meditation (non-breath focused, such as loving-kindness), and (3) non-meditative control condition.

Most meditation practices involve directing attention towards the present moment in a non-judgmental manner (Hölzel et al., 2011). Su-soku meditation required participants to count their breaths, to reduce drowsiness induced by meditation. It could be argued that breath counting is a form of placing judgment or additional cognitive load; therefore, this delivery affects the ecological validity of the results, as it does not reflect common meditative practice. On the other hand, increasing one's attentive state by breathing and counting during meditation could provide additional benefits (van Son et al., 2019). As a result, further comparative studies are necessary to determine whether breath counting facilitates or hinders the effects of meditative practice.

8.3.2 Experimental measures

As aforementioned in previous chapters, utilising a respiratory belt would improve the respiratory phase angle analysis accuracy and allow the opportunity to explore the influence of other respiratory measures, such as oxygen saturation (SPO_2) and lung function capacity.

The event-related potential analysis of cognitive performance focused on changes in N200 and P300 on correct trials, as there were not enough incorrect trials to make a statistically valid comparison (reflected in the accuracy analysis). While this may have provided interesting comparisons, there is little evidence to suggest meditation influences error processing, and therefore such analysis may be conducted in vain (Bailey et al., 2019; Larson, Steffen, & Primosch, 2013).

8.3.3 Research field

As a general limitation, research in the field has broadly explored a range of meditative approaches; while this may be useful for drawing parallels between different meditation types, this is not necessarily helpful for formulating hypotheses for specific meditation effects.

8.4 Concluding remarks

8.4.1 Research overview

Meditation has been practised for thousands of years, yet we are only beginning to understand the neuroscience behind its therapeutic effects. In this thesis, I presented several studies that explored the interaction between neural and visceral signals during breath-focused meditation. My work suggests that neural-visceral integration can be optimised, and this may provide some mental well-being benefits. The novel application of circular statistics indicated that this optimisation might be reflected in the temporal relationship between the respiratory cycle phase and oscillatory amplitude. In addition, resonance breathing may facilitate this process. These findings add to the surging interest in "Embodied cognitive neuroscience"; in terms of both theoretical development and potential therapeutic applications for mental health (Allen & Friston, 2018; Azzalini et al., 2019; Barrett et al., 2016; Critchley & Garfinkel, 2018).

Philosophical and scientific debates surrounding the nature of the brain-body connection have been long-standing (James, 1884; Lacey & Lacey, 1970; Lange, 1922; Porges, 2009). Technological advances in neuroimaging have enabled scientists to investigate physiological mechanisms associated with cognitive function; however, understanding how these mechanisms interact with more meta-physical processes, such as interoception, is still in its infancy (Critchley & Garfinkel, 2018; Kubota et al., 2001; Thayer & Lane, 2000). Based on my findings and previous work, I argue that meditative practice may provide a window of opportunity to modulate one's interoceptive awareness and, consequently, the ability to regulate information processing (Hinterberger et al., 2019; Kluger et al., 2021; Leganes-

Fonteneau et al., 2021; Rassi et al., 2019). The concept of interoception borders close to what many may regard as an aspect of conscious processing: the ability to perceive sensory sensations. However, given the vastness of what it could mean to be “conscious”, we have a long way to go before we understand the complexity of interconnections between the brain and body, let alone how this translates to mental health.

While this thesis argues meditative practice may influence re-orientation of attention, as indicated by changes in respiratory phase angle of oscillatory amplitude across meditation blocks, there were no significant changes in flanker task performance and mood state after meditation. These null findings could be explained by task design. We may have seen different results with a cognitive task involving emotional processing or sustained long-term meditative practice (Jo et al., 2016; Quaglia et al., 2019). This thesis suggests neural oscillatory amplitude can become respiratory phase locked; however, it is unclear how this influences attention regulation and, consequently, an individual’s mental state.

8.4.2 Future directions

Based on the work presented in this thesis, future studies should consider the impact of respiratory-phase effects on attention and investigate the therapeutic potential of resonant breathing for mental health. Such work could have many implications for psychiatry and cognitive psychology. If an optimal state varies according to task demands and respiration can influence attention deployment, one could speculate the effects of the respiratory phase on neural amplitude are task-dependent. Further understanding in this area could be beneficial for assessing and enhancing cognitive control for various mental health disorders, such as ADHD, mood, and anxiety disorders. Clinical applications of embodied cognition could be advanced by assessing the additional benefits of biofeedback resonance breathing vs standard mindfulness meditation practice. In Appendix D, I provide a proposal for a potential future study that would focus on the clinical benefits of this area.

This thesis investigated the mechanistic interaction of neural-visceral signals during breath-focused meditation by applying novel statistical methods to analyse electrophysiology data. Several studies reported in this thesis propose breath-focused meditation (Su-soku), which modulates neural oscillatory amplitude in multiple frequency bands, enhances the vagal tone and may alter feedforward-feedback regulation, as indicated by neural oscillatory changes across the respiratory cycle. Some exploratory findings suggest these changes may coincide with enhancements in interoceptive awareness and mood state; however, further research is necessary. While there are notable limitations, this thesis provides a foundation for pursuing new directions in developing personalised treatments for mental health that utilise the knowledge of neural-visceral integration.

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Appendices

Appendix A

Table of key studies

Below are all the key studies that were utilised for developing the literature review in Chapter 2.

Studies	Summaries
1. Azevedo et al (2013) 10.1002/hbm.22133	**Study type:** Comparing intergroup empathy **Quality:** 5.39 impact factor, 199 h-index **Subjects:** 27: 14 white and 13 black-african **Neuroimaging method:** fMRI **Autonomic method:** pupil dilation **Emotion method:** emotion video clips **Outcomes:** Haemodynamic and autonomic responses greater when observing pain experienced by own race than other race models.
2. Balconi et al (2015) 10.1016/ j.bandc.2015.02.001	**Study type:** Exploring the integration of central and autonomic processes during emotion processing. **Quality:** 2.4 impact factor and 131 h index **Subjects:** 20 subjects (12 female and 8 male) **Neuroimaging method:** fNIRS and EEG **Autonomic method:** SCR and HR **Emotion method:** Emotional cue paradigm **Outcomes:** Lateralisation effects correlated with autonomic responsiveness.
3. Berger et al (2017) 10.1007/s00702-015-1491-4	**Study:** Investigate the effects of rTMS frequencies over right dlPFC on heart rate, skin conductance during processing of emotional stimuli in healthy subjects. Single-blind, placebo controlled, cross-over design experiment **Quality:** 3.85 impact, 116 h-index **Subjects:** 40 healthy women **Neuroimaging method:** rTMS vs sham rTMS **Autonomic method:** SCR, phasic cardiac response **Emotion method:**emotional pictures **Outcomes:** Heart rate deceleration significantly increased during presentation of negative and neutral valence after rTMS.
4. Brugnera et al (2018) 10.1027/0269-8803/a000183	**Study:** Explore psychophysiological underpinnings of emotion experience. **Quality:** 2.88 impact factor, 48 h-index **Subjects:** 28 subjects **Neuroimaging method:** fNIRS **Autonomic method:** ECG, HF-HRV. HR **Emotion method:** Anger and happiness recall **Outcomes:** HR and HF-HRV highest and lowest points induced by anger recall. NIRS bilateral increase during both, highest during verbal phases.
5. Coen et al (2009) 10.1053/j.gastro.2009.02.052	**Study type:** Exploring the link between negative emotional state and abnormal visceral sensations by investigating the effects of negative emotion on brain processing of esophageal sensation. **Quality:** 22 impact factor, 423 h index. **Subjects:** 12 male volunteers (21 to 32 years) **Neuroimaging method:** fMRI **Autonomic method:** Induced nonpainful and painful distentions to the esophagus **Emotion method:** Affective music: negative and neutral. Subjective ratings of emotional perception. **Outcomes:** No significant differences in subjective perceptions of emotion between painful and nonpainful stimulation. Following painful stimulation, brain activity increased in the right hemisphere during negative emotion and was localized to the

	anterior cingulate cortex (ACC; BA24/32), anterior insula, and inferior frontal gyrus. Following nonpainful stimulation during negative emotion, brain activity increased in the right anterior insula and ACC (BA24 and 32).
6. Critchley et al (2000) http://dx.doi.org/ 20024039	**Study:** Identifying brain areas involved in somatic arousal **Quality:** 6.7 impact factor, 471 h-index **Subjects:** 6 subjects (3 male and 3 female) **Neuroimaging method:** fMRI **Autonomic method:** SCR **Emotion method:**decision making task (reward and punishment) **Outcomes:** Found areas involved in emotion and attention are differentially involved in SCR responses.
7. Critchley et al (2002) https://doi.org/ 10.1016/s0896-6273(02)00588-3	**Study:**Examine influence of perceptual awareness of CS on brain activity and how is this modulated by autonomic arousal in fear conditioning **Quality:** 17.1 impact factor, 493 h-index **Subjects:** six healthy control subjects, four patients with autonomic failure **Neuroimaging method:** fMRI **Autonomic method:** Comparing with autonomic deficit patients **Emotion method:** emotional faces unpaired or paired with CS stimuli for fear conditioning **Outcomes:** Insula modulated by perceptual awareness in both groups. Patients with autonomic denervation associated with decreased conditioning related activity in insula and amygdala. Expression of conditioning is modulated by awareness and autonomic arousal states.
8. Critchley et al (2002) https://doi.org/ 10.1006/ nimg.2002.1147	**Study:**Explore neural mechanisms of volitional intent, self-representation, and autonomic states of arousal in relation to biofeedback performance. **Quality:** 7.4 impact factor, 381 h-index **Subjects:** 17 healthy subjects **Neuroimaging method:** fMRI **Autonomic method:** electrodermal **Emotion method:** biofeedback **Outcomes:** anterior cingulate and insula modulated by biofeedback and variation in anterior insula reflected accuracy and sensitivity of feedback.
9. Critchley et al (2000) https://doi.org/ 10.1111%2Fj.1469-7793.2000.t01-1-00259.x	**Study:**Identifying brain regions respond to changes in heart rate and blood pressure common to exercise and mental stress task states of autonomic arousal **Quality:** 6.23 impact factor, 249 h index **Subjects:** six healthy subjects **Neuroimaging method:** PET **Autonomic method:** arterial blood pressure and heart rate **Emotion method:**mental stressor tasks and isometric exercise **Outcomes:**Exercise and stressor tasks increases MAP and HR. Increased rCBF in cerebellar vermis, brainstem and right anterior cingulate and right insula covariates with MAP. rCBF in pons, cerebellum and right insula covaried with HR.
10. Cuthbert et al (2000) https://dx.doi.org/ 10.1016/S0301-	**Study:**Investigate if ERP potentials and slow waves occurs in response to affective stimuli is determined by emotional engagement. **Quality:** 2.62 impact factor, 132 h-index

0511(99)00044-7	**Subjects:** 37 subjects (18-24 yrs) **Neuroimaging method:** EEG **Autonomic method:** EKG **Emotion method:** affective pictures: pleasant, unpleasant and neutral IAPS **Outcomes:** Positive shift 200-300 after picture onset not related to content type but accentuated for pictures for increased autonomic response and reports of greater affective arousal
11. Davidson et al (2000) https://doi.org/10.1016/s0301-0511(99)00044-7	**Study:** Comparing EEG and autonomic activity in phobics vs controls when anticipating public speech **Quality:** 13.38 impact factor, 333 h index **Subjects:** 18 social phobics, 10 healthy controls **Neuroimaging method:** EEG **Autonomic method:** heart rate and blood pressure **Emotion method:** anticipated public speech, self-reports of anxiety and affect **Outcomes:**Phobics increased anxiety and negative affect during anticipation. HR elevated in phobics. Increased right-side activation in anterior temporal and lateral prefrontal regions.
12. De voogd et al (2016) 10.1093/scan/nsw055	**Study:**Disentangle roles of noradrenergic-sympathetic arousal and amygdala emotional declarative memory **Quality:** 4.23 impact factor, 109 h index **Subjects:** 24 subjects (12 female, 12 male) **Neuroimaging method:** fMRI **Autonomic method:** skin conductance, pupil dilation **Emotion method:** subsequent-memory/fear-conditioning, 128 items Hemera photo-objects. Excluded higher threat value e.g lions and snakes. Electrical shocks for conditioning. **Outcomes:** Skin conductance and pupil dilation differential conditioning effect but did not predict item memory. Amygdala did not show differential conditioning but predicted item memory for CS trials.
13. Drabant et al (2011) 10.1016/j.neuroimage.2010.11.040	**Study:** Examined bio-behavioural impact of varying anticipatory anxiety. **Quality:** 7.4 impact factor, 381 h-index **Subjects:** Two studies of 95 and 51 healthy female participants **Neuroimaging method:** fMRI BOLD **Autonomic method:** electrodermal **Emotion method:** shock anticipation task, rating anxiety experience **Outcomes:** Stepwise pattern of responding anxiety and electrodermal responses. Shock anticipation brain regions: hypothalamus, periaqueductal gray, caudate, precentral gyrus, thalamus, insula, ventrolateral PFC, dorsomedial PFC, and ACC. Modulated by neuroticism.
14. Feeser et al (2014) https://doi.org/10.1016/j.brs.2013.08.006	**Study:**Investigate whether tDCS would modulate emotional arousal ratings. Evaluate effect of tDCS on autonomic responses during cognitive apprasial.Double blind, between subject, sham-controlled trial **Quality:** 9.18 impact factor, 81 h-index **Subjects:** 48 subjects (25 women and 23 men) 20-47 yrs **Neuroimaging method:** tDCS over right dlPFC vs sham **Autonomic method:** skin conductance **Emotion method:** Emotional arousal ratings of affective images.

	Cognitive appraisal types: downregulation, upregulation, negative maintain, neutral maintain. **Outcomes:**tDCS over right dlPFC facilitates cognitive reappraisal by increasing or decreasing responsiveness depending on regulation goal.
15. Garfinkel et al (2016) 10.1093/scan/nsv099	**Study:** Investigating physiological and brain data to understand how subliminal emotional cues may affect decision making **Quality:** 3.66 impact factor, 109 h index **Subjects:** 21 subjects **Neuroimaging method:** fMRI **Autonomic method:** blood pressure **Emotion method:** Emotional word letter-strings for priming and lexical decision task. **Outcomes:** Anger primes delayed reaction time for decisions compared to relax primes. But high trait anger individuals were sped up by anger primes. Anger primes increases blood pressure and magnitude of reaction time prolongation. Evoked enhanced activity in dorsal pons and an attenuation of activity within visual occipitotemporal and attentional parietal cortices. Periaqueductal grey matter, occipital and parietal regions increased linearly with evoked blood pressure changes
16. Goodman et al (2013) https://doi.org/10.1016/j.ijpsycho.2012.09.008	**Study:** Investigate predictive power of trait and state frontal asymmetry during emotion regulation to manage arousal and facilitate cognitive performance. **Quality:** 2.88 impact factor, 135 h index. **Subjects:** 30 participants (12 women). **Neuroimaging method:** EEG **Autonomic method:** skin conductance, heart rate and eyeblink startle amplitude **Emotion method:** 2-back working-memory task with increasing levels of mental stress. Self report for emotional affect. **Outcomes:** Threat of shock in participants with higher state asymmetry greater emotion regulation than lower score indexed by eyeblink magnitude. Trait asymmetry did not predict emotion regulation.
17. Gray et al (2012) 10.1037/a0025083	**Study:** How does phasic baroreceptor activation alter the appraisal of emotional stimuli within cardiac cycle. **Quality:** 4.32 impact factor, 152 h index **Subjects:** 37 subjects (9 male and 28 female) **Neuroimaging method:** fMRI **Autonomic method:** ECG heart rate **Emotion method:** emotion facial stimuli presented at different points of the cardiac cycle **Outcomes:** Facial expressions disgust judged more intense at systole, rebound heart rate increase after expressions of disgust and happiness. Neural activity prefrontal cortex correlated emotional ratings. Periaqueductal gray matter reflected both emotional ratings and their interaction with cardiac timing. Activity within regions including prefrontal and visual cortices correlated with increases in heart rate evoked by the face stimuli, while orbitofrontal activity reflected both evoked heart rate change and its interaction with cardiac timing.
18. Henderson et al (2012)	**Study:**Investigate brain regions responsible for emotionally evoked increases in sympathetic nerve activity.

10.1016/j.neuroimage.2012.04.049	**Quality:** 6.55 impact factor, 381 h index. **Subjects:** 13 subjects (10 male, 3 female) **Neuroimaging method:** fMRI **Autonomic method:** skin response **Emotion method:** affective images **Outcomes:** Increase sympathetic activity associated brain activity in amygdala, pons, thalamus, accumbens and cerebellum. Decrease in orbitofrontal, frontal and precuneus cortices.
19. Hofmann et al (2005) 10.1037/1528-3542.5.4.464	**Study:**Investigate cortical and autonomic indicators of anxiety **Quality:** 4.31 impact factor, 152 h index **Subjects:** 40 subjects **Neuroimaging method:** EEG **Autonomic method:** ECG RSA, skin conductance **Emotion method:** public speaking task, worry induction, distress rating scale **Outcomes:**Worry greater HR and lower RSA. Worry had higher skin conductance levels but lower than anticipation. Trait speaking anxiety correlated with left frontal activity.
20. Kleint et al (2015) 10.1371/journal.pone.0133164	**Study:**Investigate neural activity while listening to heartbeat sounds and how this overlaps with interoceptive and fear networks. **Quality:** 3.75 impact factor, 367 h index. **Subjects:** 39 healthy subjects **Neuroimaging method:** fMRI **Autonomic method:** skin conductance **Emotion method:** subjective measures of state and trait anxiety. Heartbeart interoceptive task. **Outcomes:** Heartbeat sounds triggered activation of bilateral insular cortices, the inferior frontal operculum, and the middle frontal gyrus. Functional connectivity between middle frontal gyrus(seed region) and bilateral insular cortices, the left amygdala and the supplementary motor area. Right anterior insular correlated with autonomic arousal.
21. Kraus et al (2007) 10.1007/s00702-007-0755-z	**Study:** Investigate brain response to tVNS. **Quality:** 3.50 impact factor, 116 h index **Subjects:** 22 healthy subjects (20-37 ages) **Neuroimaging method:** fMRI **Autonomic method:** left canal tVNS stimulation, heart rate, blood pressure and microcirculation **Emotion method:** mood scale **Outcomes:** BOLD decreases in limbic brain areas, including amygdala, hippocampus, parahippocampal, and gyrus. Increased activation inslua, precentral gyrus and thalamus. No change to heart rate, blood pressure and microcirculation. Improved mood after stimulation.
22. Kyeong et al (2017) 10.1038/s41598-017-05520-9	**Study:** Identify the effects of gratitude and resentment meditation on neural networks and physiological state. **Quality:** 4.99 impact factor, 242 h index. **Subjects:** 32 subjects **Neuroimaging method:** fMRI **Autonomic method:** heart rate and pulse oximetry **Emotion method:** gratitude and resentment meditation. Emotion and motivation scales **Outcomes:** Average HR lower in gratitude than resentment

	meditation. Temporostriatal FC positive correlation with HR in gratitude intervention. After gratitude meditation, resting-state FC amygdala, right dorsomedial prefrontal cortex and left dorsal anterior cingulate cortex were positively correlated with anxiety scale and depression scale.
23. Lane et al (2001) Brain and Cognition. 47. 97-100.	**Study:** Explored neural substrates of autonomic regulation during emotion. **Quality:** 2.43 impact factor, 131 h index **Subjects:** 12 female subjects **Neuroimaging method:** PET rCBF **Autonomic method:** HF HRV **Emotion method:** Film and autobiographical recall: happy, sad, disgust and neutral **Outcomes:** Correlations between HR-HRV and rCBF were observed in medial prefrontal cortex and left posterior orbitofrontal/anterior insular cortices.
24. Lane et al (2013) 10.1016/ j.jad.2013.02.005	**Study:** Investigate the role of the sgACC in affective state and cardiac vagal control **Quality:** 4.83 impact factor, 205 h index **Subjects:** 11 healthy subjects and 8 depressed subjects. **Neuroimaging method: f**MRI **Autonomic method:** ECG **Emotion method:** emotional counting stroop task **Outcomes:** Positive correlations between cardiac vagal control and BoLD intensity in sgACC. Depressed subjects no significant correlations. Depression-specific emotion blocks to neutral blocks, correlation between BOLD BA25 and cardiac vagal control change was significantly greater in controls than depressed subjects.
25. Linnman et al (2012) 10.1016/ j.biopsycho.2011.12.012	**Study:** Investigating whether resting brain metabolism predicts autonomic and neuronal responses during fear conditioning **Quality:** 2.63 impact factor, 132 h index **Subjects:** 20 subjects **Neuroimaging method:** PET, fMRI **Autonomic method:** skin conductance **Emotion method:** fear conditioning **Outcomes:** resting dorsal anterior cingulate positively predicted skin conductance. Midbrain and insula resting metabolism negatively predicted functional reactivity.
26. Lutz et al (2008) https://doi.org/ 10.1371/ journal.pone.0001897	**Study:** Investigating the effects of meditation expertise on neural circuity involved in emotion processing. **Quality:** 3.75 impact factor, 367 h index. **Subjects:** 16 healthy volunteers and 15 experts **Neuroimaging method:** fMRI **Autonomic method:** pupillometry **Emotion method:** compassion meditation, emotional sounds **Outcomes:** Insula activity during meditation greater during negative sounds than positive or neutral. Strength activation insula linked to self-reported intensity meditation. Both groups increased activation in amygdala, right temporo-parietal junction (TPJ), and right posterior superior temporal sulcus (pSTS).
27. Lutz et al (2009) https://doi.org/	**Study:** Investigated relationship between insula and cardiac function during compassion meditation.

10.1016%2Fj.neuroimage.2009.04.081	**Quality:** 7.4 impact factor, 391 h index **Subjects:** 10 Buddhist practitioners and 13 healthy volunteers **Neuroimaging method:** fMRI **Autonomic method:** heart rate, HRV and RSA. **Emotion method:** Presentation of emotional sounds, compassion meditation **Outcomes:** BOLD activation in insula associated with heart rate across groups and states.
28. Mackinnon et al (2013) 10.1007/s10484-013-9226-5	**Study:** Using heartbeat evoked potentials to investigate cardiac regulation of vagal afferents during emotion induction and resonant breathing. Quality: 2.59 impact factor, 67 h index **Subjects:** 30 subjects (23-35 yrs) **Neuroimaging method:** EEG, HEPs **Autonomic method:** ECG: HR and HRV **Emotion method:** subjective measures of emotion, emotion induction by autobiographical scripts of happiness and sadness. Diaphragmatic breathing at six breaths per minute **Outcomes:** Evoked potentials most attenuated during negative emotion. HRV affects the strength of HEPs during resonant breathing.
29. Makovac et al (2015) 10.1038/npp.2015.10	**Study:** Investigate whether manipulating afferent cardiovascular signals alters the central processing of emotional information (fearful and neutral facial expression). **Quality:** 8.29 impact factor, 227 h index. **Subjects:** 21 subjects **Neuroimaging method:** fMRI **Autonomic method:** ECG, pulse oximetry, respiration Carotid stimulation vs sham stimulation **Emotion method:** emotional ratings of faces (fearful and neutral) **Outcomes:** Significant expression-by-stimulation interaction in amygdala during emotional processing. Reductions in insula,amygdala, hippocampus correlation stimulation evoked change in emotional ratings of fearful faces. Individual differences in autonomic state predicted extent carotid stimulation influenced neural response to fearful faces.
30. Makovac et al (2018) 10.1016/ j.cortex.2017.07.002	**Study:** Does processing of emotional information of faces differ between right and left carotid afferents **Quality:** 4.02 impact factor, 122 h index **Subjects:** 20 subjects **Neuroimaging method:** fMRI **Autonomic method:** ECG HRV, RMSSD **Emotion method:** automated neck suction device and emotional stimuli: fearful and neutral faces. **Outcomes:** Right and left carotid stimulation influenced opercular regions Right stimulation increased perceived intensity of fear.
31. Mandrick et al (2016) 10.1016/ j.biopsycho.2016.10.002	**Study:** Studying the effect of stress on working memory performance under variations of task difficulty **Quality:** 2.63 impact factor, 132 h index **Subjects:** 14 subjects **Neuroimaging method:** fNIRS **Autonomic method:** pupil diameter, cardiovascular and prefrontal oxygenation measurements.

	Emotion method: mental arithmetic n-back task anxiety questionnaire, unpredictable loud sounds to induce stress. **Outcomes:** Higher task difficulty degraded performance and induced increased tonic pupil diameter, heart rate and activity in lateral prefrontal cortex, decreased phasic pupil response and HRV.
32. Mather et al (2017) 10.1016/ j.neuroimage.2017.02. 025	**Study:** Investigated the relationship between LC neuromelanin and HF-HRV. Also examined the role of age and trait anxiety in contributing to the relationship between LC-MRI and HRV. **Quality:** 7.4 impact factor, 381 h index **Subjects:** 27 younger adults (18-34yrs) and 18 older adults (60-75yrs). **Neuroimaging method:** LC-MRI contrasts: structural and functional MRI **Autonomic method:** HF-HRV, heart rate **Emotion method:** trait anxiety, fear conditioning and spatial detection tasks **Outcomes:** greater LC-MRI contrast negatively associated with HF-HRV during fear conditioning and spatial detection tasks. This was not accounted for by the differences in age or anxiety.
33. Marci et al (2007) 10.3758/CABN.7.3.243	**Study:** Advance emotion research by investigating relationship between central-autonomic activity during emotional recall. **Quality:** 3.5 impact factor, 104 h index **Subjects:** 10 healthy subjects **Neuroimaging method:** PET **Autonomic method:** skin conductance, HR and HF HRV **Emotion method:** Autobiographical scripts: anger, neutral, happiness and sadness **Outcomes:** Only significant changes in central and autonomic measures were noted for anger when compared to neutral script. Changes noted in rCBF in prefrontal cortex.
34. Miskovic et al (2010) 10.1016/ j.ijpsycho.2009.12.015	**Study:** Explored extent that psychophysiological markers predict biased attention. **Quality:** 2.88 impact factor, 135 h index **Subjects:** 30 participants **Neuroimaging method:** EEG **Autonomic method:** Cardiac vagal tone **Emotion method:** emotional images of faces. **Outcomes:** Right frontal EEG asymmetry and low cardiac vagal tone predicted 37% of variability in attention vigilance for angry faces.
35. Nguyen et al (2016) 10.1016/ j.neuroimage.2015.08. 078	**Study:** Investigated the mechanisms of interoceptive integration during free listening emotionally salient film. First study to show the insular hierarchy for interoceptive processing during a natural emotional experience. **Quality:** 7.4 impact factor, 381 h index **Subjects:** 20 participants **Neuroimaging method:** fMRI **Autonomic method:** Physiological data (IBI, HF, LF HRV). **Emotion method:**Emotional film **Outcomes:** Cardiac activity synchronized across participants and was represented in the posterior insula. Connectivity analysis

	revealed anterior insula to be tuned to emotionally salient momnts in audio stream.
36. O’connor et al (2007) 10.1038/sj.npp.1301342	**Study:** Investigate the association between brain activity during grief and baseline parasympathetic activity **Quality:** 8.29 impact factor, 227 h index **Subjects:** 8 women who had experienced death of a loved one in the last 18 months **Neuroimaging method:** fMRI **Autonomic method:** ECG RSA **Emotion method:** Presented with personalised pictures and words that evoked grief **Outcomes:** Positive correlation between vPCC and parasympathetic tone during emotional processing and episodic memory tasks. Bilateral cuneus and parahippocampus were associated with higher baseline parasympathetic tone.
37. Ohira et al (2006) 10.1016/j.neuroimage.2005.08.047	**Study:** Identify neural circuity associated with regulation of peripheral physiological responses during voluntary emotion suppression **Quality:** 7.4 impact factor, 381 h index **Subjects:** 10 female subjects **Neuroimaging method:** PET **Autonomic method:** heart rate, skin conductance and endocrine **Emotion method:** Positive, neutral and negative pictures in attending and suppression task. **Outcomes:** Left amygdala and right anterior temporal pole activated during attending task, whereas left lateral prefrontal cortex, medial prefrontal and medial orbiofrontal activated in suppression task. Amygdala activation and MOPFC positively correlated with SCR and ACTH response. Emotion suppression enhanced SCR and correlation with MOFC.
38. Papousek et al (2001) https://doi.org/10.1016/s0167-8760(01)00131-3	**Study:** Investigate association between cortical activation asymmetries and sympathetic system. Examine inter individual differences in cortical-electrodermal relationship in relation to psychological stress/anxiety and depression. **Quality:** 2.88 impact factor, 135 h index **Subjects:** 50 healthy subjects **Neuroimaging method:** EEG **Autonomic method:** skin conductance **Emotion method:** depression inventory, psychological stress and state anxiety assessments. **Outcomes:** Asymmetry in orbital and dorsolateral frontal reflect two different cortical sub-systems regulating electrodermal activity and these are modulated by emotional factors.
39. Park et al (2012) 10.3758/s13415-012-0111-0	**Study:**ERP study exploring the connection between differences in cardiac vagal tone (HRV) and ERPs in response to fearful and neutral faces. **Quality:** 3.53 impact factor, 104 h index **Subjects:** 36 participants **Neuroimaging method:** ERP EEG **Autonomic method:** Cardiac vagal tone (HRV) **Emotion method:**104 emotional face images **Outcomes:** Low resting heart rate variability was associated with greater N200 activity in response to fearful faces and greater LPP response to neutral faces. This was also linked to increased source

	activity in visual areas.
40. Pollatos et al (2007) https://doi.org/ 10.1002/hbm.20258	**Study:** Examined the association between bodily perception and emotional intensity, and associated neural activity. **Quality:** 5.04 impact factor, 199 h index **Subjects:** 32 subjects **Neuroimaging method:** EEG **Autonomic method:** Subjective valence and arousal ratings, ECG **Emotion method:** Emotional picture presentation (60 images from IAPS), heartbeat perception task **Outcomes:** Positive relationship between heartbeat awareness and emotional intensity. P300 amplitudes in response to pleasant and unpleasant images were enhanced in subjects with high interoceptive awareness. This was source localised to insula and somatosensory cortices and anterior cingulate and prefrontal.
41. Pollatos et al (2005) 10.1016/ j.cogbrainres.2005.09.019	**Study:** Investigating relationship between interoceptive awareness and emotion regulated brain activity. **Quality:** 3.25 impact factor, 212 h index **Subjects:** 44 subjects **Neuroimaging method:** EEG **Autonomic method:** Subjective emotional valence and arousal rating. **Emotion method:** 60 affective images from IAPS (pleasant, neutral and unpleasant), heartbeat perception task. **Outcomes:** Good heartbeat perceivers reported higher arousal ratings and no difference in emotional valence. No difference between groups for visual evoked P300 and slow wave latency. Higher p300 amplitude in good heartbeat perceivers across all images. Perception scores correlated significantly with mean arousal rating and p300 amplitude and slow wave window.
42. Pollatos et al (2007) 10.1016/ J.BRAINRES.2007.01.026	**Study:** Identify brain structures that are mutually activated during interoceptive awareness of heartbeats and during cardiovascular arousal. **Quality:** 3.25 impact factor, 212 h index **Subjects:** 20 subjects **Neuroimaging method:** fMRI **Autonomic method:** interoceptive awareness task **Emotion method:** trait anxiety and personality tests **Outcomes:** Degree of interoceptive awareness predicted degree of activation in insula and medial frontal/dorsal cingulate. Negative feelings correlated BOLD response to interoceptive awareness condition.
43. Rabellino et al (2007) 10.1002/hbm.23702	**Study:** Investigate the neural underpinnings associated with HF-HRV during trauma exposure cues in PTSD. **Quality:** 5.39 impact factor, 199 h index **Subjects:** 18 subjects with PTSD, 18 healthy controls. **Neuroimaging method:** fMRI **Autonomic method:** HF-HRV **Emotion method:** Sub and supraliminal processing of personlized trauma-related words **Outcomes:** PTSD exhibited decreased HF-HRV reactivity in response to sub and supraliminal cues. Subliminal processing PTSD subjects showed decreased neural response associated with HF-HRV in left dorsal anterior insula. In contrast supraliminal processing decreased neural activity associated with HRV in

	posterior insula and increased neural activity associated with HF-HRV in left centromedial amygdala in PTSD compared to controls.
44. Ruiz-Padial et al (2018) 10.1016/ j.biopsycho.2018.06.008	**Study:** Investigated signal complexity (fractal dimension) EEG with positive and negative states **Quality:** 2.63 impact factor, 132 h index **Subjects:** 21 participants **Neuroimaging method:** EEG fractal dimension **Autonomic method:**HRV **Emotion method:** Eight video clips related to four different types of emotion induction: disgust, humour, fear and neutral. **Outcomes:** Humour elicited highest FD score in EEG and highest HRV, whereas fear produced the lowest score in both measures.
45. Sakai et al (2007) 10.1016/ j.autneu.2007.01.001	**Study:** Investigate autonomic and neural changes from specific acupuncture sensations. **Quality:** 2.24 impact factor, 86 h index **Subjects:** 16 subjects (22-30 yrs) **Neuroimaging method:** EEG **Autonomic method:** heart rate, blood pressure **Emotion method:** acupuncture **Outcomes:** Acupuncture increased power of all spectral bands but gamma. Changes in HF and total power of HRV positively correlated with theta, alph and gamma while LF of SBPV and LF/HF of HRV were negatively correlated with changes in power of all spectra bands.
46. Schaefer et al (2014) 10.1016/ j.biopsycho.2013.12.011	**Study:** To examine the biological correlates of normal and phobic fear **Quality:** 2.63 impact factor, 132 h index **Subjects:** 21 snake phobic and 21 non-phobic controls **Neuroimaging method:** fMRI **Autonomic method:** electrodermal, pupil dilation **Emotion method:** Videos of snakes to elicit fear, self-report affect measures **Outcomes:** Phobia subjects recruited more brain regions and increased pupil dilation, electrodermal and self-report to snakes than control. Phobia greater between and within subject concordance with these measures.
47. Schirmer et al (2010) 10.1016/ j.clinph.2009.09.029	**Study:** Investigate the relationship between mismatch negativity and emotion processes associated with the detection of change. **Quality:** 3.61 impact factor, 191 h index **Subjects:** 23 subjects **Neuroimaging method:** EEG mismatch negativity **Autonomic method:** heart rate **Emotion method:** stimulus material spoked with neutral or angry prosody, state-trait anxiety inventory. **Outcomes:** MMN to angry voice deviants were larger than neutral deviants and correlated with heart rate acceleration. This also increased with increasing state anxiety.
48. Takahashi et al (2005) 10.1016/ j.ijpsycho.2004.07.004	**Study:** Investigate psychophysiological parameters during zen meditation **Quality:** 2.88 impact factor, 135 h index. **Subjects:** 20 normal adults **Neuroimaging method:** EEG **Autonomic method:** HRV

	Emotion method: Su-soku zen meditation, harm avoidance measures **Outcomes:** Slow alpha in frontal was negatively correlated with nuLF and LF/HF. Frontal theta correlated nuHF and harm avoidance score.
49. Thome et al (2017) 10.1002/hbm.23340	**Study type:** Characterize autonomic response patterns and related neuronal patterns in individuals with PTSD at rest. **Quality:** 5.40 impact factor, 199 h index Subjects: 98 participants: 57 PTSD diagnosis and 41 healthy controls **Neuroimaging method:** fMRI **Autonomic method:** HRV **Emotion method:** Subjective rating scales **Outcomes:** PTSD individuals exhibited lower HRV. PTSD CAN regions associated connectivity in regions related to emotional reactivity. CAN connectivity in controls was strongly related to higher HRV. This was absent in PTSD subjects.
50. Tupak et al (2014) 10.1016/ j.neuroimage.2013.09.066	**Study:** Investigate if VLPFC activation during labelling is due to cognitive picture evaluation alone or specific to implicit regulation of salient emotional stimuli. **Quality:** 7.4 impact factor, 381 h index **Subjects:** 35 healthy subjects **Neuroimaging method:** fNIRS **Autonomic method:** skin conductance **Emotion method:** 36 neutral and 36 threatening images from IAPS, matching vs labelling condition **Outcomes:** Increased VLPFC activation during threat labelling but not neutral pictures. VLPFC activated by cognitive but not perceptual evaluation of threat. Perceptual processing activate emotional regulation in high anxious subjects.
51 Urry et al (2009) 10.1016/ j.neuroimage.2009.05.069	**Study:** Examine prefrontal-autonomic physiology associations as participants used reappraisal to regulate unpleasant emotion. **Quality:** 7.4 impact factor, 381 h index **Subjects:** 26 subjects **Neuroimaging method:** fMRI **Autonomic method:** Electrodermal, pupil diameter, cardiac acceleration signals **Emotion method:**54 unpleasant images from IAPS in spontaneous emotion reactivity task, then modulate affective response with reappraisal. **Outcomes:** EDA greater when increasing and lower when decreasing compared to maintaining response. Pupil diameter and cardiac acceleration were higher in increasing and decreasing compared to maintaining. Reappraisal-related activation in two medial prefrontal regions was correlated greater cardiac acceleration and changes in EDA. Emotional arousal effects meditated by right amygdala.
52. Valenza et al (2016) 10.1098/ rsta.2015.0176	**Study:**Investigating brain-heart interactions during emotion perception. **Quality:** 6.7 impact factor, 174 h index **Subjects:** 22 subjects **Neuroimaging method:** EEG **Autonomic method:** heart rate, HRV

	Emotion method: 110 affective images from IAPS **Outcomes:** EEG oscillations in theta were significantly influenced by arousal-dependent changes between positive and negative stimuli.
53. Wager et al (2009) 10.1016/ j.neuroimage.2009.05.044	**Study:** Examine brain-heart relationships during social evaluative threat. **Quality:** 7.4 impact factor, 381 h index. **Subjects:** 18 subjects **Neuroimaging method:** fMRI **Autonomic method:** heart rate **Emotion method:** Subjective anxiety rating, speech task **Outcomes:** Heart rate responses were meditated by rostral dorsal anterior cingulate cortex and de-activated by ventromedial prefrontal cortex. HR responses were mediated by PAG. Anxiety weakened the connections of vmPFC, dorsomedial prefrontal cortex and lateral frontal cortex.
54. Weissman et al (2018) 10.1016/ j.neuroimage.2018.08.069	**Study:** Examine neural response to emotions with attentional focus in different ways **Quality:** 7.4 impact factor, 381 h index. **Subjects:** 135 participants **Neuroimaging method:** fMRI **Autonomic method:** skin response and ECG **Emotion method:**emotional face task **Outcomes:** Greater RSA withdrawal associated increased ventromedial prefrontal cortex response. More skin conductance associated with decreased activation in prefrontal regions and increased activation in left hippocampus. Coupling of brain-cardiac interactions also predicted autonomic responses at baseline.
55. Williams et al (2001) 10.1006/ nimg.2001.0904	**Study:** Investigated neural-autonomic response of fear perception **Quality:** 7.4 impact factor, 381 h index. **Subjects:** 11 subjects (all male, 30 years) **Neuroimaging method:** fMRI **Autonomic method:** skin conductance **Emotion method:** Blocks of fear vs neutral **Outcomes:** Fmri arousal/without arousal provided distinct differentiation of amygdala and hippocampal networks. This was associated with differences in visceral experience vs declarative fact processing of fear.
56. Williams et al (2004) 10.1016/ j.cogbrainres.2004.06.005	**Study:** Investigating the relationship between brain and autonomic response to fear across experimental trials. **Quality:** 3.3 impact factor, 56 h index **Subjects:** 22 subjects **Neuroimaging method:** fMRI **Autonomic method:** Skin conductance **Emotion method:** Fear and neutral facial expression **Outcomes:** Phasic SCRs followed a U-shape pattern across early, middle and late presentations of fear stimuli. fMRI data revealed a concomitant temporal sequence of preferential somatosensory insula, dorsomedial prefrontal cortex and left amygdala engagement.
57. Xia et al (2017) 10.1162/jocn_a_01087	**Study:** Investigated how task-related skin conductance responses can predict variance in arousal experience. **Quality:** 3.47 impact factor, 32 h-index **Subjects:** 39 subjects

	Neuroimaging method: fMRI **Autonomic method:** skin conductance **Emotion method:** Subjective rating of emotional experience, emotionally evocative images **Outcomes:** Greater skin conductance reactivity to emotional images and stronger intrinsic salience network connectivity predicted more intense experiences of arousal.
58. Yang et al (2007) 10.1016/j.neulet.2007.09.039	**Study:** Examined the relationship between ANS and amygdala in adolescents **Quality:** 3.05 impact factor, 177 h index **Subjects:** 18 adolescents **Neuroimaging method:** fMRI **Autonomic method:** heart rate **Emotion method:** Facial expression task **Outcomes:** Significant amygdala activation for all facial expressions. Right amygdala for fearful faces was significantly correlated with heart rate. Coupling between processing emotional events and ANS.
59. Yasui et al (2010) 10.1016/j.autneu.2010.03.011	**Study:** Examining the effects of thermotherapy on neural-autonomic systems. **Quality:** 2.25 impact factor, 86 h index **Subjects:** 13 female subjects **Neuroimaging method:** NIRS **Autonomic method:** ECG: HF, HF/LF **Emotion method:** heat and steam generating sheets on the neck (therapeutic intervention) **Outcomes:** Decreased Oxy-Hb concentration in the anterior-dorsal region of the medial prefrontal cortex (adMPFC), while increasing parasympathetic nervous activity and decreasing sympathetic nervous activity.
60. Yoshihara et al (2016) 10.1016/j.neuroimage.2016.04.040	**Study:** Investigating functional brain connectivity related to sympathetic response to fearful stimuli **Quality:** 7.4 impact factor, 381 h index **Subjects:** 32 subjects **Neuroimaging method:** fMRI **Autonomic method:** skin conductance **Emotion method:** Horror films vs control films **Outcomes:** Brain activity correlated with change of rate of fingertip temp. Connectivity analysis revealed greater positive functional connectivity between amygdala and ACC when watching horror film than control film. Whole-brain physiological interaction functional connectivity between left amygdala and ACC was modulated according to fear rating.
61. Zaki et al (2012) https://doi.org/10.1016/j.neuroimage.2012.05.012	**Study:** Examining the neural convergence of interoception and emotional experiences. **Quality:** 7.4 impact factor, 381 h index **Subjects:** 16 participants (11 females) **Neuroimaging method:** fMRI **Autonomic method:** ECG, heartbeat monitoring **Emotion method:** Emotional autobiographical events in video format **Outcomes:** Heartbeat monitoring engaged insular and inferior frontal operculum. This was also engaged when subjects rated emotions and correlated with emotional experience.

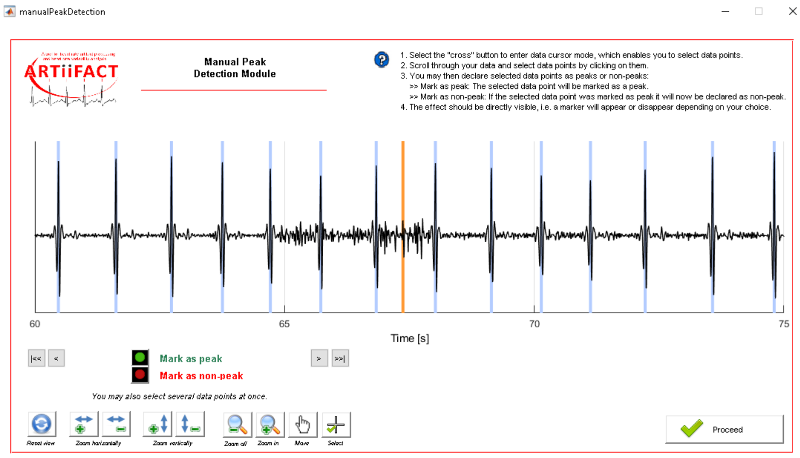
62. Tang et al (2009) 10.1073/ pnas.0904031106	**Study:**Compared rest, during and after 5 days of meditation (body-mind) and relaxation training **Quality:** 12.78 impact factor, 771 h index. **Subjects:** 86 subjects, 46 experiment 1 and 40 experiment 2 **Neuroimaging method:** EEG and SPECT **Autonomic method:** HR, respiratory and skin conductance **Emotion method:** meditation **Outcomes:** Connection between HRV and theta during meditation

Appendix B

Verifying HRV analysis accuracy

To assess the accuracy of the in-house MATLAB script for generating HRV values, I compared the results with values generated from Kubios HRV (version 2.2, University of Eastern Finland, Finland).

The Kubios data was pre-processed using an automatic QRS detection with ARTiiFACT 2.0 (Kaufmann et al., 2011). This software package was recommended by Laborde et al. (2017), as they state R waves can be verified manually after automatic detection, which enables faster and accurate identification of waveform peaks (see Figure B.1). To enhance the automated detection of R peaks in the ECG data, a high pass filter at 10Hz was applied and global threshold was set to 50. Artifacts in the data, such as ectopic beats, were corrected using cubic spline interpolation (Malik & Camm, 1990).



1. Select the "cross" button to enter data cursor mode, which enables you to select data points.
2. Scroll through your data and select data points by clicking on them.
3. You may then declare selected data points as peaks or non-peaks:
   >> Mark as peak: The selected data point will be marked as a peak.
   >> Mark as non-peak: If the selected data point was marked as peak it will now be declared as non-peak.
4. The effect should be directly visible, i.e. a marker will appear or disappear depending on your choice.

You may also select several data points at once.

Figure B.1: Image representing the manual inspection of identified IBI's using ARTiiFact. The orange highlighted section shows manual marking of an incorrectly identified beat. Blue highlighted sections reflect the automatic detection of R waves based on criteria set by the user. After this process, the software calculates the time between each R wave, referred to as inter-beat intervals.

After ensuring this data was adequately cleaned, Kubios HRV was used to calculate nonlinear heart rate variability measures for the three blocks of Su-soku meditation. To derive nonlinear measures, Kubios produced a Poincaré plot for individual participants, which plots IBIs against previous IBIs to assess the correlation between consecutive intervals.

As this study is primarily interested in parasympathetic tone, the relative percentage change in RMSSD between in-house and Kubios data was first assessed.

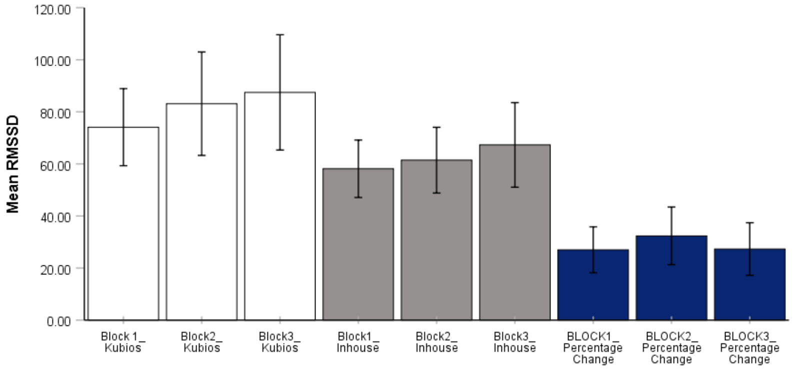


Figure B.2: Bar plot for visually comparing mean RMSSD for Kubios and In-house output.

Figure B.2 suggests that Kubios provides a greater RMSSD outputs than in-house, however it also appears to have greater variance as indicated by the larger error bars. We can also note from this figure there is a similar pattern between Kubios and in-house across meditation blocks, whereby RMSSD appears to increase across meditation blocks. These two observations indicate that both outputs appear to measure the same variable, however the outputs from Kubios may be subject to lower signal-to-noise ratio. For this reason, the definitive analysis used HRV output from the in-house script.

Appendix C

Python script example

```
# Resampling data at 1000
import numpy as np
import os
import pandas as pd, numpy as np
import matplotlib.pyplot as plt

input_directory = "Wavelet_AVG_frequency"
output_directory = "downsampled_Waveletdata"

def resample(df, target_freq, unit_str):
    resample_str = "{:.4g}{}".format(len(df)/target_freq, unit_str)
    return df.resample(resample_str).mean()

for file_name in os.listdir(input_directory):
    data = np.loadtxt(f"{input_directory}/{file_name}")
    df = pd.DataFrame(data)
    df.index = pd.TimedeltaIndex(df.index, unit='S')
```

```
    df_resampled = resample(df, 1000, "S")

np.savetxt(f"{output_directory}/{base_filename}_downsample.txt",
df_resampled, fmt="%5.4f", delimiter=" ")


#Getting radian values for data samples
Samples_degrees = (np.linspace(0,1, 1000))*360
radian_Values = np.radians(Samples_degrees)
shaped_radian = radian_Values.reshape(-1, 1)

import cmath
import os
import pandas as pd

input_directory='/home/natasha/Downloads/Susoku2/downsampled_woodland'
output_directory = '/home/natasha/Downloads/Susoku2/Complex_files'

import math

data = list()

for file_name in os.listdir(input_directory):
    # Get data into correct shape array
    data_breath = np.loadtxt(f"{input_directory}/{file_name}")
    shaped_breath = data_breath.reshape(-1, 1)

    # Get arrays of data as complex numbers in rect and polar form
    cns = np.vectorize(cmath.rect)(shaped_breath, shaped_radian)
```

```

    # Find the average (again, rect and polar form)
    avg_cn = np.mean(cns)
    avg_phase = cmath.phase(avg_cn)
    avg_radius = abs(avg_cn)

    # Circular statistics based on mean resultant length,
    #which is avg_radius. circular and angular std are in radians
    circ_var = 1-avg_radius
    ang_var = 2*(1-avg_radius)
    ang_std = math.sqrt((2*(1-avg_radius)))
    circ_std = math.sqrt((-2*(math.log(avg_radius))))

    # Work out exactly which data we were working with!
    subject = file_name.split('_')[0].upper()
    block = file_name.split('_')[1].upper()
    electrode = file_name.split('_')[2].upper()
    frequency = file_name.split('_')[3].upper()

    # Add raw data for this subject
    data.append({
        "Subject": subject,
        "Frequency": frequency,
        "Circular variance": circ_var,
        "Angular variance": ang_var,
        "Circular std": circ_std,
        "Angular std": ang_std,
        "Average complex number": avg_cn,
        "Avg Phase Angle": avg_phase,
```

```
        "Avg Radius": avg_radius
    })

df = pd.DataFrame(data)
sorted_df = df.sort_values(["Subject","Frequency"]).reset_index(drop=True)

sorted_df.to_csv(f"{output_directory}/woodland_complex.txt")
```

Appendix D

Proposed study

Below outlines a potential future study that could be used to further develop themes explored in this thesis.

Project proposal: Developing a resonance-breathing mobile application for mental health.

In moments of stress, we sometimes become increasingly aware of every breath we take in, and then expel. Just by the act of taking notice of our breathing, we feel a rising sensation of calm. Our minds once clouded by panicking thoughts are lifted, and we feel more able to make accurate and thoughtful decisions. By reflecting on this subjective experience, we realise our own innate potential to "gain back" control over situations. Perhaps if we could consistently tap into this phenomenon, we could find respite from the restlessness of our thoughts and protect our mental health.

Research suggests that our breathing rate can set the tone for our physiological state. As we breathe in, our heart rate tends to accelerate, then decelerate again as we breath out (Angelone & Coulter, 1964). This coupling of heart-rate (HR) change to respiratory cycle is called the respiratory sinus arrhythmia (RSA). The amplitude of the RSA reflects activity in the parasympathetic nervous system, the "rest and digest" branch of the autonomic nervous system that promotes relaxation and repair. Research has shown that increasing RSA amplitude can have restorative effects on autonomic function (Niizeki & Saitoh, 2012; Sakakibara & Hayano, 1996; Sasano et al., 2002; Tonhajzerova, Mestanik, Mestanikova, & Jurko, 2016).

An individual's peak RSA amplitude tends to be greatest when within the respiratory cycle, the slowest heart-rate occurs when the lungs are empty and the fastest when the lungs are full – in other words, when the phase-lag between the RSA and the respiratory cycle is near zero (Berntson, Cacioppo, & Quigley, 1993). This may be because at zero phase lag, there is "resonance" between the respiratory frequency and the frequency of the regulatory feedback loop between blood pressure sensors and autonomic regulation of heart-rate (Buchner, 2019; Saul, Berger, Chen, & Cohen, 1989). Paced-breathing therapies have been developed to help people maximise their RSA amplitude by finding the breathing rate at which this resonance occurs (Park & Jung, 2020). Typically this is about six to ten breaths per minute i.e at between 0.1 – 0.15Hz (Lehrer, Vaschillo, & Vaschillo, 2000; Steffen, Austin, DeBarros, & Brown, 2017).

Respiration also influences neural oscillations in the brain. A study by Hinterberger and colleagues (2019) showed that slow brain oscillations were entrained with the respiratory cycle, with maximum phase-locked amplitude occurring at a breathing rate of 10 breaths per minute. In that study, maximum RSA amplitude was also found at 10 breaths per minute. Our own work shows that at higher frequencies, brain oscillation amplitudes nest within the phase of the respiratory cycle ("phase-amplitude coupling"). We have found this in delta (~2 Hz), theta (~6 Hz) alpha (~12 Hz) and beta brain oscillatory frequencies (15-30 Hz), with the largest amplitudes occurring shortly after the beginning of inspiration. This may reflect phase-amplitude coupling with phase-locked slow oscillations that have become entrained to the breathing cycle.

Brain oscillatory activity affects our cognitive processes, including the way we distribute our attention between the external and internal worlds – between perception and interoception. Finding a breathing rate that hits our "sweet spot" where heart and brain are locked in resonance with each other may be key to unlocking our ability to "gain back control" over our thoughts and actions in everyday life.

In this project you will develop a therapeutic mobile application that will help users learn to achieve this state of calm control at will. At its simplest, it would use input from an optical pulse detection device to provide a visual or audio representation of the RSA amplitude, together with a breath-pacing signal designed to seek the breathing rate that maximises RSA. A more sophisticated system could make use of input from an oximeter to feedback the user's actual breathing rate, andpotentially a dry electrode EEG headset that could help the user learn to entrain their cortical rhythms with the their respiratory-cardiac cycle.

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