IMPERIAL COLLEGE LONDON

DOCTORAL THESIS

Enabling the Quantification of Human Stress from Physiological Responses

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A thesis submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy

in the

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I, Tricia Akua Boakyewaa ADJEI, declare that this thesis, titled "Enabling the Quantification of Human Stress from Physiological Responses", is the result of original research, undertaken by myself, unless otherwise stated.

I have clearly cited all quotes, contributions and publications from others.

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Date: 20th February 2020

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Abstract

Faculty of Engineering

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Doctor of Philosophy

Enabling the Quantification of Human Stress from Physiological Responses

by Tricia Akua Boakyewaa ADJEI

The stress response primes the human body to overcome a challenge or threat, and is governed by the sympathetic (SNS) and parasympathetic nervous systems (PNS). However, there is a great need to objectively identify stress, as overactivation of the SNS is dangerous to health. This PhD aimed to produce a heart rate variability (HRV) based measure of stress, to outperform the state-of-the-art and objectively identify levels of stress. Classification Angle (ClassA), a nonlinear framework based on second-order-difference plots of HRV, was developed to achieve this aim; ClassA produces four metrics, which include measures of SNS and PNS dynamics, and was tested on the HRV of ten males and ten females, exposed to periods of rest and mental (arithmetic) stress. The framework was compared to the state-of-the-art temporal, spectral and nonlinear measures. Only the heart rate of the males produced significant differences (p-value < 0.01) between the HRV from the rest and stress epochs. In contrast, two of the ClassA metrics were able to discern stress in both the males and females, with three-dimensional plots of the ClassA metrics spatially separating the HRV from the rest and stress epochs. The ability of the framework to distinguish between levels of stress was then tested on the cardiac signals of 28 day-surgery patients, recorded before and during surgery. Pain was defined as a stressor, and the ClassA framework produced significant differences between the HRV from the patients who experienced extreme pain, and those who did not. Furthermore, the ClassA framework has proved unique in accounting for sex-differences in the stress response, and in analysing only ten seconds of HRV data, whilst the state-of-the-art require five minutes. The development of the ClassA framework therefore achieves the PhD aim, and is the first step in the creation of a tool to objectively identify stress.

Acknowledgements

It is still surreal that I have come to the end of my PhD studies... I will certainly never forget the many times I sat at my desk, in front of red MATLAB errors, praying silently under my breath "God, why is it not working!?", which was always shortly followed by a quick "Thank you Jesus!" once the computation worked. As the wise King Solomon put it "A person's heart plans their way, but the Lord determines their steps" Proverbs 16.9.

I will also forever remember the many chats I shared with Professor Mandic. I will always be grateful to Professor Mandic for his support and guidance; what I have learnt from him is invaluable.

Lastly, but by no means least, I thank the Engineering and Physical Sciences Research Council (EPSRC) for funding my PhD studies. The EPSRC also funded a ten-week studentship, which I completed between the third and fourth years of my undergraduate studies at the University of Surrey. Had my then personal tutor, Dr. Daniel Abásolo, not offered me the studentship, I may never have discovered my love for research.

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List of Abbreviations

ANS	Autonomic nervous system
ApEn	Approximate entropy
ClassA	Classification angle
ECG	Electrocardiogram
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
LF	Low frequency
nHF	Normalised power of high frequency components of heart rate variability
nLF	Normalised power of low frequency components of heart rate variability
PEP	Pre-ejection period
PermEn	Permutation entropy
PNS	Parasympathetic nervous system
PSHP	Presurgical data from patients who experienced high pain
PSLP	Presurgical data from patients who experienced low pain
P^{Q1}	Cardiac deceleration proportion
$P^{Q2,4}$	Proportion of balanced increases and decreases in heart rate variability
P^{Q3}	Cardiac acceleration proportion
RAS	Real angle sum
SampEn	Sample entropy
SDNN	Standard-deviation-of-the-NN-interval
SHP	Surgical data from patients who experienced high pain
SLP	Surgical data from patients who experienced low pain
SNS	Sympathetic nervous system
SODP	Second-order-difference-plot

Chapter 1

Introduction

1.1 What is Stress?

From heart disease, high blood pressure and stroke, to low immunity and asthma, chronic stress is known to cause a multitude of health conditions (Maunder and Cameron, 2016), all of which inevitably have a negative impact on an individual's well-being, wider society, and the economy.

In a patient information sheet provided by the United Kingdom's (UK) National Health Service, stress was described as a psychological and physiological response elicited to deal with a threat to an individual's person or integrity (Health Psychology Service, 2012). Stress can therefore manifest in all spheres of life; it can reduce quality-of-life, often manifests alongside anxiety, and can lead to depression if prolonged, whereby individuals may no longer be able to adapt to face life's challenges (Bystritsky and Kronemyer, 2014). For reference, anxiety differs from stress as it is defined as a specific fearful reaction to an imminent or uncontrollable event (Bystritsky and Kronemyer, 2014).

Worryingly, the reduction in quality-of-life caused by stress has now been identified in all sections of the population. For example, statistics from the UK show that stress is no longer a preserve of adult-life, with more and more pupils in the classroom experiencing dangerous levels of pressure; there was a 15% increase in the number of exam counselling sessions delivered to teenagers by the UK's child safety welfare service, Childline, from 2016/17 to 2017/18 (National Society for the Prevention of Cruelty to Children, 2018). Similarly, in the workplace, employees are experiencing ever greater levels of stress. Of all cases of work-place illness, the 2017/18 UK Labour Force Survey reported that the prevalence of stress, depression and anxiety cases was 44%, whilst in 2016/17, this value was lower at 40.5% (Labour Force Survey, 2018).

The high personal cost of stress is further reflected in a high financial cost to the economy. It was also reported in the 2017/18 UK Labour Force Survey that out of a total of 26.8 million workdays lost to ill-health, 15.4 million were lost to stress, depression and anxiety (Labour Force Survey, 2018). However, in spite of the staggering impact of stress, combating stress at a societal level is complicated by the fact that stress is a *state of being* as opposed to a sickness with a single cause. The experience of stress is also heavily influenced by its frequency of occurrence- individuals who have experienced prolonged stress are more likely to find daily hassles stressful (Epel et al., 2018). Therefore, responses to stress vary from person to person, where what one person perceives as distressing, another perceives as motivating, and is able to cope well.

Colloquially, the lack of general distinction between stress that can motivate and stress that impairs diminishes the perception of the seriousness of stress. Yet, the distinction between good stress and bad stress is fundamental to the physiological understanding of stress, and its important role in ensuring the survival of humans in threatening situations. It is well known in both psychology and biology that some of the exact same mechanisms which prime humans to perform, and manifest as good stress, are the very same mechanisms that in others, manifest as bad stress and can cause potentially dangerous health problems. For example, it has been found that whilst small concentrations of cortisol, a hormone released in response to stress, can improve memory, larger concentrations can cause the degeneration of brain structures, impairing memory (Blascovich and Mendes, 2010; Epel et al., 2018). The realisation that a degree of stress may be required to perform optimally in certain situations has generated a need to objectively assess stress levels. The development of a stress assessment tool which is both objective and accurate is however, not trivial. The difficulty lies in the subjectivity of the appraisal of stress-inducing situations or tasks (referred to as stressors), where humans naturally vary in the cognition and the assessment of whether a stressor poses demands which exceed available physiological resources (Epel et al., 2018).

The ability to objectivity assess stress levels would enable the introduction of interventions to prevent highly stressed individuals becoming further stressed; however, psychological and biological research has not been able to achieve the identification of good stress from bad stress in a way that is both quantitative and noninvasive. The work presented in this thesis introduces a quantification of stress which can identify a state of excessive stress from cardiac signals, noninvasively.

1.1.1 History of Stress Research

The concept of a human stress response is relatively recent. The link between a physiological response to threats dates back to a book published in 1908, by psychologist William McDougall from the University of Oxford, who speculated why humans would often flee from fearful situations (McDougall, 1908). McDougall's work was later developed and refined by Walter Cannon, a physiologist from Harvard University; Cannon is credited with defining the bodily responses to threats as physiological adaptations to prepare the body for either "fighting or flight" (Cannon (1915), p. 211). Some twenty years later, in his seminal letter to Nature, biologist and the father of modern stress research, Hans Selye, referred to the initial physiological response to threats as an alarm reaction, representing an organism's efforts to adapt to new conditions (Selye, 1936).

By 1955, Selye had taken the word *stress* from the physical sciences to describe the many manifestations of the reactions to disease, and had produced a comprehensive paper discussing the link between stress and disease (Selye, 1955). It was highlighted in Selye (1955) that given the etymology of the word disease, dis-ease essentially encapsulated what it meant to be in a stressed state. Selye consequently inferred that the scientific community at large, had at that point, neglected to thoroughly research stress due to the fact that it did not have one specific cause (Selye, 1955).

1.1.2 Physiology of the Stress Response

Despite much of the early research into the stress response having an endocrinological bias, focussing on the action of hormones, Selye (1955) became interested in the role of the nervous system in the stress response; the nervous system is now at the centre of stress research.

The link between emotions and the nervous system have become well established, as emotional reactions are known to be coordinated by a network of brain structures, collectively called the limbic system (Faller et al., 2004). A structure of the limbic system, the hypothalamus, coordinates the autonomic nervous system (ANS) which is responsible for involuntary actions, such as metabolism (Faller et al., 2004). The ANS is comprised of the sympathetic and parasympathetic nervous systems, as well as the enteric nervous system (Faller et al., 2004). The enteric nervous system is responsible for the action of the gastrointestinal tract (Faller et al., 2004), whilst the sympathetic nervous system (SNS) is associated with the mobilisation of energy resources to respond to emergencies, and the parasympathetic nervous system (PNS) is associated with the conservation of energy resources in the body (Strominger et al., 2012); an outline of the physiology of the response of the autonomic

nervous system to stress is shown in Figure 1.1 (Faller et al., 2004; Norris, 2006; Strominger et al., 2012).

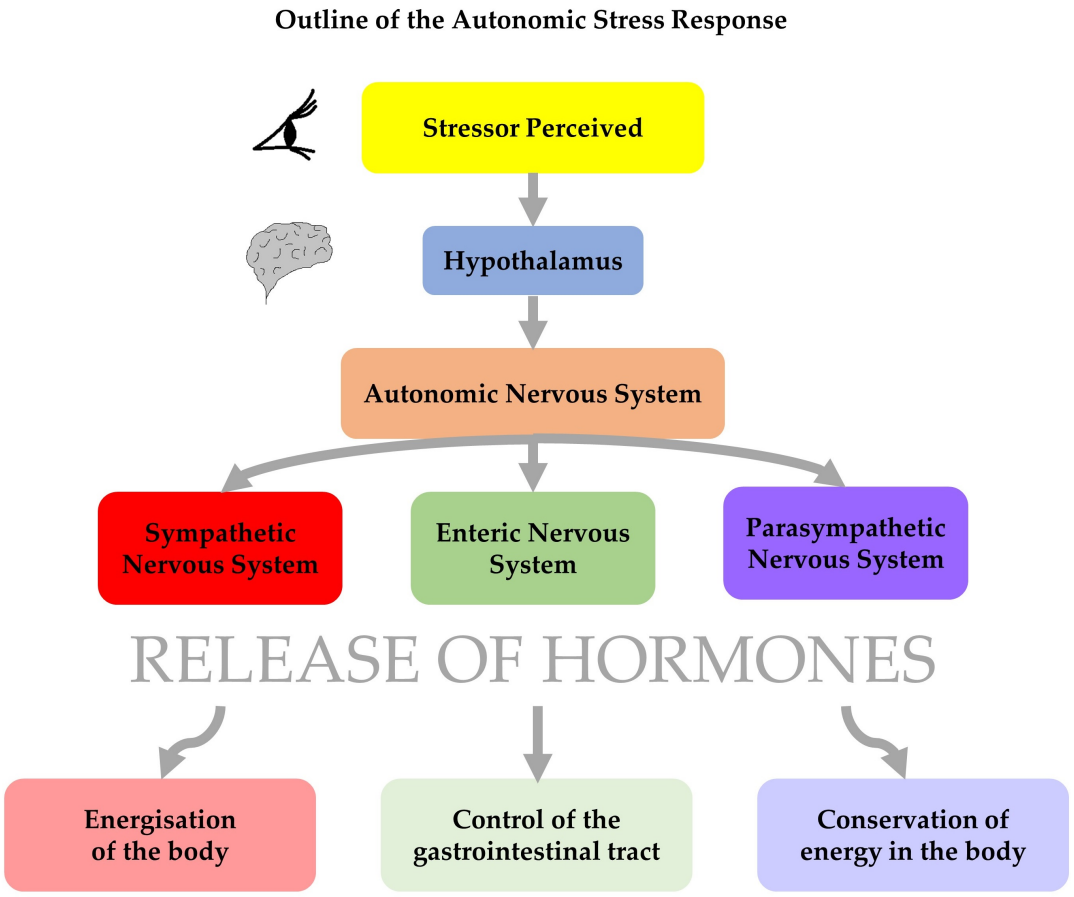


FIGURE 1.1: Outline of the physiology of the stress response (Faller et al., 2004; Norris, 2006; Strominger et al., 2012).

The PNS dominates during instances of calm, for example, through maintaining low heart rates and respiratory rates (Faller et al., 2004). Conversely, the SNS is accepted to be activated during instances of stress, in order to either 'fight' or 'take flight' in response to a stressor (Cannon, 1915; Faller et al., 2004). Muscles will often have greater energy and oxygen requirements during instances of fight-or-flight, so amongst other actions, the SNS acts to increase both the heart rate and the metabolism of sugar (Faller et al., 2004). However, despite the complementary roles of the SNS and PNS, several modes of SNS and PNS activation were identified and summarised in Berntson et al. (1991), p. 461; a recreation of the summary can be found in Table 1.1. It was reported in Berntson et al. (1991) that although coupled reciprocal activity between the SNS and PNS could be expected during the activation of the stress response, so as to maximise the dynamic range of target organs, SNS and PNS activity could be nonreciprocal. Depending on the context, nonreciprocal modes, such as coactivation

or coinhibition, may be exhibited; for example fear is known to induce SNS activation to increase heart rate and blood pressure, whilst simultaneously inducing PNS activation to empty the bowels, or bladder (Cannon, 1939; Berntson et al., 1991).

TABLE 1.1: Modes of autonomic control, recreated from Berntson et al. (1991), p. 461.

Autonomic Modes			
Autonomic Response	**PNS Increase**	**PNS No Change**	**PNS Decrease**
SNS Increase	Coactivation	Uncoupled SNS activation	Reciprocal SNS activation
SNS No Change	Uncoupled PNS activation	Baseline	Uncoupled PNS withdrawal
SNS Decrease	Reciprocal PNS activation	Uncoupled SNS withdrawal	Coinhibition

The SNS and PNS transmit signals to relevant organs using hormonal messengers such as epinephrine (formally adrenaline) and norepinephrine (formally noradrenaline) (Faller et al., 2004); due to their chemical structure, both epinephrine and norepinephrine are classified as catecholamine hormones (Strominger et al., 2012). A three-stage stress response was outlined in Selye (1955), based on the action of such stress hormones, and their effects on the SNS and PNS.

Although the specifics of the stress response outlined in Selye (1955) are not biologically investigated in this PhD research, Selye's stress response can aid in the understanding of how and why high levels of stress can become dangerous. Selye's outline of the stress response begins with the **(i)** initial alarm reaction, and is followed by **(ii)** the resistance stage and ends with the **(iii)** the exhaustion stage (Selye, 1955).

The initial alarm reaction can be characterised by an initial release of catecholamines, to control the SNS and PNS, and the release of another class of hormones called glucocorticoids (Selye, 1955; Norris, 2006). Glucocorticoids can regulate metabolism and inhibit the immune system (Faller et al., 2004). The most widely researched glucocorticoid is cortisol, which regulates the conversion of proteins into glucose, and decreases the number of immune system cells in circulation in the body, thus ensuring an efficient use of the body's resources during instances of stress (Faller et al., 2004). The resistance stage is characterised by the prolonged release and action of glucocorticoids in response to a persistent stressor (Selye, 1955; Norris, 2006); glucocorticoids are associated with the ability to

withstand extreme stress, such as starvation (Faller et al., 2004). Lastly, the exhaustion stage is characterised by a prolonged reaction to a persistent stressor, which can lead to death (Norris, 2006).

The Selye (1955) stress response is summarised in Figure 1.2, with endocrinological explanations from Faller et al. (2004), Norris (2006), and Strominger et al. (2012). However, despite the wide acceptance of Selye's stress response, an alternative theory describing the long-term effects of stress has been proposed in Dienstbier (1989).

In Dienstbier (1989), it was posited that not all forms of stress would have the potential to cause physiological damage, but instead frequent exposure to intermittent stressors would lead to an improved ability to cope when faced with new stressors. Following a review of the physiology of coping, Dienstbier identified positive correlations between catecholamine levels and cognitive/ physical performance (Blascovich and Mendes, 2010); he argued that intermittent stressors initiated a process which resulted in an SNS arousal which was low at basal levels, but strong and responsive when a stressor was encountered (Dienstbier, 1989).

Although it was argued in Dienstbier (1989) that researchers had overlooked the natural process of acquiring the ability to better cope, due to the inherent difficulty in assessing the effects of intermittent stress in laboratory settings, the work presented in this thesis will include an assessment of the relationship between basal autonomic activity and the intensity of stress-induced SNS activation; is low basal SNS and PNS activity seen alongside strong SNS activation?

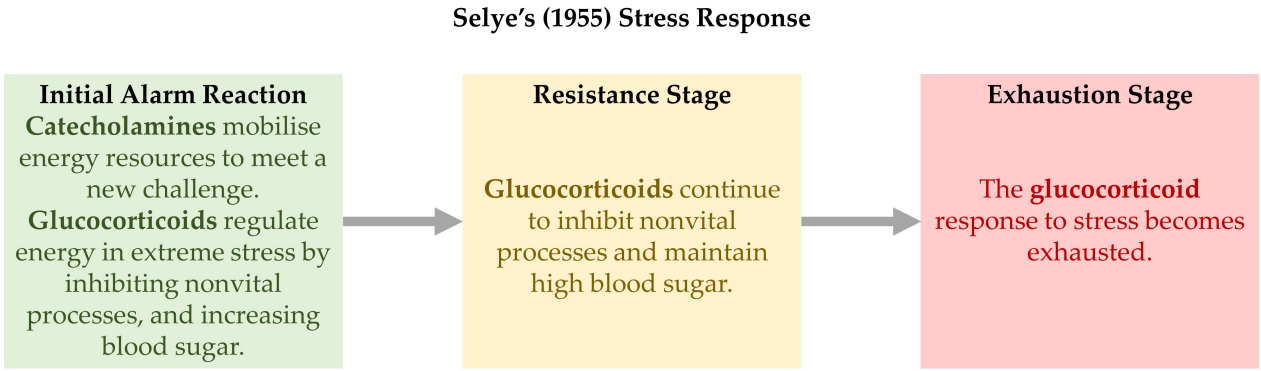


FIGURE 1.2: Summary of Selye's (1955) stress response, with endocrinological explanations from Faller et al. (2004), Norris (2006), and Strominger et al. (2012).

1.2 Assessment of Stress

Stress can be assessed both quantitatively and qualitatively.

Psychological assessments of stress are largely qualitative, and can take the form of observations or self-report questionnaires (Figueroa-Fankhanel, 2014). Such assessments are therefore subjective assessments, from either the perspective of a trained professional, or the subject themselves (Figueroa-Fankhanel, 2014). The majority of questionnaires used to qualitatively assess stress are standardised and verified in literature. For example, the scores from one of the most widely deployed anxiety questionnaires, the State-Trait Anxiety Inventory for Adults, were verified over 1838 adults (Spielberger, 2015). Thus, standardised questionnaires are an attractive and low cost means to assess stress, with proven validity and reliability (Figueroa-Fankhanel, 2014) (it is not advised to use custom, self-devised questionnaires which have not been validated). However, the subjectivity of questionnaires limits their use, particularly in culturally diverse study cohorts, as the understanding of stress can be based on culture (Figueroa-Fankhanel, 2014). Furthermore, not only may the responses to questionnaires not correlate with physiological responses (Epel et al., 2018), but, qualitative measures of stress often only offer a time-discrete snapshot of an individual's stress levels (Shi et al., 2007).

In contrast, quantitative measures of stress have been made popular by the time-continuous and objective assessments they offer (Shi et al., 2007). The quantitative assessments are typically derived from measurable physiological data, often assessing SNS driven responses, such as catecholamine and glucocorticoid levels, galvanic skin response and body temperature, cardiac dynamics, respiratory rate or blood pressure (Figueroa-Fankhanel, 2014; KrKovic et al., 2018).

Assessments of cardiac dynamics are amongst the most common quantitative assessments of stress, as increases in heart rate can be a noninvasive measure of SNS activation. Heart rate is commonly measured using electrocardiography (recordings of the electrical activity of the heart) or other measures of pulse, such as photoplethysmography (Figueroa-Fankhanel, 2014). Given the wide range of factors affecting cardiac dynamics, the main limitation of cardiac assessments of stress is the difficulty in the derivation of physiologically meaningful results.

Figure 1.3, adapted from Faller et al. (2004, p. 224) depicts a typical healthy electrocardiogram (ECG) signal in which the distinctive peaks within the trace have been named with the conventional PQRST annotations; a heart beat is defined as a the contraction of the left ventricle of the heart, which appears as an R peak in an ECG trace, where the time interval between two successive R peaks is termed an

RR interval (Faller et al., 2004; Sörnmo and Laguna, 2005). A series of RR intervals, or a series of intervals between two successive P, Q, S, or T waves, are termed heart rate variability (HRV) signals (Sörnmo and Laguna, 2005). Heart rate variability can be used to assess SNS and PNS dynamics (Figueroa-Fankhanel, 2014). An in-depth description of HRV based analyses of stress can be found in Chapter 2.

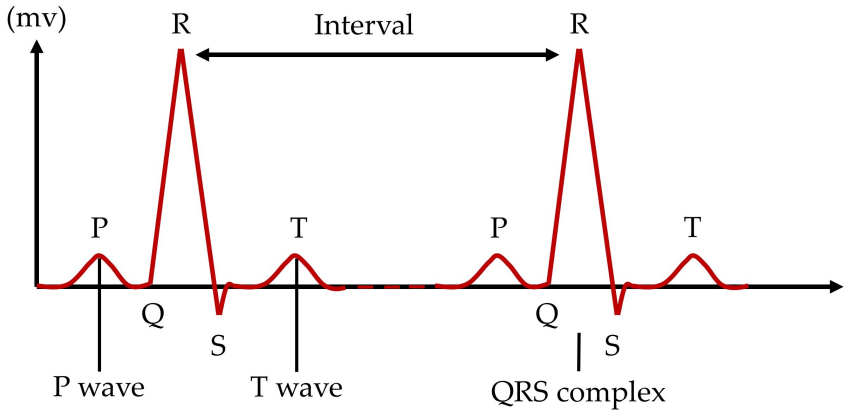


FIGURE 1.3: Illustration of a healthy electrocardiogram signal, with PQRST annotations, adapted from Faller et al. (2004, p. 224).

In addition to cardiac dynamics, activation of the sympathetic nervous system is also reflected in increases in respiratory (breathing) rate and can be measured through the assessment of chest expansions, using either observations of chest rises, respiratory-inductive-plethysmography chest bands, strain gauges, or through measurements of nasal/oral airflow (Figueroa-Fankhanel, 2014). Although the assessments of chest expansions can be low cost, they are often impractical in real-world ambulatory settings as they are inevitably sensitive to movement artefact. The measurement of airflow is also impeded by the often impractical use of masks which must cover the nose and mouth.

Blood pressure is another quantity which can be used to indicate stress levels. Short-term stress raises blood pressure, to increase the flow of blood to muscles, whilst chronic stress leads to a prolonged elevation of blood pressure, which contributes considerably to the prevalence of cardiovascular diseases (Figueroa-Fankhanel, 2014; Munakata, 2018). However, although the relationship between stress and blood pressure is well-researched, the exact physiological mechanisms which govern the relationship are not fully understood (Munakata, 2018), with both SNS overactivity and PNS underactivity being linked to chronic high blood pressure (Mancia and Grassi, 2014). Moreover, gold standard blood pressure is measured invasively and directly from blood vessels, and the assessment of stress from

blood pressure in real-world scenarios is limited by the difficulty in noninvasively acquiring continuous measurements. For these reasons, other cardiac measures are employed more often than blood pressure in the assessment of stress.

Another quantitative measure of stress is the measurement of hormone levels, and in particular, the measurement of catecholamines, such as epinephrine. Catecholamines are released immediately upon the detection of a stressor (Romero and Butler, 2007), whilst the blood concentration of the glucocorticoid cortisol peaks approximately 30 minutes post-stressor (Lundberg, 2005). Blood assays of 660 heart disease patients have shown that epinephrine levels rise approximately 75% just five minutes after exposure to a mental stressor (Hammadah et al., 2017). However, although blood epinephrine levels have also been found to positively correlate with heart rate and blood pressure in response to mental stress (Hammadah et al., 2017), the assessment of blood catecholamine levels is complicated by the requirement to complete blood assays.

Arguably, the most accurate measures of stress are measures of sympathetic and parasympathetic nerve activity. These can be measured directly via the insertion of microelectrodes into nerves, or indirectly, through the measurement of cardiac dynamics following the administration of pharmaceutical nerve blockades (Victor et al., 1987; Montano et al., 1994). In microneurography, microelectrodes with tips as narrow as 1 μm, are inserted into nerves to record nerve traffic (Victor et al., 1987), however, the invasiveness of the use of such microelectrodes undoubtedly limits the range of experiments which can be undertaken using microneurography.

Less invasively, pharmaceutical nerve blockades have been widely used to assess cardiac dynamics; it was found in Victor et al. (1987) that the blocking of sympathetic nerve receptors, through the administration of pharmaceutical drugs, eliminated an expected stress-induced rise in heart rate. Such a finding enabled a confirmation that an increase in heart rate would have been caused by SNS activity (Victor et al., 1987).

A summary of the above described common measures of stress is shown in Table 1.2 (Victor et al., 1987; Montano et al., 1994; Shi et al., 2007; Romero and Butler, 2007; Mancia and Grassi, 2014; Figueroa-Fankhanel, 2014; Hammadah et al., 2017; Epel et al., 2018; Munakata, 2018).

TABLE 1.2: Summary of common measures of stress (Victor et al., 1987; Montano et al., 1994; Shi et al., 2007; Romero and Butler, 2007; Mancia and Grassi, 2014; Figueroa-Fankhanel, 2014; Hammadah et al., 2017; Epel et al., 2018; Munakata, 2018).

Common Measures of Stress		
Measure	**Advantages**	**Disadvantages**
Questionnaires	These qualitative measures offer a low-cost means to assess stress, where all standard questionnaires are verified in literature, and offer standardised interpretations.	Questionnaires are inherently subjective, cannot be used to continuously measure stress, and offer no means of assessing the physiology of the stress response.
Heart Rate	This simple quantitative measure is assumed to provide a noninvasive assessment of sympathetic nerve activity, where sympathetic activation leads to an increase in heart rate.	Stress is one of many causes of heart rate increases, meaning heart rate is not an accurate or direct means of assessing stress-induced sympathetic nerve activity.
Heart Rate Variability	Temporal, spectral and nonlinear algorithmic characterisations of heart rate variability signals provide a quantitative, noninvasive and continuous measure of stress.	Conventional analyses of heart rate variability signals are not always accurate in indicating the separate activities of the sympathetic and parasympathetic nervous systems.
Respiratory Rate	This quantitative measure provides an indirect and noninvasive assessment of sympathetic nerve activity, where sympathetic activation leads to an increase in respiratory rate.	The acquisition of accurate respiratory rate is notoriously difficult, as measures of chest expansions have a high sensitivity to movement artefact, whilst the measurement of oral or nasal airflow requires the use of impractical facial apparatus.
Blood Pressure	This quantitative measure provides an indirect and noninvasive assessment of sympathetic nerve activity, where sympathetic activation leads to an increase in blood pressure.	The use of blood pressure to assess stress is complicated by the poorly-understood physiology of blood pressure control, and the difficulty in obtaining continuous and accurate measures of blood pressure.
Blood-Hormone Concentrations	This quantitative measure provides a direct and accurate assessment of the release of stress hormones.	Assays to determine the concentration of stress hormones in blood cannot be used to assess stress continuously. Also, not all stress hormones are released immediately upon the detection of a stressor.
Nerve Activity using Nerve Blockades	The quantitative assessment of nerve activity is achieved through the assessment of autonomically driven responses, such as heart rate, following the administration of a pharmaceutical sympathetic or parasympathetic nerve blockade.	The use of nerve blockades to indirectly measure sympathetic and parasympathetic nerve activity is often not practical in the assessment of stress in real-world ambulatory scenarios.

Continued on next page

TABLE 1.2: (continued)

Measure	Advantages	Disadvantages
Direct Nerve Activity	Nerve activity is used to provide the gold-standard in the quantitative measurement of the stress response. Activation of the stress response can be assessed directly through invasive measurements of sympathetic and parasympathetic nerve activity, using needle electrodes.	Direct measures of nerve activity require the invasive and uncomfortable insertion of microelectrodes into the sympathetic and parasympathetic nerves.

In brief, it can be seen that whilst many of the quantitative measures of stress assess responses driven by the sympathetic nervous system, many of the measures are not suited to continuous recordings in ambulatory real-world scenarios. Heart rate variability is an exception, as it has the potential to continuously and accurately measure sympathetic and parasympathetic activity, using wearable sensors in real-world scenarios.

A time series of heart rate variability can be analysed with basic mathematical operations and statistics, or advanced signal processing techniques, such as temporal, spectral, and nonlinear algorithms. However, an accurate and statistically verified signature of stress, which can indicate the presence and severity of stress is yet to be derived from heart rate variability.

1.2.1 PhD Focus

The continuous nature of a time series of HRV, the practical ease at which a time series of HRV can be acquired, and the potential to determine both sympathetic and parasympathetic activity from a single HRV signal demonstrates the advantages of HRV in stress analysis. The research described in this thesis will therefore focus on the derivation of signatures from HRV signals, which *are* able to accurately indicate both the presence and severity of stress.

1.3 Aims, Objectives and Hypothesis

The aim of this research is to produce a quantitative measure of stress, derived from heart rate variability signals, which is able to outperform the state-of-the-art and distinguish between moderate levels of stress and distress.

The objectives of this PhD are as follows;

1. To assess the accuracy of the state-of-the-art measures of stress from heart rate variability signals.
2. To propose a novel algorithmic solution which accurately identifies stress from heart rate variability signals, and statistically show that the performance of the proposed method surpasses those of the state-of-the-art measures.
3. To verify the performance of the proposed algorithmic solution in the discernment of moderate levels of stress from distress.

It is hypothesised that signatures within basal HRV will indicate whether an individual will experience excessive or moderate levels of stress once exposed to a stressor.

For clarity, the performances of the measures of stress presented in this thesis will be quantified using statistical tests. The tests assess the ability of stress measures to separate different groups of data (such as data recorded during instances of stress, and data recorded in the absence of stress), by testing a so-called null hypothesis, which proposes that the groups of data are not significantly different to one another (Campbell and Swinscow, 2009). The probability that a null hypothesis has been incorrectly rejected is termed the *p*-value (Campbell and Swinscow, 2009). A *p*-value is defined as statistically significant if it is less than a specified significance level, usually set at 0.05 or 0.01, whereby a statistically significant *p*-value indicates a statistically significant difference between the tested groups of data (Campbell and Swinscow, 2009).

1.4 Outline of Thesis

An in-depth review of the literature on current stress analyses from HRV signals is presented in Chapter 2, Review of Literature, in which the need for an accurate HRV measure of stress is justified. The review of relevant literature is followed by Chapters 3 to 5, in which the analyses surrounding the development of an HRV based measure of stress are detailed and explained. Chapters 3 to 5 respectively address each research objective listed in Section 1.3. Chapter 6 concludes this thesis, and includes a discussion on the results, limitations, contributions and implications of the work presented here.

The key findings of this thesis are displayed in Figure 1.4.

1.5 Structure and Key Findings of Thesis

1.5.1 Publications

The research described in this thesis has resulted in several journal publications; each publication and its relevance to the chapters of this thesis is described below.

The study described in Chapter 3 was detailed in *Adjei, T, J Xue, and D P Mandic (Nov. 2018). "The Female Heart: Sex Differences in the Dynamics of ECG in Response to Stress". In: Frontiers in Physiology 9.1616, pp. 1–8. DOI: 10.3389/fphys.2018. 01616. URL: https://www.frontiersin.org/articles/10.3389/fphys.2018.01616/full*. The results from the study showed that sex differences existed in the characterisations of HRV signals to identify stress. Adjei et al. (2018) showed that stress could lead to an increase in the long-term univariate entropy of HRV signals in females.

The study described in Chapter 4 was detailed in *Adjei, T, W v Rosenberg, T Nakamura, T Chanwimalueang and D P Mandic (Apr. 2019). "The ClassA Framework: HRV Based Assessment of SNS and PNS Dynamics Without LF-HF Controversies". In: Frontiers in Physiology 10.505, eCollection 2019. DOI: 10.3389/fphys.2019.00505. URL: https://www.frontiersin.org/articles/10.3389/fphys. 2019.00505/full*. This article introduced the three-dimensional HRV-based measure of stress, called the ClassA Framework, presented in this thesis.

The dataset of HRV signals analysed in Chapter 5 was previously presented in *Powezka, K, T Adjei, W von Rosenberg, P Normahani, V Goverdovsky, N J Standfield, D P Mandic and U Jaffer (May 2019). "A pilot study of preoperative heart rate variability predicting pain during local anesthetic varicose vein surgery". In: Journal of Vascular Surgery: Venous and Lymphatic Disorders 7.3, pp.382-386. DOI: 10.1016/j.jvsv.2018.08.008. URL: https://www.sciencedirect.com/science/article/abs/pii/S2213333X18304050*. The pilot study described in Powezka et al. (2019) demonstrated that the stress-induced trends in the HRV from premenopausal females conflicted with the trends in HRV from males and postmenopausal females, and therefore showed that the HRV from premenopausal females should be analysed separately.

Enabling the Quantification of Human Stress from Physiological Responses

CHAPTER	CONTENTS	KEY FINDINGS
1| Introduction	Background to stress research	Generally accepted that the stress response is driven by the SNS (Selye, 1955).
2| Review of Literature	Review of temporal, spectral and nonlinear HRV analyses	**State-of-the-art HRV based measures:** Heart Rate, Standard-deviation-of-the-NN-interval, Low Frequency Power, High Frequency Power, Sample Entropy and Permutation Entropy.
3| Original Research: Performance of Traditional Stress Measures and Sex Differences	Evaluation of the statistical significance of stress measures in discerning stress from rest	▪ Only the heart rate of the males was able to produce a statistical significance (p-value < 0.01) in the discernment of stress from rest. ▪ First ever report of a stress-induced rise in univariate HRV entropy.
4| Original Research: The Classification Angle Framework	Nonlinear ClassA framework which produces four metrics: $\underline{PQ^1}$- PNS dominance $\underline{PQ^{2,4}}$- Balance between increases and decreases in HRV sequences $\underline{PQ^3}$- SNS dominance $\underline{RAS}$- Trend in HRV	▪ Three ClassA metrics produced a statistical significance (p-value < 0.01) in the discernment of stress from rest in the males. ▪ Two ClassA metrics produced a statistical significance (p-value < 0.01) in the discernment of stress from rest in the females. ▪ Three-dimensional plots of $\underline{PQ^{2,4}}$ vs $\underline{PQ^3}$ vs $\underline{PQ^1}$ spatially separated HRV from stress and rest epochs. ▪ $\underline{RAS}$ produced a statistical significance (p-value < 0.01) in the discernment of stress from rest in the males and females.
5| Original Research: The Characterisation of Surgical Pain using the Classification Angle Framework	Evaluation of the statistical significance of the ClassA metrics in discerning between HRV from patients with low and high sensitivities to pain, using data from the presurgical and surgical periods.	A high sensitivity to pain in the Group 1 patients was characterised by: ▪ Low parasympathetic tone. ▪ High sympathetic tone. ▪ Decreasing HRV. A high sensitivity to pain in the Group 2 patients was characterised by: ▪ High parasympathetic tone. ▪ Low sympathetic tone. ▪ Increasing HRV. The HRV signatures of high and low sensitivities to pain differ according to age and sex.
Chapter 6| Project Conclusion		

FIGURE 1.4: Structure and key findings of this PhD thesis.

Chapter 2

Review of Literature

2.1 Temporal and Spectral Stress Analyses of Heart Rate Variability

In 1996, the Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology released guidelines on the use and interpretation of HRV signals in clinical investigations (Malik et al., 1996). The Task Force cited a measure called the standard-deviation-of-the-NN-interval as the simplest temporal HRV measure, however the simplest method is arguably heart rate (*HR*) itself. The division of 60 seconds by an HRV value produces a corresponding *HR* value in beats per minute, and a sustained rise in *HR* can be interpreted as being induced by stress. Yet, in spite of this, as *HR* is regulated by the actions of both the SNS and PNS, it cannot be used to determine the separate dynamics of the sympathetic and parasympathetic nervous systems. The simplicity of *HR* is also its downfall.

As mentioned, the standard-deviation-of-the-NN-interval (*SDNN*) is another simple temporal method used to assess HRV, where the NN interval is a term referring to the intervals between QRS complexes in a healthy ECG signal (Malik et al., 1996). The method evaluates the standard deviation of HRV, and decreases in *SDNN* can be interpreted as stress-induced (Malik et al., 1996). The Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology described the *SDNN* as representing "all cyclic components responsible for variability in the recorded period" (Malik et al.,1996, p. 355), meaning its performance as a measure of stress is sensitive to the length of the signal analysed; the Task Force advised that the lengths of signals analysed in a single study should therefore be standardised. It was also stated that ideally 24 hours of HRV should be analysed to fully capture circadian low frequency and high frequency variations, but that for practical reasons many studies had opted to use a minimum of five-minutes

of HRV (Malik et al., 1996). To better capture the long-range circadian rhythms of HRV, the Task Force endorsed temporal analyses over spectral analyses, and at least 18 hours of HRV data was recommended to best capture the long range dynamics in *SDNN* analyses (Malik et al., 1996). However, in addition to the data-length dependence of *SDNN*, the method is limited by the fact that, similar to *HR*, alone, it cannot indicate the separate activities of the SNS and PNS.

In contrast, spectral, or frequency-based, analysis has been widely used to assess the separate activities of the SNS and PNS. One of the first studies to quantify the responses of the SNS and PNS using spectral analysis was a study detailed in Montano et al. (1994), in which 22 study participants were subjected to physical disturbance in the form of an orthostatic tilt. The ECG signals of healthy subjects were recorded whilst they were secured on an electrical tilt table in the supine position, and tilted upright at 15°, 30°, 45°, 60° and 90° (Montano et al., 1994). The results from the spectral analyses of the HRV series derived from the recorded ECG signals suggested that not only was the frequency of respiration coupled to the power of the higher frequency components of the HRV signals, but that when normalised by the total power of the signal, the power of the lower frequency and higher frequency components produced strong correlations of 0.78 and -0.72, respectively, with the degree of tilt (Montano et al., 1994). Noticeably, the absolute power of the low frequency components did not produce a strong correlation with the degree of tilt, whilst the absolute power of the high frequency components did, with a correlation coefficient of -0.41 (Montano et al., 1994). As tilting was known to induce changes in the balance between the SNS and PNS, the findings from the study were used as empirical evidence to show that spectral analysis could be a proficient non-invasive method to explore the sympathetic and parasympathetic modulations of the heart (Montano et al., 1994). The results suggested that upright tilt, which was known to induce sympathetic activation, caused an increase in the power of the lower frequency components of HRV, and a decrease in the power of the higher frequency components (Montano et al., 1994).

It is now also often cited that the low and high frequency bands of HRV correspond to the speeds of sympathetic and parasympathetic nerves respectively, as sympathetic nerves are known to elicit a response in ≥ 5 seconds, whilst parasympathetic nerves elicit a response in ≤ 1 second (Nunan et al., 2010). Therefore, in the majority of literature, the low frequency (LF) components of HRV have been widely accepted to represent the activity of the sympathetic nervous system, and have been defined to be the spectral components between 0.04 to 0.15 Hz (Burr, 2007). Conversely, the high frequency (HF) components of HRV have been widely accepted to represent the activity of the parasympathetic

nervous system, and have been defined to be the spectral components between 0.15 to 0.4 Hz (Burr, 2007). From here, the powers within the low frequency and high frequency bands of HRV will be simply referred to as 'LF power' and 'HF power' respectively.

However, the transferability of the results presented in Montano et al. (1994) to assessments of psychological stress is limited by a debate surrounding how similar the physiological responses to physical stressors or disturbances, such as tilt, are to psychological stressors (Houtveen et al., 2002).

Three years after the publication of the paper from Montano et al. (1994), researchers from the same group published another stress study (Pagani et al., 1997). In Pagani et al. (1997), sympathetic nerve microneurography, electrocardiography, and respiratory measurements were used to analyse the physiological responses of one female and seven male participants during sympathetic activation and inhibition; sympathetic activation and inhibition were induced pharmaceutically. It was found that the power of the low frequency components of the heart rate variability signals increased as the amplitudes of sympathetic nerve activity increased (Pagani et al., 1997). In contrast, during sympathetic inhibition, the authors found a decrease in LF power and notably, an increase in HF power (Pagani et al., 1997). The authors likened the inducement of sympathetic inhibition to parasympathetic activation, which suggests they employed an assumption of reciprocity between the sympathetic and parasympathetic activities (Pagani et al., 1997). It was also suggested that the simultaneous increase and decrease in HF power and LF power respectively, were demonstrative of an intimacy between the interaction of the sympathetic and parasympathetic nervous systems' oscillatory structures (Pagani et al., 1997). However, it is known that sympathetic and parasympathetic nerve activities are not always coupled nor reciprocal (Berntson et al., 1991).

Furthermore, the studies of both Montano et al. (1994) and Pagani et al. (1997) did not account for sex-differences in the stress response. Sex-differences in the stress response are now of importance as it has been theorised that the female response to stress can be different to the male response (Taylor et al., 2000). The exact sex ratio of the study population in Montano et al. (1994) was not reported, and it was only remarked that there were "volunteers of either sex" (p. 1826), which means it cannot be seen if the ratio of male to female participants affected the conclusions drawn from the study. Similarly, the study cohort in Pagani et al. (1997) only included one female, which again means that any possible sex differences between the male and female SNS and PNS physiologies could not have been revealed.

The growing speculations regarding sex differences in the physiology of the stress response have been fuelled by the realisation that males have long had a higher prevalence of cardiovascular disease, compared to females (Ahs et al., 2009).

Ramaekers et al. (1998) is study in which sex-differences in the spectral components of HRV were investigated; the authors assessed the temporal and spectral characteristics of a total of 276 HRV signals (135 from females), recorded from subjects aged 18 to 71. It was hypothesised that the females would exhibit greater power in the HF band of HRV, due to greater parasympathetic dominance (Ramaekers et al., 1998). Employing a statistical significance level of 0.05, statistically significant differences between the HF powers computed from the males and females were not found; instead, significantly higher power in the LF band was found amongst the males (p-value=0.002) (Ramaekers et al., 1998). It was speculated that this finding indicated that cardiac dynamics in males were modulated more greatly by the SNS than in females (Ramaekers et al., 1998). Importantly, this difference in LF power was only present between the females aged below 40 years and the males. It was explained in Ramaekers et al. (1998) that this age dependence suggested that the lower rates of cardiovascular diseases in females were due to the effects of feminising hormones, called oestrogens, the levels of which decrease after menopause. Oestrogens were described as 'cardioprotective' (Ramaekers et al., 1998).

The age-dependence of the sex differences in the stress response, reported in Ramaekers et al. (1998), can now be explained using conclusions presented in Kajantie and Phillips (2006), in which the benefits of the cardioprotection provided by oestrogens were reviewed. It was concluded that high concentrations of oestrogens could alter the stress response, such that a growing foetus would be protected from exposure to the harmful effects of stress hormones. Thus, the natural fluctuations in the levels of oestrogens throughout the menstrual cycle would inevitably mean that any cardioprotective effects of oestrogens would largely benefit premenopausal females, as opposed to postmenopausal females (Kajantie and Phillips, 2006).

In addition to the findings of sex differences in the stress response, there are further challenges to the understanding of the spectral characterisation of stress.

Burr composed a review of the physiological interpretation of normalised LF and HF powers (Burr, 2007). The normalisation method which was reviewed was of the form where the absolute LF and HF powers were each divided by the summation of the LF and HF powers (Burr, 2007). It was

therefore found that mathematically, such a normalisation would lead to a redundancy where both the normalised LF and normalised HF powers would contain the same information (Burr, 2007). Despite the influence of Burr (2007), it cannot be overlooked that the normalisation method focussed upon made no mention of the total signal power, and was therefore not the same as the method used during the formulation of the theory connecting the LF and HF powers to the SNS and PNS, such as that in Montano et al. (1994). This inherently undermines the applicability of the conclusions drawn upon in Burr (2007), but also demonstrates that caution must be heeded when deciding upon a normalisation method to use in the analysis of the LF and HF powers of HRV.

Furthermore, some years following the critique in Burr (2007), the physiological interpretations of the LF and HF powers were questioned in Billman (2013). Billman reviewed and critiqued the common usage of the ratio of LF power to HF power to represent autonomic dynamics and cast serious aspersions over the physiological meaning of the spectral characterisations of HRV. The speculations reported in Billman (2013) contradicted the previously undisputed concepts that LF power could indicate the level of sympathetic nerve activity (Pagani et al., 1997). Billman also highlighted that the sympathetic and parasympathetic nervous systems could have a nonreciprocal relationship. It was further pointed out that not only was a considerable proportion of the variability within the LF band of unknown origin, but that prevailing heart rate and the mechanics of respiration could affect the LF and HF powers, such that they could not be physiologically defined with any great confidence (Billman, 2013).

The similarity between the spectral manifestations of physical and mental stressors have also been questioned, and in a bid to address this query, a study was conducted, Houtveen et al. (2002), in which 11 males and 11 females were asked to complete a task to elicit mental stress, before entering a relaxed state, and then completing mild exercise. Cardiac parameters were recorded throughout the experiment, and it was found that exercise produced larger decreases in both the LF and HF powers of HRV than mental stress (Houtveen et al., 2002). It was speculated that this difference in the physiological response to mental and exercise stress could have been caused by variations in respiratory frequencies (Houtveen et al., 2002). Furthermore, the authors opted to use the unconventional frequency bands of 0.0625- 0.125 Hz and 0.125- 0.5 Hz to define the LF and HF bands respectively, meaning, their results are not fully transferable to typical spectral stress studies (Houtveen et al., 2002).

A cardiac measure called pre-ejection period (PEP) was also employed in Houtveen et al. (2002) to estimate sympathetic modulation. Unlike spectral analysis, PEP is derived from cardiac impedance signals, and large changes in PEP are defined to represent sympathetic modulation of the heart (Sherwood et al., 1990). Furthermore, a separate review paper on cardiac impedance found that the application of ensemble averaging to impedance signals effectively filtered out the effects of respiration from the signals (Sherwood et al., 1990). Ensemble averaging was therefore employed in the analysis of the recorded impedance signals in Houtveen et al. (2002), and it was found that reductions in PEP were significantly larger during mental stress when compared to the exercise response (p-value=0.022). Thus, in light of the sensitivity of the LF and HF powers to the effects of respiration, the PEP results from Houtveen et al. (2002) suggest that there are indeed differences between the cardiac responses to mental and physical stress, which are not solely related to differences in respiration.

In a very recent deviation from standard LF and HF power based analyses of stress, SNS and PNS activities have also been modelled in Valenza et al. (2018); in the study, ECG signals were recorded from seven healthy participants who had been administered a pharmaceutical parasympathetic nerve blockade. The subjects were asked to change posture to induce changes in autonomic balance, following which sympathetic coefficients, or kernels, were derived through the convolution of the subjects' HRV signals through functions of time, known as Laguerre functions (Valenza et al., 2018). Likewise, parasympathetic kernels were derived from seven subjects who had been administered a sympathetic nerve blockade (Valenza et al., 2018). The performances of the derived sympathetic and parasympathetic kernels in the tracking of the SNS and PNS activities were validated on 236 ECG signals, recorded from participants completing several stress-inducing tests, including tilts and handgrips. The authors employed between-group statistical tests to assess the performance of the sympathetic and parasympathetic kernels in the tracking of autonomic dynamics in response to the stress-inducing tests; the sympathetic and parasympathetic kernels were found to statistically outperform the LF and HF powers in separating the basal HRV from the instances of stress (Valenza et al., 2018). In spite of the positive results reported in Valenza et al. (2018), the authors admitted that naturally, the performance of any model based assessment of autonomic dynamics would be dependent upon the fit of the model, and the quality and representativeness of the data used to train the model.

Therefore, in brief, it is evident that the identification of stress from temporal and spectral analyses of HRV signals is not always straightforward. A summary of the temporal and spectral stress studies

described above, in which study volunteers were subjected to a form of stress, can be seen in Table 2.1.

TABLE 2.1: Summary of human stress studies employing temporal and spectral analyses of heart rate variability.

Stress Studies Employing Temporal and Spectral Analyses		
Study	Strengths	Weaknesses
Montano et al. (1994): Twenty-two healthy volunteers were subjected to upright tilt in a bid to induce physical stress.	**The degree of tilt positively correlated with the power of the low frequency components of HRV, and negatively correlated with the power of the high frequency components of HRV.**	The experimental design could only induce physical stress. Sex differences in the activities of the SNS and PNS were not investigated.
Pagani et al. (1997): Pharmaceutical nerve blockades were administered to eight healthy participants before their HRV signals were assessed. Microelectrodes were used to record the activities of the participants' sympathetic and parasympathetic nerves.	**It was confirmed that the power of the low frequency and high frequency components of HRV correlated with the activities of the SNS and PNS.**	Sex differences in the activities of the SNS and PNS were not investigated.
Houtveen et al. (2002): The difference between the cardiac responses to mental stress and physical stress was investigated in 22 healthy participants, subjected to a mental stress task, quiet rest and an exercise task.	**It was found that whilst the large changes in respiration rate during exercise were reflected in the LF and HF powers, the reduction in the preejection period during mental stress was significantly larger than that of physical stress. Therefore, mental stress was found to have induced greater sympathetic activation than physical stress.**	Sex differences in the responses to stress were not investigated.
Valenza et al. (2018): Heart rate variability kernels were derived from seven subjects to model sympathetic and parasympathetic activity.	**The kernels were found to statistically outperform the LF and HF powers in tracking sympathetic and parasympathetic nerve activities.**	The performance of the kernels is entirely dependent upon the representativeness of the ECG signals recorded from only seven subjects.

In conclusion, since the findings of research such as Montano et al. (1994), the spectral analysis of heart rate variability signals has been widely used to quantify stress. Yet, as thoroughly explored in

Billman (2013), not only are the physiological interpretations of the LF and HF components of HRV power spectra inaccurate, but, sympathetic and parasympathetic activities are not strictly reciprocal; these misinterpretations are further confounded by possible sex differences in the stress response (Ramaekers et al., 1998).

These limitations of spectral analyses have led to the creation of novel developments such as the model produced in Valenza et al. (2018); however attempts to model SNS and PNS dynamics could suffer from model over-fitting. It is therefore imperative to explore other approaches to HRV analysis, to produce accurate characterisations of HRV and the stress response, such as nonlinear analyses.

2.2 Nonlinear Analysis and Complexity Science

The definition of a nonlinear method of analysis is one in which there are no assumptions regarding the underlying dynamics of a signal, instead, a signal is analysed to reveal particular characteristics (Mandic et al., 2008). This is in contrast to linear methods, which assume the underlying dynamics of a signal; for example, in the computation of the LF and HF powers, it is assumed that a signal can be deconstructed into a superimposition of periodic sine waves (Akin and Kiymik, 2000). Schematics showing the differences in linear and nonlinear analysis approaches are depicted in Figures 2.1 and 2.2; it can be seen that whilst linear methods of analysis aim to decompose and describe a signal using defined equations, nonlinear methods aim to only describe specific features of a signal (Akin and Kiymik, 2000; Gautama et al., 2004; Mandic et al., 2008). Nonlinear methods of analysis therefore offer an unbiased means to analyse signals which contain nonlinearities, and are not comprised of linearly superimposed functions (Mandic et al., 2008). Nonlinearities in signals are defined as signal dynamics which do not have a Gaussian probability distribution (Mandic et al., 2008).

Heart rate variability signals have been found to contain nonlinearities, which indicates that HRV should ideally be analysed using a nonlinear method (Gautama et al., 2004).

Complexity analysis is a branch of nonlinear analysis which is gaining more and more popularity in the processing of physiological signals, and is a field of analysis which aims to assess the level of complexity within a system (Tononi et al., 1998). Information theory approaches to complexity were reviewed in Tononi et al. (1998), in which it was made clear that the definition of complexity, and thus how exactly to asses it, was not straightforward. It was argued that a truly complex system was "neither completely regular nor completely random" (p. 478); complexity was further described as a

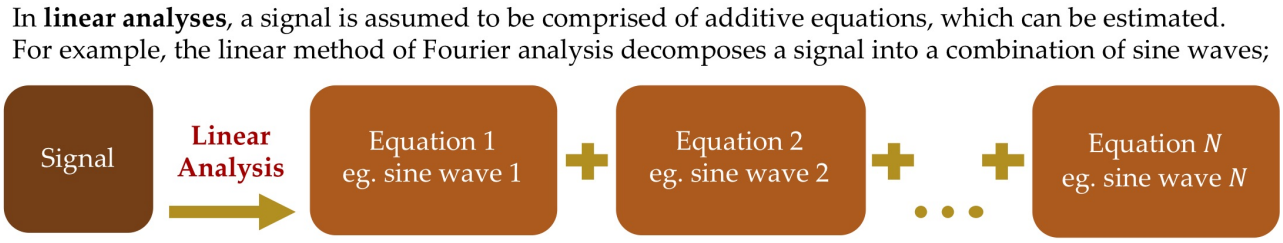


FIGURE 2.1: Schematic of a linear analysis approach (Akin and Kiymik, 2000; Gautama et al., 2004; Mandic et al., 2008)

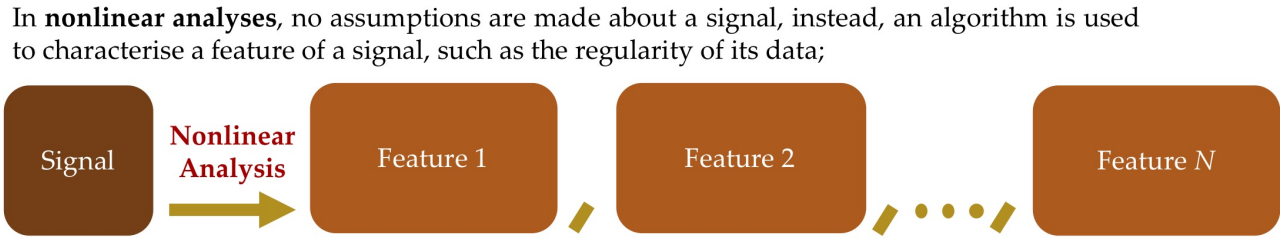


FIGURE 2.2: Schematic of a nonlinear analysis approach (Akin and Kiymik, 2000; Gautama et al., 2004; Mandic et al., 2008)

measure which "captures both functional segregation and integration" (Tononi et al., 1998, p. 481). Notably, the importance placed on system integration in assessments of complexity in Tononi et al. (1998), is mirrored in an influential paper, Goldberger et al. (2002).

Goldberger et al. (2002) is a paper which discussed physiological complexity, in which it was suggested that the complexity within physiological systems could be a result of the interaction between the many feedback loops which regulate the systems. Central to Goldberger's paper is the complexity-loss theory, which proposes that healthy physiologies are able to adapt quickly to changes due to their inherent long range correlations and nonlinear interactions.

It was posited in Goldberger et al. (2002) that physiological constraints, such as disease and ageing reduced the adaptive abilities of physiologies, hence reducing their complexity. However, there are differing definitions of complexity, with some researchers evaluating a system's complexity through the computation of the entropy in signals which have been recorded from the said system; pioneers in the analysis of signal entropy such as Pincus, have likened complexity to entropy and signal irregularity, stating that the more irregular a time series, the higher the entropy, and the higher the complexity (Pincus, 1991). Yet it was specifically stated in Goldberger et al. (2002) that measures of entropy, citing Pincus (1991), were not necessarily measures of physiological complexity.

Nevertheless, due to convention, it is still common to employ measures of entropy to assess signal complexity. This is particularly true in stress research, where many studies have employed entropies to investigate the effects of stress on HRV, as discussed in Section 2.2.1 of this chapter. However, before discussing the applications of entropy to stress research in Section 2.2.1, it is important to understand the scale of the debate surrounding the use of entropies to investigate complexity, and one study which demonstrates this controversy well is Vaillancourt and Newell (2003).

Vaillancourt and Newell (2003) is a highly cited study, which assessed the entropy of measured biomechanical force signals from 30 participants. The participants were asked to first place their hand on a force plate, and track a straight line with their index finger at a constant force, and then track a sinusoidal line (Vaillancourt and Newell, 2003). Of the 30 participants, there were equal numbers of those aged between 20-24 years, 64-69 years and 75-90 years, and the popular entropy measure called approximate entropy (*ApEn*), from Pincus (1991), was used to assess the complexity of the measured forces (Vaillancourt and Newell, 2003).

Pincus introduced *ApEn* in 1991 as a measure of complexity, as an alternative to the more traditional approaches of chaos modelling. Approximate Entropy was described as a family of statistics to measure "the [logarithmic] likelihood that runs of patterns that are close remain close on next incremental comparisons" (Pincus, 1991, p. 2299). The measure of *ApEn* also has limitations. Firstly, it assumes stationarity within a time series, so as the statistical characteristics within a signal, such as the mean and standard deviation are assumed to not change with time (Pincus, 1991); this is a considerable limitation as physiological signals are notoriously nonstationary (Goldberger et al., 2002). Also, approximate entropy does not address the nonlinear components within signals (Goldberger et al., 2002).

Nonetheless, it was reported in Vaillancourt and Newell (2003) that during the task to track the straight line, there was a significant decrease in *ApEn* with ageing (p-value < 0.01) in accordance with the complexity-loss theory, but counter to the complexity-loss theory, there was also a significant increase in *ApEn* with ageing during the task to track the sinusoid (p-value < 0.05) . It was therefore concluded in Vaillancourt and Newell (2003) that the increase in entropy seen with age during the sinusoid tracking task contradicted the original complexity-loss theory from Goldberger. Vaillancourt and Newell cited their previous advancement of the complexity-loss theory, described in Vaillancourt and Newell (2002), to explain the age-related increase in signal entropy. It was theorised in Vaillancourt and Newell (2002) that the direction of changes in physiological complexity

would depend on the external demands of a task.

The fact that a physiological constraint such as ageing was able to both increase and decrease signal entropy in Vaillancourt and Newell (2003) begs the question, will the physiological constraint of stress cause consistent changes in HRV entropy?

It is therefore evident that there is great interest and merit in employing entropies to investigate the directional changes in HRV entropy, caused by stress. Also, since the originators of the complexity-loss theory stated that measures of entropy could not be accurate measures of complexity (Goldberger et al., 2002), it is of particular interest to investigate whether stress will consistently cause decreases in the entropy of HRV, in the same way it could be expected to cause a decrease in complexity.

2.2.1 Nonlinear Analyses in Stress Research

Stress and anxiety are both responses to threats, and are therefore variants of the same phenomenon (Bystritsky and Kronemyer, 2014), and in a study reported in Bornas et al. (2006), the entropies of ECG signals, recorded from anxious flyers in a flight simulation, were analysed. In the study, 21 male and 40 female flight phobics, along with 20 male and 38 female controls, participated in a simulated aircraft take-off, during which their ECG signals were recorded for five-minutes (Bornas et al., 2006). The authors introduced the investigation as a pioneering study of an anxiety disorder using multiscale entropy (Costa et al., 2002; Bornas et al., 2006).

First introduced in Costa et al. (2002), multiscale entropy is an analysis approach in which entropy is computed at different temporal scales within a signal. The process of accessing a signal at different temporal scales is called coarse-graining, and is analogous to filtering a signal to obtain lower frequency components. An illustration of the coarse-graining procedure can be found in Figure 2.3, adapted from Costa et al. (2002, p. 2) and Lu et al. (2012, p. 2401), where the data points, $x(n)$ from a signal, are averaged in nonoverlapping windows of the same length as the temporal scale, τ. A signal at temporal scale 1 is therefore the original signal. The coarse-grained data points, $y(i)$, are found as follows in Equation 2.1, where i and n respectively denote the indices of the coarse grained signal and the indices of the original signal, and N represents the length of the original signal (Costa et al., 2002, p. 2);

$$y_\tau(i) = \frac{1}{\tau} \sum_{n=(i-1)\tau+1}^{i\tau} x(n) \qquad 1 \leq i \leq \frac{N}{\tau}. \tag{2.1}$$

Analysis at different temporal scales is a preferred approach in many physiological signal characterisations as many of the feedback loops which regulate different physiologies function over many temporal scales (Goldberger et al., 2002). The entropy method employed in Costa et al. (2002) was the successor to *ApEn*, sample entropy (*SampEn*), which was in turn introduced in Richman and Moorman (2000). Richman and Moorman argued that *ApEn* was inherently biased as it included self-matches in the assessment of the probabilities used in its entropy calculation. Sample entropy however was described as "the negative natural logarithm of the conditional probability that two sequences similar for m points remain similar at the next point, where self-matches are not included in calculating the probability" (Richman & Moorman, 2000, p. H2039). A full description of the computation of sample entropy can be found in Algorithm 1, on page 53. The length of the sequences to be compared in the algorithm, m, is termed the embedding dimension, and the deviation within which similarities are counted as matches is termed the tolerance, r. It is recommended that the value of r should be a proportion of the standard deviation of the signal, although this introduces inaccuracies in the analysis of nonstationary signals (Pincus, 1991).

Referring back to Bornas et al. (2006), sample entropy was computed using an m of 2 and an r of 0.2 multiplied by the standard deviation of the data, from temporal scale 1 to scale 40. It was found that at scale 35 the mean entropy values computed from the ECG signals from the flight phobics were significantly lower than those from the control group (p-value ≤ 0.05) (Bornas et al., 2006). Bornas et al. (2006) therefore provides support for the multiscale analysis of cardiac signals, although no physiological explanation was given as to why temporal scale 35 specifically would provide statistically significant results.

Furthermore, despite the explanation given in Bornas et al. (2006), that autonomic reactivity is more commonly assessed through HRV, no comment was given as to why the authors opted to compute the entropy of the ECG signals, as opposed to the corresponding HRV series; this raises the possibility that an analysis of the HRV series instead may have yielded greater reductions in entropy. Also, the decision in Bornas et al. (2006) to employ *SampEn* in the assessment of entropy could be seen as questionable given the fact that *SampEn* also assumes that a signal is stationary (Lu et al., 2012).

In a more recent study, Williamon et al. (2013), HRV series from a 57-year-old male pianist were extracted from 20-minute long ECG signals, recorded during both a low stress concert in front of the authors alone, and a high stress concert in front of an audience of 400. Williamon et al. assessed the entropy within the HRV series in windows of seven minutes in length, at temporal scales 1 and 2, using the multiscale entropy method from Costa et al. (2002); the multiscale entropy was computed using an m of 2, and an r of 0.15. Williamon et al. found that at temporal scale 2 in particular, the high stress HRV series was more regular than that during the low stress performance. With respect to the LF and HF bands within the HRV series, it was found that the entropy analysis of the HF signal components produced lower entropy values during the high stress performance, whilst the LF band analysis produced similar entropies for both the high stress and low stress performances (Williamon et al., 2013); however, the statistics indicating the significance of the results were not reported.

Notwithstanding, the results from Williamon et al. (2013) do show that stress can reduce overall HRV entropy, and the entropy of the HF component of HRV; the generalisability of the results are however greatly compromised as only one male subject was assessed in the study. Also, again, any signal analysis which employs *SampEn* is limited by the nonstationarity of physiological signals (Lu et al., 2012).

In a similar vein to the study reported in Williamon et al. (2013), Chanwimalueang et al. detailed how *SampEn* was employed to model stress in HRV signals, which had been extracted from the ECG signals of two males presenting at an academic conference. The first recording was from a poster presentation, 170-minutes in length, whilst the second recording was from a podium presentation, 60-minutes in length (Chanwimalueang et al., 2016). The *SampEn* analyses for the poster and podium presentations were undertaken in windows of nine- and three-minutes in length, respectively (Chanwimalueang et al., 2016). Upon visual inspection of plots of the so-obtained *SampEn* results, the authors did not find any stark changes in the entropies related to the poster presentation, but they did find that at the very beginning of the podium presentation, there was a sharp decrease in entropy, which was cited as indicating high stress (Chanwimalueang et al., 2016). Indeed, the conclusion that the podium presentation was more stressful than the poster presentation is perhaps intuitive and to be expected. It is widely reported in psychological literature that an element of social evaluation induces stress (Dickerson and Kemeny, 2004), and a podium presentation can involve more direct social evaluation than a poster presentation. A review of 208 psychological stress studies found that

stressors which involved social-evaluation, with the threat of negative judgement, produced heightened cortisol responses, compared to stressors without an element of social-evaluation (Dickerson and Kemeny, 2004).

However, given that the results presented in Chanwimalueang et al. (2016) were produced from only two subjects, both of which were male, the generalisability of the results are compromised. Furthermore, the stress-induced reductions in entropy were only presented graphically, and were not quantified or presented statistically.

In another study of entropy-based stress analysis, series of RR intervals were extracted and analysed from the ECG signals of 10 males and 13 females, recorded whilst they were at rest for 5 minutes, and whilst they undertook an arithmetic task for 5 minutes (Vuksanović and Gal, 2007). Vuksanovic and Gal computed the entropy within each HRV series, using the *SampEn* method with an embedding dimension of 2 and a tolerance of 0.2 multiplied by the standard deviation of the data. Vuksanović and Gal (2007) found that when comparing rest to the arithmetic task, there was a significant decrease, (p-value < 0.01), in the computed *SampEn* from 10 participants, who also exhibited a significant decrease in the HF power proportion of their RR interval series. Conversely and perhaps even more intriguingly, the remaining participants instead exhibited increases in their HF power under test conditions, did not produce significant reductions in entropy (Vuksanović and Gal, 2007). The results from Vuksanović and Gal (2007) are striking given the fact that stress is rarely associated with an increase in HF power, and that two distinct stress-induced trends in HF power and entropies were found (some participants exhibited stress-induced decreases in HF power and entropy, and some did not).

It cannot be ignored that the numbers of the participants who experienced these two distinct responses matched the respective numbers of males and females recruited in the study. However, throughout Vuksanović and Gal (2007), there was no indication as to how many males were amongst those who exhibited stress-induced decreases in HF power and entropy, and how many females were amongst those who did not exhibit the same stress response, so it cannot be inferred whether these distinct responses were driven by sex; a sex-driven response can be supported in literature, and is discussed in depth in Chapter 3.

In an alternative study, Mohr et al. (2002), the manifestations of stress in bovine HRV signals were examined. Mohr et al. recorded the HRV series from 18 unstressed calves, 17 externally stressed

calves (who were subjected to warm conditions), and 17 internally stressed calves (who were suffering from diarrhoea). Nonlinear analyses were then completed through Recurrence Quantification Analysis, which uses recurrence plots, and importantly, is unaffected by nonstationarity within signals (Webber and Zbilut, 1996; Mohr et al., 2002). The recurrence plots were created using the distances between vectors in a matrix of RR intervals (Mohr et al., 2002). It was found that the determinism within the signals from the externally and internally stressed calves was significantly higher (p-value < 0.05) than that within the signals from the unstressed calves [1]. The internally stressed calves produced the greatest increase in determinism. Mohr et al. interpreted the loss of stochasticity seen during stress as being indicative of a loss of freedom within cardiovascular regulation. Mohr et al. also aligned stress to load, describing the increase in determinism as being indicative of the animal having to focus more on the load. However, although Recurrence Quantification Analysis provides a robust characterisation of nonlinear, nonstationary data, the performance of the method is heavily dependent on at least three user defined plot parameters (Birleanu et al., 2011). Mohr et al. employed parameters which had been previously recommended in literature, however, naturally, caution must be heeded when implementing any algorithm which has a performance which is sensitive to user-defined parameters. Without analysing a signal iteratively, using different parameters, the true performance of the algorithm cannot be determined. In a study evaluating the use of recurrence plots in the analysis of physiological data, Birleanu et al. (2011), it was explained that there was no consensus regarding the choice of the parameters, and that it was down to the discretion of the researcher.

That said, the results from the analysis of bovine data in Mohr et al. (2002) show that there is merit in the analysis of HRV using nonlinear methods which are not based on entropy. This is an important finding, as the vast majority of nonlinear HRV analyses of stress add to the literature on tried-and-tested entropy-based analyses, without necessarily adding novelty. Additionally, popular one-dimensional nonlinear methods, such as entropy measures, are not always able to indicate the separate activities of the SNS and PNS.

In 2007, a novel nonlinear HRV measure was introduced in Porta et al. (2007), from members of the research group which published some of the initial work on the LF and HF powers and autonomic balance (Montano et al., 1994; Pagani et al., 1997); the method introduced in Porta et al. (2007) was described as being able to indicate the separate activities of the SNS and PNS. The new symbolic

[1] In brief, a signal is deterministic if it can be described using equations, and it is stochastic if it cannot (Mandic et al., 2008).

method was introduced to produce results which were able to track the activities of the SNS and PNS during graded tilt tests. Symbols based on the assessment of the relationship between three consecutive HRV data points (that is for example, three data points increasing in value, or three data points decreasing in value etc.) were extracted from the HRV series of 17 participants (Porta et al., 2007). One group of symbolic indices were suspected to reflect the activity of SNS, and another was suspected to reflect the activity of the PNS; it was found that the indices derived from the symbols were better correlated to the angles of tilt than the spectral LF and HF measures (Porta et al., 2007).

However, Porta et al. admitted that given the slower speed of SNS dynamics, it was possible that symbols of three data points in length would not have been long enough to fully capture longer range SNS dynamics.

The nonlinear HRV stress investigations described above, in which human study volunteers were subjected to a form of stress, are summarised in Table 2.2.

Thus, in brief, Bornas et al. (2006), Vuksanović and Gal (2007), Williamon et al. (2013) and Chanwimalueang et al. (2016) all employed the use of sample entropy from Richman and Moorman (2000) in their stress analyses, and found that stress typically induced reductions in entropy. Yet despite the effectiveness of these approaches, they were all limited by the fact that they were one-dimensional and could not assess individual SNS and PNS dynamics. Conversely, a symbolic approach was also developed by one of the originators of the LF and HF spectral analyses of stress, and demonstrated the feasibility of the assessment of the separate dynamics of the SNS and PNS using nonlinear signal processing (Porta et al., 2007). Therefore, it cannot be disputed that if the quantification of stress is to truly evolve and uncover never seen before relationships between HRV-derived variables, there must be a continued effort to further explore alternative nonlinear analyses which can identify the separate activities of the SNS and PNS.

TABLE 2.2: Summary of human stress studies employing nonlinear analyses of heart rate variability.

Stress Studies Employing Nonlinear Analyses		
Study	Strengths	Weaknesses
Vuksanović and Gal (2007): Sample entropy and spectral analyses were used to characterise HRV signals from 10 males and 13 females who completed a mental arithmetic test.	**It was found that 10 of the subjects exhibited a stress-induced decrease in HF power and a decrease in sample entropy, whilst 13 exhibited negligible change in sample entropy and an unexpected stress-induced increase in HF power.**	The authors did not specify the sex of the subjects who exhibited the two distinct stress-induced trends in sample entropy and HF power. It therefore cannot be seen if the trends were driven by sex.
Porta et al. (2007): Three-digit long symbols were derived from the HRV signals of 17 healthy subjects subjected to upright tilts.	**It was found that the derived symbols were able to track sympathetic activation.**	The authors admitted that three-digit symbols may not have been long enough to truly track long-range sympathetic activity.
Williamon et al. (2013): Sample entropy was computed on an HRV signal from a male pianist who gave a low-stress performance and a high-stress performance.	**Sample entropy was shown to indicate stress in a real-world scenario; it was found that the high-stress performance induced a reduction in sample entropy**	The HRV of only one male subject was assessed.
Chanwimalueang et al. (2016): Sample entropy was computed on the HRV signals from two male presenters at an academic conference.	**Sample entropy was shown to indicate stress in a real-world scenario; it was found that the podium presentations induced a sharp decrease in sample entropy.**	Not only were the HRV of only two male subjects assessed, but the stress-induced reductions in entropy were not quantified.

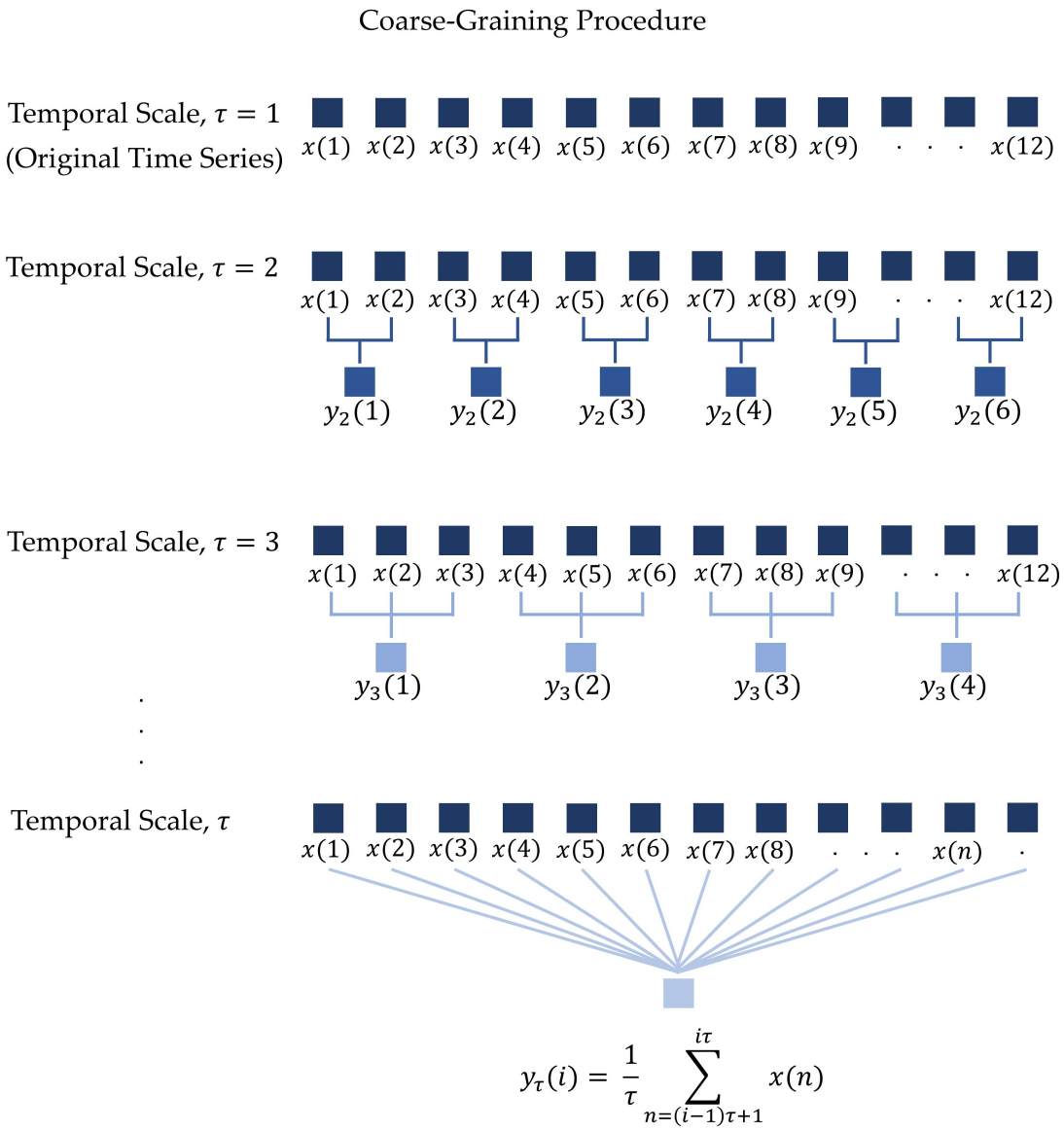


FIGURE 2.3: Illustration of the coarse-graining procedure, to access a time series at higher temporal scales, adapted from Costa et al. (2002, p. 2) and Lu et al. (2012, p. 2401).

Chapter 3

Performance of Traditional Stress Measures in Males and Females

As it can be read from Chapter 2, signal analysis of HRV to assess stress has largely constituted temporal and spectral analyses, with nonlinear approaches being more recent investigations.

The assessment of the state-of-art in the characterisation of stress from HRV is the first objective of this research, in order to achieve the overall research aim, of the development of a quantitative measure of stress, which has a reliable performance.

3.1 Chapter Summary

The human physiological response to mental stress has been widely assessed using measures derived from heart rate variability. However, the performances of these measures in identifying stress have not proven to be reliable. The unreliability of these measures is further confounded by sex differences in the cardiac responses to mental stress, which are often erroneously ignored. To this end, the work presented in this chapter shows that the understanding of the stress response is fundamentally altered once sex differences are taken into account. This is achieved by comparing heart rate variability signals, acquired during instances of mental stress induced by mental arithmetic, from 10 females and 10 males of similar arithmetic ability. The heart rate variability signals were analysed using temporal, spectral and nonlinear signal processing techniques, which revealed significant statistical differences between the sexes. The results showed that whilst the measure of heart rate was able to discern the state of rest from the arithmetic test in the males (p-value=0.008), none of the measures were able to significantly discern rest from arithmetic in the females. This difference between

the males and females demonstrates the need for a quantitative measure of stress which performs well in both sexes. Overall, stress produced an expected increase of 35% in the power of the low frequency component of HRV in the males, but produced a decrease of 7% in the females. Similarly, stress induced a decrease of 18% in the power of the high frequency component of HRV in the males, but caused a large increase of 40% in the females. It is also shown, that stress induced an expected decrease in long-term univariate sample entropy in the males, but caused an increase in the females. This finding is noteworthy, as a stress-induced increase in complexity has only been reported in Valente et al. (2018), in which a method called conditional entropy was applied to short HRV segments, consisting of only 300 heart beats. In the analyses of long-term HRV, mental stress has always been understood to be manifested in a decrease in sample entropy. These findings of a stress-induced rise in entropy in females not only highlight the pitfalls in ignoring sex in the formation of a physiological hypothesis, but also suggest, for the first time, that the conventionally cited cardiac changes attributed to the fight-or-flight stress response are not always applicable to females. Instead, the pilot study detailed in this chapter provides an alternative interpretation of cardiac responses to stress in females, which could indicate closer alignment to the behavioural tend-and-befriend response to stress.

3.2 Chapter Background

Sex differences in the stress response have been attributed to the effects of oestrogens on cardiac dynamics (Ramaekers et al., 1998; Kajantie and Phillips, 2006), however the exact mechanisms by which oestrogens affect cardiac dynamics remain unknown. Saleh and Connell investigated the activities of the sympathetic and parasympathetic nerves following the injection of oestrogens into nerve centres in rodent brains, and found that the injections could enhance parasympathetic nerve activity and attenuate sympathetic nerve activity (Saleh and Connell, 2003). The extension of the finding in Saleh and Connell (2003), of an oestrogen-induced attenuation of sympathetic activity, is in support of Kajantie and Phillips (2006), where as mentioned previously, it was explained that an oestrogen-induced attenuation of sympathetic nerve activity would be advantageous in females as it would protect a growing foetus from the harmful effects of sympathetic stress hormones.

As also mentioned, any modulation of the stress response caused by oestrogens would present most prominently amongst premenopausal females, who experience monthly elevations in oestrogens, and in particular females who are in the follicular phase of their menstrual cycle (Dart et al., 2002).

The follicular phase spans the first two weeks of the menstrual cycle, and is characterised by rapidly increasing levels of oestrogens (Dart et al., 2002). To demonstrate the modulating effect of oestrogens on HRV, Liu et al. assessed *HR* and the spectral components of HRV from premenopausal females, postmenopausal females, postmenopausal females using oestrogen hormones replacement therapy, and males. It was found that the HRV from the premenopausal females contained the greatest HF power, followed by the postmenopausal females using hormone replacement therapy, then the postmenopausal females, and lastly, the males. Statistically, the difference between the HF power of the premenopausal females and the postmenopausal females using hormone replacement therapy was not significant (p-value > 0.05), whilst the corresponding difference between the premenopausal females and the postmenopausal females not using hormone replacement therapy was significant (p-value $\leq$ 0.05) (Liu et al., 2003). Moreover, the statistical difference between the HF power of the postmenopausal females not using hormone replacement therapy and the males was not significant (p-value > 0.05) (Liu et al., 2003). It is also of note that the premenopausal females had the highest heart rate, despite having the greatest HF power (Liu et al., 2003); this finding is contrary to the expectation that a high HF component in HRV would indicate parasympathetic dominance, and hence low heart rate. It is therefore evident that high levels of oestrogens can drastically alter the autonomic manifestation of stress, and necessitates the need for a quantitative measure of stress which accounts for both males and females of all ages.

In the pilot study reported in this chapter, the state-of-the-art temporal HRV-based stress measures of *HR* and *SDNN*, the spectral measures of the LF and HF powers, and the nonlinear measures of *SampEn* and permutation entropy (*PermEn*) were applied to HRV series, derived from ECG signals which were recorded from male and female subjects whilst they completed a standardised stress test.

All computed measures were statistically analysed to assess the performance of the traditional measures in the discernment of states of stress from states of rest. The measures computed from the male and females subjects were analysed and compared statistically, to identify sex-specific trends in the characterisation of stress, and therefore verify previously reported, but not widely known, sex differences in the temporal and spectral measures (Liu et al., 2003), as well as uncover sex differences in the long-term univariate nonlinear entropy measures, in response to stress.

3.3 Materials and Methods

3.3.1 Subjects

Ten male and ten female subjects, aged 28.6±5.6 years and 23.3±1.3 years respectively, were recruited in this study. The calendar method was used to establish that all females were in the follicular phase of their menstrual cycle, so as to ensure that the effects of oestrogens on the stress response could be analysed (Dart et al., 2002).

3.3.2 Experimental Procedure

The subjects took part in a custom designed stress test, inspired by the Trier Social Stress Test (Birkett, 2011), in pairs. The custom designed test consisted of an initial 15 minutes of sitting rest, a one minute break, and then 15 minutes of a mental arithmetic test. To increase their stress levels, the subjects were told that they were competing with their opponent in the mental arithmetic test; all subjects were of a similar arithmetic ability, and all pairs of subjects were matched in English language ability and sex.

The standardised stress test was commenced at 2 pm for all pairs of subjects. This precaution was implemented to ensure that the results from the study would not be influenced by differences in the time of data recording, as the SNS and PNS are both affected by circadian rhythms (Montano et al., 1994). It has been found that sympathetic activity peaks in the morning, and is lowest at night, whilst parasympathetic activity peaks at night and is lowest in the morning (Furlan et al., 1990; Montano et al., 1994). Similarly, normalised powers of the low frequency components of HRV have been found to be lowest at night and highest in the morning, whilst normalised powers of the high frequency components of HRV have been found to be lowest in the morning and highest at night (Furlan et al., 1990; Montano et al., 1994).

The subjects' ECG signals were recorded using a custom-designed amplifier named the iAmp, developed by Dr Valentin Goverdovsky (Kanna et al., 2018) (see Figure 3.1). The ECG signals were recorded from one channel at a sampling frequency of 1000 Hz, with one electrode on the lowest left rib, and the other on the lowest right rib, representing a so-called Lead I ECG configuration (Society for Cardiological Science & Technology, 2014); the ground electrode was placed adjacent to the right-side electrode.

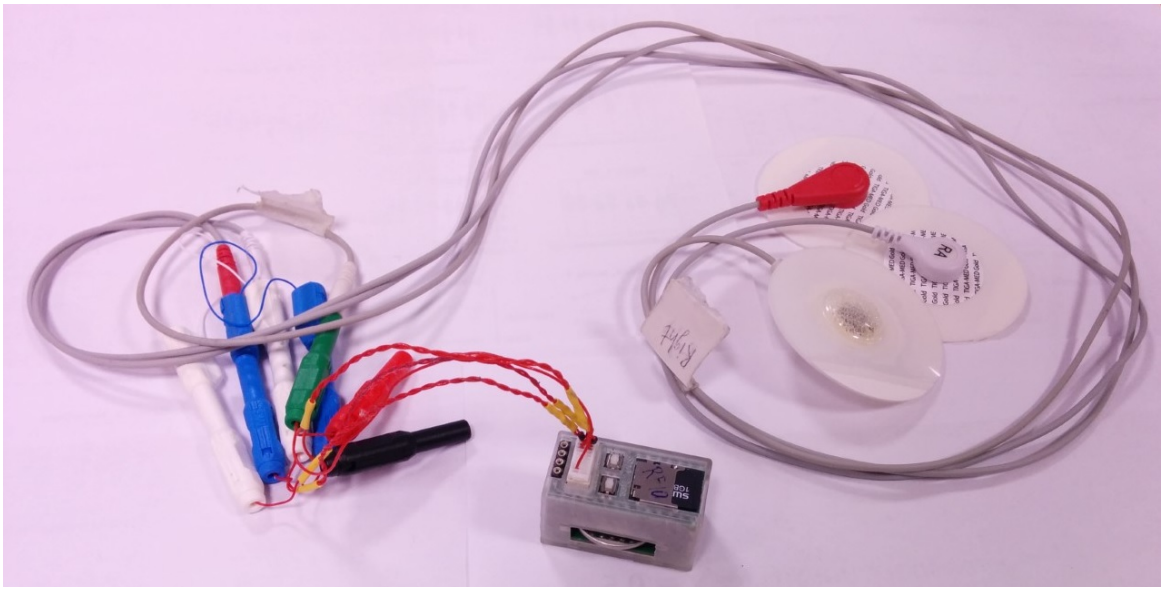


FIGURE 3.1: A photograph of the iAmp, used to record electrocardiogram signals.

3.3.3 Data Analysis

All data analyses were undertaken in the MATLAB programming environment. Heart rate variability was extracted from the recorded ECG signals using the algorithm introduced in Chanwimalueang et al. (2015), and resampled at a frequency of 4 Hz. The temporal measures of *HR* and the *SDNN*, the spectral measures of the LF and HF power components and the nonlinear measures of *SampEn* and permutation entropy (*PermEn*) were then computed from the derived HRV signals.

All measures were computed from the subjects' HRV; the analyses were completed on windowed segments of HRV, 5 minutes in length. Once each window of data was analysed, an increment of one second was applied before windowing the next segment of HRV. The use of a 5-minute window is in accordance with the minimum data length recommendation for analysis given in Malik et al. (1996). In each window of analysis, outliers were defined as values which were either less than 1.5 times the 25th percentile of data, or greater than 1.5 times the 75th percentile of data; all outliers were replaced with the median of the interquartile range of the data.

Temporal Analyses

Heart rate in beats-per-minute (bpm) was computed as shown in Equation 3.1, where N represents the number of data points, $x(n)$, in the window of analysis.

$$HR = \frac{\sum_{n=1}^{N}(\frac{60}{x(n)})}{N} \tag{3.1}$$

The standard-deviation-of-the-NN-interval in milliseconds (ms) was computed as shown in Equation 3.2, where $\overline{x(n)}$ signifies the mean (Vollmer, 2015; Kim et al., 2018).

$$SDNN = \sqrt{\frac{\sum_{n=1}^{N}(x(n) - \overline{x(n)})^2}{N-1}} \tag{3.2}$$

Spectral Analyses

The periodogram method with a Hamming window the same length as the windowed HRV series was used to estimate the power spectrum of the signal. Equation 3.3 outlines the computation of the periodogram, where $X(\omega_k)$ is the Discrete Fourier Transform of the signal, $x(t)$, of length N, at time t; the angular frequency is denoted by ω_k, and the imaginary number is denoted by j (Akin and Kiymik, 2000).

$$X(\omega_k) = \sum_{t=0}^{N-1} x(t) e^{\frac{-j\omega_k t}{N}} \tag{3.3}$$

The spectral power, Pf_k, for a frequency, f_k, is computed as shown in Equation 3.4, where f_s is the sampling frequency of the signal (Akin and Kiymik, 2000).

$$Pf_k = \frac{f_s}{N} |X(k)|^2 \tag{3.4}$$

The absolute powers of the low and high frequency components of the HRV signals were computed as the powers of the 0.04 to 0.15 Hz and 0.15 to 0.4 Hz bands respectively, with units of millisecond squared (ms^2) (Burr, 2007). The absolute LF and HF powers were then normalised by the power of the 0.04 to 0.5 Hz band, termed N_p, to produce *nLF* and *nHF* respectively; see Equations 3.5 and 3.6.

$$nLF = \frac{absoluteLF}{N_p} \tag{3.5}$$

$$nHF = \frac{absoluteHF}{N_p} \tag{3.6}$$

The power of the 0.04-0.5 Hz band was used to normalise the absolute LF and HF powers for the following reasons:
(i) There is no clear physiological interpretation for HRV frequencies below 0.04 Hz (Malik et al., 1996).
(ii) The rate at which the heart typically contracts is 1 Hz (that is, 60 bpm), so in accordance with the Nyquist theorem, the useful information content of HRV will be contained below 0.5 Hz (Kuusela, 2013).

Nonlinear Analyses

The nonlinear measures of *SampEn* and *PermEn* were computed from the HRV signals.

As described in Chapter 2, Section 2.2.1, sample entropy is a measure of signal regularity, introduced in Richman and Moorman (2000) as an unbiased improvement to the approximate entropy method from Pincus (1991). Sample entropy was described as "the negative natural logarithm of the conditional probability that two sequences similar for m points remain similar at the next point, where self-matches are not included in calculating the probability" (Richman & Moorman, 2000, p. H2039), so the higher the *SampEn* value, the more irregular the signal. Sample entropy is limited by its dependence on the length of m; it was warned in Pincus (1991) that the predecessor to *SampEn*, approximate entropy, was unstable when m was greater than four. This limitation applies to *SampEn* too.

The computation of *SampEn* is outlined in Algorithm 1 on page 53 (Costa et al., 2002; Costa et al., 2005; Song et al., 2012).

Permutation entropy was introduced in 2002 by Bandt and Pompe, as a measure of regularity which reaches a maximum for a highly irregular signal, and is unaffected by signal amplitude. Permutation entropy is a symbolic measure, in which the relative frequency of unique symbols, of length m, within a signal are computed (Bandt and Pompe, 2002). It was recommended in Bandt and Pompe (2002) that m should be in the range of three to seven.

The computation of *PermEn* is outlined in Algorithm 2 on page 54 (Bandt and Pompe, 2002; Bian et al., 2012).

The computed *HR*, *SDNN*, *nLF*, *nHF*, *SampEn* and *PermEn* were segmented to correspond to the test epochs of rest and mental arithmetic. The segmented measures corresponding to the rest epoch were then statistically compared to those from the arithmetic epoch, using either the paired t-test, or the

Algorithm 1: Sample Entropy

1 A windowed signal, x, of length N is segmented using an embedding dimension, m, to create $(N-m+1)$ segments. Each such segment, X_m, is of length m. In this work, m was defined as $m = 2$.

2 A tolerance level of $r = 0.15 \times std$, where std is the standard deviation in the data window, is defined.

3 The maximum difference, d_{max}, between the scalar components of two consecutive segments, $X_m(i)$ and $X_m(j)$, is computed as

$$d_{max} = \max_{k=0,\ldots,m-1}(|x(i+k) - x(j+k)|) \tag{3.7}$$

4 For each $X_m(i)$, the event $d_{max} < r$ is defined as a match, and a count of such matches is denoted by A_i. The probability of the matches, $A_i^m(r)$, for $X_m(i)$ is calculated as

$$A_i^m(r) = \frac{A_i}{N-m-1} \tag{3.8}$$

Note: The denominator, $N-m-1$, ensures that the segment $X_{m+1}(i)$ can be included in the computation of the probability.

5 Then, the sum of the probability of matches for all segments, Φ, is defined as

$$\Phi^m(r) = \frac{\sum_{i=1}^{N-m} A_i^m(r)}{N-m} \tag{3.9}$$

6 The embedding dimension, m, is increased to $m+1$, and Step 1 to Step 5 are repeated; the sum of the probability of matches for all segments when $m = m+1$ are defined as Ψ, and the sample entropy is computed as

$$SampEn(m,r) = ln(\frac{\Phi}{\Psi}) \tag{3.10}$$

7 Sample entropy for x is computed such that one *SampEn* value is obtained for each data window.

Wilcoxon test, sometimes referred to as the Wilcoxon signed rank test (Campbell and Swinscow, 2009); the normality of the measures were tested using the Lilliefors test, such that the normally distributed measures were compared using the parametric paired t-test, and the measures not normally distributed were tested using the nonparametric Wilcoxon test. The segmented measures from the male subjects were also statistically compared to those from the female subjects, using either the parametric two-sample t-test for normally distributed data, or the nonparametric Mann-Whitney-U test for data not normally distributed. A statistical significance level of 0.01 was assumed in all statistical

Algorithm 2: Permutation Entropy

1 A windowed signal, x, of length N is segmented using an embedding dimension, m, to create $(N - m + 1)$ segments. Each such segment, X_m, is of length m. In this work, m was defined as $m = 6$.

2 The elements in X_m are ranked into ascending order,
$x(n + j_1 - 1) \leq x(n + j_2 - 1) \leq \ldots \leq x(n + j_m - 1)$ where j is the time index of each data point, $x(n)$, in x. Note that if $x(n + j_1 - 1) = x(n + j_2 - 1)$, the segments are ordered in accordance with their time indices.

3 The time indices of the re-ordered vectors are then used to convert each segment into a symbol

$$A(n) = (j_1, j_2, \ldots, j_m) \tag{3.11}$$

4 The total number of unique symbols is denoted as k, and the relative frequency of each unique symbol is found as P_n.

5 Permutation entropy (PE) is computed as the Shannon entropy of the relative frequencies of the unique symbols,

$$PE(m) = -\sum_{n=1}^{k} P_n ln P_n \tag{3.12}$$

6 The computed $PermEn$ is then normalised to enable comparisons with results from signals of different lengths. The normalised PE is denoted by $PermEn$, and is given by

$$PermEn = \frac{PE(m)}{ln(m!)} \tag{3.13}$$

tests.

The Lilliefors test assesses how closely the distribution of a dataset is to a normal distribution (Machiwal and Jha, 2012). The paired t-test tests the null hypothesis that there is no difference between the means of paired datasets, whilst the two-sample t-test assesses the standard deviation of two groups to determine whether the two groups come from the same distribution (Campbell and Swinscow, 2009). The Wilcoxon test ranks the differences between paired samples from two groups and tests the null hypothesis that the differences between the paired samples have a mean of zero, whereas the Mann-Whitney-U test ranks all data within two groups, and assesses the ranks to determine whether the two groups are drawn from the same population (Campbell and Swinscow, 2009).

3.4 Results

The means and standard deviations of the computed *HR*, *SDNN*, *nLF*, *nHF*, *SampEn* and *PermEn* from the males are displayed in the second and third columns of Table 3.1. The fourth column shows the *p*-values indicating the statistical significances of the comparisons between the measures from the rest and arithmetic epochs, and the fifth column displays the means of the percentage changes in the computed measures from the rest epoch to the arithmetic epoch, where increases are displayed in green and decreases are shown in red. The corresponding results from the females are displayed in Table 3.2, and Table 3.3 shows the *p*-values computed from the comparisons of the *HR*, *SDNN*, *nLF*, *nHF*, *SampEn* and *PermEn* from the male and female subjects.

Statistically significant *p*-values (≤ 0.01) are shown in bold; those computed using the nonparametric Wilcoxon test or Mann-Whitney-U test are marked with an asterisk, *, and those computed using the parametric paired t-test or the two-sample t-test are displayed without an asterisk.

TABLE 3.1: Computed measures from the male subjects. Statistically significant *p*-values (*p*-value ≤ 0.01) are shown in bold; those computed using the Wilcoxon test are marked with an asterisk, *, and those computed using the paired t-test are displayed without an asterisk.

Measure	Epoch		*p*-value	Mean of Percentage Changes (%)
	Rest	Arithmetic		
HR (bpm)	74.24±13.72	90.05±18.90	**0.008**	+22.12
SDNN (ms)	44.87±20.99	54.18±16.99	0.043	+32.40
nLF	0.59±0.20	0.72±0.097	0.041	+35.18
nHF	0.41±0.19	0.28±0.094	0.040	-17.54
SampEn	0.72±0.13	0.61±0.14	0.024	-14.55
PermEn	0.50±0.045	0.55±0.041	0.017	+8.80

TABLE 3.2: Computed measures from the female subjects. Statistical *p*-values computed using the Wilcoxon test are marked with an asterisk, *, and those computed using the paired t-test are displayed without an asterisk.

Measure	Epoch		*p*-value	Mean of Percentage Changes (%)
	Rest	Arithmetic		
HR (bpm)	82.46±12.62	89.95±17.45	0.38*	+9.97
SDNN (ms)	56.06±21.08	43.56±11.25	0.076	-14.81
nLF	0.77±0.11	0.71±0.10	0.084*	-7.40
nHF	0.22±0.10	0.29±0.10	0.065*	+39.64
SampEn	0.60±0.088	0.64±0.079	0.13	+6.56
PermEn	0.50±$\leq$ 0.010	0.52±0.035	0.18	+4.69

TABLE 3.3: Statistical *p*-values from the comparisons of the computed measures from the male and female subjects; those computed using the Mann-Whitney-U test are marked with an asterisk, *, and those computed using the two-sample t-test are displayed without an asterisk.

Measure	***p*-value**	
	Rest	**Arithmetic**
HR (bpm)	0.31*	0.99
SDNN (ms)	0.25	0.12
nLF	0.031*	0.80
nHF	0.021*	0.76
SampEn	0.026	0.64
PermEn	0.77	0.12

The measures computed from the male subjects, displayed in Table 3.1, show that only *HR* produced significantly different values from the rest and arithmetic epochs (p-value≤ 0.01), although *SampEn* and *PermEn* produced marginally significant p-values of 0.024 and 0.017 respectively. The measures of *HR*, *SDNN*, *nLF* and *PermEn* increased from the rest epoch to the arithmetic epoch with mean percentage changes of approximately +22%, +32%, +35% and +9% respectively, whilst *nHF* and *SampEn* decreased from the rest epoch to the arithmetic epochs with mean percentage changes of approximately -18% and -15% respectively. The biggest increase was therefore found for the *nLF* measure at $\approx$+35%, and the biggest decrease was found for the *nHF* measure at $\approx$-18%.

The results from the females, displayed in Table 3.2, show that none of the measures produced significantly different values from the rest to arithmetic epochs. Furthermore, in contrast to the males, the measures of *HR*, *nHF*, *SampEn* and *PermEn* all increased from the rest epoch to the arithmetic epoch with approximate mean percentage increases of +10%, +40%, +7% and +5% respectively, whilst *SDNN* and *nLF* decreased from the rest epoch to the arithmetic epoch, with approximate mean percentage decreases of -15% and -7% respectively. Therefore, the biggest increase was found for the *nHF* measure at $\approx$+40%, and the biggest decrease was found for the *SDNN* measure at $\approx$-15%.

The p-values displayed in Table 3.3 show that the *nLF*, *nHF* and *SampEn* measures from the rest epochs revealed large but statistically nonsignificant differences between the male and female subjects, with p-values of 0.076, 0.084 and 0.065 respectively, whilst the measures from the arithmetic epoch produced comparatively large p-values in the range of 0.12 to 0.99.

For visual assistance in the assessment of the differences between the males and females during the rest and arithmetic epochs, Figures 3.2 to 3.7 display boxplots of the computed measures of *HR*,

SDNN, *nLF*, *nHF*, *SampEn* and *PermEn*. Figure 3.2 contains annotations to explain the structure of a boxplot.

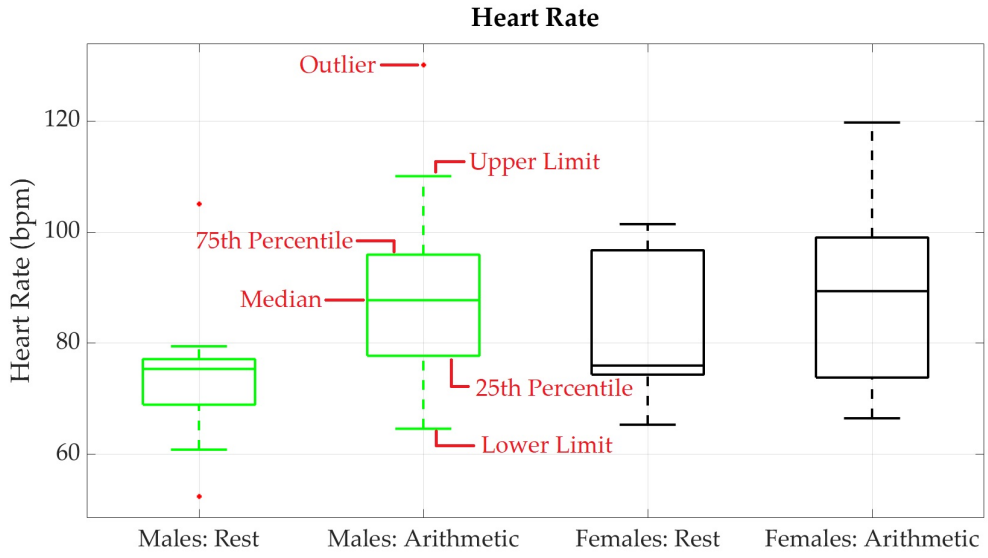


FIGURE 3.2: Boxplots of the heart rate responses during the test epochs of rest and arithmetic.

3.5 Discussion

The measures of *HR*, *SDNN*, *nLF*, *nHF*, *SampEn* and *PermEn* computed from 10 males and 10 females have demonstrated distinct trends in HRV following 15 minutes of sitting rest and 15 minutes of a mental arithmetic test, highlighting often missed sex differences in HRV dynamics. All of the females were in the follicular phase of their menstrual cycle, and these results are the first to show sex differences in the *SampEn* and *PermEn* responses to stress in HRV.

The finding of a statistically significant increase in *HR* amongst the males from the rest to arithmetic epochs (p-value=0.008), shown in Table 3.1, alongside the finding of a nonsignificant increase in *HR* amongst the females (p-value=0.38) provides conclusive evidence that a large rise in HR, elicited by mental stress is not universal, proving that whilst *HR* is perhaps a good measure of stress in males, it is not in females. This is reflected in a mean percentage rise in *HR* from the females of ≈+10%, which was less than half of that seen in the males, at ≈+22%. *The HR results from this study confirm that sex-differences exist in the cardiac response to an identical mental stressor, which therefore calls for further investigation into how stress is understood to affect cardiac dynamics in females during the follicular phase of their menstrual cycle, and in addition, the luteal phase of the menstrual cycle, and during menstruation.*

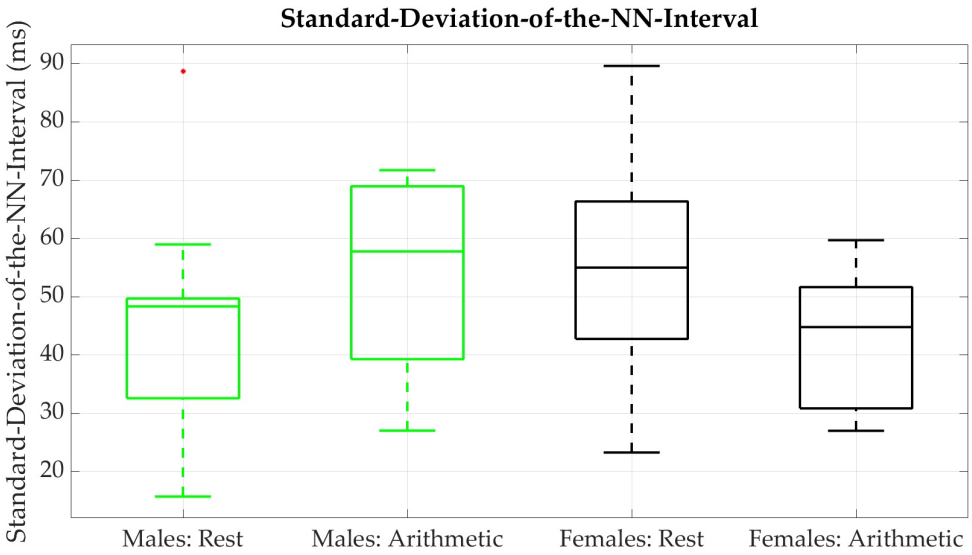


FIGURE 3.3: Boxplots of the standard-deviation-of-the-NN-interval responses during the test epochs of rest and arithmetic.

The immersion of a hand in hot water is a physiological stressor, and in Tousignant-Laflamme et al. (2005) the cardiac responses to the immersion of a hand in hot water in 19 males and 20 females was assessed and it was found that whilst the males produced a significant positive correlation between heat intensity and their cardiac activity, with a correlation coefficient of 0.77 (p-value$\leq$ 0.01), the females produced a nonsignificant weak correlation coefficient of -0.02. Although the authors of Tousignant-Laflamme et al. (2005) did not obtain information regarding the menstrual cycle phases of their subjects, the mean ages of the subjects were reported as 23.1$\pm$4.4 years and 27.5$\pm$7.2 years respectively for the males and females, indicating that it is highly likely that the female subjects were premenopausal, and would have experienced a large exposure to oestrogens.

The effects of oestrogens on catecholamine hormones, in reference to *HR*, have been widely investigated in mammals. In a review on the effects of oestrogens on the response to stress, Ueyama et al. (2008), it was found that the removal of the ovaries in rodents enabled greater increases in *HR*, compared to the increases in *HR* seen in rodents whose ovaries had been removed, but were supplemented with oestrogens. It was concluded in Ueyama et al. (2008) that oestrogens reduced the flow of sympathetic stress hormones from the central nervous system. It was also reported in Ueyama et al. (2008) that oestrogens reduced the reactivity of the heart to catecholamines, in order to protect the heart from the effects of stress hormones. The extension of these findings to humans would explain why the females in this present study exhibited smaller increases in *HR* during the mental arithmetic

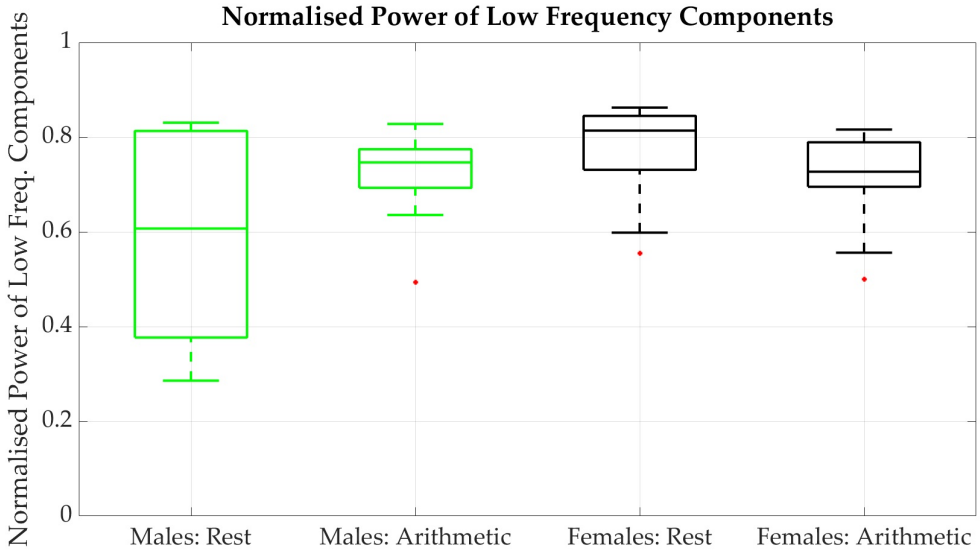


FIGURE 3.4: Boxplots of the power of the low-frequency components of heart rate variability during the test epochs of rest and arithmetic.

test, compared to the males.

The *SDNN* results also reveal an undeniable sex difference, whereby mental stress induced a large increase in *SDNN* in the males (≈+32%), but induced a decrease in the females (≈-15%). As *SDNN* is a measure of variance in HRV, these results suggest that the HRV of the females approached a steady state value during the mental arithmetic test. In contrast, stress increased the variance of the HRV in the males, which indicates that their *HR* did not reach a steady state, and continued to rise throughout the arithmetic epoch (Castrillón et al., 2017). Of note, a decrease in *SDNN* is the expected response to a stressor, and would typically represent regular HRV (Kim et al., 2018).

Furthermore, the nonsignificant *p*-values produced from the comparison of the *SDNN* values from the rest to arithmetic epochs of 0.043 and 0.076, for the males and females respectively, show that *SDNN* in this study was not an accurate identifier of stress. This less than optimal performance of *SDNN* could be explained by the length of data analysed since it has been suggested that *SDNN* performs best in identifying changes in cardiac activity which contains circadian dynamics (Malik et al., 1996).

The results from the spectral analyses in this study also reveal sex differences. Not only were there contrasting changes in the *nLF* and *nHF* responses from the males and females from the rest to arithmetic epochs (in which the males exhibited the expected stress-induced increase in *nLF* and the females exhibited a lesser known stress-induced increase in *nHF*), but the large rise in *nLF* in the males

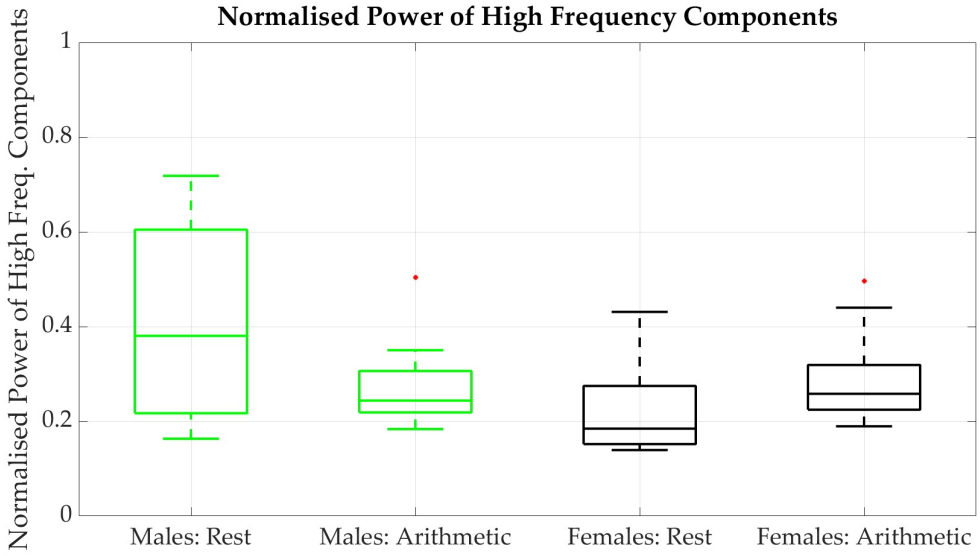


FIGURE 3.5: Boxplots of the power of the high-frequency components of heart rate variability during the test epochs of rest and arithmetic.

of ≈+35% was comparable to the rise in *nHF* in the females of ≈+40%. *The stress-induced increase in the power of the high frequency component of HRV in the females is contrary to the conventional understanding that stress induces a rise in the power of the low frequency component of HRV.* This finding exemplifies the need to appreciate sex differences and analyse HRV from males and young females separately, as the conflation of the male and female responses to stress could lead to the possible misinterpretation of experimental results. For example, a key paper, Berntson et al. (1994), published over two decades ago, assessed the reciprocity of SNS and PNS activities, and reported that no correlation was found between the two autonomic branches. However, the study was published at a time of little awareness of sex differences in the stress response, so although the study cohort was made up entirely of females, the conclusions drawn from the study were formed following the comparison of the results with those from studies with entirely male cohorts. Perhaps ironically, Berntson et al. verified that the subjects recruited for their study were in the follicular phase of the menstrual cycle, although this was not to assess the effects of oestrogens, but to avoid the testing of a potentially pregnant subject. Conflations of the male and female responses to stress, as in Berntson et al. (1994), could therefore undeniably cause the misinterpretation of experimental findings.

Furthermore, it cannot be ignored that the controversy over the physiological interpretations of the LF and the HF components of HRV remain largely unresolved. In summary, the LF component has been speculated to:

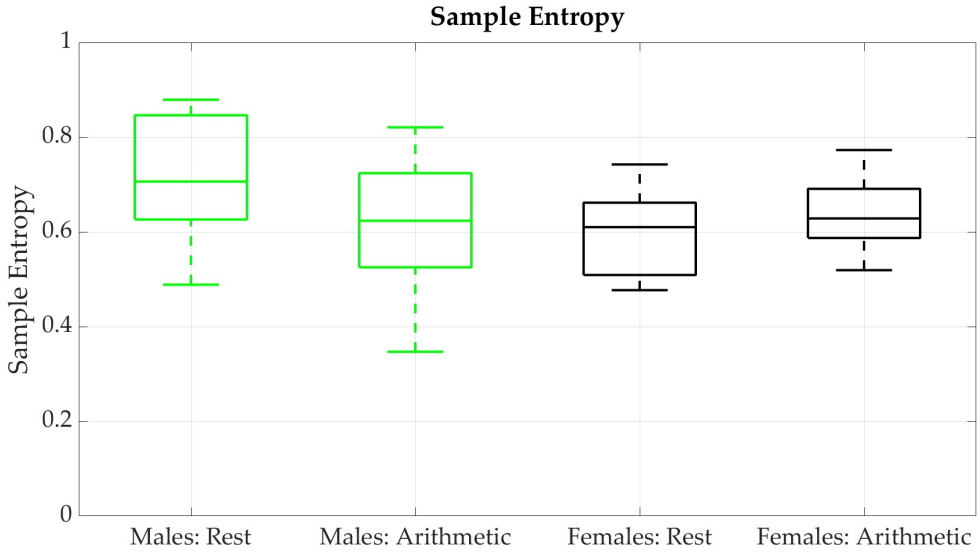


FIGURE 3.6: Boxplots of the sample entropy responses during the test epochs of rest and arithmetic.

(i) Represent the modulation of the sympathetic and parasympathetic nervous systems (Malik et al., 1996).

(ii) Be influenced by the frequencies of slow breathing (Brown et al., 1993).

(iii) Be influenced by the frequencies of the contraction of blood vessels (Kenwright et al., 2008).

Similarly, the high frequency component of HRV has also been suggested to be influenced by typical breathing frequencies (Kenwright et al., 2008). Thus, the physiological interpretation of the LF and HF components of HRV cannot be verified without conducting a thorough neurological and endocrinological study in which respiration is also controlled (Stacey et al., 2018).

The results from the nonlinear analyses also reveal sex differences in the dynamics of HRV in response to stress. In Bornas et al. (2006), Vuksanović and Gal (2007), Williamon et al. (2013) and Chanwimalueang et al. (2016), it was reported that mental stress induced decreases in the long-term univariate entropy of cardiac signals, however, the results presented in Table 3.2 establish that stress caused increases in both the *SampEn* and *PermEn* of the HRV from the female subjects, and an increase in the *PermEn* of the HRV in the males. The stress-induced increase in the irregularity of the HRV from the females further confirm that they experienced a stress response which differed from the stress response experienced by the males. These results also contradict the complexity-loss theory described in Goldberger et al. (2002), which could support the view from Goldberger et al. that entropies are not true measures of physiological complexity.

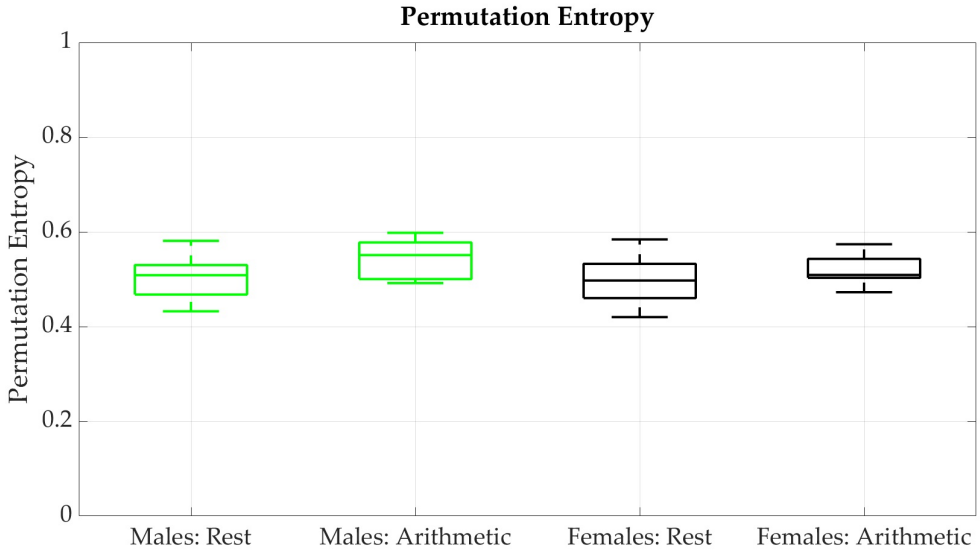


FIGURE 3.7: Boxplots of the permutation entropy responses during the test epochs of rest and arithmetic.

Noticeably, stress induced relatively small increases in *PermEn* in both the males and females, at ≈+9% and ≈+5%, respectively, whilst *SampEn* decreased in the males by ≈-15%, and increased in the females, by ≈+7%. *These findings show a stress-induced rise in long-term univariate HRV entropy, and also differences in the sample entropy computed from males and females*. Furthermore, these findings also show that permutation entropy did not behave in an identical manner to sample entropy. The difference in the directional changes of sample entropy and permutation entropy in the males suggest that the two methods were not measuring the same characteristic. This is perhaps not surprising given the aforementioned controversy in determining whether entropies measure complexity (Goldberger et al., 2002). Intriguingly, the originators of *SampEn* described the metric as a measure of regularity (Pincus, 1991; Richman and Moorman, 2000), and although the originators of *PermEn* also introduced their metric as being able to distinguish signal regularity from irregularity, they introduced *PermEn* as a metric which is able track system dynamics (Bandt and Pompe, 2002; Dimitriev et al., 2016).

The sex-differences in the stress response also have potential explanations, as a behavioural stress response which is specific to females has been suggested in Taylor et al. (2000). It was hypothesised that whilst males experience the conventional *fight-or-flight* response to all stressors, females can exhibit a *tend-and-befriend* response, in which social coping could be employed to combat stress (Taylor et al., 2000). The tend-and-befriend response was posited to be driven by oestrogens, and the nurturing hormone, oxytocin (Taylor et al., 2000). Although the oxytocin levels of the female subjects

could not be assessed or gauged in this present study, the measurement of the female subjects in the follicular phase of their menstrual cycles enables an assumption that the subjects were exposed to high levels of oestrogens. The results from this study therefore reveal, again for the first time, cardiac trends which could characterise the behavioural tend-and-befriend response to the stress induced by the arithmetic test, that is, a small increase in HR, a decrease in nLF, an increase in nHF, and increases in HRV regularity and dynamics, as shown by the mean of the percentage changes of the measures shown in Table 3.2. In contrast, the stress-induced percentage changes displayed in Table 3.1, could be used to characterise the fight-or-flight response, by a large increase in HR, an increase in nLF, a decrease in nHF, and a decrease in HRV regularity, and an increase in HRV dynamics; these trends are illustrated in Figure 3.8.

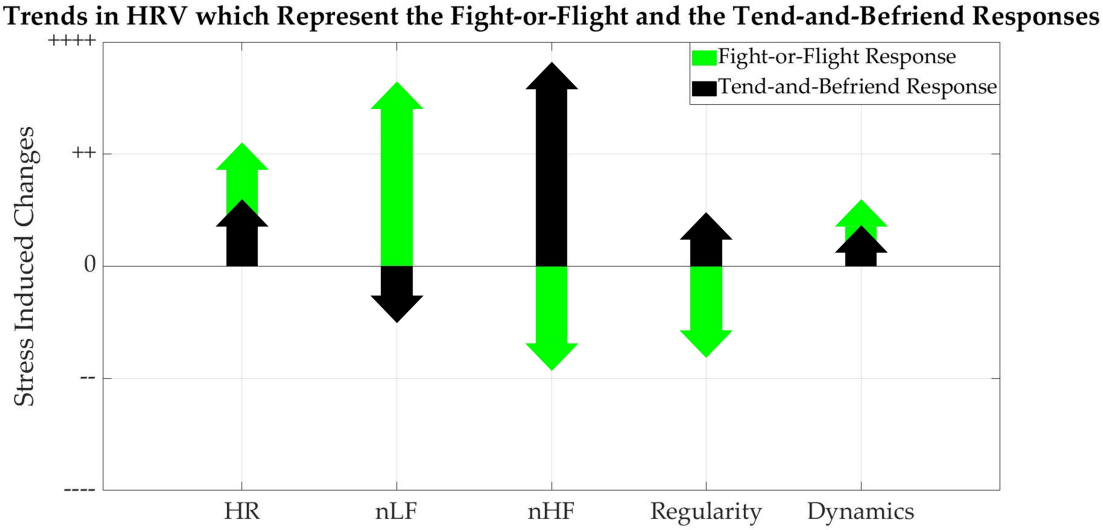


FIGURE 3.8: Proposed characterisations of heart rate variability for the manifestations of the fight-or-flight and the behavioural tend-and-befriend responses to stress.

In comparison to corresponding studies of mental stress in humans, mental stress has been repeatedly found to cause inconsistent changes in the LF power of HRV; an expected increase in LF power was reported in Cohen et al. (1998), negligible changes in the LF power of HRV were reported in Vuksanović and Gal (2007), and a stress-induced decrease in LF power was reported in Dimitriev et al. (2019). A summary of these studies can be found in Table 3.4; it can therefore be seen that the spectral characterisation of HRV is not a reliable means to identify stress, often producing inconsistent results.

TABLE 3.4: Summary of corresponding heart rate variability based studies of mental stress.

Corresponding Stress Studies	
Study	Summary
Cohen et al. (1998):	Nine subjects with post traumatic stress disorder and nine healthy subjects participated in a study in which they were asked to recount a previous trauma. The subjects' HRV signals were assessed using spectral analyses. **RESULTS:** The recounting of a previous trauma induced an increase in the percentage of power in the LF band of HRV, and a decrease in the percentage of power in the HF band of HRV.
Vuksanović and Gal (2007):	Twenty-three healthy subjects participated in a mental arithmetic task. The subject's HRV were analysed using linear and nonlinear methods. **RESULTS:** Mental arithmetic was found to produce negligible change in LF, but induced a decrease in HF in 10 subjects and an increase in HF in 13 subjects. There was a decrease in sample entropy in 10 subjects, but negligible change in sample entropy in the remaining 13 subjects.
Dimitriev et al. (2019):	One-hundred and sixty-two healthy subjects participated in a mental arithmetic task. The subjects' HRV were analysed using temporal and spectral measures. **RESULTS:** Mental arithmetic induced increases in HR and decreases in SDNN, the power of the LF band of HRV, and the power of the HF band of HRV.

The results produced in this chapter provide large scope for further study. For example, the use of the calendar method to determine the menstrual cycle phases of females is convenient, but not always accurate. In future investigations of the female response to stress, hormonal assays should be acquired so as to quantitatively verify the female subjects' levels of oestrogens, in addition to the expansion of the female cohort to include females in the later half of the menstrual cycle, the luteal phase, to enable a fuller understanding of the female stress response.

However, in addition to establishing sex differences in the cardiac stress response, the results shown in Tables 3.1 and 3.2 demonstrate that *HR* was the only measure which was able to discern between the rest and arithmetic epochs in the males, with statistical significance, whilst in the females, none of the measures were able to do so. Therefore in reference to the first objective of this PhD research, *to assess the accuracy of the state-of-the-art measures of stress from heart rate variability signals*, it is clear that there is a need to develop a quantitative measure of stress which is effective for both males and

females.

3.6 Conclusion

The long-overlooked sex differences in the cardiac response to stress, through temporal, spectral and nonlinear heart rate variability (HRV) based signatures have been investigated, in signals recorded from 10 males and 10 females in the follicular phase of the menstrual cycle. The signals were recorded during 15 minutes of sitting rest, and 15 minutes of a mental arithmetic test, and heart rate, standard-deviation-of-the-NN-interval, the normalised powers of the low frequency and high frequency components, sample entropy, and permutation entropy were computed from the subjects' HRV and statistically compared. The normalised spectral powers and sample entropy have revealed marginally significant differences (p-values of 0.031, 0.021 and 0.026, respectively) between the males and females during rest. In the males, mental stress was found to induce large increases in heart rate, the standard-deviation-of-the-NN-interval, the power of the low frequency components of HRV and decreases in the power of the high frequency components of HRV and sample entropy. In the females, mental stress was found to induce decreases in the standard-deviation-of-the-NN-interval and the power of the low frequency components of HRV, a smaller increase in heart rate than in the males, and increases in the power of the high frequency components of HRV and sample entropy. Stress induced comparable increases in permutation entropy in both the males and females, at +9% and +5% respectively.

The differences between the HRV signatures from the males and females suggest that oestrogens modulate the cardiac response to stress in females. Not only can the *results presented here challenge the development of scientific hypotheses which do not account for sex differences*, but the stress-induced *increases* in the long-term univariate sample entropy of HRV (seen in the females) challenge the common assumption that sample entropy is a reliable measure of signal complexity which always decreases in HRV in response to stress (Bornas et al., 2006; Vuksanović and Gal, 2007; Williamon et al., 2013; Chanwimalueang et al., 2016).

The results from this pilot study have established that the stress-induced cardiac trends which are commonly reported, such as increases in heart rate and the power of the low frequency components of HRV, are the cardiac manifestations of the *fight-or-flight* response in males, whereas smaller increases in heart rate and large increases in the power of the high frequency components of HRV

may characterise the behavioural *tend-and-befriend* response to stress seen in females. The results presented here also demonstrate the need to develop an HRV-based stress metric which is able to perform well in both males and females.

Chapter 4

The Classification Angle Framework

The need for a reliable quantitative HRV-based measure of stress is undeniable.

Results from the previous chapter, presented in Tables 3.1 and 3.2, have highlighted the following limitations of the state-of-the-art HRV-based stress measures:

Limitation Heart rate was found to only discern between states of rest and mental stress with statistical significance (p-values ≤ 0.01) in male subjects, and not in females. Also, *HR* was not able to indicate the activities of the SNS and PNS due to its one-dimensionality.

Limitation The standard-deviation-of-the-NN-interval was found to not discern between states of rest and mental stress with any statistical significance. Also, *SDNN* was not able to indicate the individual activities of the SNS and PNS.

Limitation The physiological interpretation of the power of the low frequency band of HRV remains unclear (Malik et al., 1996), rendering its use controversial.

Limitation The physiological interpretation of the power of the high frequency band of HRV remains unclear (Malik et al., 1996), rendering its use controversial.

Limitation Long-term univariate sample entropy has been found to produce unconventional and contradictory trends in the identification of stress, by decreasing in the males and increasing in the females, and furthermore, *SampEn* could not indicate the activities of the SNS and PNS.

Limitation Permutation entropy increased in both males and females during mental stress, but could not indicate the activities of the SNS and PNS due to its one-dimensionality.

It has also been recommended that an HRV series should be at least 5-minutes in length to compute *HR*, *SDNN*, *nLF* and *nHF*, as 5-minutes is considered a suitable standardised length which is able to

capture at least ten times the wavelength of the component being investigated (Malik et al., 1996). However, more than 24 hours of data would ideally be required to compute meaningful *SDNN* (Malik et al., 1996).

This chapter presents a novel algorithmic framework, introduced in Adjei et al. (2019), which accurately identifies stress from heart rate variability signals, and therefore, achieves the second objective of this PhD research, 'to propose a novel algorithmic framework which accurately identifies stress from heart rate variability signals, and statistically show that the performance of the proposed method surpasses those of the state-of-art measures'. This is achieved by overcoming the above listed limitations of *HR*, *SDNN*, *nLF*, *nHF*, *SampEn* and *PermEn*.

4.1 Chapter Summary

The powers of the low frequency and high frequency components of heart rate variability series have become popular measures of stress and the respective activities of the sympathetic and the parasympathetic nervous systems. However, the widely adopted, but questionable physiological interpretations of the low frequency and high frequency components of heart rate variability in the assessment of sympathetic and parasympathetic balance put under serious scrutiny any stress assessment which employs the components. To avoid these controversies, this chapter introduces the novel Classification Angle framework, which yields a family of metrics which are able to quantify cardiac dynamics in three-dimensions. This is achieved using a finite-difference plot which displays successive rates of change of heart rate variability; the framework is demonstrated to provide sufficient degrees of freedom to determine cardiac deceleration and/or acceleration.

The robustness and accuracy of the Classification Angle framework is verified using the dataset of heart rate variability signals from 10 males and 10 females recorded during the standardised stress test, consisting of an epoch of rest and an epoch of mental arithmetic. This dataset was introduced in Chapter 3.

Comparative statistical testing demonstrated that unlike the existing low frequency and high frequency components of heart rate variability, the Classification Angle metrics were capable of distinguishing mental stress epochs from the epochs of rest, with statistical significance (p-value ≤ 0.01). The Classification Angle results also revealed that mental stress induced a decrease in cardiac deceleration and an increase in cardiac acceleration (representing a decrease in parasympathetic tone, and

an increase in sympathetic tone, respectively) in the males, but not in the females. Finally, the analyses and the experimental results provide conclusive evidence that the nonlinear Classification Angle framework quantifies cardiac activity from heart rate variability through the resolution of three critical obstacles to the state-of-the-art in heart rate variability stress assessments:
(i) The Classification Angle framework is not based on controversial assumptions of balance between the SNS and PNS, and hence does not assume balance between the powers of the low frequency and high frequency components of HRV.
(ii) The temporal resolution of the Classification Angle framework, when estimating parasympathetic dominance is as little as ten seconds of a heart rate variability series, while only one minute is required to estimate sympathetic dominance.
(iii) Unlike the analyses of the powers of the low and high frequency components of heart rate variability, the Classification Angle framework does not require the prohibitive assumption of signal stationarity.
The Classification Angle framework is unique in offering stress analysis based on heart rate variability in three-dimensions.

4.2 Chapter Background

As described in the previous chapters, the endeavour to objectively discern states of stress from rest using only HRV has long been a subject of research, since many qualitative stress assessments, such as questionnaires, are inherently subjective. The subjectivity of such qualitative measures restricts their use in practical applications. For example, a subjective measure of stress cannot be relied upon to assess a soldier's mental state before action, neither can a subjective measure be used to assess if subjects with poor verbalisation, such as the very young, are experiencing excessive levels of stress. The ability to objectively and quantitatively identify stress states would therefore facilitate the deployment of tools for the monitoring and management of stress in everyday life.

However, the unsatisfactory attempts to *quantitatively* identify stress, from physiological signals using temporal (Castrillón et al., 2017), spectral (Montano et al., 1994) and nonlinear (Vuksanović and Gal, 2007) measures, reflect the complexity of the human stress response, the manifestations of which are yet to be fully understood. As described in Chapter 1, Section 1.1.2 on page 16, since the publication of Cannon (1915), the stress response has been largely attributed to the sympathetic energisation of the body; for example, heart rate, the most basic measure of stress, is naturally expected to rise,

and was indeed found to increase in both males and females in the study detailed in Chapter 3 (Adjei et al., 2018). However, it is also well established that the responses to stress are not exclusively sympathetic, whereby parasympathetic reactions to stress include the production of tears and the emptying of the bladder (Berntson et al., 1991; Avnon et al., 2004). It is therefore advantageous for a measure of stress to assess both SNS and PNS activities separately.

The spectral analyses of HRV to determine SNS and PNS dynamics are amongst the most widely used techniques in the quantitative assessment of stress. As described in Chapter 2, the spectral analyses have been inspired by the known differences in the speeds of parasympathetic and sympathetic nerves (parasympathetic nerves elicit a much faster ($\leq$ 1 second) response than sympathetic nerves ($\geq$ 5 seconds) (Nunan et al., 2010)), and by experimental findings, such as those published in Montano et al. (1994), in which orthostatic tilt was found to correlate positively with LF power, and negatively with HF power. However, to restate from Chapter 2, despite concerns regarding the validity of the use of the LF and HF powers to assess autonomic dynamics (Eckberg, 1997), the LF and HF powers were quickly accepted to become popular measures of stress. This popularity continued until the limitations of the physiological interpretations of the LF and HF powers were critiqued in Billman (2013). To reiterate, it was highlighted in Billman (2013) that the SNS was not the sole contributor to the variability of the LF peak, and that the PNS was not the sole contributor to the HF peak in HRV power spectra. These findings have led to the realisation that the use of the LF and HF powers to assess SNS and PNS activities could be erroneous and inadvisable, and as a result nonlinear measures of stress have increased in popularity, such as the use of entropies. To reiterate, this rise in popularity can be attributed to findings that physiological signals, particularly those from unhealthy subjects, can contain nonlinearities (Gautama et al., 2004).

Adjei et al. (2018) showed stress-induced increases in long-term univariate HRV sample entropy, contrary to the stress-induced reductions in sample entropy reported in Bornas et al. (2006), Vuksanović and Gal (2007), Williamon et al. (2013) and Chanwimalueang et al. (2016). However, although entropies may be able to identify instances of stress, they can be computationally inefficient, or leave poor temporal resolution. They also provide no indication as to the physiological mechanisms which lead to the stress response, that is, SNS or PNS activation.

Furthermore, other nonlinear symbolic measures of stress, such as that introduced in Porta et al. (2007), in which the occurrence of unique symbols (or patterns) in HRV were counted, can be restricted by the length of symbol analysed. More precisely, the length of the symbol analysed may

not account for long-range SNS dynamics as well as shorter range PNS dynamics (Porta et al., 2007). Similarly, model-based algorithms to assess SNS and PNS dynamics are limited by their dependence upon the fit of a model (Valenza et al., 2018).

In light of the above described inaccuracies associated with the current measures of stress, this chapter details an investigation into the usefulness of second-order-difference-plots in the assessment of cardiac deceleration and acceleration, from HRV signals. The developed framework aims to assess the spread of the finite-differences in HRV series, from which, a family of metrics collectively termed Classification Angle (ClassA) is introduced. A multiscale analysis is employed to account for the long-range variations of SNS activity, and the short-range variations of PNS activity, thus ensuring that the metrics pertaining to SNS and PNS activity are not assumed to be balanced. Without an assumption of balance, the ClassA metrics are better able to provide insight into the separate dynamics of the SNS and PNS. The Classification Angle framework is capable of separating different stress states, and is comprised of four metrics, three of which offer a three-dimensional assessment of stress. The four metrics are:

Real Angle Sum (*RAS*) The mean of the sum of the angles between HRV rates of change and the abscissa of a scatter plot of finite differences.

Cardiac Deceleration Proportion (P^{Q1}) The proportion of HRV rates of change which fall into the first quadrant of a scatter plot of finite-differences.

Proportion of HRV Balance ($P^{Q2,4}$) The proportion of HRV rates of change which fall into the second and fourth quadrants of a scatter plot of finite differences.

Cardiac Acceleration Proportion (P^{Q3}) The proportion of HRV rates of change which fall into the third quadrant of a scatter plot of finite differences, computed from a higher temporal scale than that of P^{Q1}.

For rigour, the performance of the ClassA metrics in distinguishing between rest and stress is investigated using the HRV dataset introduced in Chapter 3, enabling a direct comparison with the state-of-the-art measures. This serves to conclusively demonstrate the statistical performance of ClassA in the ability to discern stress from rest; two of the ClassA metrics are shown to be significantly more accurate in the characterisation of stress than the current state-of-the-art measures of the *SDNN*, *nLF*, *nHF*, *SampEn* and *PermEn*.

4.3 Classification Angle

4.3.1 Second-Order-Difference-Plots

The Classification Angle framework is based on a scatter plot of HRV finite-differences, a second-order-difference-plot (SODP), first introduced in Cohen et al. (1996). The original SODP was a plot of $(x(n+2) - x(n+1))$ against $(x(n+1) - x(n))$, where $x(n)$ represented data points in an HRV series, x, (Cohen et al., 1996). The SODP is therefore a graph of successive HRV rates of change plotted against one another, and can be interpreted as displaying the correlation between successive HRV rates (Cohen et al., 1996; Kamath, 2012; Santos et al., 2015). The SODP was first introduced as a means to assess the degree of variability within HRV signals, and was found to be more robust than the more commonly used Poincaré plot (Cohen et al., 1996).

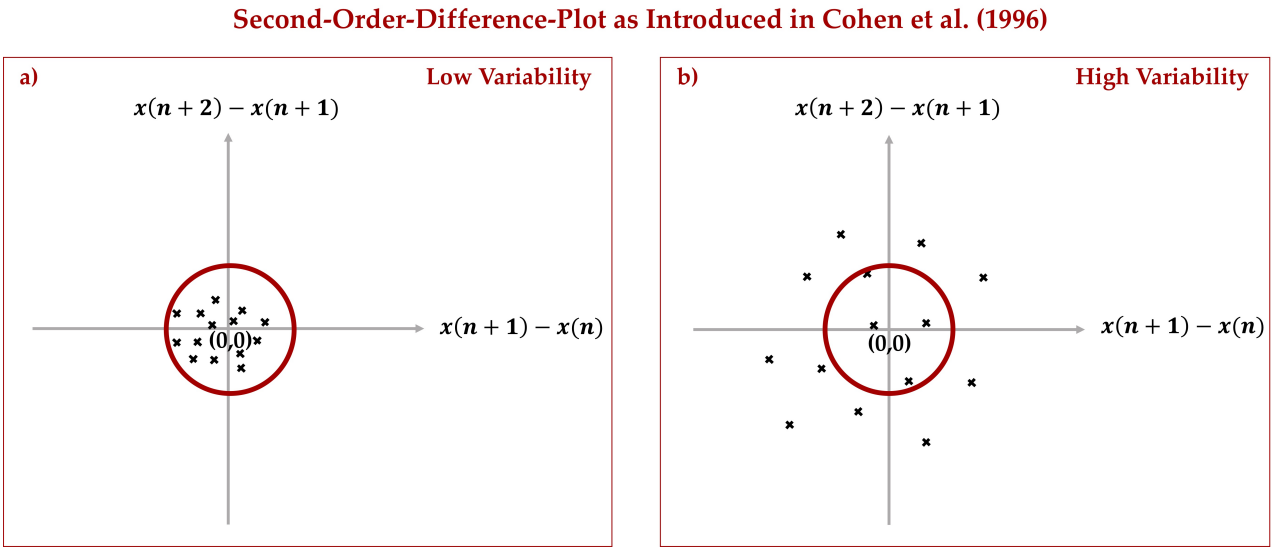


FIGURE 4.1: The second-order-difference-plot as introduced in Cohen et al. (1996), in which the central tendency measure was used to quantify the dispersion of data points. The central tendency measure was defined as the proportion of data points falling within a specified radius around the origin of the plot (Cohen et al., 1996). The red circles in the second-order-difference-plots indicate a specified radius; plot **a** illustrates the second-order-difference plot of an heart rate variability signal with low variability, whilst plot **b** illustrates the second-order-difference plot of an heart rate variability signal with high variability. It was found in Cohen et al. (1996) that the heart rate variability signals of congestive heart failure patients exhibited greater variability than those of healthy controls.

The authors of Cohen et al. (1996) computed second-order-difference-plots of HRV signals from 54 subjects, 26 of which were congestive heart failure patients, and 28 were healthy controls, and found that the data points within the SODPs of the congestive heart failure patients exhibited more variability than those of the healthy controls. Figure 4.1 displays an SODP, as introduced in Cohen et al.

(1996). The authors also introduced the central tendency measure to quantify an SODP. The central tendency measure is a measure of the proportion of data points which fall within a particular radius around the origin of an SODP (Cohen et al., 1996); a radius of 0.1 was employed in Cohen et al. (1996). Extensions of the SODP and the central tendency measure have been employed to characterise other physiological signals, such as intracranial pressure signals (Cohen et al., 1996; Santamarta et al., 2012). Furthermore, the SODP is not limited by the use of standard Gaussian-based mathematical models, which are not guaranteed to provide the best descriptors of HRV dynamics (Cohen et al., 1996).

Also, given the potential for nonlinearities in HRV (Gautama et al., 2004), the SODP has the advantage that it is a nonlinear measure, whilst the spectral methods typically used in HRV-based stress analyses to compute the LF and HF powers are linear. To demonstrate the potential of SODPs in the analysis of physiological data, a summary of studies employing SODPs in the identification of pathologies can be found in Table 4.1. In a similar fashion to Cohen et al. (1996), Thuraisingham (2009) employed SODPs to classify the HRV signals of congestive heart failure patients from those of healthy subjects, and achieved an accuracy of 100%, whilst Martínez et al. (2015) employed SODPs to assess HRV signals so as to identify oncoming episodes of atrial fibrillation, and achieved an accuracy of 80%. The features of SODPs have also been applied in classification models to differentiate the HRV signals of healthy subjects from those of diabetics in Pachori et al. (2016). The application of SODPs in stress research is therefore unique.

Despite the applicability of the original SODP and the central tendency measure to nonlinear signals, their use in stress research would be limited by their one-dimension of analysis; the proportion of data points within an arbitrarily defined radius of an SODP could not offer separate indications of sympathetic and parasympathetic dominance.

However, the quadrants of an SODP, as illustrated in Figure 4.2 on page 77, can be interpreted physiologically (Kamath, 2012); the first quadrant represents cardiac deceleration (parasympathetic tone), the second and fourth quadrants represent a balance of increases and decreases in an HRV sequence, whilst the third quadrant represents cardiac acceleration (sympathetic tone) (Kamath, 2012). The physiological interpretation of the SODP quadrants at different temporal scales forms the basis of the ClassA framework.

The use of a data point dispersion method (although not an SODP) in the assessment of autonomic functioning is supported by findings in Makowiec et al. (2017). Makowiec et al. used a visualisation

TABLE 4.1: Summary of physiological studies employing second-order-difference-plots to distinguish between health and sickness.

Corresponding Stress Studies	
Study	Summary
Cohen et al. (1996):	The SODPs of the HRV signals from 28 healthy subjects and 26 congestive heart failure patients were compared. **RESULTS:** The SODPs from the healthy subjects showed less variability than the SODPs of the congestive heart failure patients.
Thuraisingham (2009):	The HRV signals of 36 healthy subjects, and 36 congestive heart failure patients were analysed. The scatter of data points in SODPs were used to classify the subjects as either healthy or patients with congestive heart failure. **RESULTS:** The SODP features produced an accuracy of 100% in the classification of the healthy subjects from the congestive heart failure patients.
Martínez et al. (2015):	The HRV signals of 46 atrial fibrillation patients were assessed using the scatter of data points in an SODP. The scatter of data points in the SODPs were used to characterise the signals before an episode of atrial fibrillation. **RESULTS:** The scatter of data points in SODPs were able to identify the signal epochs immediately prior to an episode of atrial fibrillation, with an accuracy of 80%.
Pachori et al. (2016):	The HRV signals of 15 healthy subjects, and 15 diabetics were analysed using five HRV parameters, including the elliptical area covered by the scatter of points in an SODP. The classification ability of the combined parameters was computed. **RESULTS:** A classification accuracy of over 95% was achieved in the classification of HRV from the healthy subjects and the diabetics.

network tool, which was similar to a Poincaré plot, to assess increments in HRV and autonomic drive, and reported that autonomic supervision of the heart caused larger increments in HRV when compared to the function of the heart in the absence of autonomic supervision.

4.3.2 Classification Angle Framework

Given the physiological interpretability offered by SODPs of HRV, and therefore the potential to assess sympathetic and parasympathetic dominances, an SODP was chosen as the methodology upon which to derive an accurate measure of stress. However, it was quickly realised that the SODP, as introduced in (Cohen et al., 1996), could be improved upon for stress analysis. The two features of

an SODP which were investigated for improvement were;

(i) The method of finite differencing employed in a plot.

(ii) The method of quantification employed in an SODP to enable the separate quantifications of sympathetic and parasympathetic dominance.

Improvement of Finite Differencing: Finite differencing is a means to estimate the first derivative of a signal (Mandic and Goh, 2009), and is commonly estimated using the first difference method of $(x(n+1) - x(n))$ (Cohen et al., 1996), where $x(n)$ is an HRV data point; however, the first difference method of plotting $(x(n+2) - x(n+1))$ against $(x(n+1) - x(n))$ is known to amplify low frequency noise occurring from data point to data point (Mandic and Goh, 2009).

The amplification of low frequency noise can be avoided through the use of alternative approximations of the first derivative; one such approximation is the three-point forward derivative approximation of $(4x(n+1) - 3x(n) - x(n+2))/2$ (Butt, 2010). By incorporating more data points into the approximation of the derivative, the error in the estimation of the derivative is reduced, as the low frequency noise occurring from data point to data point cannot be amplified (Mandic and Goh, 2009). In preliminary investigations of the ClassA framework, it was empirically found that the three-point forward derivative approximation was sufficient in producing an approximation of the derivative with a low error, without introducing computational inefficiency. For this reason, SODPs of $[(4x(n+2) - 3x(n+1) - x(n+3))/2]$ plotted against $[(4x(n+1) - 3x(n) - x(n+2))/2]$ were employed in the ClassA framework, as shown in Algorithm 3 on page 78.

Method of Quantification: In the quest to obtain separate assessments of the sympathetic and parasympathetic nervous systems, it was realised that an SODP would have to be analysed without an assumption of reciprocity between the SNS and PNS. This necessitated that the SNS and PNS dynamics were estimated from different SODPs. Thus, the ClassA framework analyses HRV series at two temporal scales, where one SODP is derived from HRV at a specific temporal scale, and another SODP is derived from HRV at another temporal scale.

Signal fluctuations over different temporal scales are common in physiological systems (Zhang, 1991; Costa et al., 2002), and are of particular relevance in HRV analysis, given that parasympathetic nerves are faster than sympathetic nerves (Nunan et al., 2010). The coarse-graining procedure employed in the ClassA framework is the same as that displayed in Figure 2.3 on page 45 and described in Equation 2.1 on page 39 (Costa et al., 2002, p. 2 and Lu et al., 2012, p. 2401).

An HRV signal at its original temporal scale should be treated as beat-to-beat HRV, and contains short-range PNS dynamics, whereas longer range SNS dynamics are only evident at higher temporal scales. However, as the coarse-graining procedure inevitably shortens a signal, a longer window of analysis must be used when analysing a signal at a higher temporal scale. Therefore, as a rule in the ClassA framework, HRV signals must be coarse-grained to a scale that is sufficiently high to capture longer range SNS dynamics, but that also retains a comparable number of data points to that of the windowed signal at its original temporal scale.

Several methods were explored for the quantification of the SODPs produced in the ClassA framework, including the calculation of the area covered by SODP data points, the summation of the distances between the SODP data points and the origin, and also the mean of the gradients of lines connecting the SODP data points to the origin. However, these methods lacked the accuracy and interpretability offered by the computation of the proportion of data points in the quadrants of an SODP.

In the ClassA framework, the data points in the first quadrant, and the data points in the second and fourth quadrants of an SODP of an HRV signal are computed at the original temporal scale, to represent the degree of parasympathetic dominance and the degree of balance between increasing and decreasing sequences in the HRV signal, respectively. Whereas, the points in the third quadrant of an SODP for a coarse-grained HRV series represent the degree of sympathetic dominance.

In addition to P^{Q1}, P^{Q3} and $P^{Q2,4}$, the ClassA framework also computes a trend detection measure, the Real Angle Sum (*RAS*). The Real Angle Sum is the mean of the sum of the angles in the anticlockwise direction between every data point in the SODP and the abscissa; for an illustration of the principle see Figure 4.3 on page 78. The Real Angle Sum is measured in degrees, °, and is interpreted using the quadrants of an SODP, such that the value of *RAS* indicates the overall trend in data:

Real Angle Sum between 0-90° indicates a predominantly increasing HRV sequence.

Real Angle Sum between 90-180° or 270-360° designates a predominantly balanced HRV sequence.

Real Angle Sum between 180-270° reflects a predominantly decreasing HRV sequence.

The metric of *RAS* is used to quantify stress levels with a single value, and it is hypothesised that *RAS* will tend to a value in the range of 180-270° during stress, as the values in an HRV series decrease.

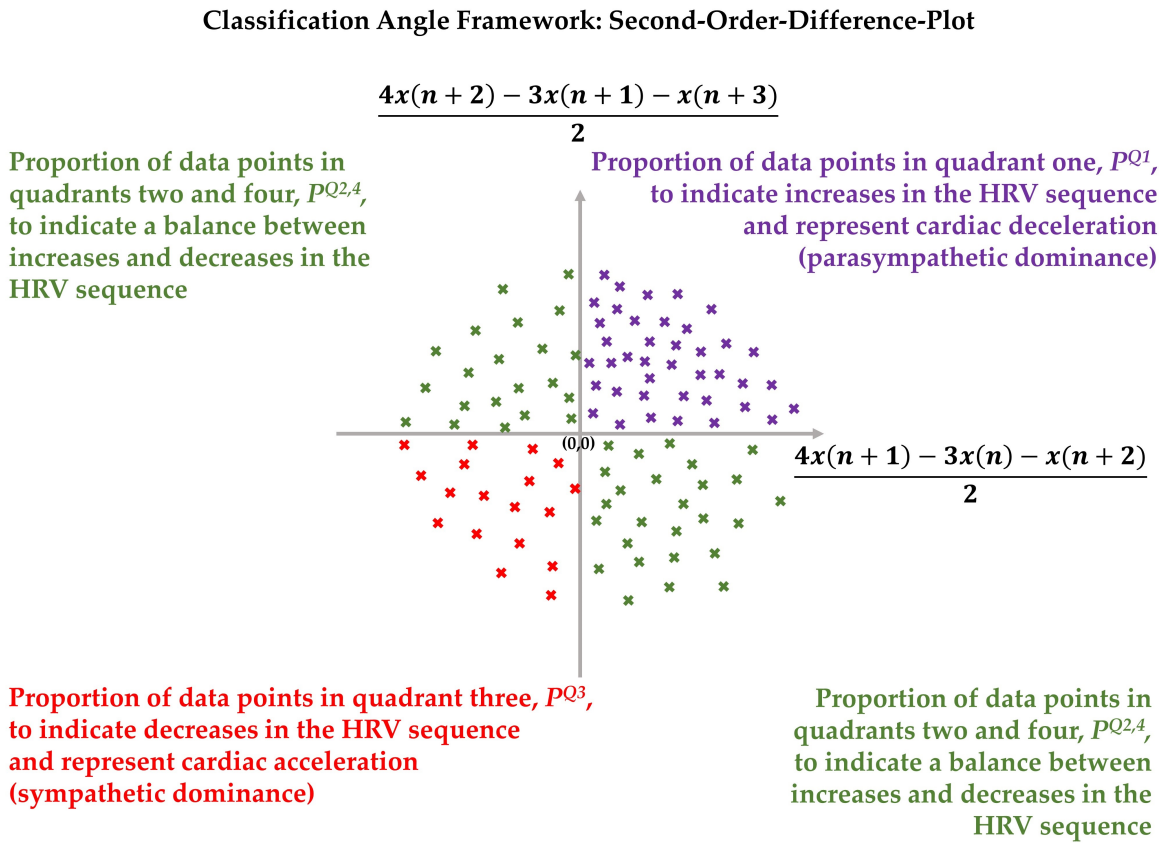


FIGURE 4.2: Second-order-difference-plot employed in the Classification Angle framework (with physiological interpretations of the quadrants of the plot (Kamath, 2012)).

It is important to note that whilst the ClassA framework is not the only advancement in signal-based stress analysis, it is unique in the physiological interpretability offered by its results. Many of the recent advancements rely upon machine learning models to discern stress, often using several signal types (Chen et al., 2017). However, such models are used to recognise signal patterns with accuracies which are entirely dependent upon the quality and representativeness of the data used to create the model. For example, in Chen et al. (2017), stress was identified in drivers, using the multimodal machine learning of ECG, galvanic skin response, and respiratory signals, whilst in Nicholson et al. (2019), machine learning was applied to neurological images to distinguish between images from healthy control subjects, and those from patients with posttraumatic stress disorder. Although both studies reported the proficiency of machine learning approaches in the identification of signal patterns indicative of stress, the interpretability of the results produced can only be guaranteed if the data used to initially train the models were of a high quality.

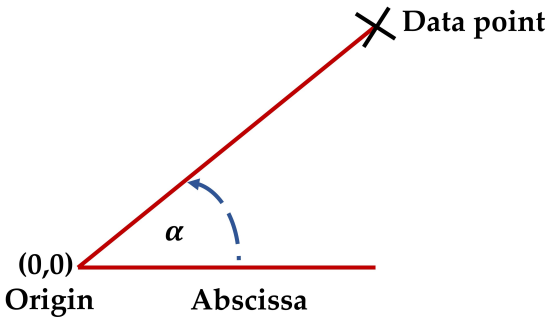


FIGURE 4.3: Angle between each data point on the Classification Angle second-order-difference-plot and the abscissa.

Algorithm 3: Classification Angle (ClassA)

1 Create a scatter plot of first differences within HRV by plotting $[(4x(n+2) - 3x(n+1) - x(n+3))/2]$ against $[(4x(n+1) - 3x(n) - x(n+2))/2]$ for signal data points, $x(n)$.

2 Compute, in the anticlockwise direction, the angle, α_n, that each point in the scatter plot makes with the abscissa, such that data points in the first quadrant of the scatter plot will make acute angles with the abscissa, those in the second quadrant will make obtuse angles, and those in the third and fourth will make reflex angles.

3 Sum the angles, α_n, computed in Step 2 and divide the total by the number of data points, N, in the windowed HRV signal. Designate this total as the Real Angle Sum (RAS), $RAS = \frac{\sum \alpha_n}{N}$.

4 Count the number of points in the scatter plot which fall within the first quadrant, and denote this as Q^1N.

5 Divide Q^1N by N to find the proportion of the total number of points that lie in the first quadrant, P^{Q1}, that is $P^{Q1} = \frac{Q^1N}{N}$.

6 Repeat Steps 4 and 5 to compute the proportion of points in the second and fourth quadrants together, $P^{Q2,4}$.

7 Course-grain the HRV signal to access the signal at a higher temporal scale, and repeat Steps 4 and 5 to compute the proportion of points in the third quadrant, P^{Q3}. In this study, the HRV was coarse-grained to temporal scale 7.

8 The metrics RAS, P^{Q1}, $P^{Q2,4}$ and P^{Q3} are the four outputs of ClassA.

9 Create a three-dimensional plot of $P^{Q2,4}$ against P^{Q3} against P^{Q1} to identify different states of stress. The overall trend in the data is indicated by RAS.

4.4 Materials and Methods

4.4.1 Subjects

The performance of the ClassA framework was verified on the dataset of ECG signals recorded from 10 males and 10 females introduced in Chapter 3, Section 3.3.1 on page 49.

4.4.2 Experimental Procedure

All the subjects took part in a standardised stress test consisting of 15 minutes of sitting rest and 15 minutes of a mental arithmetic test, as described in Chapter 3, Section 3.3.2 on page 49.

4.4.3 Data Analysis

The subjects' HRV were extracted from their ECG signals, as described in Chapter 3, Section 3.3.3 on page 50.

The ClassA framework was applied to each HRV signal, and the results were segmented in accordance with the test epochs of rest and mental arithmetic.

The ClassA metrics of P^{Q1}, $P^{Q2,4}$ and *RAS* were computed within a window of 10 seconds in length, with a 1 second increment, whilst the metric P^{Q3} was computed from HRV coarse-grained to a temporal scale, τ, of 7, within a window of 60 seconds in length, with a 1 second increment. Therefore, regarding the respective windows of analyses, P^{Q1}, $P^{Q2,4}$ and *RAS* were computed from 40 data points, whilst P^{Q3} was computed from a comparable 34 data points.

Similar to the analyses used to obtain *HR*, *SDNN*, *nLF*, *nHF*, *SampEn* and *PermEn*, outliers were not included in the ClassA analyses. The outliers within each window of analysis were replaced with the median of the interquartile range of the windowed data; outliers were defined as data values which were either 1.5 times smaller than the 25th percentile of data, or 1.5 times greater than the 75th percentile of data.

Although the key and unique feature of the ClassA framework is the three-dimensional identification of stress offered by plots of $P^{Q2,4}$ against P^{Q3} against P^{Q1}, the individual performances of the metrics were also assessed and compared to the one-dimensional state-of-the-art measures of *HR*, *SDNN*, *nLF*, *nHF*, *SampEn* and *PermEn*, so as to demonstrate the framework's performance, and to enable

a direct comparison with the performance of the state-of-the-art measures reported in Chapter 3, Section 3.4.

The mean of the ClassA metrics from the rest epochs were therefore compared to those from the epochs of the mental arithmetic test. The normality of the ClassA results were also assessed using the Lilliefors test; for normally distributed data, the paired t-test was used to compare the ClassA metrics from the rest epochs to those from the arithmetic epochs, whilst the Wilcoxon test was used to compare the results which were not normally distributed. To enable a comprehensive assessment of the ClassA metrics, the metrics from the males were also compared to those from the females, using either the two-sample t-test for normally distributed data, or the Mann-Whitney-U test for data which were not normally distributed.

Similar to the study reported in Chapter 3, a statistical significance level of 0.01 was assumed in all statistical analyses.

Furthermore, to demonstrate the three-dimensions of analysis offered by the ClassA framework, the ClassA characterisation of stress in males was produced by first computing mean P^{Q1} series, mean $P^{Q2,4}$ series and mean P^{Q3} series across the rest epoch data and across the arithmetic epoch data from the 10 males. The number of data points within the so-obtained series were then reduced by averaging the data points in nonoverlapping windows of 30 seconds in length, before plotting the averaged signals, as in $P^{Q2,4}$ against P^{Q3} against P^{Q1}. A ClassA characterisation of stress in females was also computed in the same way.

4.5 Results

The second and third columns of Table 4.2 display the males' mean ClassA metrics of P^{Q1}, $P^{Q2,4}$, P^{Q3} and *RAS* from the rest and arithmetic epochs respectively, whilst the fourth column displays the *p*-values indicating the statistical significance of the differences between the ClassA metrics from the rest epochs and the arithmetic epochs. Statistically significant *p*-values are shown in bold (p-value $\leq$ 0.01); all ClassA results were found to be normally distributed, so the *p*-values were computed using the parametric paired t-test. The last column displays the means of the percentage changes in the metrics from the rest to arithmetic epochs; increases are shown in green, and decreases are shown in red. Table 4.3 displays the results from the females.

The metrics computed from the male subjects, displayed in Table 4.2, show that of P^{Q1}, $P^{Q2,4}$ and P^{Q3}, P^{Q1} produced the largest proportions; the proportions of $P^{Q2,4}$ and P^{Q3} were approximately half that of P^{Q1}, and were both comparable during the rest and arithmetic epochs.

The only ClassA metric to not produce significantly different results from the rest epochs to the arithmetic epochs was P^{Q3} (p-value= 0.066), despite having the largest mean percentage increase, of +31%, from rest to arithmetic; this indicates a probability of 0.066 that the rise seen in P^{Q3} was due to chance (Campbell and Swinscow, 2009). The metric of P^{Q1} was the only ClassA metric to decrease from rest to arithmetic, with a mean percentage decrease of ≈-9%, whereas $P^{Q2,4}$ and RAS produced mean percentage increases of ≈+21% and ≈+5%.

In contrast, the results from the females, displayed in Table 4.3, show some sex-differences in the stress-induced changes in the ClassA metrics. Namely, unlike the males' results, P^{Q1} increased with stress and RAS decreased with stress. Also, whilst both $P^{Q2,4}$ and P^{Q3} remained approximately half that of P^{Q1}, the proportions of P^{Q3} were slightly larger than those of $P^{Q2,4}$.

The mean of the stress-induced percentage changes in the metrics reflect stark differences with the males' results, as P^{Q1} in the females increased by ≈+5%, and P^{Q3} decreased by a very small and negligible -0.5%; the metric of $P^{Q2,4}$ increased by +9% and RAS decreased by ≈-2%.

The p-values indicating the statistical significance of the differences between the male and female results from the rest and arithmetic epochs are shown in Table 4.4; the p-values were computed using the parametric two-sample t-test, and statistically significant values are shown in bold (p-value $\leq$ 0.01). It can be seen from Table 4.4 that although there were no significant differences between the ClassA metrics from the males and females during the rest epochs, the P^{Q1} values from the arithmetic epochs from the females were significantly larger than those from the males (p-value= 0.0068). Also, the RAS values from the arithmetic epochs from the females were significantly smaller than those from the males (p-value= 0.0037).

The above mentioned sex differences in the ClassA characterisations of stress are reflected in Figures 4.4 and 4.5, which display three-dimensional plots of the averaged signals of $P^{Q2,4}$ against P^{Q3} against P^{Q1} from the males and females respectively. The signals from the rest epoch are shown in blue, and those from the arithmetic epoch are shown in red. The figures show that the ClassA results from the females occupied different regions within the plot, compared to the males.

TABLE 4.2: Metrics computed from the Classification Angle framework for the male subjects. All p-values were computed using the paired t-test, and statistically significant values (p-value ≤ 0.01) are shown in bold.

Measure	Epoch		p-value	Mean of Percentage Changes (%)
	Rest	Arithmetic		
P^{Q1}	0.44±0.039	0.40±0.015	**0.0036**	-9.34
P^{Q3}	0.18±0.052	0.21±0.019	0.066	+31.04
$P^{Q2,4}$	0.18±0.026	0.22±0.027	**0.0063**	+20.93
$RAS(°)$	134.29±6.48	141.39±2.33	**0.0038**	+5.47

TABLE 4.3: Metrics computed from the Classification Angle framework for the female subjects. All p-values were computed using the paired t-test, and statistically significant values (p-value ≤ 0.01) are shown in bold.

Measure	Epoch		p-value	Mean of Percentage Changes (%)
	Rest	Arithmetic		
P^{Q1}	0.42±0.042	0.44±0.040	**0.01**	+4.55
P^{Q3}	0.21±0.042	0.21±0.039	0.73	-0.50
$P^{Q2,4}$	0.18±0.032	0.20±0.029	0.18	+9.02
$RAS(°)$	137.40±6.67	134.09±6.53	**0.0066**	-2.38

TABLE 4.4: Statistical p-values from the comparisons of the Classification Angle metrics from the male and female subjects. All p-values were computed using the two-sample t-test, and statistically significant values (p-value ≤ 0.01) are shown in bold.

Measure	p-value	
	Rest	Arithmetic
P^{Q1}	0.27	**0.0068**
P^{Q3}	0.15	0.55
$P^{Q2,4}$	0.091	0.13
RAS	0.30	**0.0037**

4.6 Discussion

The p-values displayed in Tables 4.2 and 4.3 demonstrate the effectiveness of the ClassA framework in distinguishing between the epochs of stress and rest. Namely, P^{Q1}, $P^{Q2,4}$ and RAS all produced significant differences between the test epochs for the males, with p-values of 0.0036, 0.0063 and 0.0038 respectively, whilst P^{Q1} and RAS produced significant differences with p-values of 0.01 and 0.0066 for the females. This is in striking contrast with the results produced by the state-of-the-art measures displayed in Table 3.1 and Table 3.2 on page 55, in which only the males' HR was able to

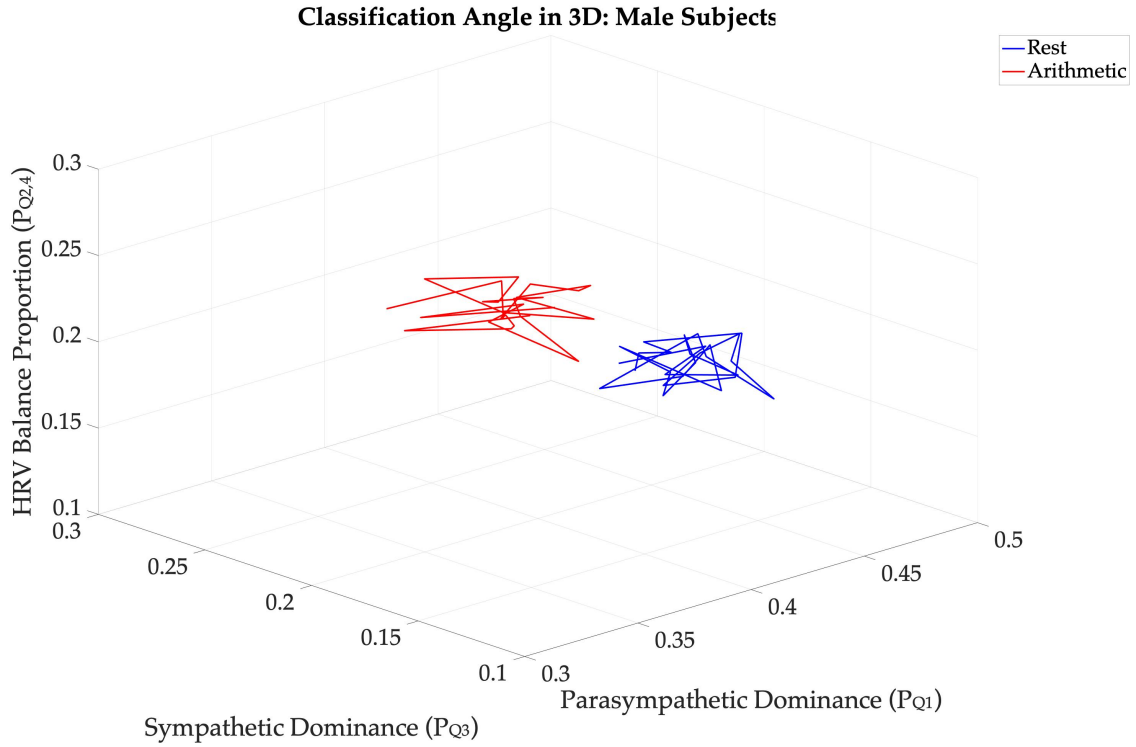


FIGURE 4.4: Plots of the Classification Angle metrics of the proportion of HRV balance ($P^{Q2,4}$) against the cardiac acceleration proportion (P^{Q3}) against the cardiac deceleration proportion (P^{Q1}), from the male subjects.

produce a statistically significant difference between the rest and arithmetic epochs (p-value of 0.008).

The physiological interpretation of the ClassA metrics also confirm noticeable sex differences in the response to stress. Firstly, as P^{Q1} is computed from the first quadrant of an SODP of beat-to-beat HRV, it represents cardiac deceleration and is interpreted to represent the activity of the parasympathetic nervous system (Kamath, 2012). Thus, the statistically significant decrease in P^{Q1} in the males from the rest to the arithmetic epochs (Table 4.2) indicates that PNS withdrawal had a large contribution to the stress-induced statistically significant increase in *HR* (shown in Table 3.1). It would therefore be expected that a significant decrease in PNS tone would have been seen alongside a significant increase in SNS tone, since the conventional understanding of the stress response often assumes a reciprocal relationship between the PNS and SNS (Billman, 2013). However, as the third quadrant in an HRV SODP is defined to represent cardiac acceleration, and hence SNS dominance (Kamath, 2012), the statistically nonsignificant increase in P^{Q3} in the males, from rest to arithmetic, suggests that the activity of the SNS and PNS in this experiment can not be *accurately* termed reciprocal sympathetic activation (Berntson et al., 1991). It must also be noted that given the exceptionally large mean of the percentage rises in P^{Q3}, it is evident that large average rises in a variable do not always constitute a

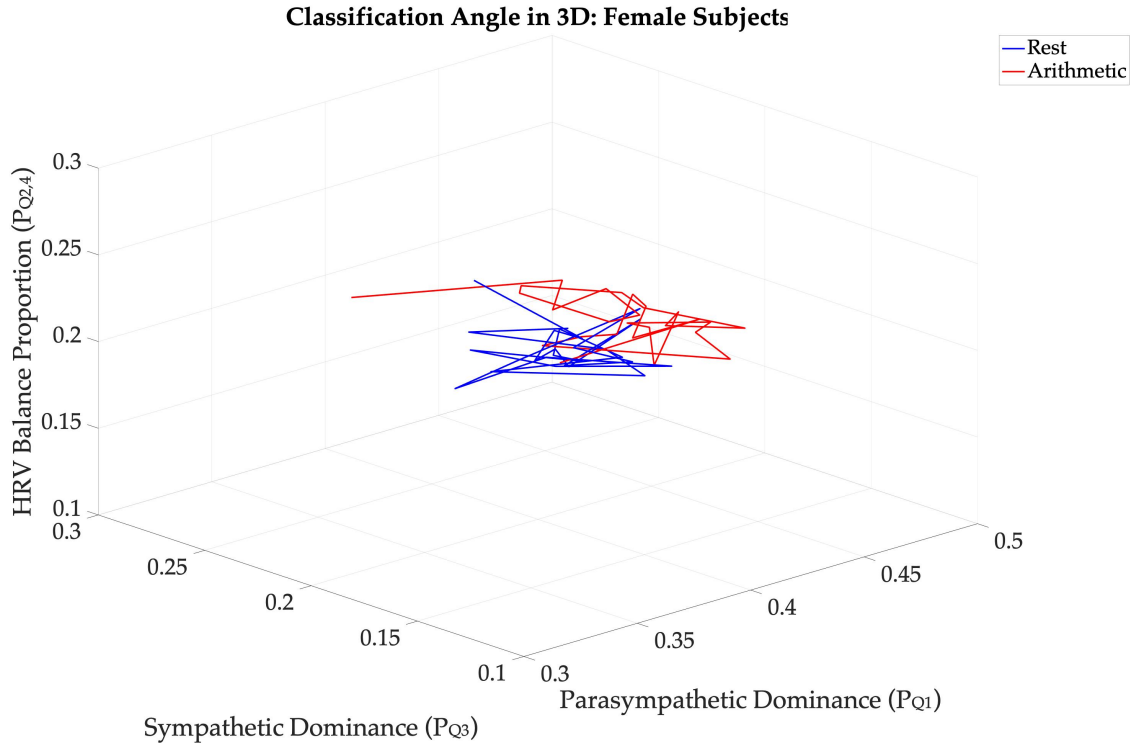


FIGURE 4.5: Plots of the Classification Angle metrics of the proportion of HRV balance ($P^{Q2,4}$) against the cardiac acceleration proportion (P^{Q3}) against the cardiac deceleration proportion (P^{Q1}), from the female subjects.

quantifiable significant trend, and could still be the result of chance (Campbell and Swinscow, 2009). However, the significance of the drop in P^{Q1} enables the confirmation that the males experienced parasympathetic withdrawal (Berntson et al., 1991). A parasympathetic withdrawal which is more consistent than a sympathetic activation is not often associated with the stress response, although a substantial parasympathetic withdrawal response was widely reported as far back as 1966 in Robinson et al. (1966). In Robinson et al. (1966), the effects of pharmaceutical nerve blockades on autonomic activity were investigated, and it was reported that initial rises in the *HR* of males in response to exercise stress were caused by parasympathetic withdrawal, not sympathetic activation; sympathetic activation was only reported after prolonged stress (Robinson et al., 1966). The P^{Q1} and P^{Q3} results reported here could therefore reflect the predominance of PNS withdrawal over SNS activation during short term stress in males. *Therefore, the ClassA signal metrics can offer an identification of whether the activities of the PNS and SNS are coupled*.

In total contrast to the males, the increase in parasympathetic tone in the females, represented by a significant increase in P^{Q1} from the rest to arithmetic epochs contradicts the majority of literature concerning stress. This further confirms sex-differences in the stress response, and demonstrates the

proficiency of the ClassA metrics in discerning between the states of stress and rest in females; none of the state-of-the-art measures were able to do so with any statistical significance. The significant increase in P^{Q1}, the negligible change in P^{Q3} (Table 4.3) alongside the nonsignificant increase in *HR* in the females (Table 3.2 on page 55) can be aligned to the conclusions in Saleh and Connell (2003), that oestrogens attenuate sympathetic nerve activity and enhance parasympathetic nerve activity.

However, unlike P^{Q1} and P^{Q3}, $P^{Q2,4}$ produced the same trend in both the males and females, by increasing from the rest to arithmetic epochs. As $P^{Q2,4}$ represents the proportion of balance between increases and decreases in the HRV, that is, consecutive increases and decreases in HRV, a low $P^{Q2,4}$ can be interpreted to indicate the presence of segments of HRV which either consistently increased or decreased. The increase seen in $P^{Q2,4}$ in the males may be reflective of HRV which became less dominated by increases, and the increase seen in the females may be reflective of HRV which became less dominated by decreases.

It can also not be ignored from Tables 4.2 and 4.3 that P^{Q3} produced the highest p-values of all the ClassA metrics. This comparatively poorer performance of P^{Q3} could be caused by the choice to compute P^{Q3} from the seventh temporal scale of HRV. It was found heuristically that temporal scale seven provided the best compromise between the ability to capture longer-range SNS dynamics, whilst still retaining high temporal resolution. This trade-off in deciding upon the temporal scale at which to compute P^{Q3} is a limitation of the Classification Angle framework.

The ability of the fourth ClassA metric of *RAS* to separate the epochs of stress from no stress was next verified. The metric *RAS* provides a single value to describe the overall trend of HRV and although if used alone it could not indicate the degree of parasympathetic and sympathetic dominance, it was able to separate the two test epochs of rest and arithmetic with significant p-values of 0.0038 and 0.0066 for the males and females respectively. In complete contrast, none of the other one-dimensional measures of stress, investigated in Chapter 3, were able to do so in *both* the males and females. Of note, similar to P^{Q1}, the stress-induced changes in *RAS* from the males and females were in different directions; this is reflected in the p-values displayed in Table 4.4, where only *RAS* and P^{Q1} were able to statistically discern between the males and females. The *RAS* results shown in Table 4.2 indicate that whilst the males' HRV remained largely balanced during both the rest and arithmetic epochs, the increase in *RAS* suggests that HRV tended towards a decrease (cardiac acceleration) following the induction of stress. Similarly, the HRV of the females remained largely balanced, but tended towards an increase (cardiac deceleration) during the arithmetic epoch. The ability of *RAS* to

significantly quantify the trend within a signal with a single value is unique. This metric therefore has the potential to be employed when stress must be identified, using a simple one-dimensional measure; this is not dissimilar to measures of entropies in stress research, although neither sample entropy nor permutation entropy were able to identify stress with any significance (see Tables 3.1 and 3.2 on page 55). Therefore the significance of the *RAS* results in discerning between stress and rest demonstrates its performance, compared to sample and permutation entropy. Moreover, *RAS* has the advantage that it is computationally inexpensive.

The computational efficiency of the ClassA framework is extremely advantageous as it enables an increase in the temporal resolution of stress analysis from HRV. Not only have the results presented in this chapter demonstrated the effectiveness of ClassA on only 10 and 60 seconds of HRV data to assess PNS and SNS dynamics respectively, but also, the framework is not mathematically complicated, and could be evaluated and fine-tuned by a practitioner who is new to programming. For comparative purposes, the times taken to compute the ClassA metrics and the traditional stress measures, described in Chapter 3, from 15 minutes of HRV are shown in Table 4.5. The ClassA metrics of P^{Q1}, $P^{Q2,4}$ and *RAS* were computed from a 1 minute data window, with a 1 second increment, whilst P^{Q3} was computed from a 360 second data window, in order to accommodate the course-graining required in its computation. Similarly, and despite the recommendation to compute the traditional HRV-based stress measures from HRV data windows of at least 5 minutes in length (Malik et al., 1996), the traditional measures were also computed from a 1 minute data window with a 1 second increment for comparative purposes. The results shown in Table 4.5 demonstrate that in spite of the computation of the traditional measures in window lengths which were a fifth of the recommended size (Malik et al., 1996), and the computation of the ClassA metrics in window lengths six times greater than those recommended in Adjei et al. (2019), the computation of all four ClassA metrics was faster than the computations of nLF, nHF and $SampEn$.

It must also not be overlooked that the ClassA metrics of P^{Q1}, $P^{Q2,4}$ and P^{Q3} were not derived to be used as one-dimensional measures; the multidimensional separation ability of the ClassA metrics are demonstrated in Figures 4.4 and 4.5. The three-dimensional plots of the ClassA metrics, averaged across the subjects, demonstrate that there are clear regions within the plots which represent the different psychophysiological states experienced during the stress test. Figure 4.4 shows that the three metrics of P^{Q1}, $P^{Q2,4}$ and P^{Q3} were able to differentiate the epochs of rest and arithmetic from the males with clear separation. Figure 4.5 shows that there was also separation of the rest from

TABLE 4.5: Times taken to compute the Classification Angle metrics and traditional heart rate variability measures, from 15 minutes of heart rate variability, using a minute-long data window with a 1 second increment.

Measure	Time Taken in Seconds
HR	0.15
SDNN	0.16
nLF	0.78
nHF	0.95
SampEn	1.05
PermEn	0.33
ClassA Metrics	0.61

arithmetic epochs amongst the females, although the regions within the plot pertaining to the rest and arithmetic epochs were closer than in Figure 4.4.

It is evident that the three-dimensional representation of HRV offered by the ClassA metrics can be used to identify different pschyophysiological states.

It must also be noted that although the significant p-values displayed in Tables 4.2 and 4.3 demonstrate the precision of the males' P^{Q1}, $P^{Q2,4}$ and RAS results and the females' P^{Q1} and RAS results in the discernment between epochs of mental arithmetic and rest, the experimental design limits the quantification of the accuracy of the ClassA metrics. The quantification of the accuracies of the ClassA metrics in the measurement of stress would ideally require a comparison of the metrics to quantitative measures of sympathetic and parasympathetic activities; this could be achieved through the acquisition of hormone samples from the subjects, taken whilst they completed the experimental protocol, or through direct measures of electrical nerve activities. Without such measures of SNS and PNS activities, the proficiency of the ClassA metrics to measure stress can only be assessed qualitatively through the fact that the study subjects verbally confirmed a feeling of stress following the mental arithmetic tests, and that the metrics were statistically found to discern between stress states (as indicated by the significant p-values).

A further limitation of this current study is that the respiratory rates of the subjects were not recorded, so the effects of respiration on the ClassA framework could not be evaluated; it is possible that the performance of stress measures could be altered by the removal of the respiratory effects from the HRV signals, but the difficulty in recording accurate respiration in real-world scenarios prevented its inclusion in this study.

4.7 Conclusion

The Classification Angle (ClassA) framework has been introduced to capture the stress-specific dynamics of the autonomic nervous system from HRV. An assessment of cardiac dynamics was achieved using a modified second-order-difference-plot of an HRV series. The framework has been shown to identify the dominant branch of the autonomic nervous system, without inheriting any of the controversies associated with the low and high frequency components of HRV. The comprehensive ClassA framework has been designed to produce four metrics to assess autonomic dynamics.

Three of the four ClassA metrics have been able to distinguish between epochs of rest and mental arithmetic; this was established over experiments on ten males and ten females in the follicular phase of the menstrual cycle, with statistically significant p-values in the range of 0.0036 to 0.01. In contrast, of the state-of-the-art measures of heart rate, standard-deviation-of-the-NN-interval, the powers of the low frequency and high frequency components of HRV, sample entropy and permutation entropy, assessed in Chapter 3, only the males' heart rate was able to discern between the rest and mental arithmetic epochs with any statistical significance. None of the state-of-the-art measures were able to discern stress in the females. The ClassA framework has been able to discern and characterise stress in both the males and females.

Furthermore, unlike the state-of-the-art HRV measures, the proposed ClassA metrics offer a three-dimensional interpretation of parasympathetic and sympathetic dynamics. *An HRV measure that can indicate whether the activities of the sympathetic and parasympathetic nervous systems are coupled has the potential to transform quantitative stress research; the ClassA framework has been verified as one such metric.*

In summary, the proposed ClassA framework achieves the second objective of this project (see page 24), by accurately identifying stress from HRV signals with a performance that surpassed those of the state-of-the-art. The ClassA framework has been demonstrated to be robust and accurate in discerning stress from rest, and offers a three-dimensional analysis; its unique features include operations on nonstationary data, and its metrics admit physiological interpretation. The framework is also computationally inexpensive, and can be computed over as little as 10 seconds of HRV data, which is a substantial increase in temporal resolution, over the 5-minute standard.

Chapter 5

The Characterisation of Surgical Pain using the Classification Angle Framework

Chapter 3 described a study which realised the first objective of this PhD research, by assessing the state-of-the-art HRV-based measures of stress, and Chapter 4 achieved the second objective, in introducing the ClassA framework to assess stress and out-perform the state-of-the-art measures.

Whilst Chapter 4 assessed the ability of ClassA to distinguish states of stress from rest, the work presented in this chapter aims to go a step further by distinguishing between different levels of stress. The third objective is therefore accomplished, such that the performance of the ClassA framework is verified in the identification of moderate levels of stress from distress. The stressor in this chapter was defined as pain during day-surgery, whereby patients who suffered from excessive pain during surgery were defined as experiencing distress and those who did not find the surgery extremely painful were defined as experiencing moderate levels of stress. Not only does the work described in this chapter characterise moderate stress from distress using the ClassA framework, but it is also shown that the ClassA framework has the potential to *identify* which patients will experience distress during surgery, from HRV recorded *before* surgery.

5.1 Chapter Summary

The possibility of identifying pain from heart rate variability signals could transform pain management. However, to date, the accurate quantification of pain using HRV has not been achieved due to previous limitations in obtaining reliable spectral characterisations of stress from HRV, as well as

sex-differences in autonomic dynamics. This chapter presents a characterisation of pain sensitivity in day-surgery patients, which has the potential to be used to predict pain sensitivity.

Electrocardiogram signals were recorded from 28 day-surgery patients, both before and during surgery to treat varicose veins. The surgeries were conducted under local anaesthetic, and the patients were asked to numerically report the pain they experienced during surgery. Heart rate variability series were derived from the patients' ECG signals, and the ClassA framework was used to extract signatures of autonomic function. Low parasympathetic tone before surgery and a decreasing HRV sequence were found to be predictive of high levels of pain during surgery in males and older females (older females were categorised as being either postmenopausal, or going through the menopause). Furthermore, a separate characterisation of pain sensitivity in young female patients, categorised as being premenopausal, was undertaken, and in this group high presurgical parasympathetic tone was found amongst the patients who reported high levels of pain.

The results from this chapter demonstrate not only that presurgical predictions of pain sensitivity amongst patients, including young females, is feasible, but also, the results from the ClassA analysis of HRV during surgery have confirmed sex-differences in the autonomic manifestation of pain. The findings presented in this preliminary study have the potential to transform clinical pain assessment, enabling clinicians to determine the sensitivity to pain of their patients, both male and female, and hence tailor anaesthesia to their patients before surgery.

5.2 Chapter Background

The ability to feel pain in our everyday life is vital to survival, as it alerts us to impending illness or injury. Conversely, the induction of pain during clinical treatment is often unwanted and unwelcome. The need to avoid the induction of pain during clinical treatment is highlighted by the United Nations' Special Rapporteur on the right to health, who states that the "failure to ensure access to controlled medicines for the relief of pain and suffering threatens fundamental rights to health and to protection against cruel, inhuman and degrading treatment" (Méndez (2013), p. 13). Pain during surgical procedures must therefore be avoided, rendering the administration of anaesthesia during clinical treatment imperative, from routine procedures, through to life saving emergency surgeries. Day-surgeries to treat varicose veins are treatments commonly undertaken under a local anaesthetic, yet despite the fact that anaesthesia blocks the communication between pain receptors and sensory

centres in the brain (Royal College of Anaesthetists, 2015), pressure receptors are not affected by the anaesthetics, and patients undergoing surgery under local anaesthesia can continue to feel pressure (NHS Yeovil District Hospital, 2013). Some patients perceive such surgical pressure as painful. Clinicians may therefore decide to administer a general anaesthetic to patients with a high sensitivity to pain, who are expected to find the surgery under local anaesthesia painful, in spite of the often increased health risks and costs associated with general anaesthesia (Locke et al., 2018).

The objective identification of patients with a high sensitivity to pain is therefore a challenge, and as such, clinicians would benefit enormously from tools to identify patients who would be better suited to the administration of a general anaesthetic, from those with a low sensitivity to pain who would not find pressure during surgery painful. The work presented in this chapter therefore aims to help mitigate this challenge, through the use of the Classification Angle framework to:

(i) Demonstrate the feasibility of the objective identification of a high or low sensitivity to pain in patients due to have varicose vein surgery, from presurgically recorded ECG signals.

(ii) Demonstrate the feasibility of the objective identification of a high or low sensitivity to pain in varicose vein surgery patients, from ECG signals recorded during surgery, and in so doing, assess the autonomic manifestation of surgical pain.

5.2.1 The Autonomic Nervous System and Pain

Pain is a stressor, and elicits a physiological stress response (Figueroa-Fankhanel, 2014), such as the 'fight-or-flight' response described by Walter Cannon in Cannon (1915).

As with other forms of stressors, the sympathetic and parasympathetic branches of the autonomic nervous system govern the initial reaction to pain (Figueroa-Fankhanel, 2014), and the activities of the SNS in the specific response to pain have been widely researched. In a review on the effect of pain on the SNS, Schlereth and Birklein (2008), it was concluded that the short-term activation of the SNS suppresses pain through the inhibition of the transmission of pain signals in the spinal cord, although the direct molecular interactions between the activity of the SNS and pain remain unknown. In contrast, long-term activation of the SNS, oversensitises the nervous system, and has been found to exacerbate chronic pain (Schlereth and Birklein, 2008; Bantel and Trapp, 2011).

The relationship between the PNS and nociception, the perception of pain, has also been widely researched, and it is accepted that PNS activation can inhibit nociception, and hence reduce pain

(Bantel and Trapp, 2011). For example, in an investigation reported in Botha et al. (2014), an increase of parasympathetic tone, through the initiation of deep breathing, increased the thresholds to pain in 37 healthy adults subjected to electrical stimulation. Conversely, in a further investigation reported in the same paper, the administration of a pharmaceutical parasympathetic nerve blockade prevented the increase in pain thresholds which had been previously induced by deep breathing (Botha et al., 2014).

Furthermore, the autonomic response to pain has been found to be variable, where the greater the level of pain psychologically perceived (irrespective of the level of pain actually sensed by nerves), the greater the level of autonomic activation (Mischkowski et al., 2018). High-pass filtered HRV has also been found to indicate autonomic activity in response to pain; in Allen et al. (2018), the correlations between HRV and the ability to continue with daily activities in spite of pain were investigated in 20 subjects with chronic pain. It was found that the subjects who exhibited greater high frequency power in HRV were better able to continue with their daily activities, compared to those with reduced high frequency power (Allen et al., 2018).

This interaction between the autonomic nervous system and pain has also long been utilised to monitor the conditions of patients under general anaesthesia during surgery, where anaesthetists routinely monitor sympathetic driven responses, such as heart rate and blood pressure, to assess the depth of a patient's sedation (Bantel and Trapp, 2011). However, the signatures of autonomic activation which are indicative of the sensitivity to pain are yet to be fully explored, and are a subject of the work in this chapter.

In the study reported in this chapter, the novel Classification Angle (ClassA) framework, introduced in Adjei et al. (2019), was applied to HRV signals from 28 hospital day-surgery patients, so as to assess the differences in the characteristics of the HRV from patients with low and high sensitivities to pain. An ECG signal was recorded from each patient before and during varicose vein surgery; local anaesthesia was administered to all patients, and post-surgery, the patients were asked to numerically rate the severity of the pain they experienced during the surgery. It is demonstrated that not only is the ClassA framework able to discern between the cohorts of patients with low and high sensitivities to pain, using only the data recorded before the surgery, but the results also clearly show that the characteristics of HRV in response to pain in young females (categorised as premenopausal) differs from that of males and older females.

5.3 Materials and Methods

5.3.1 Patient Cohort

Twenty-eight varicose vein surgery patients were recruited in this study; of the 28, there were 9 males aged 54 $\pm$ 15.54 years, and 19 females aged 45.79 $\pm$ 13.68 years.

It is important to note that of the research into various forms of stressors, research into pain has produced the largest body of literature to suggest that oestrogens cause sex-differences in the stress response (Pfleeger et al., 1997; Riley et al., 1999; Craft et al., 2004; Craft, 2007). Therefore, in the study reported in this chapter, data from the females aged below 49 years were analysed separately to the data from the females aged 49 or older and the males. The age of 49 was reported to be the median age of menopause in the UK (Pokoradi et al., 2011), meaning the modulating effects of oestrogens on cardiac dynamics could be expected to be most pronounced in females aged below 49. However, the retrospective nature of the data analysis in this study prevents the verification of the female subjects' menstrual status.

The HRV dataset from the females aged $\geq$ 49 and the male patients was termed Group 1, and the dataset from the females aged < 49 was termed Group 2. Group 1 consisted of the data from 16 patients (9 males and 7 females) aged 56.63 $\pm$ 13.66 years, and Group 2 consisted of the data from 12 patients aged 37.5 $\pm$ 7.66 years. Group 1 was comprised of 17 ECG recordings, as one male patient underwent two separate surgeries on separate days.

The surgeries on the 28 patients consisted of the ablation and/or avulsion of varicose veins in the legs, under local anaesthesia. The patients' ECG signals were recorded using the iAmp, developed by Dr Valentin Goverdovsky (Kanna et al., 2018), pictured in Figure 3.1 on page 50, both before and during the surgeries. Figure 5.1 illustrates an ECG recording from a patient, using the iAmp. The presurgical periods had a mean length of 34 minutes and 51 seconds ($\pm$ 21 minutes and 55 seconds), whilst the surgical periods had a mean length of 24 minutes and 59 seconds ($\pm$ 9 minutes and 18 seconds).

Following the surgery, the patients were asked to rate how painful they found the surgery on a scale of one to ten, where one indicated a low intensity of pain, and ten indicated an extremely high intensity of pain. Patients who reported a pain score of seven or more were defined as having a high sensitivity to pain by the surgical team.

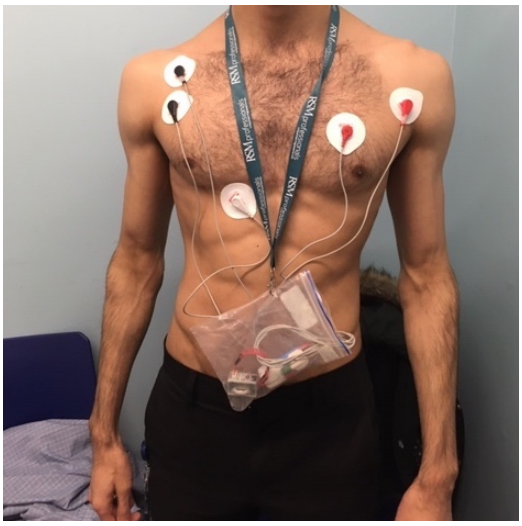


FIGURE 5.1: A photograph illustrating the measurement arrangement employed to record the patient electrocardiogram signals.

5.3.2 Data Analysis

The patients' ECG signals were recorded at a sampling frequency of 1000 Hz, from the torso, whereby the electrical potentials across the body were measured using electrodes placed on the right, and electrodes placed on the left (Society for Cardiological Science & Technology, 2014). The recorded ECG signals were analysed in the MATLAB programming environment, and the HRV signals were derived from the ECG signals using the algorithm introduced in Chanwimalueang et al. (2015); the HRV signals were resampled at 4 Hz. The HRV signals were then analysed retrospectively to reveal signatures which were indicative of a low or high sensitivity to pain. The HRV signals from the presurgical and surgical periods were analysed separately.

Heart rate variability signatures were extracted using the ClassA framework, to explore the separability of the HRV from the patients with a low or high sensitivity to pain. The HRV signatures were therefore based on the ClassA metrics of P^{Q1}, $P^{Q2,4}$, P^{Q3} and RAS, assessing parasympathetic dominance, balance between increases and decreases in an HRV sequence, sympathetic dominance and the overall trend within the HRV sequence, respectively.

The ClassA metrics of P^{Q1}, $P^{Q2,4}$ and RAS, were computed from HRV at the first temporal scale, from a window 10 seconds in length, with a 1 second increment, and P^{Q3} was computed from HRV which had been coarse grained to the seventh temporal scale, using a window 60 seconds in length, with a 1 second increment.

Similar to the analyses in Chapters 3 and 4, outliers were identified in each window of analysis. Outliers were defined as HRV values which were either 1.5 times smaller than the 25th percentile of the data, or 1.5 times greater than the 75th percentile of the data, and were replaced by the median of the interquartile range of the data in the window of analysis.

The computation of the ClassA framework is outlined in Algorithm 3 on page 78. The following eight groups of data were created, such that the data of all the patients belonging to each group were averaged using the mean; this resulted in mean P^{Q1}, $P^{Q2,4}$, P^{Q3} and *RAS* series for each group:

Group 1 *PSLP* Presurgical data from the Group 1 patients who experienced low pain.

Group 1 *SLP* Surgical data from the Group 1 patients who experienced low pain.

Group 1 *PSHP* Presurgical data from the Group 1 patients who experienced high pain.

Group 1 *SHP* Surgical data from the Group 1 patients who experienced high pain.

Group 2 *PSLP* Presurgical data from the Group 2 patients who experienced low pain.

Group 2 *SLP* Surgical data from the Group 2 patients who experienced low pain.

Group 2 *PSHP* Presurgical data from the Group 2 patients who experienced high pain.

Group 2 *SHP* Surgical data from the Group 2 patients who experienced high pain.

To reiterate, each of the patients' P^{Q1}, $P^{Q2,4}$, P^{Q3} and *RAS* series corresponding to the eight above-described groupings were averaged, to produce single P^{Q1}, $P^{Q2,4}$, P^{Q3} and *RAS* series for each group. Only the first 7 minutes of surgical data were used in the analyses as the shortest surgery was found to be 7 minutes long. For consistency, only the last 7 minutes of presurgical data were also analysed.

5.3.3 Statistics

The statistical significance of the abilities of the averaged P^{Q1}, $P^{Q2,4}$, P^{Q3} and *RAS* series to distinguish between patients with a high sensitivity to pain and patients with a low pain sensitivity were tested using the two-sample t-test, for normally distributed data, and the Mann-Whitney-U test, for data which were not normally distributed. In contrast, the abilities of the P^{Q1}, $P^{Q2,4}$, P^{Q3} and *RAS* series to distinguish between presurgical and surgical data were tested using the paired t-test, for normally

distributed data, and the Wilcoxon test, for data which were not normally distributed. Again, the Lilliefors test of normality was employed to determine the use of either a parametric or nonparametric test; a statistical significance level of 0.01 was employed in all analyses.

The ClassA metrics from Groups 1 and 2 were also compared to verify that separate ClassA characterisations were indeed required for the two groups of patients, using the parametric two-sample t-test or the nonparametric Mann-Whitney-U test.

5.4 Results

Six of the patients in Group 1 and seven of the patients in Group 2 reported high pain; their mean pain scores were 8 ± 1.26 and 7.71 ± 0.76, respectively. The mean of the P^{Q1}, P^{Q3}, $P^{Q2,4}$ and *RAS* values from Group 1 are shown in Table 5.1, and those from Group 2 are shown in Table 5.2.

Of P^{Q1}, P^{Q3} and $P^{Q2,4}$, the measure of PNS dominance, P^{Q1}, was the largest in both Groups 1 and 2 during the presurgical and surgical periods; the measures of SNS dominance, P^{Q3}, and balance between increases and decreases in the HRV sequence, $P^{Q2,4}$, produced respective ranges of 0.20-0.23 and 0.18-0.22. The *RAS* results were in the range of 134-143°.

The equality of the ClassA metrics were statistically analysed and the *p*-values from the tests of equality between the metrics from the patients with a low or high sensitivity to pain ('*PSLP* vs. *PSHP*' and '*SLP* vs. *SHP*') are shown in Table 5.3, the *p*-values from the statistical comparisons of the metrics from the presurgical and surgical periods ('*SLP* vs. *PSLP*' and '*SHP* vs. *PSHP*') are shown in Table 5.4, and the *p*-values from the comparisons of the metrics from Group 1 and Group 2 ('Group 1 *PSLP* vs. Group 2 *PSLP*', 'Group 1 *SLP* vs. Group 2 *SLP*', 'Group 1 *PSHP* vs. Group 2 *PSHP*' and 'Group 1 *SHP* vs. Group 2 *SHP*') are shown in Table 5.5. The *p*-values computed using the nonparametric statistical tests are displayed with an asterisk, *, and those computed using the parametric tests are displayed without an asterisk; significant *p*-values (≤ 0.01) are shown in bold.

Of the results shown in Table 5.3, the only ClassA metric to not produce a statistically significant difference between the patients with a low or high sensitivity to pain was the Group 1 presurgical $P^{Q2,4}$ data (Group 1 *PSLP* vs *PSHP*). Of the results shown in Table 5.4, the difference between the Group 1 low pain presurgical and surgical P^{Q3} (Group 1 *SLP* vs *PSLP*), the difference between the Group 2 low pain presurgical and surgical P^{Q3} (Group 2 *PSLP* vs *PSHP*), and the the difference between the Group 2 high pain presurgical and surgical $P^{Q2,4}$ (Group 2 *SHP* vs *PSHP*) were not significant; the remaining

TABLE 5.1: Mean of the averaged Classification Angle metrics of parasympathetic dominance (P^{Q1}), sympathetic dominance (P^{Q3}), balanced increases and decreases in HRV ($P^{Q2,4}$) and the Real Angle Sum (RAS), computed from Group 1.

Group 1 Cohort	$\boldsymbol{P^{Q1}}$	$\boldsymbol{P^{Q3}}$	$\boldsymbol{P^{Q2,4}}$	RAS
PSLP	0.43 ± 0.033	0.20 ± 0.021	0.20 ± 0.016	137.52 ± 5.91
SLP	0.41 ± 0.035	0.20 ± 0.024	0.20 ± 0.019	139.61 ± 5.93
PSHP	0.41 ± 0.050	0.22 ± 0.028	0.20 ± 0.027	140.18 ± 8.63
SHP	0.39 ± 0.041	0.23 ± 0.032	0.21 ± 0.023	142.47 ± 7.30

TABLE 5.2: Mean of the averaged Classification Angle metrics of parasympathetic dominance (P^{Q1}), sympathetic dominance (P^{Q3}), balanced increases and decreases in HRV ($P^{Q2,4}$) and the Real Angle Sum (RAS), computed from Group 2.

Group 2 Cohort	$\boldsymbol{P^{Q1}}$	$\boldsymbol{P^{Q3}}$	$\boldsymbol{P^{Q2,4}}$	RAS
PSLP	0.41 ± 0.043	0.22 ± 0.032	0.22 ± 0.029	140.12 ± 6.93
SLP	0.39 ± 0.039	0.22 ± 0.032	0.22 ± 0.028	141.86 ± 6.95
PSHP	0.45 ± 0.043	0.21 ± 0.032	0.18 ± 0.019	134.36 ± 7.52
SHP	0.43 ± 0.034	0.20 ± 0.027	0.18 ± 0.022	136.34 ± 5.76

TABLE 5.3: Statistical p-values from the comparisons of the Classification Angle metrics from patients who experienced either low or high pain. Statistically significant p-values (p-value ≤ 0.01) are shown in bold; those computed using the Mann-Whitney-U test are marked with an asterisk, *, and those computed using the two-sample t-test are displayed without an asterisk.

Cohort	$\boldsymbol{P^{Q1}}$ **p-value**	$\boldsymbol{P^{Q3}}$ **p-value**	$\boldsymbol{P^{Q2,4}}$ **p-value**	RAS **p-value**
Group 1 *PSLP* vs. *PSHP*	$\ll$ **0.01**	$\ll$ **0.01***	0.033*	$\ll$ **0.01**
Group 1 *SLP* vs. *SHP*	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***
Group 2 *PSLP* vs. *PSHP*	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***
Group 2 *SLP* vs. *SHP*	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***

TABLE 5.4: Statistical p-values from the comparisons of the Classification Angle metrics from the presurgical and surgical periods. Statistically significant p-values (p-value ≤ 0.01) are shown in bold; those computed using the Wilcoxon test are marked with an asterisk, *, and those computed using the paired t-test are displayed without an asterisk.

Cohort	$\boldsymbol{P^{Q1}}$ **p-value**	$\boldsymbol{P^{Q3}}$ **p-value**	$\boldsymbol{P^{Q2,4}}$ **p-value**	RAS **p-value**
Group 1 *SLP* vs. *PSLP*	$\ll$ **0.01***	0.050*	$\ll$ **0.01***	$\ll$ **0.01**
Group 1 *SHP* vs. *PSHP*	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***
Group 2 *SLP* vs. *PSLP*	$\ll$ **0.01***	0.36*	$\ll$ **0.01***	$\ll$ **0.01***
Group 2 *SHP* vs. *PSHP*	$\ll$ **0.01***	$\ll$ **0.01***	0.93*	$\ll$ **0.01***

TABLE 5.5: Statistical p-values from the comparisons of the Classification Angle metrics from Group 1 and Group 2. Statistically significant p-values (p-value ≤ 0.01) are shown in bold; those computed using the Mann-Whitney-U test are marked with an asterisk, *, and those computed using the two-sample t-test are displayed without an asterisk.

Cohort	$\boldsymbol{P^{Q1}}$ **p-value**	$\boldsymbol{P^{Q3}}$ **p-value**	$\boldsymbol{P^{Q2,4}}$ **p-value**	RAS **p-value**
Group 1 *PSLP* vs. Group 2 *PSLP*	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***
Group 1 *SLP* vs. Group 2 *SLP*	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01**
Group 1 *PSHP* vs. Group 2 *PSHP*	$\ll$ **0.01**	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01**
Group 1 *SHP* vs. Group 2 *SHP*	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***	$\ll$ **0.01***

differences were statistically significant (p-value $\ll 0.01$). All comparisons of the data from Group 1 and Group 2 were also found to be significant (see Table 5.5).

It is shown in Table 5.3 that the *RAS* values computed from the patients with a low or high sensitivity to pain were significantly different to one another. It is also shown in Table 5.4 that the Group 1 *RAS* values were significantly higher (p-values $\ll 0.01$) during the surgical period compared to the presurgical period. The results from Group 2 showed the same trend of a higher *RAS* during the surgical period compared to the presurgical period (p-values $\ll 0.01$).

5.5 Discussion

5.5.1 Group 1

The results presented in this study show that it is feasible to discern between a low or high sensitivity to surgical pain from presurgical HRV, and that a low or high sensitivity to pain can also be characterised using HRV from the surgical period.

The mean P^{Q1} values presented in Table 5.1 and the statistical p-values, shown in Tables 5.3 and 5.4 demonstrate that the level of PNS dominance was approximately twice that of the SNS, but noticeably, P^{Q1} decreased slightly in both the patients with a low sensitivity to pain, and those with a high sensitivity to pain, from the presurgical to surgical periods. The finding of a stress-induced decrease in parasympathetic tone is supported by the physiology of cardiac control, as the PNS is known to dominate during times of rest, and then exhibit withdrawal at times of stress (Gordan et al., 2015). The p-values from the comparisons of P^{Q1} for *SLP* vs. *PSLP* and *SHP* vs. *PSHP*, shown in Table 5.4, were both statistically significant (Table 5.4, Group 1 P^{Q1} *SLP* vs. *PSLP*: p-value $\ll 0.01$, *SHP* vs. *PSHP*: p-value $\ll 0.01$). However, as the *PSLP* P^{Q1} data were significantly larger than the *PSHP* P^{Q1}

data (Table 5.3, Group 1 P^{Q1} *PSLP* vs. *PSHP*: p-value $\ll 0.01$), and remained larger throughout the surgery (Group 1 P^{Q1} *SLP* vs. *SHP*: p-value $\ll 0.01$), *it can therefore be concluded that high parasympathetic tone was a signature of a low sensitivity to pain in Group 1.* This finding is supported in literature; for example, not only has it been found that a parasympathetic nerve blockade prevented an expected analgesic effect of parasympathetic stimulation (Botha et al., 2014), but also, the therapeutic effects of parasympathetic nerve stimulation have been verified and consequently approved to help treat depression in the European Union and in Canada (Bonaz et al., 2013).

The mean P^{Q3} values shown in Table 5.1 and the statistical p-values shown in Tables 5.3 and 5.4 indicate that the changes in P^{Q3} differed according to the patients' sensitivity to pain. There was a nonsignificant increase in sympathetic tone in the patients with a low sensitivity to pain from the presurgical to the surgical periods, with a p-value of 0.05 from the comparison of Group 1 P^{Q3} *SLP* vs. *PSLP*, shown in Table 5.4, whilst there was a significant increase in presurgical to surgical P^{Q3} in the data from the patients with a high sensitivity to pain (Table 5.4, Group 1 P^{Q3} *SHP* vs. *PSHP*: p-value $\ll 0.01$), which suggests the activation of the SNS during surgery. However, the significantly larger P^{Q3} from the presurgical data from the patients with a high sensitivity to pain, compared to the presurgical data from the patients with a low sensitivity to pain (Table 5.3, Group 1 P^{Q3} *PSLP* vs. *PSHP*: p-value $\ll 0.01$) indicates that *the patients with a high sensitivity to pain experienced high sympathetic tone before the surgery even started*. The exact reason for the high presurgical sympathetic tone cannot be determined, but it is known that the feeling of anxiety exacerbates pain (Rhudy and Meagher, 2000), and it could be that the patients with a high sensitivity to pain felt anxious about the surgery beforehand.

Whilst P^{Q1} and P^{Q3} indicate the dominance of parasympathetic and sympathetic tone, a lower mean $P^{Q2,4}$ indicates the presence of sequences in HRV which either consistently increased or decreased, and higher values indicate successive increases followed by decreases in HRV. Therefore, the lower presurgical values of $P^{Q2,4}$ shown in Table 5.1 and the significant increases in $P^{Q2,4}$ from the presurgical to the surgical periods support the finding from the P^{Q3} results, that surgery introduced decreases in the patients' previously predominantly increasing HRV (Table 5.4, Group 1 $P^{Q2,4}$ *SLP* vs. *PSLP*: p-value $\ll 0.01$, *SHP* vs. *PSHP*: p-value $\ll 0.01$).

The *RAS* results also reveal differences between the patients with a high and low sensitivity to pain, as the mean values and the statistical p-values, presented in Tables 5.1, 5.3 and 5.4, show that whilst all the values of *RAS* indicated an HRV series which overall, neither increased or decreased, the

HRV from the patients with a high sensitivity to pain tended more towards a decrease than the HRV from the patients with a low sensitivity to pain. This suggests that greater pain is associated with decreasing HRV, which is pronounced enough to distinguish a low from a high sensitivity to pain. This claim is supported by the fact that both the data from the patients with a low and high sensitivity to pain exhibited a significant increase in *RAS* from the presurgical to surgical periods (Table 5.4, Group 1 *RAS SLP* vs. *PSLP*: p-value $\ll 0.01$, *SHP* vs. *PSHP*: p-value $\ll 0.01$).

In summary, **in a comparison of the data from the patients with a low sensitivity to pain, a high sensitivity to pain was associated with a lower parasympathetic tone, a higher sympathetic tone, and an HRV which tended towards a decrease.** These trends were consistent both presurgically and surgically, and are in line with the fight-or-flight response (Cannon, 1915; Selye, 1955).

5.5.2 Group 2

The results presented in this study establish that the manifestation of a high sensitivity to pain in HRV series, identified using the ClassA framework, for young females differed considerably to that of Group 1. The statistically significant p-values displayed in Table 5.5 also verify that the metrics from Group 1 and Group 2 differed greatly.

Firstly, although the mean P^{Q1} values presented in Table 5.2 and the statistical p-values shown in Tables 5.3 and 5.4 indicate that there was a significant decrease in parasympathetic tone from the presurgical to surgical periods in the patients with a low sensitivity to pain (Table 5.4, Group 2 P^{Q1} *SLP* vs. *PSLP*: p-value $\ll 0.01$), the patients with a low sensitivity to pain also had lower overall PNS tone than the patients with a high sensitivity to pain (Table 5.3, Group 2 P^{Q1} *PSLP* vs. *PSHP*: p-value $\ll 0.01$, *SLP* vs. *SHP*: p-value $\ll 0.01$). The higher presurgical parasympathetic tone found in the patients with a high sensitivity to pain, compared to the patients with a low sensitivity to pain, contradicts the corresponding result from Group 1, in which higher parasympathetic tone was found amongst the patients with a low sensitivity to pain. This finding from Group 2 verifies that the hormonal makeup of the younger females had an effect on their PNS dynamics, differing it from those of the males and older females. This finding is supported by the conclusions from Pfleeger et al. (1997), Craft et al. (2004), and Craft (2007), in which it was reported that oestrogens modulated the physiological manifestation of pain in females. However, this present study goes further than verifying sex-differences in the stress response to pain; *this study is the first to show that there are HRV-based signatures of pain, specific to young females, which can be used to not only characterise pain in young*

females, but have the potential to also predict their sensitivity to pain before a surgical procedure begins. More precisely, a high presurgical PNS tone appears to be indicative of a high sensitivity to pain.

The mean P^{Q3} values shown in Table 5.2 and the statistical p-values shown in Tables 5.3 and 5.4 show that the data from the young females with a low sensitivity to pain produced P^{Q3} values which were both larger than those of the males and older females of Group 1 who had a low sensitivity to pain, and did not change significantly from the presurgical to the surgical periods (Table 5.4, Group 2 P^{Q3} *SLP* vs. *PSLP*: p-value=0.36). However, P^{Q3} from the Group 2 patients with a high sensitivity to pain decreased significantly from the presurgical to the surgical periods (Table 5.4, Group 2 P^{Q3} *SHP* vs. *PSHP*: p-value $\ll 0.01$), which suggests that *the patients with a low sensitivity to pain did not exhibit changes in sympathetic tone in response to the surgery, whilst those with a high sensitivity to pain did*.

The mean values of $P^{Q2,4}$ shown in Table 5.2, and the statistical p-values shown in Tables 5.3 and 5.4 suggest that there were more consecutive increases and decreases in the HRV sequences of the patients who had a lower sensitivity to pain, compared to the patients who had a higher sensitivity to pain (Table 5.3, Group 2 $P^{Q2,4}$ *PSLP* vs. *PSHP*: p-value $\ll 0.01$, *SLP* vs. *SHP*: p-value $\ll 0.01$).

Furthermore, the mean *RAS* values shown in Table 5.2 and the statistical p-values, shown in Tables 5.3 and 5.4 show that unlike Group 1, the *RAS* results from the Group 2 patients with a high sensitivity to pain were significantly smaller than those from the patients with a low sensitivity to pain (Table 5.3, Group 2 *RAS PSLP* vs. *PSHP*: p-value $\ll 0.01$, *SLP* vs. *SHP*: p-value $\ll 0.01$), indicating that the HRV of the Group 2 patients with a low sensitivity to pain tended more towards a decrease than that of the patients with a high sensitivity to pain. However, *similar* to Group 1, both the patients with the low and high sensitivity to pain produced *RAS* values which increased from the presurgical to the surgical periods.

In summary of the Group 2 results, **in a comparison of the data from the patients with a low sensitivity to pain, a high sensitivity to pain was associated with a higher parasympathetic tone, a lower sympathetic tone, and an HRV which tended towards an increase; these trends are in total contrast to the trends seen in the Group 1 results.** These results show that the markers used in any objective tool to identify young females with a high sensitivity to pain, both presurgically and surgically, should differ to those used in males and older females.

This study is however limited by the retrospective categorisation of the females aged 49 years or older as perimenopausal/postmenopausal, as this categorisation cannot be verified. Additionally,

although pain is a subjective sensation, the use of subjective numerical scores of pain in this study could lead to inaccuracies, as it is well known in psychology that some individuals are labelled as 'repressors', and routinely repress emotions and sensations (Myers and Derakshan, 2004).

Nevertheless, this study has also enabled an assessment of the accuracy of the theory of the stress response, presented in Dienstbier (1989) (described on page 19). To recap, it was posited in Dienstbier (1989) that a good response to stress, that is, the ability to cope well and not experience distress, could be identified by a low basal SNS tone, and a strong SNS activation when stressed. However, neither the patients with a low sensitivity to pain from Groups 1 or 2, exhibited this reaction; indeed both groups of patients with a low sensitivity to pain produced nonsignificant changes in P_{Q3} from the presurgical to surgical periods, indicating nonsubstantial changes in SNS tone. The findings from the present study instead suggest that substantial changes in SNS tone are a marker of high sensitivity to pain in both males and females, though the direction of the change differed between Group 1 and 2.

A high sensitivity to pain amongst the Group 1 patients was associated with an increase in SNS tone, whilst amongst the Group 2 patients with a high sensitivity to pain, there was an unexpected decrease in SNS tone. This finding supports the conclusion that oestrogens attenuate sympathetic nerve activity (Saleh and Connell, 2003), although the findings must also be verified biologically, using pharmaceutical nerve blockades and hormonal assays.

5.6 Conclusion

This study aimed to characterise the heart rate variability signals from day-surgery patients, to assess the autonomic manifestation of low and high sensitivities to pain. The heart rate variability signals were extracted from the ECG signals of 28 patients, recorded both before and during surgery to treat varicose veins. The patients were asked to numerically rate the intensity of the pain experienced during surgery, and the Classification Angle framework was employed to extract signatures from the heart rate variability signals which could be used to identify pain in patients. The results presented showed that in the males, and females older than 49, a low presurgical parasympathetic tone, high sympathetic tone and a decreasing heart rate variability series were indicative of a high sensitivity to pain.

Blood pressure dynamics are driven by the SNS, and the findings of a high sympathetic tone and a high sensitivity to pain are supported by findings from Fillingim and Maixner (1996), in which the blood pressure measurements of 25 males were found to be proportional to their sensitivity to pain, whilst the blood pressure measurements from 23 females were found to be inversely proportional to pain sensitivity; pain was experimentally induced by the occlusion of blood to the hand (Fillingim and Maixner, 1996). Similarly, the sympathetically driven emotion of anxiety has been found to be strongly correlated to pain intensity in males, but not in females (Frot et al., 2004). A greater influence of the sympathetic nervous system in the modulation of pain in males has also been reported in Tousignant-Laflamme and Marchand (2006).

In the females aged below 49, a high presurgical parasympathetic tone, low sympathetic tone and an increasing heart rate variability series were indicative of a high sensitivity to pain.

The results presented in this study have therefore shown that high and low sensitivities to pain are identifiable using distinct heart rate variability signatures, derived using the Classification Angle framework; this study also shows that these signatures differ according to age and sex.

The stress elicited within the patients who exhibited a low sensitivity to pain can be likened to moderate stress, as the patients were able cope well, and did not experience the distress of a high sensitivity to pain; the findings from the males and older females indicate that moderate stress can be identified by a stress reaction of negligible change in SNS tone, whilst distress can be identified by a substantial increase in SNS tone. For younger females, oestrogen appears to reduce SNS tone during distress. Therefore, it is evident that the identification of differences in the signatures of moderate stress and distress from HRV is possible using the ClassA framework, thus achieving the third objective of this PhD research. Statistically significant p-values produced following comparisons of the ClassA metrics from the patients with high and low sensitivities to pain support this claim.

Chapter 6

Project Conclusion

The structure and main findings of this thesis are outlined on Figure 1.4 on page 26.

The PhD research described in this thesis aimed to produce a quantitative measure of stress, derived from heart rate variability, which was able to outperform the state-of-the-art and distinguish moderate stress from distress. Through the achievement of the three research objectives, listed on page 24 and described below, the aim of this research has been realised.

The work detailed in Chapter 3 achieved the first objective, to assess the state-of-the-art HRV-based stress measures of heart rate, the standard-deviation-of-the-NN-interval, the powers of the low and high frequency components of HRV series, sample entropy and permutation entropy. The measures were applied to an HRV dataset from 10 adult males and 10 adult females during 15 minutes of sitting rest, and 15 minutes of a mental arithmetic task, designed to induce stress. Heart rate in the males was found to be the only measure to produce a statistically significant difference between the rest and arithmetic epochs (p-value = 0.008). The findings from this chapter show that stress can induce an increase in the long-term univariate sample entropy of HRV; prior to this investigation, stress was typically cited to cause a decrease in the long-term univariate sample entropy of HRV (Chanwimalueang et al., 2016).

The work presented in Chapter 4 achieved the second objective, to introduce the Classification Angle framework to accurately identify stress from HRV signals, in a manner which outperformed the state-of-the-art measures. The framework outputs four metrics, indicating parasympathetic dominance, P^{Q1}, sympathetic dominance, P^{Q3}, the balance between increases and decreases in HRV sequences, $P^{Q2,4}$, and a measure of data trend, the Real Angle Sum. Using the same dataset analysed in

Chapter 3, three of the four measures from the framework were able to produce a statistically significant difference between the rest and arithmetic epochs (p-value ≤ 0.01) in the males, and two of the measures were able to do so in the females. Not only is the superior performance of the framework compared to the state-of-the-art measures demonstrated, but more importantly, three of the measures (parasympathetic dominance, sympathetic dominance, and the balance of increases and decreases in HRV sequences) in a three-dimensional plot were able to spatially separate the epochs of rest from arithmetic. Furthermore, the fourth measure of data trend, the Real Angle Sum, was able to produce a statistically significant difference between the rest and arithmetic epochs (p-value ≤ 0.01) in *both* the males and females, whilst none of the measures studied in Chapter 3 were able to do so.

The work described in Chapter 5 achieved the third objective, to verify the performance of the proposed Classification Angle framework in the discernment of moderate stress from distress, where pain was taken as a stressor; the ClassA framework was used to identify HRV signatures discerning low pain from high pain. The work presented in this chapter also enabled a test of the theory presented in Dienstbier (1989), which suggested that low basal sympathetic tone, and a strong stress-induced sympathetic activation were representative of a good response to stress. However, the findings from Chapter 5 showed that neither the patients from Group 1 nor Group 2 who experienced low pain elicited such a response. Instead, in a comparison with the respective patients who experienced high pain, the patients with a low sensitivity to pain in Group 1 exhibited a lower basal sympathetic tone, with little change during surgery. A higher basal sympathetic tone, with little change during surgery was found amongst the Group 2 patients with a low sensitivity to pain.

In all three studies described in Chapters 3, 4 and 5, large differences were found in the manifestation of stress depending on the age and sex of the subjects, which prove that future HRV-based stress analyses cannot ignore sex differences, as the conflation of distinct stress responses would invalidate any derived characteristics.

6.1 Comparison of results presented in Chapter 4 and Chapter 5

There were consistent trends amongst the males from Chapter 4 and the Group 1 patients from Chapter 5 in the transitions from rest to mental arithmetic (Chapter 4) and in the transitions from the presurgical period to the surgical period (Chapter 5), as both transitions were characterised by decreases in parasympathetic dominance. This is in accordance with the conventional understanding of

the stress response, in which the parasympathetic nervous system is expected to withdraw to enable an energisation of the body (Selye, 1955).

There were further consistent trends amongst the males from Chapter 4 and the Group 1 patients from Chapter 5, who exhibited a high sensitivity to pain. Both transitions into stress were characterised by increases in sympathetic dominance, which is again in accordance with the conventional understanding of the stress response, in which the sympathetic nervous system is expected to be activated to enable the desired energisation of the body (Selye, 1955). In contrast, of the Group 1 and Group 2 patients who did not experience distress, and instead exhibited low sensitivities to pain, the level of sympathetic dominance did not change significantly from the presurgical to surgical periods, indicating that in the males and older females, the achievement of the fight-or-flight response would not have been the result of large increases in sympathetic tone, but would instead be a result of the withdrawal of the parasympathetic nervous system. The finding of a stress response not driven by the SNS has not been reported in many recent studies, but is in accordance with the concept of an uncoupled PNS withdrawal, depicted in Table 1.1 on page 18 (Berntson et al., 1991). To reiterate, an uncoupled PNS withdrawal in response to stress was reported as far back as 1966, when it was shown in a highly cited study that short-term physical stress caused a reduction in parasympathetic tone, and negligible change in sympathetic tone (Robinson et al., 1966); sympathetic activation was only reported following prolonged exposure to a stressor (Robinson et al., 1966). This is biologically feasible given the fact that the PNS is known to slow heart rate from its intrinsic speed of approximately 100 bpm to a rate which can be as slow as 60 bpm (Gordan et al., 2015), thus, the withdrawal of PNS activity alone is enough to raise heart rate by 40 bpm with minimal involvement from the SNS. *Moderate stress, and the ability to cope, therefore appears to be characterised by withdrawal of PNS activity, in the absence of substantial SNS activation.*

There were also further consistent trends in the action of the sympathetic nervous systems in the transition from rest to mental arithmetic amongst the females studied in Chapter 4, and the transition from the presurgical period to the surgical period in the Group 2 patients who exhibited a high sensitivity to pain, from Chapter 5. Both groups exhibited an absence of a stress-induced increase in sympathetic tone, which supports the findings of an oestrogen driven attenuation of the SNS (Liu et al., 2003), and completely contradicts the conventionally cited sympathetic driven response to stress (Selye, 1955). However, noticeably, the female subjects, studied in Chapter 4, produced stress-induced increases in parasympathetic tone. This stress-induced increase in parasympathetic

dominance remains unexplained (see Table 3.2 on page 55), and must be further investigated physiologically to verify the subjects' autonomic dynamics. Also, despite the fact that the Group 2 patients studied in Chapter 5 produced overall stress-induced reductions in parasympathetic tone from the presurgical to surgical periods, the patients with a high sensitivity to pain exhibited a significantly greater level of parasympathetic dominance, compared to the patients with a lower sensitivity to pain (Table 5.3, Group 2 P^{Q1} *PSLP* vs. *PSHP*: p-value $\ll 0.01$, *SLP* vs. *SHP*: p-value $\ll 0.01$). This all suggests that the parasympathetic nervous system plays a pivotal role in the stress response in young females, although a thorough search of physiological and biological literature has shown that a parasympathetic driven response to stress has not been researched to any great extent. Therefore, findings from this research demonstrate that our understanding of the stress response is greatly limited by a comparative lack of research into the dominance of the parasympathetic nervous system in young females.

6.2 Limitations and Future Work

The work presented in this thesis could be improved in several ways.

Firstly, it is widely known that deep breathing stimulates the PNS (Botha et al., 2014), and it has been advised that to mitigate against the effects of respiration, the respiratory rate of subjects should either be recorded, or subjects should be asked to breathe at controlled rates (Brown et al., 1993). However, there still remains a lack of a means to robustly measure respiration, and the requirement for subjects to breathe at controlled rates remains largely impractical in many study protocols. For these reasons, breathing was neither controlled nor measured in the studies reported in Chapters 3, 4 and 5. Yet, as the implementation of the requirements to monitor respiration would enable a comprehensive assessment of HRV-based stress measures, future assessments of the proposed Classification Angle metrics should employ a study protocol in which either the acquisition of respiration data would not be impeded by speech or movement, or in which subjects would neither have to speak or move considerably.

The interpretation of the Classification Angle metrics are also limited by the fact that in this work, the ClassA metrics were only assessed in the characterisation of two forms of stress, that is, stress caused by mental arithmetic and pain. Given the broad definition of stress, both stressors were very specific. To better prove the credibility of the ClassA framework as a measure of stress in future work, the

framework must be applied to HRV from a variety of stress-inducing activities, using larger datasets than those used in Chapters 4 and 5. In particular, although the clinical feasibility of the framework has been demonstrated in Chapter 5, in the identification of HRV signatures which are indicative of a low or high sensitivity to pain, the framework must be specifically validated in a larger study of day-surgery patients. This would enable a more precise characterisation of the ClassA metrics and pain, to in turn enable the identification of the regions within a three-dimensional plot of $P^{Q2,4}$ against P^{Q3} against P^{Q1} which would be indicative of high or low sensitivities to pain. Notably, it was advised in a letter to The BMJ (formally the British Medical Journal) that algorithmic prognostic tools should refrain from stipulating numerical thresholds for the classification of continuous variables (Wyatt and Altman, 1995). This would mean that the widespread deployment of the ClassA framework for clinical use would ideally output the position of a patient's data in an annotated three-dimensional ClassA plot, allowing the clinician themselves to make an informed decision as to the psychophysiological state of their patient; this is as opposed to the framework itself outputting a fixed label to describe a patient's psychophysiological state.

It could also be argued that the computation of the ClassA measure of sympathetic dominance, P^{Q3}, in a longer window of analysis, and at a higher temporal scale than the other ClassA metrics, complicates the use of the ClassA metrics in real time, and restricts temporal resolution.

The analyses presented in this thesis are also limited by the subjectivity in the evaluation of stress. For example, in Chapters 3 and 4, mental arithmetic was assumed to induce mental stress, without quantitative evidence. Similarly in Chapter 5, the HRV-based measures of stress were validated using subjective self-reports of pain. To overcome such subjectivity, the analyses of the HRV-based stress measures would benefit from comparisons with nerve activity. However, as the acquisition of nerve activities using needle electrodes would undoubtedly induce further stress and discomfort in subjects, in future studies, nerve activities should be indirectly and painlessly assessed using pharmaceutical parasympathetic and sympathetic nerve blockades, which would then enable a conclusive validation of the abilities of the ClassA metrics in the tracking of the PNS and SNS (Robinson et al., 1966; Victor et al., 1987; Botha et al., 2014; Valenza et al., 2018).

The characterisations of stress in this thesis should also be refined by obtaining quantitative measures of oestrogens in female subjects.

6.3 Contributions and Implications

The largest contribution presented in this thesis to stress analysis is the proposed computationally efficient HRV-based measure of stress, the Classification Angle framework. The two ClassA metrics of P^{Q1} and P^{Q3} offer *quantifiable HRV measures of parasympathetic and sympathetic tone* respectively, and could therefore potentially replace the use of the powers of the low and high frequency components of HRV in stress analysis. The replacement of the LF and HF powers is a real possibility, as stress analysis based on the spectral components of HRV has been losing popularity since the realisation that the spectral components lacked clear physiological interpretations (Billman, 2013). Also, it is worth noting that whilst simple temporal measures of stress, and the nonlinear entropy based measures are also not affected by the misinterpretation of the spectral components of HRV, they are limited by only providing one dimension of analysis; it is not possible to assess sympathetic and parasympathetic dynamics separately using only one dimension.

Furthermore, the computation of P^{Q3} at a temporal scale higher than that of P^{Q1} provides support for the use of multiscale analyses, first made famous in Costa et al. (2002). The computation of P^{Q3} at a high temporal scale demonstrates the relevance of coarse-graining physiological signals in the assessment of processes which fluctuate at different speeds. *The faster speed of parasympathetic nerves compared to sympathetic nerves necessitates analysis at different temporal scales*, but no other HRV-based measure of stress utilises analysis at different temporal scales to assess PNS and SNS activity.

Separate from achieving the original aim and objectives of this project, an unexpected contribution of this work is the verification that sex can radically alter the manifestation of stress in cardiac dynamics. The 'cardioprotectiveness' of oestrogens has been gaining more attention in the study of hormones since the 1990s (Ramaekers et al., 1998), but the work in this thesis has shown conclusively that stress cannot be studied without accounting for sex differences, and therefore shows that it is imperative to include females, ideally of all ages, in any study cohort investigating stress.

Had the data from the males and females of Chapter 3 not been separated based on sex, the resulting trends from the males would have conflicted with the trends from the females, and would have potentially prevented any precise characterisation of stress in the subjects. Likewise, the work presented in Chapter 5 also shows that had the patients not been separated based on their probable levels of oestrogens, the relationship between pain and parasympathetic/sympathetic dynamics would not have been revealed.

Also, due to the convention of using entropies to measure complexity, stress has long been cited to reduce physiological complexity (Bornas et al., 2006), and although this may be true in some instances, stress-induced increases in the long-term univariate entropy of HRV were found in the analyses in Chapter 3, and thus calls into question the use of entropy methods to assess physiological complexity. *Stress was always conventionally cited to cause a decrease in the long-term univariate entropy of HRV signals*.

6.4 Project Impact and Practical Applications

The impacts of this project range from calling into question long-standing conventions, including those championed by the originators of stress research (Cannon, 1915; Selye, 1936), such as the universality of the 'fight-or-flight' response, and the convention of using entropies to measure physiological complexity.

The project title of 'Enabling the Quantification of Human Stress from Physiological Responses' may have initially appeared far-fetched, but the results presented in Chapter 5 have demonstrated that it is possible to assess stress objectively and accurately. More precisely, the Classification Angle framework has been shown to provide a three-dimensional measure of autonomic dynamics, which the state-of-the-art HRV measures do not. Also, importantly, the framework has been shown to provide sex-specific interpretations of stress from its outputted results, which the state-of-the-art HRV measures do not account for.

The ClassA metrics also demonstrate the potential to objectively identify the propensity to be distressed, and feel pain in day-surgery patients; the work presented here therefore offers practical solutions to the personalisation of healthcare and the mitigation of distress. However, as mentioned, a clinical validation study recruiting many more patients, and acquiring information regarding the levels of oestrogens in females will be required to create accurate three-dimensional plots of the ClassA metrics for clinical pain assessment.

Therefore in conclusion, although the Classification Angle framework requires extensive validation, critically, it demonstrates that stress can indeed be identified, with some confidence, using only heart rate variability, whereby the results described in this thesis have supported the research hypothesis, to extract signatures within HRV which can identify moderate stress from distress.

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