WESTERN SYDNEY UNIVERSITY

THE TIME COURSE OF MUSCLE SYMPATHETIC AND CARDIOVASCULAR RESPONSES TO PHYSICAL AND MENTAL STRESSORS IN MALES AND FEMALES

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Statement of Original Authorship

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Khadigeh El Sayed

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Abstract

Elevated blood pressure (BP) responses to stressors in young people have been associated with greater risk of hypertension later in life. The aim of this project was to determine what drives BP responses to stress in healthy young males and females. The time course of muscle sympathetic nerve activity (MSNA), BP and heart rate (HR) responses to mental stressors (Stroop colour-word test and mental arithmetic) and physical stressors (cold pressor test (CPT), static handgrip exercise, and post-exercise ischemia) were recorded in 21 healthy young males and in 19 healthy young females. Individuals who experienced a rise in MSNA during stress were classified as positive responders, and those who experienced a fall in MSNA during stress were classified as negative responders. In ***Study 1*** it was hypothesised that negative responders to mental stress experience a more rapid rise in BP at the onset of the task than positive responders. It was also hypothesised that parallel increases in BP and MSNA occur during physical stressors and these are consistent between participants. The results indicate that that negative responders to mental stress exhibit a more rapid rise in diastolic pressure at the onset of the stressor (1.3 ± 0.5 mmHg/s), suggesting a baroreflex-mediated suppression of MSNA. In positive responders there is a more sluggish rise in BP during mental stress (0.4 ± 0.1 mmHg/s), which appears to be MSNA-driven. The physical stressors elicited large and consistent increases in BP and MSNA amongst participants. In ***Study 2***, the effects of sex on the early BP response to stress were examined in both positive and negative responders. The peak changes, time of peak, and rate of changes in BP were compared between males and females and between positive and negative responders. Consistent with the findings in the males, the female negative responders experienced a greater rate of rise in diastolic BP (1.1 ± 0.6 mmHg/s) compared to the positive responders

(0.2 ± 0.1 mmHg/s). Cardiovascular and sympathetic responses to stressors were generally consistent between males and females. However, changes in total MSNA during mental arithmetic were greater in males and changes in HR during handgrip were also greater in males ($P<0.05$). In contrast, changes in MSNA burst amplitude during Stroop test were greater in females than in males ($P<0.05$). In ***Study 3*** the effects of the menstrual cycle on cardiovascular and sympathetic responses to stressors were assessed in young, healthy females. It was concluded that the CPT, but not other stressors, elicits greater sympathetic responses during the low-hormone phase (9±2 bursts/min) compared with the high-hormone phase (5±3; $P=0.014$), but was not associated with larger elevations in BP. In summary, the work described in this thesis has uncovered novel information on the underlying physiological mechanisms responsible for the differences between responders and non-responders to mental stressors, and has also uncovered sex-based differences and the effects of female hormones on sympathetic responsiveness to stressors.

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List of Abbreviations

ACh	Acetylcholine
ANS	Autonomic Nervous System
ABP	Arterial Blood Pressure
BMI	Body Mass Index
BP	Blood Pressure
BRS	Baroreflex Sensitivity
CNS	Central Nervous System
CO	Cardiac output
CPT	Cold Pressor Test
CVLM	Caudal Ventrolateral Medulla
CVS	Cardiovascular System
DBP	Diastolic Blood Pressure
ECG	Electrocardiography
HH	High Hormone
HPAA	Hypothalmic-Pituitary-Adrenal Axis
HR	Heart Rate
LH	Low Hormone
MAP	Mean Arterial Pressure
ML	Mid Luteal
MSNA	Muscle Sympathetic Nerve Activity
MVC	Maximal Voluntary Contraction
NA	Noradrenaline
NE	Norepinephrine
NTS	Nucleus Tractus Solitaries
OCs	Oral Contraceptives
PNS	Peripheral Nervous System
RMS	Root Mean Square
RVLM	Rostral Ventrolateral Medulla
SA	Sinoatrial

SBP	Systolic Blood Pressure
SE	Standard Error
SNA	Sympathetic Nerve Activity
SNS	Sympathetic Nervous System
SSNA	Skin Sympathetic Nerve Activity
SV	Stroke Volume
TPR	Total Peripheral Resistance
VAS	Visual Analog Scale

Preface

This thesis is arranged in six chapters. ***Chapter One*** is a general introduction to the thesis and provides an overview of the relevant literature on the nervous system, with particular reference to the autonomic nervous system, and the cardiorespiratory system. Then it goes in depth into the physiological effects of physical and mental stress, blood pressure regulation in males and females and cardiovascular and sympathetic responses to stress. ***Chapter Two*** details the general methods used across the studies. ***Chapter Three*** is an investigation of the time course of blood pressure and MSNA responses to stressors in positive and negative responders. ***Chapter Four*** examines how a participant's sex can influence blood pressure and sympathetic responses to physical and mental stressors. ***Chapter Five*** investigates the effects of the menstrual cycle on cardiovascular and sympathetic responsiveness to stressors in young, healthy females. Finally, ***Chapter Six*** provides a general discussion, consisting of the main findings, limitations, conclusions and suggestions for future work. ***Appendices*** are attached at the end of the thesis, corresponding to research that I took part in during my candidature.

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Chapter 1

Introduction

PROJECT OVERVIEW

According to the Australian Institute of Health and Welfare (AIHW, 2011-12), 34% of men and 29% of women (persons aged 18 and over) in Australia suffer from hypertension. Chronic stress is associated with an increased risk of hypertension (Esler et al. 2008), and therefore recent surges in work-related stress are likely to exacerbate this problem. Stress-levels in this country are now comparable to those of the UK and the US, with 12% of Australians reporting levels of stress in the severe range (Australian Psychological Society, 2011).

It is common knowledge that inter-individual differences exist in all aspects of human life. Hence, when it comes down to sympathetic activity and cardiovascular control there are often marked differences between individuals (Wallin, 2007). Evidence from studies of inter-individual differences in muscle sympathetic nerve activity (MSNA), suggest that there are large differences in resting sympathetic outflow among humans with similar arterial pressures in the normotensive range (Sundlöf & Wallin, 1978; Skarphedinsson et al. 1997). There is also considerable variability in cardiovascular responses to stress between individuals, with a wealth of evidence linking elevated blood pressure (BP) reactivity to stressors with future hypertension (Matthews et al. 1993). However, further research is required to determine how these increases in BP are brought about. Moreover, mental stress is associated with high variability in MSNA responses. MSNA has been found to increase, decrease or remain unchanged (Carter & Goldstein, 2015). Furthermore, it has been reported that in young women, BP is typically lower than that observed in men of the same age, and that the prevalence of hypertension is higher in men than in women (Burt et al. 1995).

Evidence from previous studies suggests that there are sex differences in regulation of BP and MSNA (Narkiewicz et al. 2005, Ng et al. 1993). However, some studies have shown there are no differences; Vianna et al. (2012) showed no difference between the sexes during the vasoconstriction-induced increase in BP following spontaneous MSNA bursts. It has also been found that the menstrual cycle can influence MSNA and cardiovascular responsiveness to stress. However, this has been controversial as some studies found that the endogenous female hormones (oestrogen and progesterone) have marked effects on BP and MSNA while some studies have not shown any links. Physical stressor tasks such as the cold pressor test (CPT) and static handgrip exercise evoke robust increases in MSNA and BP (Victor, 1997), which makes them useful in assessing sympathetic responsiveness during the phases of the menstrual cycle. Some studies have found there are no differences in MSNA responses to the CPT between the high hormone phase (HH) and low hormone phase (LH) (Jarvis et al. 2011; Middlekauff et al. 2012). The effects of the menstrual cycle have been reported for handgrip exercise; however, it was during the early follicular (low hormone) and late follicular (high oestrogen) phases (Ettinger et al. 1998), rather than the midluteal (ML; high oestrogen and progesterone). In addition, in a study conducted by Carter and Lawence (2007) it was reported that mental stress (mental arithmetic) is associated with similar increases in MSNA, BP and heart rate (HR) during the LH and HH phases of the menstrual cycle. This suggests that the influence of the menstrual cycle may differ between physical and mental stressors.

Because there are still uncertainties in the literature, the aim of this project is to investigate inter-individual differences in the MSNA, BP and HR responses to mental and physical stressors in healthy young males and females.

1.1 AUTONOMIC NERVOUS SYSTEM

1.1.1. General

The autonomic nervous system (ANS), which can be defined as a motor system, is involved in the functions of the gastrointestinal tract, the lower urinary tract, the genital tract, parts of the airway (trachea and bronchi), the cardiovascular system (heart, muscular arteries and arterioles); it controls the movement of iris and lens and the movement of the hairs and the secretion of sweat in the skin. Also, by controlling blood flow, the ANS thus affects the distribution of blood to every part of the body. Furthermore, it also interacts with the endocrine and immune systems (Gabella, 2001).

The ANS operates in an automatic fashion, controlled largely via visceral reflexes, where afferent (sensory) signals from the visceral organ project to the spinal cord, brainstem or the hypothalamus and provide sensory feedback that maintains the internal state of the body constant via effector (motor) signals that are conveyed to various organs of the body. This serves the homeostatic principles that are essential for life (Macefield, 2012). The effector pathway is divided into two major divisions: the sympathetic and parasympathetic nervous systems (Shield, 1993).

In the autonomic pathways, neurons have their cell bodies in the brainstem or spinal cord and synapse onto visceral motor neurons (sympathetic or parasympathetic) in peripheral ganglia. The autonomic motor pathway to a target organ differs significantly from the somatic motor

pathways. In somatic pathways, a motor neurone in the brainstem or spinal cord issues a myelinated axon that reaches all the way to the skeletal muscle.

1.1.2. The sympathetic nervous system

The sympathetic division of the PNS is the main focus of this project due to its actions on the heart and blood vessels, hence providing one of the fundamental mechanisms for the control of blood flow and pressure (Söderström et al. 2003). The neural innervation of the heart and peripheral circulation, as illustrated in figure 1.1, originates from the intermediolateral cell column of the spinal cord (Wallin & Charkoudian, 2007). The activity of the neurones that make up the sympathetic division of the visceral motor system ultimately prepares individuals for "flight or fight" (Parati & Esler, 2012) such that in extreme circumstances, heightened levels of sympathetic neural activity allow the body to make maximum use of its resources (particularly its metabolic resources). Thus, during high levels of sympathetic activity the HR accelerates and the force of cardiac contraction is enhanced (perfusing skeletal muscles and the brain). In addition, blood vessels of the skin and gut constrict (rerouting blood to muscles, thus allowing them to extract a maximum of available energy).

1.1.3. The parasympathetic nervous system

In contrast to the sympathetic division, the parasympathetic division (as shown in figure 1.2) has a calming effect on many body functions. It regulates the ''rest and digest'' functions (McCorry, 2007), through reduced energy expenditure and normal bodily maintenance, including such functions as digestion and waste elimination, hence opposing the effects of the sympathetic nervous system (SNS) (Purves, 2008).

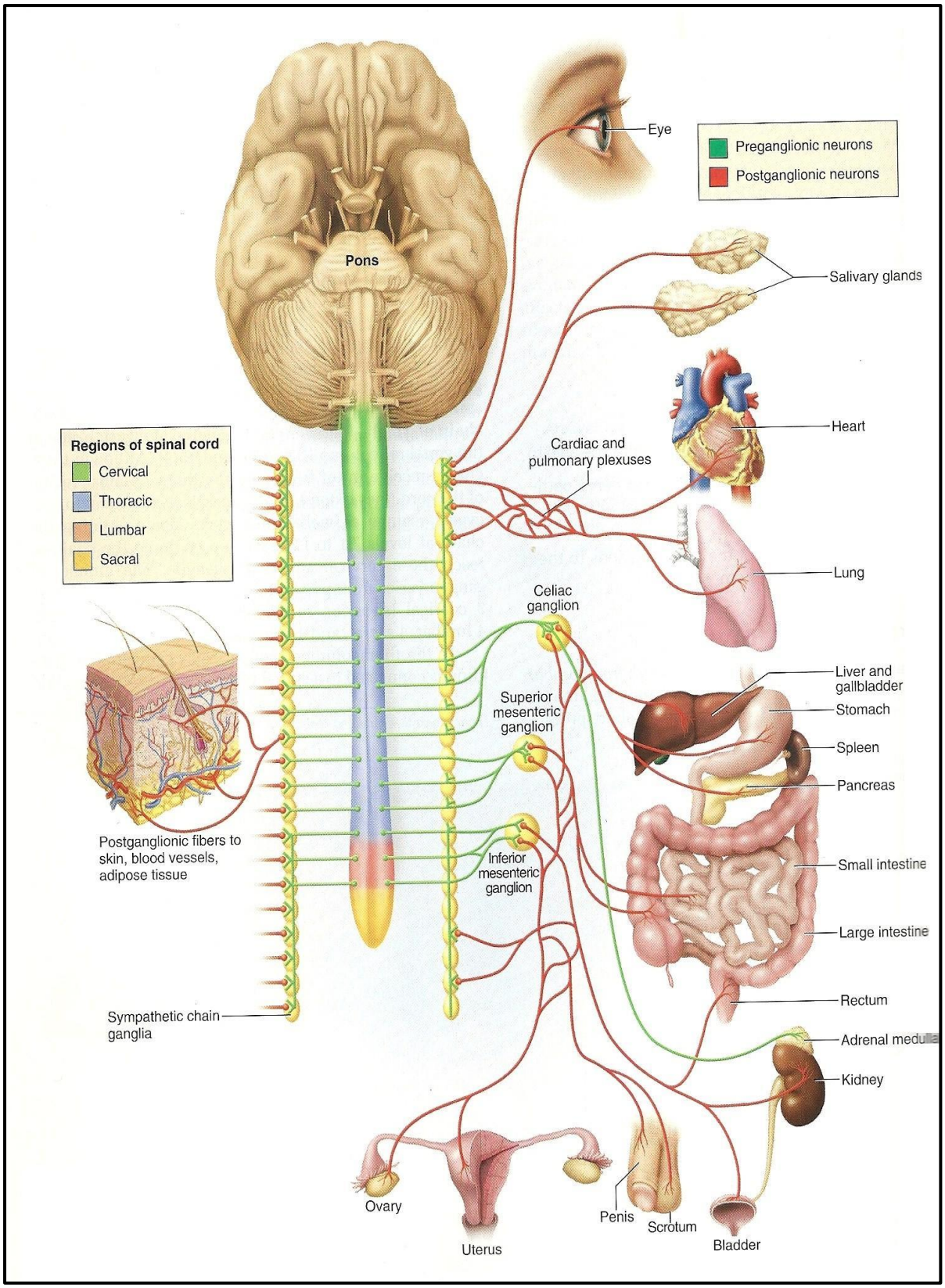

Figure 1.1: Overview of the sympathetic divisions of the visceral motor system (Saladin, 2007).

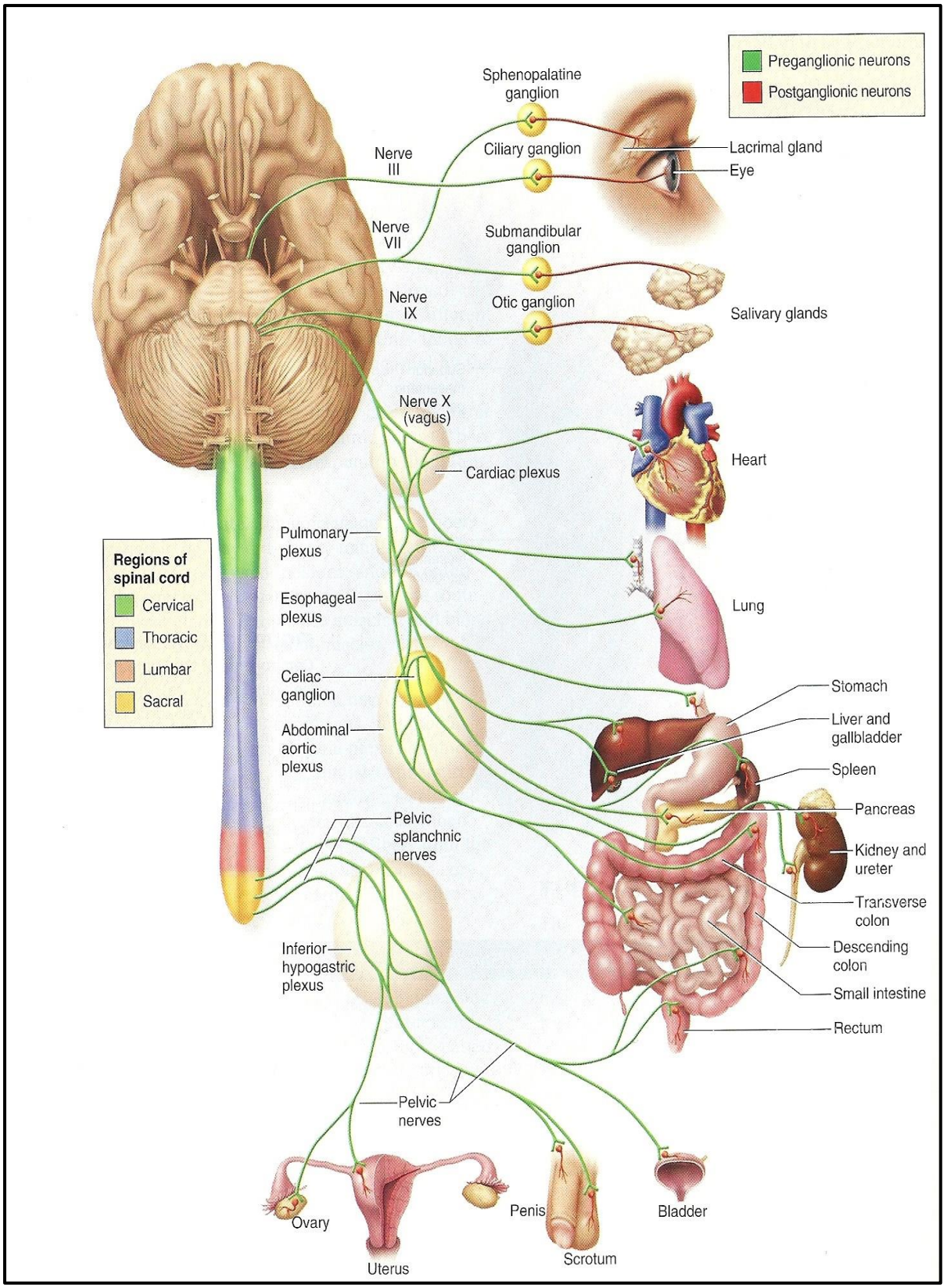

Figure 1.2: Overview of the parasympathetic divisions of the visceral motor system (Saladin, 2007).

1.2 CARDIOVASCULAR SYSTEM

1.2.1. General

The cardiovascular system (CVS) is involved with delivering blood to and from the tissues; this function can be influenced by mental activity, emotional state, posture, muscular exertion, and visceral activity. In addition to mechanisms that regulate BP, there is precise neural control of blood flow to specific organs and regions of the body (Haines, 2002). While it is known that the heart can beat independently of any external neural influences, HR and stroke volume (SV) are controlled by direct inputs to the heart from the CNS, which provides ‘‘central command’’ (Kember et al. 2011). This neural control operates via both sympathetic and parasympathetic innervation of the heart (Guyenet, 2006). These efferent inputs to the heart are a direct consequence of central neuronal processing of cardiovascular afferent feedback to medullary and spinal cord centres. These centres regulate BP in response to inputs that arise primarily from arterial baroreceptors and chemoreceptors located in the carotid arteries and aortic arch (Kember et al. 2011). Baroreceptors function to maintain BP constant, buffering BP against a sudden change in posture, for example.

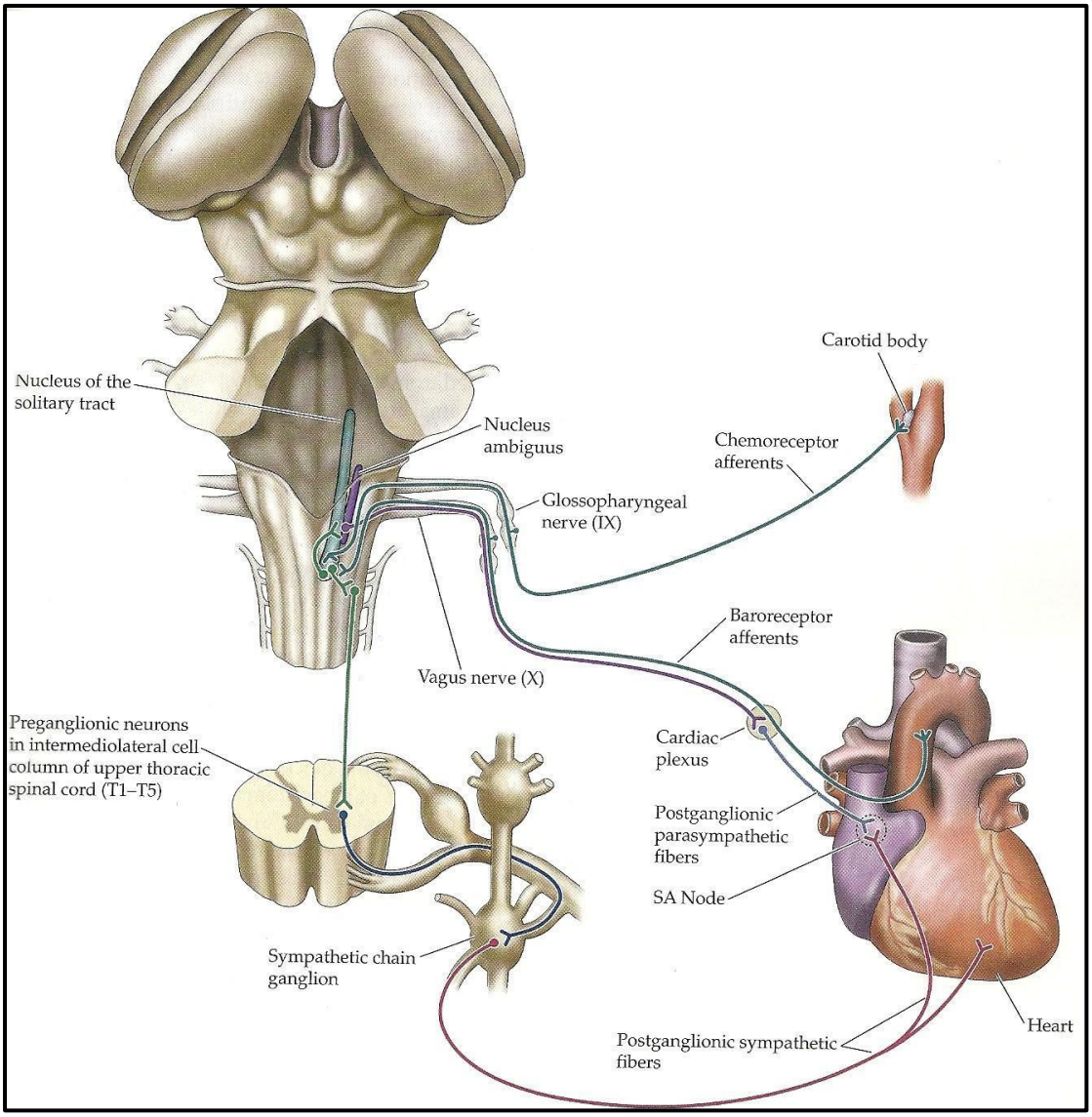

Figure 1.3: Autonomic control of cardiovascular function (Purves, 2008).

1.2.2. Blood Pressure

Blood pressure is a function of vascular resistance and cardiac output (CO), two variables that are controlled by the ANS (Guyenet, 2006). Two pressures are evident with accordance to the cardiac cycle: systolic pressure is the peak arterial BP attained during ventricular contraction, and diastolic pressure is the minimum arterial BP occurring during ventricular relaxation. Stroke volume and HR are controlled by both the sympathetic and parasympathetic branches of the ANS, while vascular resistance is controlled by the sympathetic nervous system. When there is an increase in BP, stretch-sensitive baroreceptors in the carotid sinus and aortic arch increase their activity. In turn, counter-regulatory adjustments in sympathetic and parasympathetic outflow to the heart, and sympathetic outflow to the vasculature, lead to stabilization in BP through negative feedback (Heusser et al. 2010), as illustrated in figure 1.3.

1.2.2.1. Total peripheral resistance

Peripheral resistance is the opposition to flow due to resistance that the blood encounters in blood vessels away from the heart. Total peripheral resistance (TPR) is the sum of the resistance of all peripheral vasculature in the systemic circulation. Resistance is dependent upon the radius of the vessel walls and is determined by the degree of vasoconstriction and vasodilation, which are largely under the influence of the sympathetic nervous system. Jayalalitha et al. (2008) reported the most effective factor controlling blood flow is the radius of the blood vessel, which is explained by Poiseuille's law. Applying this law allows us to study, both qualitatively and quantitatively, the effect of vessel diameter on blood flow and hence determine resistance.

In addition, the neurotransmitter noradrenaline (NA) released from the terminals of the sympathetic postganglionic neurones acts on the smooth muscle of the arterioles to increase the tone of the peripheral vessels. This results in a decrease in the radius of the vessel, ultimately causing an elevation in BP (Purves, 2008). This occurs when NA is released from the sympathetic nerve terminals and binds to α_1- or α_2-adrenergic receptors that are located on the vascular smooth muscle cells. This leads to an increase in intracellular Ca^{2+} either by causing release of Ca^{2+} from the sarcoplasmic reticulum or by increasing flux through plasmalemmal Ca^{2+} channels (Thomas, 2011), with the rise in intracellular Ca^{2+} causing contraction of the smooth muscle, resulting in vasoconstriction (McCorry, 2007).

On the other hand, acetylcholine (ACh) binds to two types of cholinergic receptors (nicotinic and muscarinic). Nicotinic receptors are found on the cell bodies of all postganglionic neurons, both sympathetic and parasympathetic, in the ganglia of the ANS. ACh is released from the preganglionic neurons and binds to these nicotinic receptors, this causes a rapid increase in the cellular permeability to Na^{+} ions and Ca^{2+} ions (McCorry, 2007).

Muscarinic receptors are found on the cell membranes of the effector tissues and are linked to G proteins and second messenger systems that carry out the intracellular effects. ACh is released from all parasympathetic postganglionic neurons and sympathetic postganglionic neurons traveling to sweat glands and the adrenal glands. In addition, muscarinic receptors can be either inhibitory or excitatory, depending on the tissue they are found on (McCorry, 2007).

1.2.3. Baroreceptors and homeostasis

Baroreceptors are specialized receptors that monitor BP, found in the carotid arteries and aortic arch. They are involved in the homeostatic control of the HR (Young et al. 2008); however, their primary control is blood pressure through their effects on the sympathetic vasoconstrictor neurons. These neurons are responsible for maintenance of TPR (Kirchheim, 1976; Raven et al. 2006), via a negative feedback system called the baroreceptor reflex. Thus they are responsible for buffering of acute fluctuations in arterial blood pressure that may occur during changes in posture, exercise, emotion, and other conditions (Benarroch, 2008), making the arterial baroreceptor reflex the most important short-term regulator of arterial pressure (Mukkamala et al. 2003).

The nerve endings in the baroreceptors are activated by stretch as the elastic elements of the vessel walls expand (Purves, 2008). Primary afferent fibres from baroreceptors in the aortic arch travel in the vagus nerve (CN X), while afferent fibres from the carotid sinus travel in the glossopharyngeal nerve (CN IX). Based on studies in anesthetized animals, as illustrated in figure 1.3, it has been determined that primary afferent fibres from arterial baroreceptors terminate in the nucleus tractus solitaries (NTS) in the medulla oblongata. Signals from the NTS are then conveyed via an excitatory pathway to GABAergic neurons in the caudal ventrolateral medulla (CVLM), which then in turn project to and inhibit spinally projecting neurons within the rostral ventrolateral medulla (RVLM) (Dampney et al. 2003), thus causing sympathetic inhibition, leading to a decrease in TPR and pressure. Additionally, in reference to figure 1.3, direct excitatory projections from NTS convey signals to the nucleus ambiguous, which supplies parasympathetic axons, conveyed by the vagus nerve, to the heart.

Release of ACh from the cardiac vagus nerve terminals onto the sinoatrial (SA) node of the heart reduces HR and SV.

1.3 MSNA AND BP CONTROL

MSNA is involved in the beat-to-beat control of BP (Macefield et al. 2013); meaning BP is influenced by the degree of peripheral vasoconstriction. Hence, its primary role in health is to buffer acute falls in BP, via the baroreflex (Macefield 2013). Some studies have found no (Sundlöf & Wallin, 1978; Kienbaum et al. 2001; Charkoudian et al. 2005, 2006a,b) or minimal relationship (Weyer et al. 2000) between baseline MSNA and BP in humans under 40 years old, but there are modest relationships between MSNA and BP in humans over 40 years old. Therefore, due to the poor relationship between the level of baseline MSNA and BP, one cannot predict the level of MSNA in a given healthy individual by recording BP alone (Joyner et al., 2008).

Direct recordings of MSNA in awake human subjects, through the technique of microneurography (see below) has shown that MSNA occurs as bursts of impulses that, through the arterial baroreflex, are strongly coupled to the cardiac cycle. A transient increase in BP causes an initial reduction in MSNA, corresponding to the phase of increased baroreceptor firing (Sundlöf and Wallin, 1978). Heusser et al. (2010) showed that electric field stimulation of carotid baroreceptors rapidly decreased MSNA and BP, and acutely reduced MSNA in a subgroup of patients with refractory arterial hypertension.

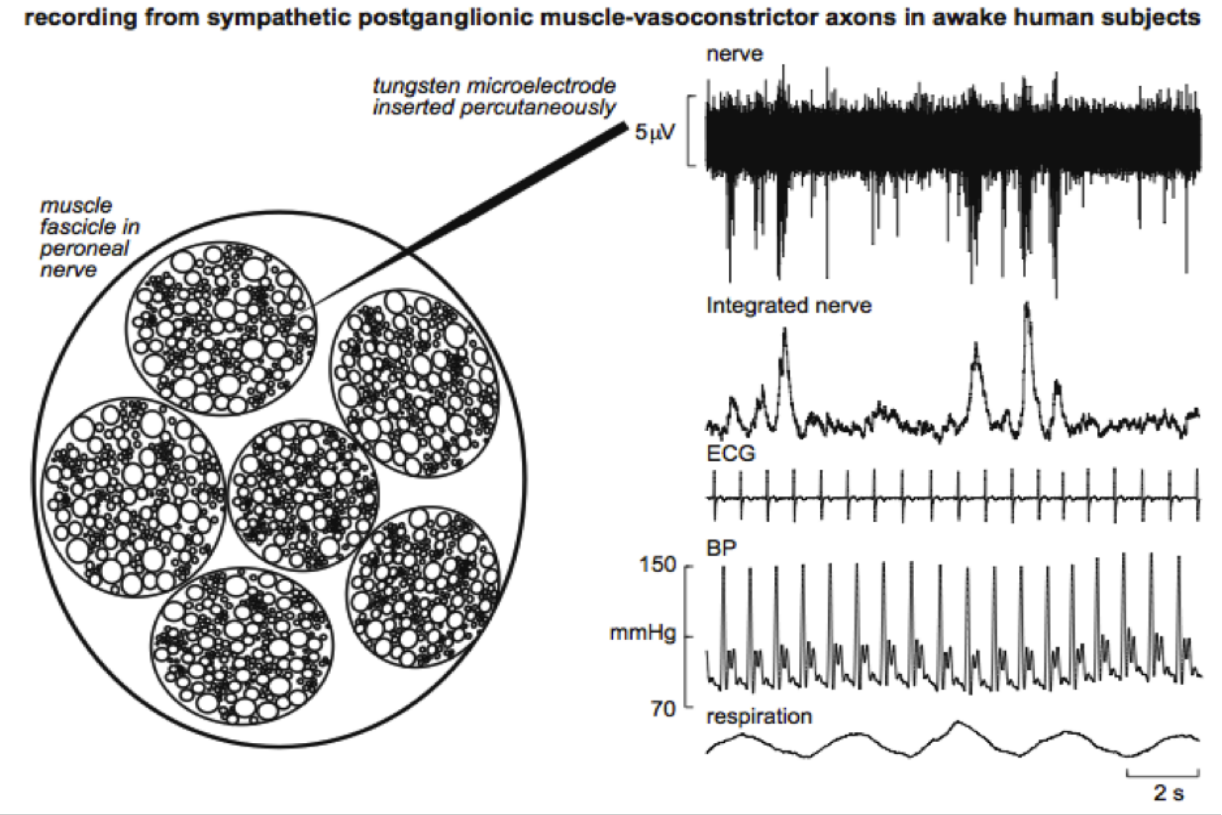

Figure 1.4: Direct MSNA recording from an awake human subject. As shown, bursts of MSNA are composed of negative-going spikes that occur synchronously with the cardiac rhythm (Macefield, 2013).

1.3.1. Recording sympathetic nerve activity in humans.

SNA in humans is typically assessed using one of the following methods:

(i) Microneurography, which involves the insertion of microelectrode percutaneously into a peripheral nerve, allows the examiner to make direct recordings of SNA.

(ii) NA spillover, using intra-arterial and intravenous lines to measure blood supply to specific organs such as the heart, can provide regional or whole-body estimates of SNA.

1.3.1.1. Microneurography

Microneurography was developed in Uppsala, Sweden 1965 - 1966 within the department of clinical neurophysiology at the Academic Hospital (Vallbo et al. 2004). It is a method in which a tungsten microelectrode is inserted percutaneously into a peripheral nerve in awake human subjects (Macefield et al. 2013). It directly measures neural traffic in myelinated and unmyelinated efferent and afferent nerves leading to and coming from muscle and skin in human peripheral nerves (Mano et al. 2006). Using this technique, afferent discharges from muscle and skin, and efferent discharges leading to muscle and skin can be recorded from human peripheral nerves to identify the sensory receptors and the effector organs. Sympathetic nerve fibres travelling in motor fascicles are generally considered to supply blood vessels in the skeletal muscles, whereas those travelling in cutaneous fascicles supply blood vessels, sweat glands, hairs and/or adipose tissue. Hence, the need for identification of the target tissue is important (Macefield et al. 2002).

To ensure reliability and validity of sympathetic recordings, laboratory conditions need to be kept within a tight range (Hart et al. 2017). For example, variations in ambient temperature can cause changes in MSNA (Fagius & Kay, 1991) and SSNA (Bini et al. 1980); hence, the ambient temperature should be kept between 21°C and 24°C. In addition, to avoid any background noise disturbances to SNA, recordings need to be made in a quiet environment (Hart et al. 2017), keeping in mind the subject should not fall asleep, as this will affect the reliability of the SNA recordings (Hornyak et al. 1991).

Mirconeurography is a robust method in measuring SNA; studies have shown it is highly reproducible within individuals (Fagius & Wallin, 1993; Fonkoue & Carter, 2015; Grassi et al. 1997; Sundlöf & Wallin, 1977; Yamada et al. 1989). Additionally, MSNA recorded in the peroneal nerve is equivalent to that recorded in the radial nerve (Rea & Wallin, 1989) and is consistent when recorded bilaterally, *i.e*. when recorded from both sides of the body (El Sayed et al. 2012).

When the electrode penetrates into a muscle fascicle of a nerve, afferent discharges can be recorded. In this case, sensory afferent discharges from muscle stretch receptors (muscle spindles) can be mechanically stimulated by tapping, squeezing, or stretching the muscle. When the electrode enters a cutaneous fascicle, afferent discharges can be recorded from cutaneous mechanoreceptors by gentle touching or tapping of the skin area innervated by the nerve.

Microneurographic studies have recorded both MSNA and skin sympathetic nerve activity (SSNA). MSNA originates from vasoconstrictor fibres, the activity of which is modulated by inhibitory inflow from the baroreceptors. Delius et al. (1972) reported an inverse relationship between the periodic fluctuations in BP and sympathetic outflow, *i.e*. rapid, large BP reductions were generally associated with the greatest increases in MSNA.

Conversely, SSNA occurs as irregular bursts that are independent of BP. They are generated by a mixture of cutaneous vasoconstrictor, vasodilator, pilomotor and sudomotor neurones, which are engaged in thermoregulation and emotional expression, and hence are activated by arousal, emotional stimuli, deep breaths, and changes of environmental temperature (Hagbarth et al. 1972).

1.3.1.2. Noradrenaline spillover

In addition to microneurography, NA spillover can be measured to record sympathetic activity. This technique was pioneered by Murray Esler and colleagues in 1979. NA is a neurotransmitter of the sympathetic nervous system, and the rationale behind the measurement of NA spillover is that at the end of each sympathetic nerve impulse NA is released from the nerve endings into the circulation (such as it "spills over" into the circulation) (Wallin & Charkoudian, 2007). In turn, NA spillover gives the rate at which released NA enters plasma.

Microneurography does not give direct access to sympathetic nerves of internal organs; this is

a limitation that is overcome by measuring NA spillover (Esler, 2000). The NA spillover technique also allows the examiner to assess both whole body sympathetic activity (Esler et al. 1979) and organ specific sympathetic activity from visceral nerves (Wallin & Charkoudian, 2007). These techniques are referred to as "total" and "regional" measurements of NA spillover (Esler et al. 1984), respectively. Both total and regional NA spillover techniques are performed by infusing radio-labeled NA and by collecting blood samples obtained from a catheter placed in an artery, or a specific artery and related vein for regional spillover.

However, NA spillover does have limitations, for example, it is an invasive technique and the use of radioisotopes is prohibited in some countries (Esler, 1993). Another disadvantage is that SNA varies in organs and thus, regional SNA has a greater analytical power than total sympathetic activity (Esler et al. 1984). Furthermore, the time resolution associated with NA spillover techniques is inferior to that of microneurography, which can provide beat-to-beat measurements of SNA.

2. Literature review

2.1 STRESS

Stress is perceived as a threat to homeostasis (Barnett at al. 1963), with responses varying in degree of specificity, depending on the type of challenge and how the individual uniquely perceives it and their ability to cope with the specific stressor. There are many types of stress such as orthostasis, cold exposure, mild blood loss, exercise, and water immersion (Bales et al. 2006; Best & Halter 1989). Other studies define stress as situations that most people would find stressful (Sergerstrom & Miller 2004).

Goldstein (2006) explains one possible response to stress. The body possesses homeostatic comparators, called homeostats that are responsible for comparing information supplied by a sensor and determined by a regulator or a set of regulator mechanisms. This response is due to an acute stressor which posed a threat to the environment, in turn resulting in the "flight or fight' response. Responses include increased delivery of oxygen and glucose to the heart and the large skeletal muscles (Sergerstrom & Miller 2004).

2.1.1. Blood pressure reactivity to stressors

Exaggerated BP reactivity to psychological stressors in young, healthy individuals has been identified as a pathophysiological risk factor in predicting the development of future hypertension and cardiovascular disease (Lovallo & Gerin 2003; Matthews et al. 2006; Olsen et al. 2013; Treiber et al. 2003). However, the responses to stress vary to a great extent between individuals.

2.1.2. Future hypertension

Blood pressure reactivity to stressor tasks, such as the Stroop colour-word test and mental arithmetic, has previously been shown to predict future hypertension in healthy, young individuals (Carroll et al. 2001; Carroll et al. 2003; Flaa et al. 2008; Matthews et al. 1993; Matthews et al. 2003; Matthews et al. 2004). Although much of the literature focuses on cardiovascular reactivity to mental stress, there is also evidence suggesting that increases in BP in response to physical stressors, such as the CPT, are positively associated with the risk of developing hypertension (Menkes et al. 1989). Matthews et al. (2004) reported that normotensive individuals who show the most marked rise in arterial pressure in response to sympathoexcitatory stress are at much higher risk for the future development of hypertension than their counterparts who can be described as 'non-responders'.

Furthermore, other studies have established that genetic factors are important in the pathogenesis of hypertension (Light et al. 1999). Normotensive young subjects with a family history (that is, both parents hypertensive) of hypertension provide an opportunity to assess early dysfunction of cardiovascular regulation. The SNS of normotensive offspring of hypertensive parents does not seem to be activated during mental stress, but a greater cardiovascular reactivity (*i.e*. BP increase) to mental stress has recently been documented in these subjects (Lambert & Schlaich, 2004). Schwartz et al. (2011) demonstrated similar results; their study showed augmented BP responses to mental stress in pre-hypertensive compared with normotensive subjects. Therefore, an augmented pressor response to stress in normotensive subjects may help to predict the development of hypertension.

In contrast to mental stress, the CPT showed contradictive results - hypertensive patients presented a markedly reduced sympathoexcitatory response to a CPT, and the same impairment was observed in young subjects genetically predisposed to hypertension. Moreover, MSNA during the CPT was markedly attenuated in subjects with established hypertension as well as in young normotensive subjects with a positive family history of hypertension (Lambert & Schlaich, 2004). Greaney et al. (2015) demonstrated greater increases in systolic BP, diastolic BP, MAP and MSNA responses to handgrip and CPT in young women with a family history of hypertension.

Whilst the capacity of mental and physical stressor responses to predict future hypertension remains controversial, the mechanisms behind the inter-individual variability in these responses remain unclear.

2.1.3. Role of sympathetic activity in hypertension

It is well known that there is a parallel relationship between the onset of high BP and elevated MSNA (Wallin et al. 1973; Esler, 2000), so an increase in MSNA is the primary determinant in establishing hypertension. Grassi et al. (2015) reported there is a sympathetic overactivity in young hypertensive patients, which is a characteristic of clinical essential hypertension. This sympathetic overdrive is also present in middle aged and elderly hypertensive patients. Moreover, other studies have used both microneurography and NA spillover techniques to show this parallel relationship between elevated SNA and hypertension (Wallin et al. 1973; Esler et al. 1989; Grassi et al. 1998). In addition, using surgical sympathectomy, early animal models of hypertension have also proven this parallel relationship; Judy et al. (1976) and

Abboud (1982) have shown that SNA is elevated in hypertensive compared with normotensive rats.

Furthermore, exactly why SNA remains elevated in hypertensive patients remains unclear and indicates that there are multiple afferent drivers causing hypertension (Hart, 2016) *i.e*. inputs instructing the brain that BP must be increased and maintained, thus driving elevated SNA (Koeners et al. 2016). These afferent signals may include input from the kidneys, carotid bodies and heart, for example, as well as endocrine modifications (Hart, 2016).

2.1.4. Physical stressors

Most prospective studies have used the CPT as a stressor task; however, several studies have argued that this task provides a poor test linking reactivity and pathology (Carroll et al. 2003). In addition, the CPT is a thermal pain test and does not elicit a β-adrenergically mediated myocardial response, which is important in early neurogenic hypertension; thus, it is not an optimal test (Matthews et al. 2004). It causes robust increases in BP and HR due to the effects of pain, and pain perception and is seen to be a poor analogue of everyday stress (Carroll et al. 2003); hence, it is not a useful tool in predicting future hypertension (Reimann et al. 2012).

In addition to the CPT, static handgrip and post-exercise ischaemia have also been used (Ettinger et al. 1996), with changes in MSNA during static exercise being brought about by central command and the accumulation of the metabolites during contraction. The latter acts

on unmyelinated and thinly-myelinated sensory endings within muscle to bring about a reflex increase in MSNA – known as the exercise pressor reflex, or metaboreflex.

Charkoudian and Wallin (2014) stated that during physical exercise, the ANS is responsible for redirecting blood flow and increasing CO via various combinations of the central command (feed-forward regulation) and the exercise pressor reflex (feed-back regulation). In addition, during post-handgrip ischemia Cui et al. (2001) concluded that the metaboreceptor stimulation was responsible for the change in the sensitivity of baroreflex control of MSNA. They also concluded that there was no significant relationship observed between MSNA responses and the perception of pain.

Studies using psychological stressors, such as mental arithmetic, have yielded more promising results than those that used the cold pressor test - as mental stressors are more effective in predicting future hypertension. Several studies of young and middle-aged adults found associations between the magnitude of BP changes during such stressors and subsequent rises in resting BP over 1 to 10 years later (Matthews et al. 2004).

2.1.5. Mental stressors

Many studies have focused on responses to mental stress tasks rather than physical stress tasks. Mental stress responses in healthy individuals include increases in HR, BP, and brachial artery dilation, as well as changes in sympathetic outflow. In most mental stress studies the test has been either the Stroop test, which involves non-verbal identification of a colour embedded in the name of a different colour (Callister et al. 1992) or mental arithmetic

using verbal serial subtractions (Callister et al. 1992; Carter et al. 2008; Carter & Ray, 2009). These mental stressor tasks have been associated with a high degree of variability, and MSNA has (for unknown reasons) been found to either increase (Callister et al. 1992; Carter et al. 2002; Ng et al. 1993), or decrease (Matthews & Solomon 2003), or remain unchanged (Matsukawa et al. 1991; Carter et al. 2008; Carter & Ray 2009) during mental stress. However, this variability has been observed in really early studies by Wallin et al. 1973 and continues to be found in recent studies (Carter & Ray 2009).

Other forms of mental stress, including speech tasks and delayed auditory feedback, have been used in previous studies (Carter & Goldstein, 2015). For the speech task the individual is given a topic and is asked to give a 5-10 minute speech. Delayed auditory feedback involves having the subjects rapidly and accurately read a book for 5-10 minutes while listening to their voices with a 200ms delay. However, these tasks are used to a lesser extent and thus information on MSNA and BP responses are limited compared with the Stroop test and mental arithmetic tasks.

Blood pressure responses to stress vary to a great extent between individuals. However, little is known about what causes some individuals to be 'positive responders', ‘negative responders’ and others 'non-responders' to stressor tasks. Based on changes in MSNA burst frequency, Carter & Ray (2009) categorised individuals according to their response to mental stress. Those individuals with an increase in MSNA of $\geq\Delta 3$ burst/min were deemed to be positive responders, while those with a decrease in MSNA ($\leq\Delta 3$ burst/min) were negative responders and those with little or no change in MSNA were classified as non-responders. Despite variable neural responses, mental stress increased mean arterial pressure (MAP) and

HR similarly in positive responders, negative responders and non-responders. These results might suggest that BP and HR responses to mental stress are not influenced by changes in MSNA. However, these results are based on mean changes in MSNA and BP and therefore do not take into account what happens to these variables on a beat-to-beat basis throughout the course of the stressor task.

It has been hypothesised that inter-individual differences in BP control during stress may be influenced by variations in baroreflex sensitivity (Lipman et al. 2002). Despite the well-known role of the baroreflex in acute BP control, its influence on the individualised responses to stressors remains uncertain. However, data collected by Lipman et al. (2002), demonstrates that greater arterial pressure responses to mental stress relates to greater carotid stiffness and lower arterial baroreflex sensitivity in middle aged and older individuals. Furthermore, a study conducted by Fauvel et al. (2000) showed that there was a lack of association between stress-induced pressor response and baroreflex sensitivity within a younger cohort. This finding does not conflict with observations in middle-aged and older subjects, because the association between baroreflex sensitivity and BP modulation may become more appreciable with increasing age (Lipman et al. 2002). Variability in BRS may provide an explanation for the inter-individual differences seen in healthy, young individuals, which ultimately may help in determining why responders are at a greater risk of future hypertension.

A study by Fonkoue and Carter (2015), found that MSNA and BP responses to mental stressors were strongly reproducible across the three experiments (two experiments on the first day and one experiment on the second day). However, results were not categorised into

'positive responders', 'negative responders' and 'non responders' according to their MSNA responses in order to compare BP responses (Carter & Ray, 2009) and predict future hypertension. Furthermore, inter-individual differences in BP control and MSNA during mental and physical stressor tasks may be influenced by other factors such as gender, the menstrual cycle and the use of the contraceptive pill.

2.1.6. MSNA during recovery from mental stress

Some studies have shown that MSNA increases after the metal task ceases when compared to baseline levels (Carter et al. 2004; Carter et al. 2005; Dishman, 2013; Ellenbogen et al. 1997; Fonkoue & Carter, 2015). Carter at al. 2005, have found that MSNA in the arm and leg remained significantly elevated in the recovery phase compared to baseline levels. They suggested that this could be due to increased circulating adrenaline (Lindqvist et al. 1996) or nitric oxide release into circulation (Dietz et al. 1994; Cardillo et al. 1997), however they did not examine the baroreflex. Another possible reason why MSNA remains elevated during the recovery period is due to the arterial baroreflexes. Callister et al (1992) found that both HR and arterial pressure decreased immediately at task cessation, whereas MSNA increased.

In addition, the SNA and the hypothalamic–pituitary–adrenal axis (HPAA) work together during mental stress. It is known that during a stressful event the HPAA (McEwen, 1998) and SNA are activated. When these systems are activated catecholamines from nerves and the adrenal medulla are released which leads to the secretion of corticotropin from the pituitary. The corticotropin, in turn, mediates the release of cortisol from the adrenal cortex. During recovery, *i.e*. when the stress is past, the systems return to baseline levels of cortisol

(McEwen, 1998). However, in some cases the SNA and HPAA fail to turn off after stress but there is limited evidence to suggest why this is the case (McEwen, 1998).

Fonkoue and Carter (2015) reported that MSNA, BP and HR during recovery from mental stressors were not repeatable across three experiments (two experiments on the first day and one experiment on the second day). Moreover, Chida and Steptoe (2010) in their meta-analysis suggested that there is a role of the cardiovascular recovery as a marker of cardiovascular risk. However, it is not known how long sympathetic activation persists after the end of mental stress (Carter & Goldstein, 2015).

2.2 BLOOD PRESSURE REGULATION IN HEALTHY YOUNG MALES AND FEMALES

Direct comparisons of MSNA between men and women demonstrated greater resting MSNA burst frequency in men than women. Some studies have found reproducible findings (Hart et al. 2009; Jones et al. 1996; Ng et al. 1993; Shoemaker et al. 2001; Yang et al. 2012), while others have found inconsistent findings (Fu et al. 2009; Fu et al. 2005; Narkiewicz et al. 2005). This inconsistency may be due to the effects of the menstrual cycle on MSNA, as only a few sex-based studies have accounted for the menstrual cycle (Hart et al. 2009; Usselman et al. 2014; Yang et al 2012). However, Usselman et al. (2014) found that MSNA burst incidence, MSNA burst frequency, baseline MAP and SBP were higher in men than women, regardless of menstrual cycle phase. Evidence from studies of inter-individual differences have shown that there are large differences in resting sympathetic activity amongst

normotensive individuals with similar BP (Sundlöf & Wallin, 1978; Skarphedinsson et al. 1997). Fagius and Wallin 1993, have shown that these differences are highly reproducible and suggested that the underlying variables are robust and may reflect important inter-individual differences in sympathetic control of BP regulation. Such variables include age (Sundlöf & Wallin 1978), sex (Ng et al. 1993), body mass index (BMI) (Scherrer et al. 1994) and menstrual cycle (Charkoudian, 2001).

Sex appears to be an important determinant in setting the resting levels of MSNA in young humans (Ng, 1993). Hart et al. (2009) reported that high levels of MSNA and high TPR are balanced by lower CO and decreased vasoconstrictor responsiveness to adrenergic stimuli in young men. However, in young women, MSNA is not correlated to either TPR or CO, thus indicating that young women regulate BP differently compared with men. Evidence suggests that the transduction of MSNA to peripheral resistance, *i.e*. the physiological translation of neurotransmitter (NA) release into vasoconstriction, may also differ between males and females. For example, Kneale et al. (2000) found that vascular transduction was lower in young females than in their male counterparts. This explains why young females typically have lower BP than males of a similar age and are at a lower risk of hypertension.

Furthermore, Schmitt et al. (2010) demonstrated that α-blockade with phentolamine induced a smaller reduction in BP in women than in men. This indicated that young women have a lower α-adrenergic support of BP compared to men of similar age. This was also reported by Hart et al. (2011), suggesting that women exhibit blunted vasoconstrictor responses to α-adrenergic stimulation, which may be related to the vasodilator effect of oestrogen. Schmitt et

al. (2010) also found a positive relationship between the resting level of MSNA and the phentolamine-induced decrease in MAP in young women but not in young men. Hence, these studies concluded that men and women rely on strikingly different integrated physiological mechanisms to maintain BP.

Hart et al. (2009) reported that in young healthy men, TPR is positively related to MSNA, suggesting that MSNA is a good index of net whole body vasoconstrictor tone. Casey et al. (2011) found that men demonstrated a positive relationship between MSNA and aortic wave reflection (pressure wave generated by the deceasing diameter of the descending aorta - during late systole, early diastole - back to the left ventricle), while this relationship was inversely related in women. Fundamental sex differences in arterial pressure regulation have been demonstrated in other studies by Shoemaker et al. (2001), Charkoudian et al. (2005) and Carter & Ray (2009).

In addition, Lambert et al. (2007) tested whether the SNS was differently affected in men and women by BMI. They found, in women, MSNA was not linked to the level BMI, whereas in men, BMI constitutes a major determinant for the level of MSNA. In addition, they conducted a 12-week weight loss diet in a small subgroup of obese subjects and found that the results were consistent with their previous findings – there was a marked effect of weight loss on MSNA in men only. These findings are supported by a study conducted by Alvares et al. (2002), where visceral fat (abdominal fat), assessed by computed tomography scans, was found to be a stronger determinant of MSNA compared with subcutaneous fat. The differences between men and women in fat distribution may account for the finding of a

relationship between MSNA and BMI in men but not in women, as visceral adiposity is more common in men. However, this area needs further research, specifically to test the influence of sex on BP, MSNA and HR in response to both physical and mental stressors.

2.3 RESPONSES IN BLOOD PRESSURE AND MSNA TO STRESSORS IN HEALTHY YOUNG MALES AND FEMALES

It has been reported that in young women BP is typically lower than that observed in men of the same age (Burt et al. 1995; Carter & Cooke, 2016; Usselman et al. 2014). This may contribute to the fact that the incidence of orthostatic hypotension and other hypotensive disorders is greater in women than in men of the same age (Fu et al. 2005; Shoemaker et al. 2001). Moreover, studies from Convertino (1998) and Barnett et al. (1999) have shown that there are lower plasma NE concentrations in women during orthostatic stress than in men, suggesting that sympathetic vasoconstrictor outflow may also be diminished. These findings suggest that there are distinct sex differences in sympathetic control when the human body is exposed to a physiological challenge stress, such as the responding to gravitational load during changes in posture. Hart et al. (2009) observed that among the factors that contribute to the overall level of TPR, the magnitude of SNA has a greater role in young men compared with young women. The authors concluded that the contribution of vasoconstrictor drive to arterial pressure regulation differs in women and men. However, in an earlier study Jones et al. (1996) reported no differences in MSNA, BP, or HR responses to the cold-pressor test and mental arithmetic between men and women.

Furthermore, previous studies have found that there are larger systolic BP responses to mental tasks in young males compared with young females. However, in response to a physical test (CPT), only DBP changes were significantly greater in males (Matthews et al. 2004). It is not clear if the menstrual cycle was controlled for. In another study Carter and Ray (2009) demonstrated that males have greater increases in BP during mental stress (mental arithmetic) compared with females, despite having lower perceived stress scores. However, changes in MSNA and HR during mental stress were not different in males and females, which in support with Jones et al. (1996).

2.4 INFLUENCE OF THE MENSTRUAL CYCLE ON BLOOD PRESSURE REGULATION AND MSNA

The menstrual cycle consists of the early follicular (low oestrogen, low progesterone) in this phase menstruation occurs, as illustrated in figure 1.5; late follicular (high oestrogen, low progesterone) in this phase ovulation occurs, usually occurring on day 14 (Mihm et al. 2011); and the ML (high oestrogen, high progesterone). Little research has been done concerning the impact of hormonal changes during the menstrual cycle on BP and cardiovascular reflexes in women. However, there is evidence that the SNS changes during the menstrual cycle (Minson et al. 2000b); it is known that the female reproductive hormones have widespread influences on sympathetic control of the circulation in humans (Charkoudian, 2001).

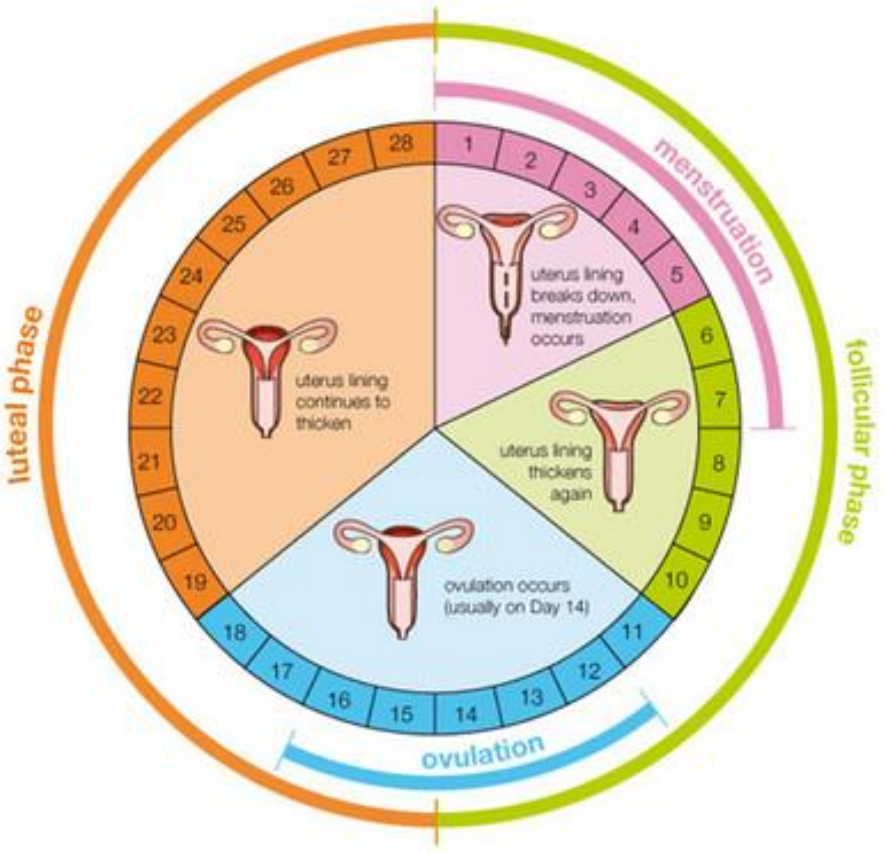

Figure 1.5: A representation of the menstrual cycle phases. (Available: http://www.fullcirclehealthcareinc.com/uploads/4/1/6/7/41671693/2808651_orig.jpg?400, Accessed 20 February 2016)

Hypertension is relatively rare in premenopausal women but increases dramatically following menopause. This low incidence of cardiovascular disease in premenopausal women has been attributed to the protective effects of endogenous female hormones (Dubey et al. 2001). The female hormones fluctuate monthly with the ovarian cycle in premenopausal women: oestrogen and progesterone levels are low during the LH phase (days 1-7 of the cycle) and reach their peak during the HH phase (days 19-23 of the cycle).

The menstrual cycle is known to influence resting MSNA. More specifically, changes in circulating sex hormone levels during the LH and HH phases have a direct effect on the resting levels of MSNA (Baker et al. 2016). There is also evidence suggesting that menstrual fluctuations in female reproductive hormones influence cardiovascular control. More specifically, several studies have demonstrated differences in the control of BP via the sympathetic nervous system during the high and low hormone phases of the menstrual cycle. It is evident that both oestrogen and progesterone have marked effects on the cardiovascular system; Minson et al. (2000a) reported that sympathetic baroreflex sensitivity (BRS) is reduced during the LH phase of the menstrual cycle, which was associated with lower resting MSNA. During the HH phase, when both oestrogen and progesterone are elevated, resting MSNA and sympathetic BRS were higher. These findings are supported by those of Usselman et al. (2014), who reported that sympathetic BRS and baseline MSNA are greater in the HH phase compared with the LH phase of the menstrual cycle. In contrast to these findings, there have been some studies reporting no difference in sympathetic baroreflex control between the two hormonal phases (Carter et al. 2009; Fu et al. 2009; Middlekauff et at. 2011). However, cardiovagal BRS was not significantly affected by the menstrual cycle (Usselman et al. 2014). Additionally, other studies have shown that the menstrual cycle alters

sympathetic neural responses to orthostatic stress in young, eumenorrheic women (Carter et al. 2009).

It has been postulated that the different phases of the menstrual cycle may influence not only resting sympathetic outflow, but BP and sympathetic responsiveness to stress. However, in a study conducted by Carter and Lawence (2007) it was reported that mental stress (in the form of a mental arithmetic task) is associated with similar increases in MSNA, BP and HR during the LH and HH phases of the menstrual cycle. As noted above, the effects of mental stress on MSNA are reported to be highly variable (Carter and Goldstein, 2015) and individuals within one study cohort may be deemed positive, negative or non-responders to mental stress with regards to the change in MSNA (Carter & Ray, 2009). Carter and Lawrence (2007) compared mean changes in MSNA during mental stress and, although a mean increase in MSNA was observed for the group, it was not clear whether all individuals experienced an increase and how consistent these responses were between menstrual phases. Furthermore, it is not known if the time course of BP and MSNA differs between low and high hormone phases.

The CPT and handgrip exercise evoke robust increases in MSNA and BP (Victor, 1987) and thus provide useful maneouvres for assessing sympathetic reactivity during the low and high hormone phases of the menstrual cycle. Previous research suggests no differences in MSNA responses to the cold pressor between the LH and HH hormone phases of the menstrual cycle (Jarvis et al. 2011; Middlekauff et al. 2012) and, although effects of the menstrual cycle have been reported for handgrip these have between the early follicular (low hormone) and late follicular (high oestrogen) phases (Ettinger et al. 1998), as opposed to ML (high oestrogen

and progesterone).

2.5 INFLUENCE OF THE CONTRACEPTIVE PILL ON BLOOD PRESSURE REGULATION AND MSNA

Often prepared as ethinyl oestradiol and progestin combinations, hormonal contraceptives are well known to have unfavorable effects on the cardiovascular system (Maguire & Westhoff, 2011). Evidence suggests that sympathetic and cardiovagal BRS and MAP are increased during the low hormone phase (*i.e*. placebo pills) compared with high hormone phase, in women taking oral contraceptives (OC) (Minson et al. 2000b). However, in contrast to this evidence, Carter et al. (2009) found that OCs did not alter sympathetic BRS, concluding that MSNA, BP, and HR responses to orthostatic stress are similar during LH and HH phases of oral contraception. Moreover, this conclusion was supported in the Minson et al. (2000b) study, which stated that OCs did not alter resting MSNA in either of the phases.

Therefore, changes in sympathetic and cardiovagal BRS when using OCs differ from the changes as seen in the normal menstrual cycle. Furthermore, a more recent study conducted by Harvey et al. (2015), indicates that women taking OCs have higher resting BP and similar MSNA during the placebo phase of OC use when compared with naturally menstruating women in the LH phase.

This review of literature highlights the lack of understanding of inter-individual differences in MSNA, BP and HR responses to mental and physical stressors in healthy young males and females. Furthermore, the time-course of these responses has yet to be studied effectively.

2.6 AIMS AND HYPOTHESES

The main aim of this project is to examine inter-individual differences in BP and MSNA responses to mental and physical stress with respect to sex and the menstrual cycle. A comprehensive series of studies was used to investigate the BP responses to mental and physical stressors, alongside direct measurements of MSNA, in an attempt to determine what drives the differences in BP reactivity in males and females. In order to do this, the following objectives were proposed:

- To investigate the time course of BP and MSNA responses to mental and physical stressors, in order to increase our understanding of the interaction between these two variables during stress. Research indicates that individuals may experience a rise (positive responders) or fall (negative responders) in MSNA during mental stress. Hence the aim is to examine the early BP response to stress in positive and negative responders and thus its influence on the direction of change in MSNA.

  Hypothesis 1: Negative responders to mental stress experience a more rapid rise in BP at the onset of the task than positive responders. Parallel increases in BP and MSNA occur during physical stressors that are consistent between participants.

- To examine the effects of gender in the early BP response to stress in both positive and negative responders and thus its influence on the direction of change in MSNA.

  Hypothesis 2: There is a greater proportion of positive responders amongst females than in males

  Hypothesis 3: The positive responders experience a reduced rate of rise in BP compared with negative responders.

- To examine what drives the increases in BP and MSNA in the LH phase by examining the time course of BP, MSNA and HR responses during a series of mental and physical stressor tasks.

  Hypothesis 4: Lower resting MSNA will provide greater capacity for an increase in sympathetic activity, and thus increases in MSNA and BP are greater in the LH phase than HH phase during physical and mental stressors.

Study 1 has been published in the Journal of Physiology and two other manuscripts are in preparation.

El Sayed, K., Macefield, V.G., Hissen, S.L., Joyner, M.J. & Taylor, C.E. 2016. Rate of rise in diastolic blood pressure influences vascular sympathetic response to mental stress. *Journal of Physiology*, 594 (24), 7465-7482. DOI: 10.1113/JP272963.

2.7 SIGNIFICANCE OF THE RESEARCH PROJECT

Knowledge of the relationship between BP and modulating factors, such as MSNA, is vital for our understanding of both short and long-term BP regulation and ultimately for the prevention and treatment of hypertension. This research will help to identify what drives the BP responses to stressor tasks and therefore what causes some individuals to be responders and others non-responders to stress; this is particularly relevant given the evidence linking BP responses to stress with future hypertension. Hence, this investigation will give us a better understanding of the key mechanisms underlying the development of hypertension in males and females.

Chapter 2

General Methods

2.1 ETHICAL APPROVAL AND PARTICIPANTS

All studies were conducted with the approval of the Human Research Ethics committee, Western Sydney University (WSU), and satisfied the Declaration of Helsinki. Written consent was obtained and participants were informed that they could withdraw from the experiment at any time.

Participants were asked to refrain from any alcohol and caffeine intake for a minimum of 12 hours before an experiment, as both have shown to induce increases in MSNA (Corti et al. 2002; Randin et al. 1995). They were also asked to refrain from any vigorous physical activity for minimum of 12 hours prior to an experiment due to the effects on resting MSNA (Ray & Hume, 1998).

The screening process included the completion of a Medical and Health Screening Questionnaire (standard questionnaire used in the Sport & Exercise Science program at WSU). Young and healthy (normotensive, non-smokers and not obese) individuals with no history of cardiovascular disease were recruited. They were also given a participant information sheet providing a description of the study, its aims, and details of the participant's involvement, including risks and benefits of partaking in the study. Contact details of the researchers were also included should they have any further questions. Due to the effects of the menstrual cycle on cardiovascular variables, pre-menopausal female participants took part in the experiment in the early follicular phase of their cycle (Minson et al. 2000a) or in the LH phase of OCs use (Minson et al. 2000b). The menstrual cycle was

assessed on the basis of a questionnaire, which allowed the subjects to calculate which day of the menstrual cycle they were on.

2.2 STUDY DESIGN

All subjects were seated in a semi-reclined posture with their legs supported in the extended position, as demonstrated in figure 2.1. Before commencing the experiments the location of the common peroneal nerve was determined via palpation of the leg at the fibular head, located at the level of the knee. This was achieved by rolling the foot medially, exposing the head of the fibular over which the common peroneal nerve laterally descends. A casting cushion was placed below the hamstrings in order to fix the leg in place and prevent the knee from rotating. Room temperature was thermostatically maintained at 22^{o}C throughout the experiments and a blanket was placed on the subject when needed, while keeping in mind that the subject should not fall asleep as this would have affected the reliability of the SNA recordings (Hornyak et al. 1991). Recordings were also made in a quiet environment to avoid any disturbances to the SNA recordings (Hart et al. 2017). The subjects then performed a series of mental and physical stressor tasks (explained in detail in section 2.5).

2.3 MEASUREMENTS

Continuous BP was recorded non-invasively, via digital arterial plethysmography (Finometer; Finapres Medical System, Amsterdam, the Netherlands), which incorporates correction for the height of the hydrostatic column, which is the difference in position of the finger sensor relative to the position of the heart. This system also calculates the haemodynamic parameters

such as SV, TPR and CO. The Finometer repeatedly calibrates the reconstructed BP wave at set intervals against brachial arterial measurements using an upper arm cuff. The BP status was confirmed using automated brachial measurements. In addition, electrocardiographic (ECG) activity was recorded with Ag-AgCl surface electrodes on the chest sampled at 2k Hz. While respiration recorded via a piezoelectric (strain-gauge) transducer around the chest, sampled at 0.4 kHz. This generates a high-level, linear signal in response to changes in thoracic circumference associated with breathing (Pneumotrace, UFI, Morro Bay, CA, USA). MSNA was recorded on a computer based data acquisition system (PowerLab 16SP, ASinstumentals, Sydney, Australia). The neurogram was sampled at 10 kHz and displayed in real-time on a computer monitor and routed to external speakers for audio feedback.

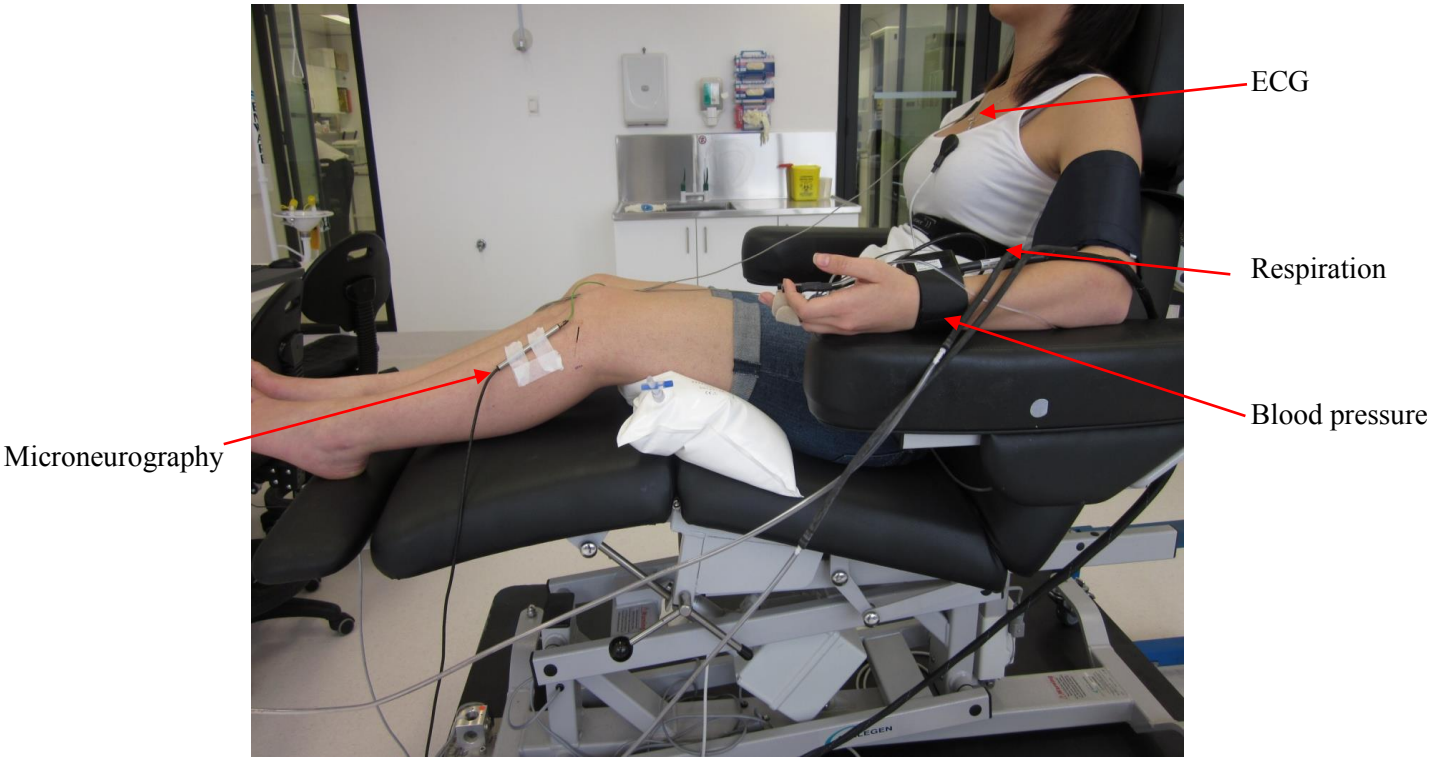

Figure 2.1: A subject during a typical experimental set-up, demonstrating microneurography on the left leg. Other parameters shown are: ECG, blood pressure and respiration.

2.4 MICRONEUROGRAPHY

The location of the common peroneal nerve was achieved by delivering weak electrical pulses (0.2 ms) of cathodal (depolarising) stimuli (<5 mA), delivered at 1 Hz to the skin at the level of knee (fibular head) using a 1 mm search probe connected to a computer-controlled, constant-current isolated stimulator (Stimulus Isolator, ADInstruments, Sydney, Australia). An Ag-AgCl surface electrode on the opposite side of the knee served as the anode. This allowed us to identify the sites on the skin overlaying the common peroneal nerve, *i.e*. the closer the nerve to the skin the lower the current required to activate the nerve and evoke muscle twitches in the muscles supplied by the nerve and paraesthesiae in the cutaneous distribution of the nerve. After identifying the optimal sites and marking the location of the nerve, the area was sterilised using alcohol swabs and then an uninsulated reference microelectrode was then inserted subcutaneously approximately 2 cm above the marked recording area. An insulated tungsten microelectrode (FHC, Bowdoinham, ME, USA) was inserted into the skin above the nerve (figure 2.2). The microelectrodes were then connected to the input terminals of an isolated amplifier headstage (NeuroAmp EX, ADInstruments, Sydney, Australia). Intraneural stimulation (0.2 ms, 1Hz, 1mA) through the recording microelectrode, relative to the reference electrode, was preformed while the experimenter advanced the microelectrode towards the nerve (figure 1.4). The subjects were then asked to report any sensations as this helped us conclude where the tip of the microelectrode was located, *i.e*. if it was approaching a cutaneous fascicle, or a muscle fascicle of the nerve. The stimulating currents were reduced progressively when these sensations increased in intensity, until we reached a current of 0.02 mA. The stimulating leads were then removed and the preamplifier and amplifier switched on; the surface

electrode on the skin opposite side of the knee served as the ground electrode. Small advances of the microelectrode caused the tip to penetrate the wall of the fascicle, which were evident by “insertion discharges” - bursts of action potentials induced by the mechanical irritation of myelinated axons. The above procedure was applied to either the left or right leg of the subject.

For the purpose of this project, only MSNA was studied. Muscle nerve fascicles was identified by the following criteria (Sundlof & Wallin, 1977):

- Weak electrical stimuli delivered through the recording electrode give rise to muscle twitches without concomitant skin paraesthesiae
- Taps on the muscle belly and passive muscle stretch evoke afferent mechanoreceptive impulses
- Regular spontaneous bursts synchronised to the cardiac cycle
- There was no response to stroking of the skin, indicating that the microelectrode was not located in a cutaneous fascicle

If the electrode was located in a cutaneous fascicle, re-adjusting took place in order to establish clear muscle afferent activity.

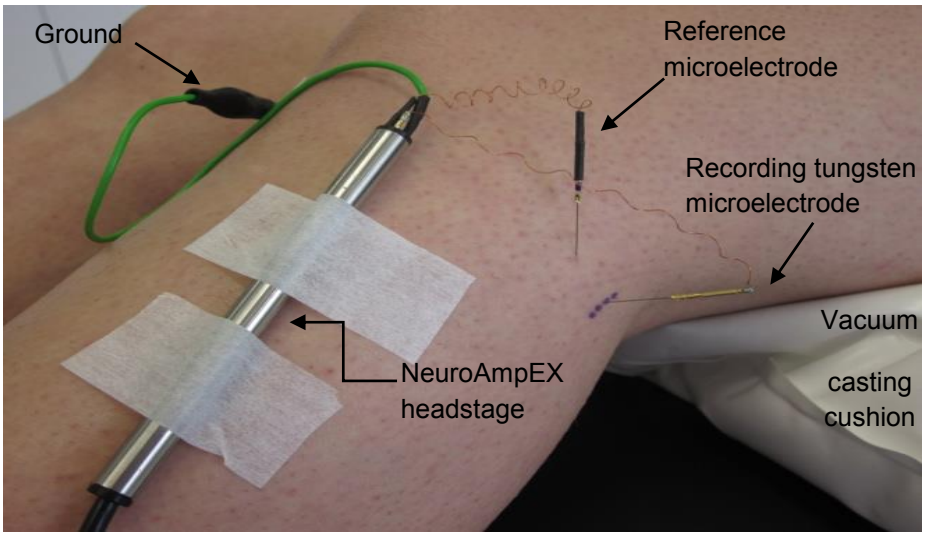

Figure 2.2: Continuous neural recording from the common peroneal nerve supplying the Tibialis Anterior muscle. The headstage is used to preamplify the raw nerve recording received immediately from the common peroneal nerve. The reference electrode inserted subcutaneously, coupled with the recording tungsten microelectrode allows the signal acquisition of the efferent motor and afferent sensory traffic. The casting cushion is placed below the hamstrings to expose the fibular head, hence facilitating the recording.

2.5 EXPERIMENTAL PROTOCOL

Once a good MSNA recording site was achieved with spontaneous neural activity, the subjects were asked to relax, while controlled baseline cardiovascular measurements were recorded for ten minutes. Following the initial 10-min baseline period, participants performed two breathing manouvres (inspiratory-capacity apnoea and end-expiratory apnoea), and then completed two mental and three physical stressor tasks. The mental tasks include: mental arithmetic and Stroop colour-word test, while the physical tasks include: static handgrip exercise, post-exercise ischaemia and a CPT. Subjects were then required to provide feedback related to these tasks, such as anxiety and difficulty scores and pain score for the CPT. Furthermore, the duration of each task lasted for two minutes and was performed in a randomized order, with an exception of post-exercise ischaemia, which immediately followed the handgrip task. All tasks started with a 2-min resting period and ended with a 2-min recovery period, and were separated by a minimum of five minutes rest to ensure that all variables were stable before commencing the next task.

Detailed descriptions of the stressor tasks are below:

Mental Arithmetic Task: Participants were given a random three-digit starting number and asked to consecutively subtract seven, verbally stating the answer for a period of two minutes. If the participant gave an incorrect answer, they were notified and reminded of the last correct number; if their answer was correct they continue without feedback. The number of correct numbers was recorded. On completion of the task, participants were asked to rate their anxiety level during the task on a scale of 0 (no anxiety) to 10 (most anxious I have felt).

Stroop colour-word test: Using an iPad with a Stroop colour-word application, participants were required to respond via touch-screen with the correct colour of the word displayed on the screen (as opposed to the colour the word spells out). The number of correct answers was recorded. On completion of the task, participants were asked to rate their anxiety levels on a scale of 0 to 10.

Cold Pressor Task: Participants were required to immerse their dominant hand in ice water for a period of two minutes. During the task the participants were asked to record their pain level using a visual analog scale (VAS); 0 describes 'no pain' while 10 describes 'the worst pain ever experienced'.

Static Handgrip Exercise: A handgrip dynamometer was calibrated according to each participant's maximal voluntary contraction (MVC). Participants were instructed to grip the handgrip dynamometer at 35% MVC for two minutes. The %MVC was displayed on the computer screen to provide feedback to the participants.

Post-exercise Ischaemia: 5 seconds prior to cessation of handgrip exercise, a cuff was inflated around the active arm to 200 mm Hg in order to occlude blood flow to the contracting muscles. The cuff remained inflated for two minutes while the participant relaxed their arm. The cardiovascular reactivity to post-exercise ischemia will be treated as a separate task to handgrip exercise. This maneuver activates the metaboreflex, in which metabolites produced during exercise excite group III and IV afferents and cause an increase in MSNA (Dampney et al. 2003), which leads to a sustained elevation in BP. Pain was not recorded.

2.6 DATA ANALYSIS

The Peak Parameters module (LabChart 7, ADInstruments, Sydney, Australia) was used to detect and measure the amplitude of individual bursts of MSNA. The nerve trace was shifted to account for the conduction delay, and adjusted for each participant to account for differences in burst latency. The average shift applied was 1.26 ± 0.01s. Mean MSNA burst amplitude and number of bursts per minute (MSNA burst frequency) were determined. MSNA was quantified by counting the number of bursts per minute (burst frequency) or per 100 heart beats (burst incidence). Total MSNA was calculated using number of bursts multiplied by the mean burst amplitude. Total MSNA and burst amplitude values were normalized to individual resting values and expressed as a percentage change from rest. For each stressor task, changes in SBP, DBP, MAP, HR, total MSNA, MSNA burst frequency and MSNA burst amplitude responses were determined across 15-s intervals throughout rest (2-min pre-stressor), task and recovery (2-min post-stressor) periods. Repeated measures ANOVAs were performed to determine the main effect of time for each variable, and post-hoc multiple comparisons were made to determine which time points were significantly different from rest. Mean changes in each variable were also determined for each stressor task by comparing to the average of the 2-min rest period prior to the stressor.

2.6.1. Positive versus negative responders

For those stressor tasks in which the direction of the change in MSNA differed between individuals, the participants were divided into groups of ‘positive’ and ‘negative’ responders. Those individuals with a mean increase in MSNA burst frequency during the task were

classified as positive responders and those with a mean decrease were classified as negative responders. The same grouping process was also performed according to mean changes in total MSNA. In order to assess whether BP responses to stressors differed between positive and negative responders, the following comparisons were made between groups:

Mean changes: The mean changes in systolic BP, DBP and MAP were compared between positive and negative responders. Mean change (mmHg) was defined as the mean BP during the task minus the mean BP during the preceding 2-min rest period.

Peak changes: The peak changes in systolic BP, DBP and MAP were compared between positive and negative responders during the first minute of the task. The first minute was chosen because evidence suggests that the majority of the increase in BP during mental stress occurs within the first minute, after which it typically plateaus (Dunn & Taylor, 2014). Peak change (mmHg) was defined as the highest BP value during the first minute of the task minus the BP value of the first cardiac cycle of the task.

Time of peak: The time of the peak changes in systolic BP, DBP and MAP during the first minute of the task were compared between positive and negative responders. Time of peak (s) was defined as the number of seconds to reach the peak BP from the start of the task.

Rate of change: The rate of change in SBP, DBP and MAP was compared between positive and negative responders. Rate of change (mmHg/s) was defined as peak change (mmHg)/ time to peak (s).

Comparisons between positive and negative responders were performed using independent t-tests. These tests were performed with individuals grouped according to changes in MSNA

burst frequency, and again for groups determined by changes in total MSNA. Since this is the first time this approach has been used, the analyses above were also performed using the peak BP in the first 30s of the task and the peak BP from the full 2-min task. There are studies that indicate that BP may rise throughout the first two minutes of mental stress (Anderson et al. 1987; Kamiya et al. 2000; Carter et al. 2013; Carter & Ray, 2009; Durocher et al. 2011; Carter et al. 2013) and recent work that suggests the first 30 seconds may be important (Greaney et al. 2015).

2.6.2. Sympathetic baroreflex sensitivity

Sympathetic BRS was assessed in all participants. The 10-min rest period at the beginning of the experimental protocol was used for quantifying sympathetic BRS via spontaneous methods (Kienbaum et al. 2001; Hissen et al. 2015). For each participant, the diastolic pressure values for each cardiac cycle were assigned to 3 mmHg bins to reduce the influence of respiratory-related oscillations (Ebert & Cowley, 1992; Tzeng et al. 2009). For each bin the corresponding MSNA burst incidence was determined (number of bursts per 100 cardiac cycles). MSNA burst incidence was plotted against the mean DBP for each bin in order to quantify sympathetic BRS via linear regression. A weighting was applied to account for the number of cardiac cycles associated with each bin (Kienbaum et al. 2001). The acceptance level for baroreflex slopes was set at $r > 0.5$ (Hart et al. 2011; Taylor et al. 2015). Independent t-tests were performed to test for differences in sympathetic BRS between positive and negative responders. All statistical analyses were performed using Prism v6.00 for Mac OS X (GraphPad software, San Diego, California, USA). A probability level of $P<0.05$ was regarded as significant. All values are expressed as means and standard error (SE).

2.7 STATISTICAL ANALYSIS

All statistical analyses were performed using Prism v6.00 for Mac OS X (GraphPad software, La Jolla, California, USA). For all statistical tests, a probability level of $P \leq 0.05$ was regarded as significant and two-tailed tests were used. All values are expressed as means and SE. Specific statistical tests performed in each study are detailed in each of the three results chapters.

Chapter 3: Study 1

Rate of rise in diastolic blood pressure influences vascular sympathetic response to mental stress

3.1 ABSTRACT

Aim: Research indicates that individuals may experience a rise (positive responders) or fall (negative responders) in MSNA during mental stress. The aim was to examine the early BP response to stress in positive and negative responders and thus its influence on the direction of change in MSNA.

Methods: BP and MSNA were recorded continuously in 21 healthy young males during 2-min mental stressors (mental arithmetic, Stroop test) and physical stressors (CPT, handgrip exercise, post-exercise ischaemia). Participants were classified as negative or positive responders according to the direction of the mean change in MSNA during the stressor tasks. The peak changes, time of peak, and rate of changes in BP were compared between groups.

Results: During mental arithmetic, negative responders experienced a significantly greater rate of rise in DBP in the first minute of the task (1.3 ±0.5 mmHg/s) compared with positive responders (0.4 ±0.1 mmHg/s; P=0.03). Similar results were found for the Stroop test. Physical tasks elicited robust parallel increases in BP and MSNA across participants.

Conclusion: It is concluded that negative MSNA responders to mental stress exhibit a more rapid rise in diastolic pressure at the onset of the stressor, suggesting a baroreflex-mediated suppression of MSNA. In positive responders there is a more sluggish rise in BP during mental stress, which appears to be MSNA-driven. This study suggests that whether MSNA has a role in the pressor response is dependent upon the reactivity of BP early in the task.

3.2 INTRODUCTION

Stress has previously been described as a 'sensed threat to homeostasis' (Goldstein & McEwen, 2002). The autonomic nervous system is responsible for responding appropriately to acute episodes of stress and, in their recent review, Carter and Goldstein (2015) discuss the possibility that variability in autonomic responses to stress may provide a unique window of insight into hypertension and other cardiovascular diseases. Noll et al. (1996) reported that BP and MSNA during mental stress increase in the offspring of hypertensives but not in the offspring of normotensives. A recent study by Fonkoue et al. (2016) substantiates and extends these findings, with reports of greater elevations in MSNA during mental stress in those with a family history of hypertension than those without, despite no differences in the blood pressure responses between groups. Although the effect of mental stress on MSNA has been the focus of a number of studies over the past forty years, many research groups have reported increases in MSNA in response to laboratory mental stressor tasks (Carter et al. 2013; Yang et al. 2013; Schwartz et al. 2011; Scalco et al. 2009; Hering et al. 2013; Heindl et al. 2006; Kuniyoshi et al. 2003), whilst others report decreases (Matsukawa et al. 1991; Halliwill et al. 1997) or no changes (Kuipers et al. 2008; Carter et al. 2008; Wasmund et al. 2002; Wilkinson et al. 1998; Jones et al. 1996). These findings suggest that considerable inter-individual variability exists in BP and MSNA responses to mental stress.

Many of the traditional sympathoexcitatory manoeuvres, such as the CPT, ischaemic handgrip exercise and lower body negative pressure, are associated with robust elevations in MSNA amongst individuals (Victor et al. 1987a; Victor et al. 1987b; Sundlöf & Wallin,

1978). In contrast, early evidence indicates that MSNA responses to mental stress are variable between participants; Wallin et al. (1973) studied MSNA responses 15 times in 9 experiments and, although the length of time under mental stress was not consistent between experiments, the authors observed increases (4 periods of stress), decreases (4 periods of stress) and no changes in MSNA (7 periods of stress). It might be expected that these MSNA responses directly influence BP in these individuals. However, BP was measured during 13 of the tests and the majority of participants experienced an increase in BP, with one showing a decrease and another no change. Since this early work, a number of studies have been published that support the idea that MSNA responsiveness to mental stress is subject to inter-individual variability (Carter & Ray, 2009; Fonkoue & Carter, 2015; Donadio et al. 2012), as highlighted by Carter & Goldstein (2015) in their recent review. Carter and Ray (2009) reported that when individuals were divided into groups according to their MSNA burst frequency response to mental stress (*i.e*. positive responders, negative responders and non-responders), all three groups demonstrated an increase in BP during the mental arithmetic task. The authors also reported no significant correlation between changes in BP and changes in MSNA burst frequency. These findings indicate that the interaction between BP and MSNA is more complex during mental stress than, for example, during rest or physical stressors. However, these results are based on mean changes for the period of mental stress and this therefore cannot inform the interaction between MSNA and BP with respect to the time course of the stressor task.

In the current study we examine the time course of BP and MSNA responses to mental and physical stressors, in order to increase our understanding of the interaction between these two variables during stress. Research indicates that individuals may experience a rise (positive

responders) or fall (negative responders) in MSNA during mental stress. The aim is to examine the early BP response to stress in positive and negative responders and thus its influence on the direction of change in MSNA. Mental arithmetic and the Stroop colour-word conflict test were used as mental stressors, and the physical tasks used were the CPT, static handgrip exercise and post-exercise ischaemia. Observations in our laboratory suggest that the initial BP response, in particular the rate of the rise in pressure, may influence the MSNA response during mental stress. The magnitude, timing and the rate of the rise in BP will be quantified using the first minute of the 2-min tasks in order to compare responses between those who experience a rise in MSNA during stress (positive responders) and those who experience a fall (negative responders). Previous research indicates that the sympathetic baroreflex is reset to higher pressures during mental stress (Durocher et al. 2011). The nature of the baroreflex negative feedback loop is such that MSNA may contribute to a rise in BP but it may also be suppressed by it. Which of these two scenarios dominates during mental stress appears to differ between individuals (*i.e*. positive and negative responders). Since the sympathetic baroreflex responds to acute increases in BP by inhibiting MSNA, it is postulated that a more rapid rise in BP at the onset of mental stress may occur concurrently with baroreflex resetting and lead to baroreflex suppression of MSNA. In contrast, a lag in the rise in BP may allow time for baroreflex resetting to occur and, with a higher set point; MSNA may increase and contribute to the elevation in BP. It is therefore hypothesised that negative responders to mental stress experience a more rapid rise in BP at the onset of the task than positive responders. It is hypothesised that parallel increases in BP and MSNA occur during physical stressors that are consistent between participants.

3.3 METHODS

3.3.1. Participants

Twenty-four healthy male participants aged between 18 and 25 years old, with no history of cardiovascular disease, were recruited for the study.

Refer to methods section 2.1 for further detail.

3.3.2. Measurements

Refer to section 2.3.

3.3.3. Experimental procedures

Refer to section 2.5.

3.3.4. Data analysis

Time course of responses to stressors

Refer to section 2.6.

Positive versus negative responders

Refer to section 2.6.1.

Sympathetic baroreflex sensitivity

Refer to section 2.6.2.

Repeatability

T-tests were performed between session 1 and session 2.

3.4 RESULTS

Of the 24 males recruited, successful recordings were obtained from 21 participants. The mean age was 22 ± 2 years old, and body mass index was 24.5 ± 0.6 kg/m^2. Mean values from the 10-min baseline for resting SBP, DBP and MAP were 129 ± 4, 61 ± 3, and 79 ± 3 mmHg, respectively. Mean resting HR was 64 ± 2 beats/min, resting MSNA burst frequency was 36 ± 1 bursts/min and resting MSNA burst incidence was 58 ± 2 bursts/100heartbeats. There were no significant differences in BP, HR or MSNA burst frequency between the rest periods prior to each stressor task (P>0.05; table 3.1). All participants completed all stressor tasks. In 11 participants, a stable MSNA recording was maintained throughout the protocol. In the remaining 10 participants adjustment of the microneurography site was required in order to recover the MSNA recording. For this reason, changes in all variables during stress were compared to the resting levels prior to the task, and MSNA burst amplitude and total MSNA are reported as percentage changes from rest.

3.4.1. Mental arithmetic

When all subjects were pooled mental arithmetic and the Stroop test were both associated with significant increases in SBP, DBP, MAP and HR (P<0.05, table 3.2). There was a significant main effect of time on total MSNA for the mental arithmetic task. Pairwise comparisons revealed that total MSNA was significantly greater than baseline during the final 15-s of the task and during the recovery. There was an increase in MSNA burst amplitude, but this did not reach statistical significance (P=0.08; table 3.2). There was no significant main effect of time on MSNA burst frequency (P=0.56).

Across the group, 13 individuals demonstrated a mean increase in MSNA burst frequency (positive responders), and 8 individuals demonstrated a mean decrease in response to mental arithmetic (negative responders). When grouped according to changes in total MSNA during mental arithmetic, 10 individuals were classified as positive responders and 11 as negative responders. There was no significant difference in the mean change in SBP, DBP or MAP between the two groups; regardless of whether they were grouped via MSNA burst frequency or total MSNA (P>0.05; table 3). Figure 3.1 illustrates the early responses to mental arithmetic in a positive and negative responder (classified via MSNA burst frequency). Whilst the magnitude of the peak changes in BP did not differ between positive and negative responders (P>0.05), the rate of rise in DBP during the first minute of mental arithmetic was significantly greater in negative responders, both when classified via response in MSNA burst frequency (P = 0.03) and total MSNA (P = 0.04; table 3.3; figure 3.2). There were significantly earlier peaks in DBP in negative responders (classified by total MSNA response) and MAP (classified by MSNA burst frequency response, P>0.05; table 3.3). The time course of BP and MSNA responses in positive and negative responders to the mental arithmetic task are illustrated in figure 3.3, in which the lag in the DBP response can be seen in the positive responders. When our analyses between positive and negative responders were repeated using the extremes, *i.e*. increases/decreases in MSNA burst frequency of ≥ 3 bursts/min (Carter & Ray, 2009), the rate of rise in DBP was still greater in negative responders (1.5 ± 0.5 mmHg/s; n=7) versus positive responders (0.4 ± 0.2 mmHg/s; n=6) but this did not reach statistical significance (P=0.09). The magnitude of the peak in diastolic BP was significantly greater in negative responders (14 ± 2 mmHg) than positive responders (7 ± 2 mmHg; P=0.03). The peak in MAP was also significantly higher in negative responders (17 ± 2 mmHg) than positive responders (9 ± 3 mmHg; P=0.04).

The analyses were repeated using the peaks in BP during the first 30s (table 3.4) and the full 2-min stressor task (table 3.5). During the first 30s of the mental arithmetic task negative responders had significantly greater peaks than positive responders (grouped by MSNA burst frequency) in SBP (27 ± 7 vs. 13 ± 3 mmHg; P=0.047), SBP (13 ± 1 vs. 6 ± 1 mmHg; P=0.0008) and MAP (16 ± 2 vs. 8 ± 2 mmHg; P=0.02). Negative responders also experienced a greater rate of rise in SBP (4.9 ± 1.3 mmHg/s) than positive responders (1.1 ± 0.5 mmHg/s; P=0.005), with a trend for a greater rate of rise in MAP (2.6 ± 0.8 vs. 1.1 ± 0.4; P=0.08). When peaks in BP were determined from the entire 2-min task, there was a significantly greater rate of rise in SBP in negative responders (2.3 ± 1.0 mmHg/s) than positive responders (0.5 ± 0.1 mmHg/s; P=0.03). There was also a trend for a greater rate of rise in DBP in negative responders (1.4 ± 0.7 mmHg/s) than positive responders (0.7 ± 0.3 mmHg/s; P=0.09).

3.4.2. Stroop test

Perceived anxiety levels (rated out of 10) were significantly higher for mental arithmetic (4.6 ± 0.4), than for the Stroop test (2.9 ± 0.6; P=0.006), which was not associated with significant changes in MSNA when the participants were pooled (P>0.05). For the Stroop test 13 individuals demonstrated a mean increase in MSNA burst frequency (positive responders), of which eight also experienced an increase during mental arithmetic. Eight individuals demonstrated a mean decrease in MSNA burst frequency during the Stroop test (negative responders), of which three also experienced a decrease during mental arithmetic. When grouped according to changes in total MSNA there were eight positive responders (six were also positive responders to mental arithmetic) and 13 negative responders (nine were also

negative responders to mental arithmetic). There was no significant difference in the mean change in SBP, DBP or MAP between positive and negative responders, whether grouped via MSNA burst frequency or total MSNA (P>0.05; table 3.3). During the first minute of the Stroop test the magnitude of the peak change and the rate of the rise in DBP was significantly greater in negative responders (classified by MSNA burst frequency response, P<0.05; table 3.3; figure 3.2). When classified by response in total MSNA negative responders experienced an earlier peak and a greater rate of rise in MAP (P<0.05). The rate of rise in DBP was also greater in negative responders although this did not reach significance (P=0.06). The time course of BP and MSNA responses in positive and negative responders to the Stroop test are illustrated in figure 3.4. When our analyses between positive and negative responders were repeated using the extremes, the rate of rise in DBP was still greater in negative responders (1.5 ± 0.5 mmHg/s; n=7) versus positive responders (0.5 ± 0.3 mmHg/s; n=9) but this did not reach statistical significance (P=0.08).

During the first 30s of the Stroop negative responders (grouped via MSNA burst frequency) experienced greater peaks than positive responders in DBP (16 ± 3 vs. 7 ± 2 mmHg; P=0.01) and MAP (18 ± 4 vs. 8 ± 2 mmHg; P=0.02; table 3.4). When peaks in BP were determined from the entire 2-min task, negative responders experienced greater peaks in DBP (32 ± 11 mmHg) than positive responders (12 ± 3 mmHg; P=0.048; table 5). There were also trends for greater peaks in SBP and MAP in negative responders, and there was a trend for a greater rate of rise in DBP in negative responders (1.2 ± 0.4 mmHg/s) compared with positive responders (0.6 ± 0.2 mmHg/s) but these did not reach significance (P=0.09).

3.4.3. Mental stressors, performance/anxiety and sympathetic baroreflex sensitivity

There was no significant relationship between mean changes in MAP and performance scores for the mental arithmetic task (r^2= 0.04; P=0.36) or the Stroop test (r^2= 0.001; P=0.87). There was also no significant relationship between mean changes in total MSNA and performance scores for mental arithmetic (r^2= 0.13; P=0.11) or the Stroop test (r^2= 0.002; P=0.84). Perceived anxiety during the stressors was not associated with mean changes in MAP or total MSNA for either task (r^2= 0.03-0.05; P>0.05). There were no significant differences in anxiety levels during mental arithmetic between positive (4.3 ± 0.6) and negative responders (5.0 ± 0.5; P=0.41). Similarly, there were no significant differences in anxiety levels during the Stroop test between positive (2.5 ± 0.3) and negative responders (3.5 ± 1.1; P=0.28). There were no significant differences in sympathetic BRS between positive (-1.4 ± 0.1 bursts/100hb/mmHg) and negative responders (-1.8 ± 0.3 bursts/100hb/mmHg; P=0.20) to mental arithmetic. There were no significant differences in sympathetic BRS between positive (-1.5 ± 0.2 bursts/100hb/mmHg) and negative responders (-1.6 ± 0.2 bursts/100hb/mmHg) to the Stroop test.

3.4.4. Physical stressors

The physical stressors elicited large and consistent increases in BP amongst participants. Typical recordings obtained during the CPT are shown in figure 3.5. It can be seen that parallel increases occurred in MSNA and BP during the test. There were significant main effects of time on BP, HR, total MSNA, MSNA burst frequency and mean MSNA burst amplitude in response to both the CPT and handgrip exercise (P<0.05, table 3.2). As

expected, during the period of post-exercise ischaemia, HR returned to baseline levels, whilst BP, total MSNA and MSNA burst amplitude remained elevated above baseline ($P<0.05$). MSNA burst frequency was not significantly different from resting levels ($P=0.13$). Figure 3.6 illustrates the time course of BP and MSNA responses during the physical stressor tasks. In the CPT, there were gradual and concurrent increases in BP and total MSNA over the 2 minutes. These increases were consistent between individuals as indicated by the small error bars (figure 3.6). The average peak pain score during the task was 6.5 ± 0.5 out of 10. Linear regression analysis revealed no significant relationship between pain score and the mean change in MAP ($r^2= 0.03$; $P=0.46$). The linear relationship between pain score and mean change in total MSNA failed to reach significance ($r^2= 0.15$; $P=0.09$). For the static handgrip task, the gradual increases in BP were paralleled by the increases in total MSNA and MSNA burst frequency. Since the CPT, handgrip and ischaemia tasks elicited consistent increases in MSNA between participants, no analyses on positive and negative responders were performed for physical stressors.

3.4.5. Repeatability

In a sub-set of individuals their responses to the stressor tasks were tested for repeatability. Ten individuals were selected and tested on the same day *i.e*. they did the stressor tasks twice within a single laboratory visit (their results are shown in table 3.6).

T-tests were performed on their mean changes in BP, MSNA and HR between session 1 and session 2. There were no significance differences between the two sessions ($P>0.05$). Figure 3.7 illustrates the repeatability of DBP responses to mental arithmetic between session 1 and session 2.

3.5 DISCUSSION

The aim was to examine the early BP response to stress in positive and negative responders and thus its influence on the direction of change in MSNA. The results indicate that physical stressors, such as the CPT, handgrip exercise and post-exercise ischaemia, are associated with significant increases in MSNA parallel to those of BP. During mental stress there are considerable inter-individual differences in the direction and magnitude of the MSNA response, despite consistent elevations in BP. Our findings indicate that negative MSNA responses to mental stress are associated with more rapid increases in DBP at the onset of the task.

The significance of these findings will be explored in the General Discussion.

Table 3.1: Resting sympathetic and cardiovascular variables prior to stressor tasks

Variable	Mental arithmetic	Stroop test	Cold pressor	Handgrip exercise / ischaemia
Systolic blood pressure (mmHg)	126 ± 4	124 ± 5	122 ± 4	125 ± 4
Diastolic blood pressure (mmHg)	65 ± 3	63 ± 3	65 ± 2	71 ± 5
MAP (mmHg)	85 ± 3	84 ± 3	84 ± 2	89 ± 4
Heart rate (beats/min)	68 ± 2	69 ± 2	73 ± 3	68 ± 2
MSNA burst frequency (bursts/min)	36 ± 2	36 ± 1	35 ± 2	37 ± 2

Table 3.2: Mean changes in sympathetic and cardiovascular variables during mental and physical stressor tasks.

Variable	Mental arithmetic	Stroop test	Cold pressor	Handgrip exercise	Post-exercise ischaemia
Systolic blood pressure (mmHg)	11 ± 3*	8 ± 3*	18 ± 4*	13 ± 3*	14 ± 3*
Diastolic blood pressure (mmHg)	5 ± 1*	4 ± 1*	11 ± 2*	11 ± 1*	9 ± 2*
MAP (mmHg)	14 ± 3*	13 ± 2*	11 ± 2*	7 ± 1*	5 ± 1*
Total MSNA (%)	21 ± 18*	0 ± 8	62 ± 11*	34 ± 11*	42 ± 12*
Heart rate (beats/min)	6 ± 2*	11 ± 2*	-0.1 ± 1*	6 ± 1*	5 ± 1*
MSNA burst amplitude (%)	22 ± 15	5 ± 8	30 ± 6*	21 ± 8*	24 ± 6*
MSNA burst frequency (bursts min^{-1})	0 ± 2	0 ± 2	7 ± 2*	3 ± 1*	4 ± 2

*Significant main effect of time ($P<0.05$); MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

Table 3.3: Peak change, time of peak, and rate of change in blood pressure in positive and negative responders to mental stressor tasks

	Peak change (mmHg)			Time of peak (s)			Rate of change (mmHg/s)		
	SBP	DBP	MAP	SBP	DBP	MAP	SBP	DBP	MAP
Mental arithmetic									
Grouped via MSNA burst freq.									
Positive responders	18 ± 3	10 ± 2	13 ± 3	15 ± 3	42 ± 6	43 ± 5	0.7 ± 0.3	0.4 ± 0.1	0.7 ± 0.3
Negative responders	31 ± 7	13 ± 2	17 ± 2	36 ± 8*	27 ± 8	23 ± 7*	2.2 ± 1.0	1.3 ± 0.5*	1.9 ± 0.7
Grouped via total MSNA									
Positive responders	15 ± 4	11 ± 3	11 ± 3	38 ± 7	52 ± 2	45 ± 6	0.7 ± 0.4	0.3 ± 0.1	0.7 ± 0.4
Negative responders	29 ± 6	13 ± 2	17 ± 3	31 ± 7	22 ± 7*	27 ± 6	1.7 ± 0.7	1.0 ± 0.3*	1.7 ± 0.5
Stroop test									
Grouped via MSNA burst freq.									
Positive responders	17 ± 4	10 ± 2	13 ± 3	39 ± 6	35 ± 6	32 ± 6	0.9 ± 0.3	0.5 ± 0.2	0.9 ± 0.3
Negative responders	33 ± 11	23 ± 6*	22 ± 6	39 ± 7	29 ± 8	29 ± 8	1.2 ± 0.4	1.4 ± 0.4*	1.3 ± 0.4
Grouped via total MSNA									
Positive responders	24 ± 12	13 ± 5	15 ± 7	47 ± 5	44 ± 5	48 ± 4	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
Negative responders	23 ± 3	16 ± 4	17 ± 4	34 ± 6	26 ± 6	20 ± 6*	1.3 ± 0.3	1.2 ± 0.3	1.5 ± 0.4*

*Significantly different from positive responders ($P<0.05$); SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

Table 3.4: Peak change, time of peak, and rate of change in blood pressure in positive and negative responders in the first 30s of the mental stressor tasks

	Peak change (mmHg)			Time of peak (s)			Rate of change (mmHg/s)		
	SBP	DBP	MAP	SBP	DBP	MAP	SBP	DBP	MAP
Mental arithmetic									
Grouped via MSNA burst freq.									
Positive responders	13 ± 3	6 ± 1	8 ± 2	15 ± 3	12 ± 3	12 ± 3	1.1 ± 0.5	1.1 ± 0.4	1.1 ± 0.4
Negative responders	27 ± 7*	13 ± 1*	16 ± 2*	10 ± 4	9 ± 2	14 ± 4	4.9 ± 1.3*	2.2 ± 0.6	2.6 ± 0.8
Grouped via total MSNA									
Positive responders	7 ± 2	6 ± 2	7 ± 3	15 ± 4	12 ± 4	12 ± 4	1.7 ± 0.9	1.5 ± 0.6	1.7 ± 0.7
Negative responders	25 ± 5*	10 ± 2	14 ± 2*	11 ± 3	10 ± 3	14 ± 3	3.8 ± 1.2	1.2 ± 0.4	1.7 ± 0.5
Stroop test									
Grouped via MSNA burst freq.									
Positive responders	14 ± 3	7 ± 2	8 ± 2	14 ± 3	13 ± 3	12 ± 3	2.7 ± 0.7	1.0 ± 0.4	1.3 ± 0.3
Negative responders	22 ± 6	16 ± 3*	18 ± 4*	14 ± 3	15 ± 4	15 ± 4	2.0 ± 0.6	1.8 ± 0.5	1.8 ± 0.5
Grouped via total MSNA									
Positive responders	15 ± 6	8 ± 3	10 ± 3	16 ± 4	17 ± 3	17 ± 3	2.2 ± 0.9	0.7 ± 0.4	1.1 ± 0.5
Negative responders	18 ± 3	13 ± 3	13 ± 3	13 ± 3	12 ± 3	10 ± 5	2.6 ± 0.6	1.7 ± 0.5	1.7 ± 0.3

*Significantly different from positive responders ($P<0.05$); SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

Table 3.5: Peak change, time of peak, and rate of change in blood pressure in positive and negative responders during the 2-min mental stressor tasks

	Peak change (mmHg)			Time of peak (s)			Rate of change (mmHg/s)		
	SBP	DBP	MAP	SBP	DBP	MAP	SBP	DBP	MAP
Mental arithmetic									
Grouped via MSNA burst freq.									
Positive responders	23 ± 3	13 ± 2	15 ± 2	71 ± 10	76 ± 9	73 ± 11	0.5 ± 0.1	0.3 ± 0.1	0.7 ± 0.3
Negative responders	35 ± 20	17 ± 3	19 ± 3	43 ± 12	64 ± 16	47 ± 13	2.3 ± 1.0*	1.0 ± 0.5	1.4 ± 0.7
Grouped via total MSNA									
Positive responders	25 ± 4	15 ± 3	15 ± 3	63 ± 11	83 ± 9	62 ± 12	0.6 ± 0.1	0.2 ± 0.1	0.9 ± 0.4
Negative responders	30 ± 6	14 ± 2	18 ± 2	58 ± 11	60 ± 13	65 ± 12	1.7 ± 0.7	0.9 ± 0.4	1.0 ± 0.5
Stroop test									
Grouped via MSNA burst freq.									
Positive responders	19 ± 4	12 ± 3	14 ± 4	53 ± 10	51 ± 11	50 ± 11	0.9 ± 0.3	0.6 ± 0.2	0.9 ± 0.4
Negative responders	36 ± 11	32 ± 11*	31 ± 10	53 ± 13	52 ± 15	59 ± 15	1.2 ± 0.4	1.2 ± 0.4	1.1 ± 0.5
Grouped via total MSNA									
Positive responders	26 ± 12	13 ± 5	16 ± 7	64 ± 12	56 ± 13	50 ± 9	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1
Negative responders	26 ± 4	23 ± 7	23 ± 7	47 ± 10	48 ± 13	55 ± 13	1.3 ± 0.4	1.1 ± 0.3	1.3 ± 0.4

*Significantly different from positive responders ($P<0.05$); SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

Table 3.6: Mean changes in sympathetic and cardiovascular variables during mental and physical stressor tasks in session 1 (S1) and session 2 (S2), n=10.

	Mental arithmetic		Stroop test		Cold pressor		Handgrip exercise		Post-exercise ischaemia	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
SBP (mmHg)	9 ± 2	6 ± 2	4 ± 3	3 ± 2	20 ± 5	12 ± 6	18 ± 3	16 ± 2	15 ± 4	17 ± 3
DBP (mmHg)	6 ± 1	4 ± 1	3 ± 2	2 ± 1	15 ± 3	21 ± 9	16 ± 4	13 ± 2	16 ± 7	10 ± 2
HR (beats/min)	7 ± 1	6 ± 2	3 ± 2	5 ± 2	8 ± 3	9 ± 3	10 ± 2	10 ± 2	1 ± 2	1 ± 2
Total MSNA (%)	0.1 ± 2	0.1 ± 2	2 ± 4	1 ± 2	56 ± 35	67 ± 34	11 ± 4	19 ± 11	3 ± 15	22 ± 10
MSNA burst frequency (bursts min-^{1})	2 ± 2	0.3 ± 2	2 ± 2	0.4 ± 2	3 ± 3	13 ± 4	3 ± 1	5 ± 2	3 ± 2	3 ± 2

SBP, systolic blood pressure; DBP, diastolic blood pressure; MSNA, muscle sympathetic nerve activity

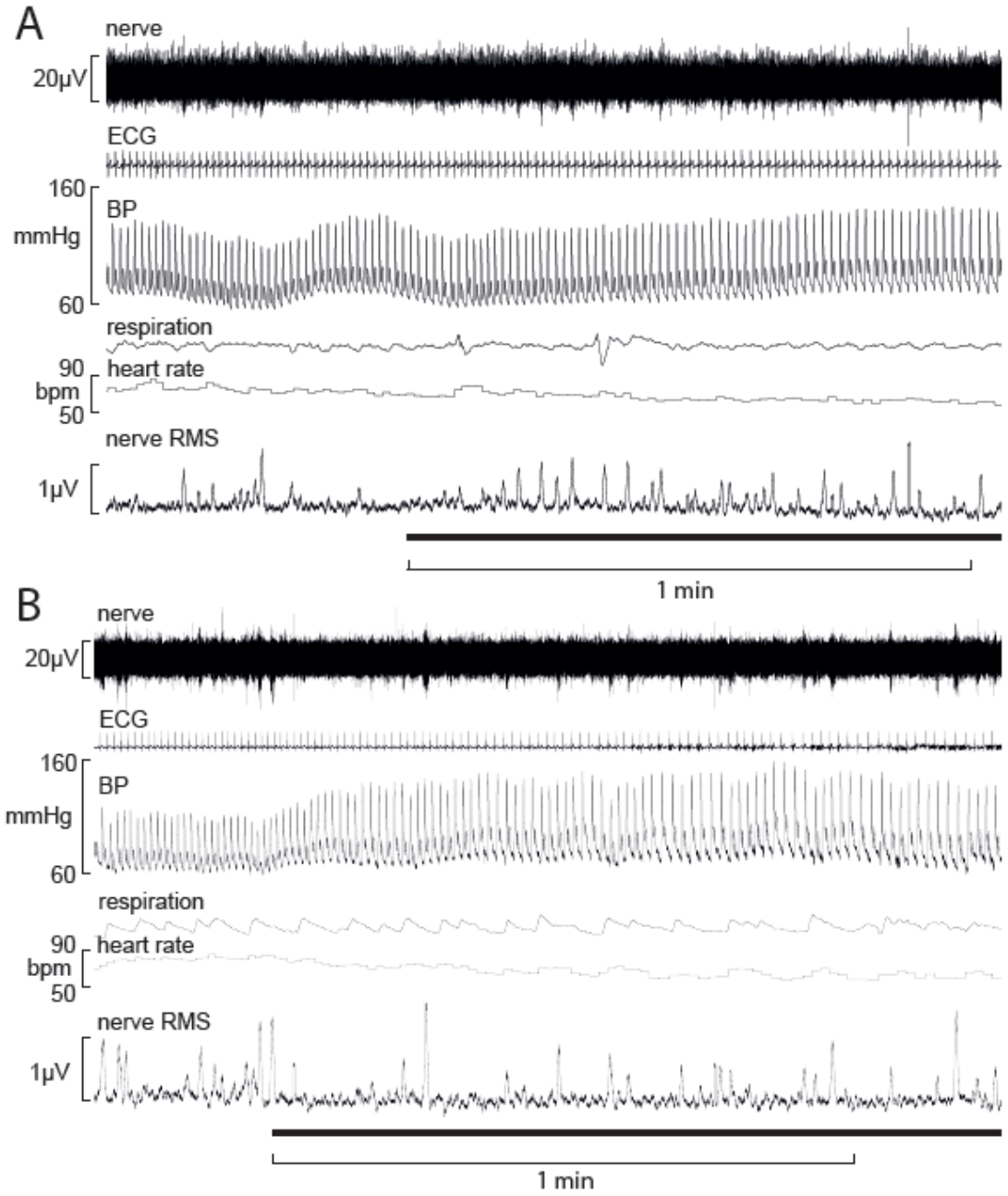

Figure 3.1: Laboratory recordings from a 22-yr old male (positive responder, A) and a 23-yr old male (negative responder, B) demonstrating the early responses to mental arithmetic (indicated by the horizontal bar). Neural activity, electrocardiogram (ECG), blood pressure (BP), respiration, heart rate and the root-mean-square (RMS)-processed nerve signal are displayed.

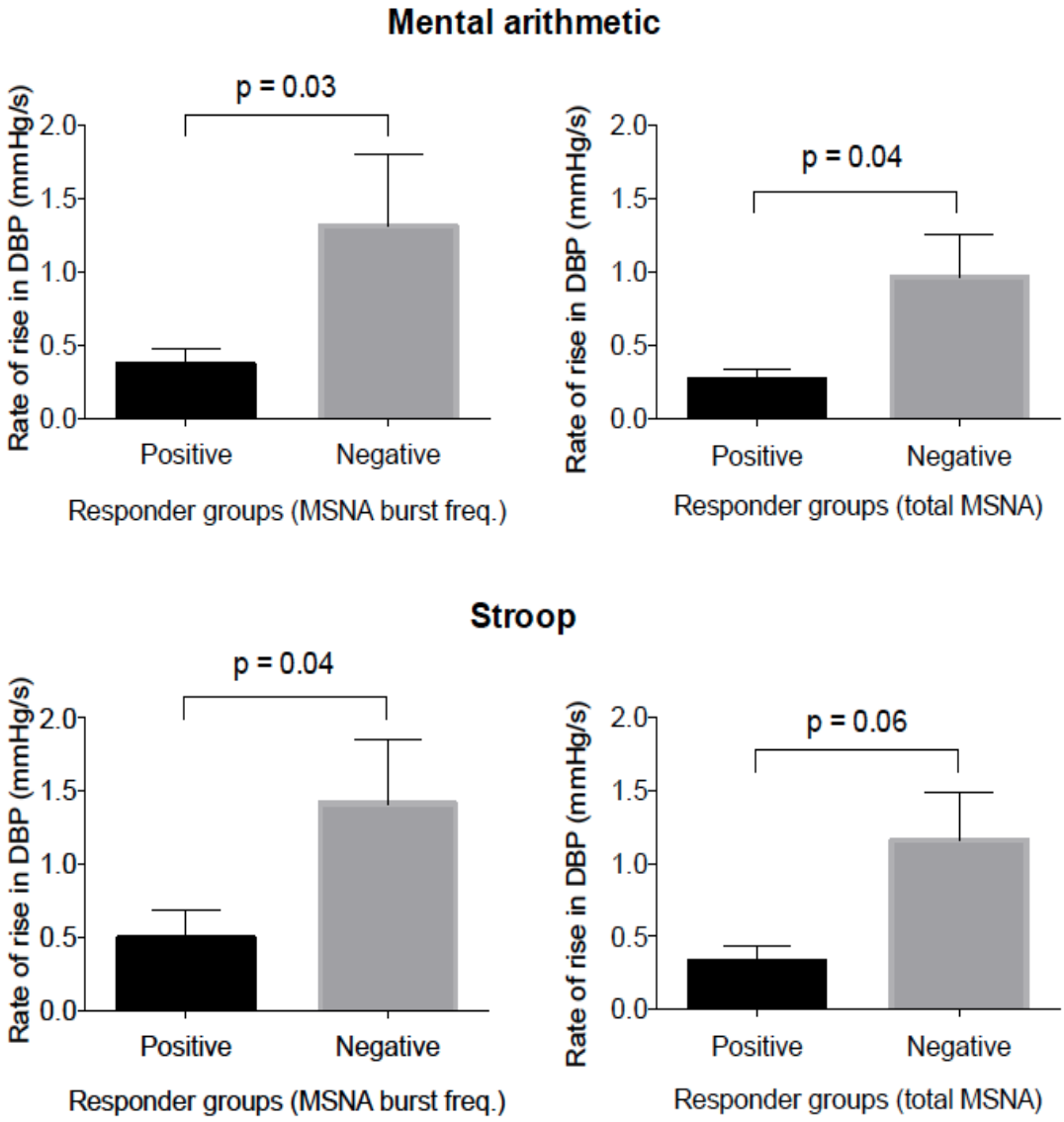

Figure 3.2: Rate of rise in diastolic blood pressure (DBP) in positive and negative responders to mental arithmetic and the Stroop test. Responders are classified according to MSNA burst frequency (left panels) and total MSNA (right panels).

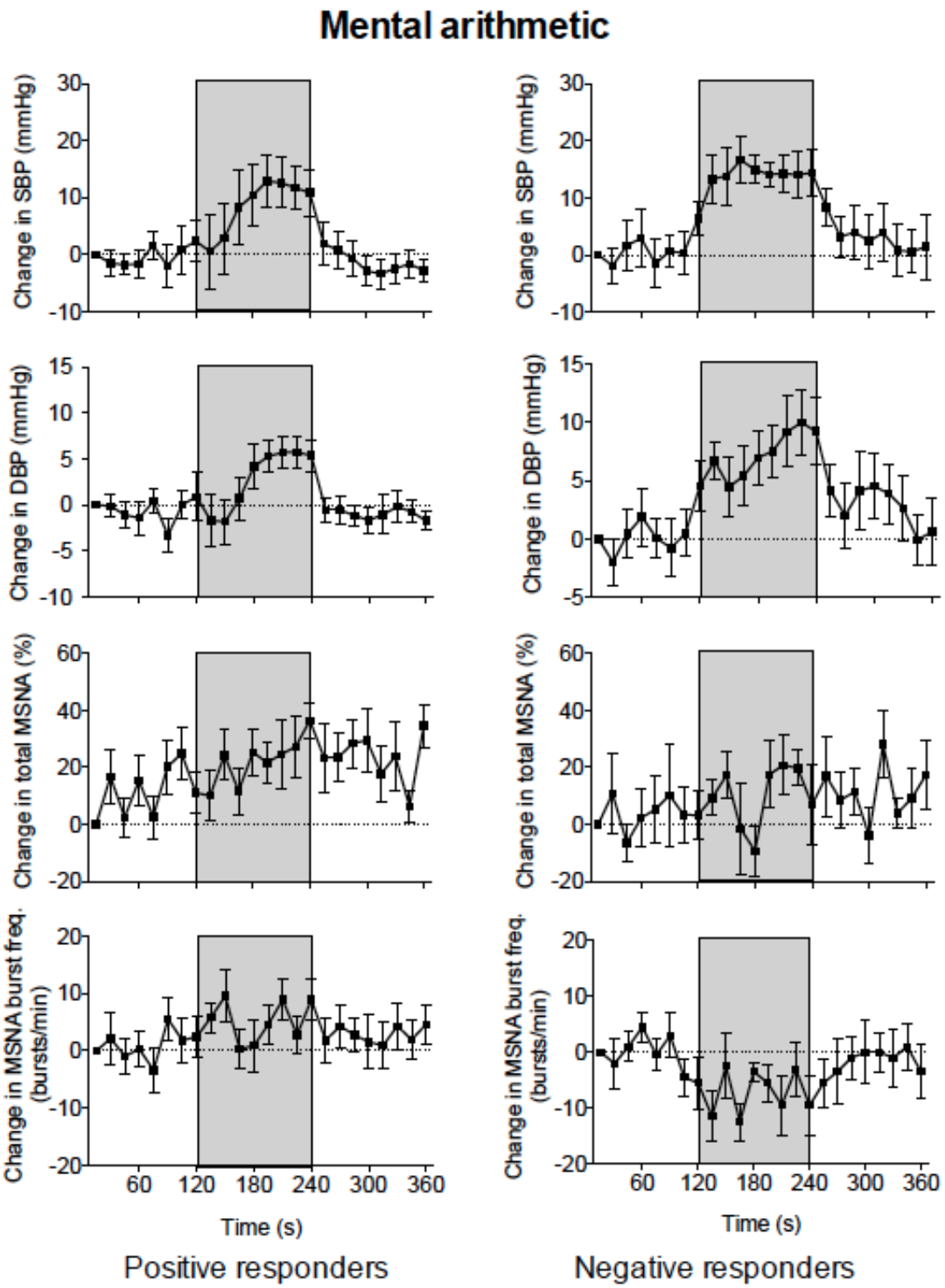

Figure 3.3: Time course of the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), total MSNA and MSNA burst frequency in positive and negative responders (grouped according to MSNA burst frequency response) during the mental arithmetic task. The grey rectangles indicate the 2-min stressor tasks.

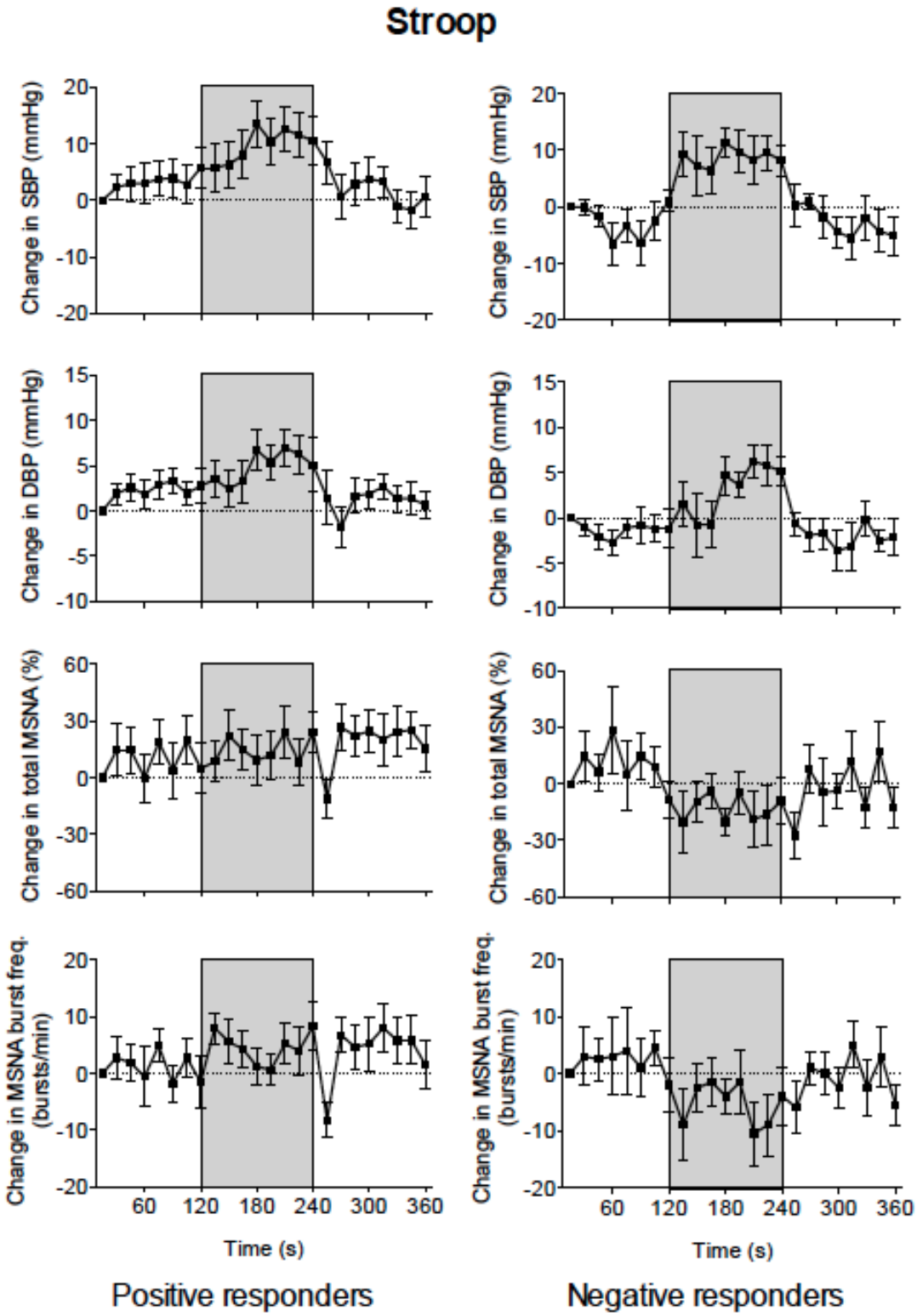

Figure 3.4: Time course of the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), total MSNA and MSNA burst frequency in positive and negative responders (grouped according to MSNA burst frequency response) during the Stroop test. The grey rectangles indicate the 2-min stressor tasks.

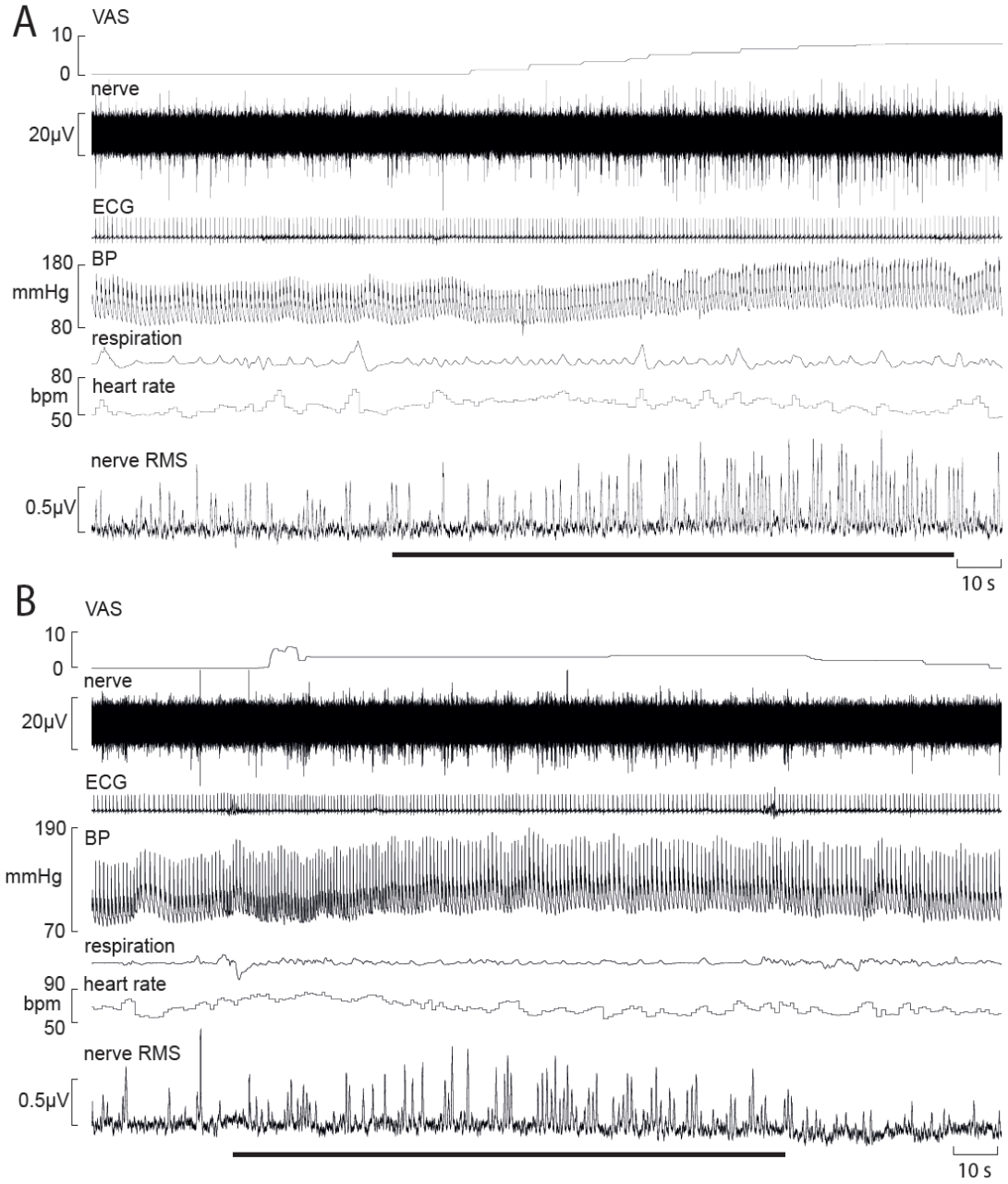

Figure 3.5: Laboratory recordings from a 25-yr old male (**A**) and a 24-yr old male (**B**) during the cold pressor test (indicated by the horizontal bar). Visual analogue pain scale (VAS), neural activity, electrocardiogram (ECG), blood pressure (BP), respiration, heart rate and the root-mean-square (RMS)-processed nerve signal are displayed during the 2-min task.

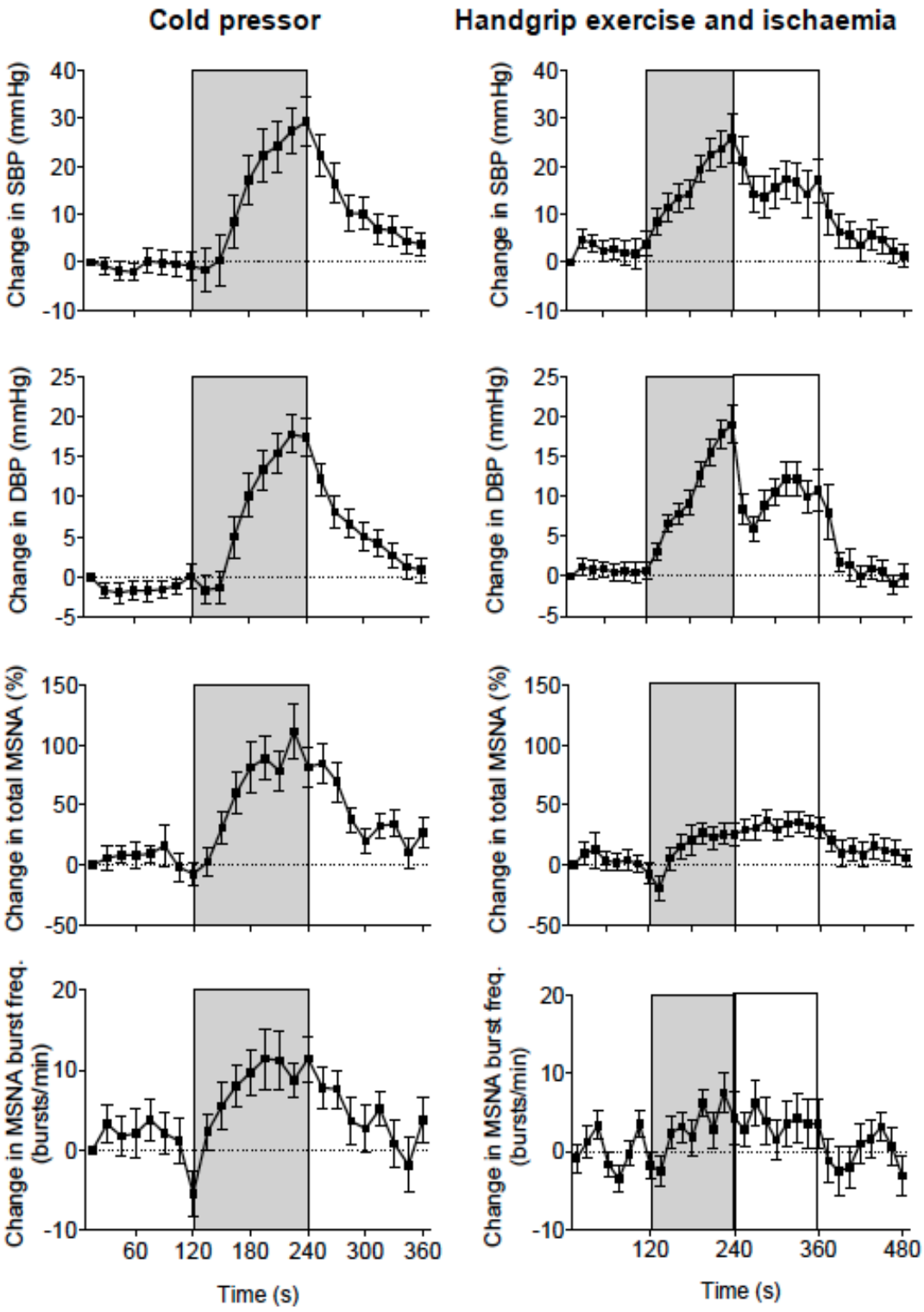

Figure 3.6: Time course of the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), total MSNA and burst MSNA frequency during the cold pressor and handgrip exercise/ischaemia tasks. The grey rectangles indicate the 2-min cold pressor and handgrip tasks; the white rectangle indicates the 2-min period of post-exercise ischaemia.

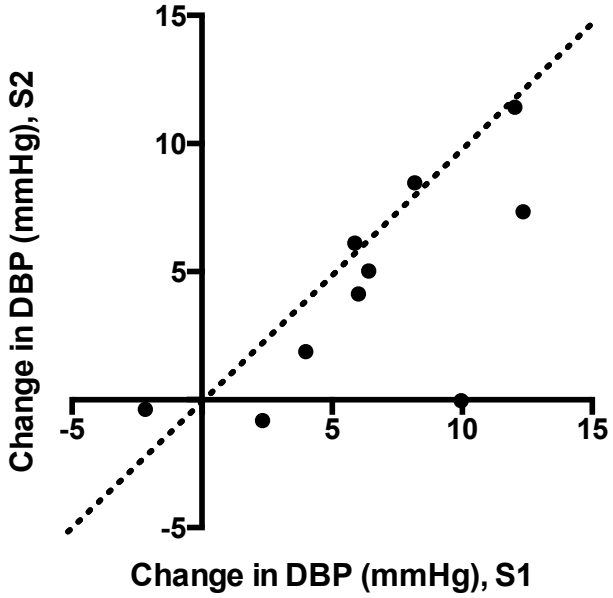

Figure 3.7: Repeatability of DBP responses to mental arithmetic between session 1 (S1) and session 2 (S2).

Chapter 4: Study 2

Rate of rise in blood pressure influences vascular sympathetic response to mental stress in males and females.

4.1 ABSTRACT

Aim: Research indicates that individuals may experience a rise (positive responders) or fall (negative responders) in MSNA during mental stress. The aim was to examine the effects of gender in the early BP response to stress in both positive and negative responders and thus its influence on the direction of change in MSNA.

Methods: BP and MSNA were recorded continuously in 21 males and 19 females during 2-min physical stressors (cold pressor and static handgrip exercise, post-exercise ischaemia) and mental stressors (mental arithmetic, Stroop test). Participants were classified as negative or positive responders according to the direction of the mean change in MSNA during the stressor tasks.

Results: The peak changes, time of peak, and rate of changes in BP were compared between males and females and between positive and negative responders.

Consistent with the findings in the males, the female negative responders experienced greater rate of rise in DBP (1.1 ± 0.6) compared to the positive responders (0.2 ± 0.1).

Conclusion: During mental arithmetic task the rate of rise in BP is greater in negative responders than positive responders. This was consistent between males and females.

4.2 INTRODUCTION:

Relatively little has been done to explore differences in reactivity to various stressors in males and females. The focus of this study was to examine the early BP response to stress in positive and negative responders in males and females and thus its influence on the direction of change in MSNA. In our previous study in young males we found that, despite consistent elevations in BP, some individuals experienced increases and others decreases in MSNA during mental stress. Negative responders exhibited rapid increases in diastolic pressure at the onset of the mental stressor, which lead to reductions in MSNA. The reductions in MSNA in the negative responders are believed to be due to the baroreflex supersession of nerve activity. In positive responders, elevations in BP were sluggish and therefore believed to be MSNA-driven. As in the previous study, the physical tasks used were the CPT, static handgrip exercise and post-exercise ischaemia. Mental arithmetic and the Stroop colour-word conflict test were used as mental stressors. Mental stressors used were the Stroop colour-word conflict test and mental arithmetic.

It is known that cardiovascular control is subject to sex differences. Briant et al. (2016) have shown that vascular transduction of MSNA is lower in young females than young males, which may lead to a more delayed rise in BP at the onset of mental stress. Based on our previous findings, this reduced rate of rise in BP may involve reduced baroreflex stimulation, thus resulting in greater sympathetic activation during the stressor. Christou et al. (2005) have shown that autonomic support of BP and baroreflex buffering is lower in young females than males, which provides further support for the hypothesis that baroreflex suppression of MSNA is reduced during mental stress in females. Therefore, it is hypothesized that there is a greater

proportion of positive responders amongst females than in males, and that this group experiences a reduced rate of rise in BP compared with negative responders.

4.3 METHODS

4.3.1. Participants

Twenty-one males and nineteen female participants aged between 18 and 29 with no history of cardiovascular disease were recruited for the study. Females were tested during the LH phase (between days 1-7) of their menstrual cycle. Out of the 19 females tested, 9 females were taking oral OCs pills.

Refer to methods section 2.1 for further detail.

4.3.2. Measurements

Refer to section 2.3.

4.3.3. Experimental procedures

Refer to section 2.5.

4.3.4. Data analysis

Time course of responses to stressors

Refer to section 2.6.

Positive versus negative responders

Refer to methods section 2.6.1, however, only peaks over the first minute were used.

Sympathetic baroreflex sensitivity

Refer to methods section 2.6.2.

Males versus females

Two-way repeated measures ANOVAs were performed to determine the main effects of sex (males versus females) and MSNA response (positive versus negative responders) for each variable (peak, time to peak, and rate of rise in systolic, diastolic and mean arterial pressure).

4.4 RESULTS

Twenty-one males and nineteen females completed the study. Subject characteristics are shown in table 4.1. The BMI for the males and females fell into the overweight category (≥25 kg/m^2), however this was not due to participants being overweight. The high BMI ranges in the participants can be explained by their body composition, which is due to the physical activity levels in the groups. The participants exercised regularly, with most undergoing resistance weight training. Thus, fat-free mass was taken: 65.5 ± 2 (males) and 46.5 ± 2 kg (females). These values are above the average for healthy, young individuals (Kyle et al. 2001), and may be explained by the physical activity levels. The participants exercised regularly (≥2 x per week), with some participating in resistance training. As shown in table 4.1, the difference in SBP between males and females did not reach statistical significance (P=0.19). However, DBP and MAP were significantly higher in females, and MSNA burst incidence was significantly higher in males (P<0.05). All subjects completed each of the stressor tasks. However, in one female the MSNA recording deteriorated during mental

arithmetic and the Stroop test and therefore the responses to mental stressors are limited to 18 female participants.

4.4.1. Mental arithmetic

The mental arithmetic task was associated with significant increases in BP, HR, total MSNA and MSNA burst amplitude over time (table 4.2; $P<0.05$). However, the post-hoc analyses revealed that total MSNA was only significantly different from rest during the recovery period. There was a significant effect of sex on total MSNA ($P=0.02$); increases were greater in males compared with the females during the task. However, females had greater increases in the recovery period compared with the males. There was an effect of time on MSNA burst frequency but this did not reach significance ($P=0.06$). For both sexes the increases in BP occurred *after* the peak in heart rate. There was a significant interaction between time and sex for HR ($P=0.0043$), with larger increases in the males than the females.

Across the groups, 13 males and 10 females demonstrated a mean increase in MSNA burst frequency (positive responders), and 8 males and 8 females demonstrated a mean decrease in response to mental arithmetic (negative responders). When grouped according to changes in total MSNA during mental arithmetic, 10 males and 8 females and were classified as positive responders while 11 males and 10 females were classified as negative responders.

When participants were grouped according to MSNA burst frequency response, there was a significant effect of MSNA response on the time to peak in DBP and MAP,

with earlier peaks in negative responders (P<0.05). This was consistent between males and females (*i.e*. no effect of sex, P>0.05). The rate of rise in SBP and MAP was significantly greater in negative responders compared with positive responders and this was consistent between males and females. There was no effect of response or sex on the rate of rise in DBP (P>0.05; table 4.3). The time course of the changes in SBP, DBP, total MSNA and burst frequency in positive and negative responders in both males and females during the mental arithmetic task, is illustrated in figure 4.1.

When participants were grouped according to total MSNA, the peak changes in SBP and MAP were significantly greater in negative responders compared with positive responders, and this was consistent between males and females. Time of peak and rate of rise in DBP and MAP were significantly earlier in negative responders (P<0.05). Rate of rise in DBP and MAP were significantly greater in negative responders (P<0.05; table 4.3). These effects were consistent between males and females (P>0.05; figure 4.2).

4.4.2. Stroop test

The Stroop colour-word conflict test was associated with significant increases in BP, HR, total MSNA and MSNA burst frequency over time, during the task (P<0.05; table 4.2). For both genders the peak increase in BP occurred after the peak increase in heart rate. There were significant effects of sex for MSNA burst amplitude (P=0.03) with greater increases in the females compared with the males.

For the Stroop test 13 males and 9 females demonstrated a mean increase in MSNA burst frequency (positive responders). Eight males and 9 females demonstrated a mean decrease in MSNA burst frequency (negative responders). When grouped according to changes in total MSNA there were eight male and 10 female positive responders. Thirteen males and 8 females were classified as negative responders.

When participants were grouped according to MSNA burst frequency response, there was a significant effect of MSNA response on the peak changes in SBP, DBP and MAP, with greater changes for negative responders compared with positive responders ($P<0.05$; table 4.3). Although this effect of response was consistent between males and females (no significant interaction between response and gender), there was an effect of sex with greater peaks in SBP, DBP (figure 4.3) and MAP in males compared with females. Time course of the changes in SBP, DBP, total MSNA and burst frequency in positive and negative responders in both males and females during the mental arithmetic task, is illustrated in figure 4.4. When participants were grouped according to total MSNA there were no effects of response or sex on peaks, time to peaks or rate of rise in SBP, DBP and MAP ($P>0.05$; table 4.3).

4.4.3. Sympathetic baroreflex sensitivity in positive and negative responders

In the males in *Study 1*, it was reported that there was no significant difference in sympathetic BRS between positive and negative responders to mental arithmetic and the Stroop test ($P>0.05$). Similarly, in females there is no significant difference in sympathetic BRS between positive (-2.0± 0.5 bursts/100hb/mmHg) and negative responders (-2.7 ± 0.6 bursts/100hb/mmHg; $P=0.42$) to mental arithmetic. There were

no significant differences in sympathetic BRS between positive (-2.4 ± 0.5 bursts/100hb/mmHg) and negative responders (-2.4 ± 0.6 bursts/100hb/mmHg, P=0.96) to the Stroop test.

4.4.4. Physical stressors

The CPT caused gradual and concurrent increases in BP and MSNA during the 2-min task. Figure 4.5 illustrates the time course of the responses to the CPT in the males and females. Significant increases in BP, HR and MSNA occurred (table 4.4; $P<0.05$), but there were no significant differences between the two groups ($P>0.05$). There was no significant difference in pain scores between males (6.5 ± 0.5) and females (7.6 ± 0.6; P=0.24). Moreover, linear regression analysis revealed no significant relationship between peak pain score and the mean change in SBP for either the males (r^2= 0.001; P=0.89) or females (r^2=0.01; P=0.70).

As expected, static handgrip exercise also resulted in significant increases in BP, HR and MSNA over time (table 4.4; $P<0.05$). Figure 4.6 illustrates the time course of the responses to the static handgrip task and post-exercise ischaemia between the males and females. There was a significant interaction between time and sex for MAP (P=0.0025), DBP (P=0.046) and heart rate (P=0.0001); there was also a significant effect of sex for HR (P=0.0142), with greater increases in males compared with females. As shown in figure 4.6, the males displayed a bigger drop in BP when handgrip exercise ceased, but it still remained significantly elevated above baseline during ischaemia for both sexes. In addition, post-exercise ischaemia was associated with significant increases in BP and MSNA over time (table 4.4; $P<0.05$). There were no significant effects of sex or interaction between time and sex for any of the variables during post-exercise ischaemia ($P>0.05$). Since the cold pressor, handgrip

and ischaemia tasks elicited consistent increases in MSNA between participants, no analyses on positive and negative responders were performed.

4.5 DISCUSSION

In this study changes in BP during mental stress in males and females were compared between negative and positive responders, *i.e*. those with an overall decrease or increase in MSNA during stress. During mental arithmetic the rate of rise in BP is greater in negative responders than positive responders, this was also observed in both males and females. Consistent with ***Study 1***, the results for the physical stressors, such as the CPT, handgrip exercise and post-exercise ischaemia, are associated with significant increases in MSNA parallel to those of BP. The results were consistent in both males and females.

The significance of these findings will be explored in the General Discussion.

Table 4.1: Baseline sympathetic and cardiovascular variables (mean ± SE) for males (n=21) and females (n=19).

Variable	Males	Females	Difference (m - f)
Age (years)	22 ± 0.4	23 ± 0.8	-1
BMI (Kg/m2)	25 ± 1	25.7 ± 2	-0.7
Fat-free mass (kg)	66 ± 2	46 ± 1	20*
Systolic BP (mmHg)	129 ± 4	120 ± 3	9
Diastolic BP (mmHg)	61 ± 3	75 ± 2	-14*
MAP (mmHg)	79 ± 3	90 ± 2	-11*
Heart rate (beats/min)	64 ± 2	70 ± 3	-6
MSNA burst frequency (bursts/min)	36 ± 1	35 ± 1	1
MSNA burst incidence (bursts/100heartbeats)	58 ± 2	51 ± 2	7*

* Significant difference between the males and females ($P<0.05$). BP = blood pressure; MAP = mean arterial pressure; MSNA = muscle sympathetic nerve activity

Table 4.2: Mean changes in sympathetic and cardiovascular variables during mental stressor tasks for the males (n=21) females (n=18).

Variable	Mental arithmetic		Stroop test	
	Males	Females	Males	Females
Systolic BP (mmHg)	11 ± 3	9 ± 2*	8 ± 3	4 ± 2*
MAP (mmHg)	7 ± 1	7 ± 1*	5 ± 1	4 ± 1*
Diastolic BP (mmHg)	5 ± 1	6 ± 1*	4 ± 1	3 ± 1*
Heart rate (beats/min)	6 ± 1	4 ± 2*‡	6 ± 1	4 ± 2*
Total MSNA (%)	5 ± 6	2 ± 8*†	-0.4 ± 8	14 ± 9*
MSNA burst ampl. (%)	8 ± 4	6 ± 5*	5 ± 8	19 ± 13†
MSNA burst freq. (bursts min-1)	0.07 ± 2	-3 ± 6	-0.52 ± 1	-0.44 ± 1*

BP = blood pressure; MAP = mean arterial pressure; MSNA = muscle sympathetic nerve activity.

*Significant main effect of time (P<0.05). †Significant effect of sex (P<0.05). ‡Significant interaction (P<0.05).

Table 4.3 (a): Peak change, time of peak, and rate of change in blood pressure in positive and negative responders to mental stressor tasks for the males (n=21) and females (n=18).

	Peak change (mmHg)					
	SBP		DBP		MAP	
	Males	Females	Males	Females	Males	Females
Mental arithmetic						
Grouped via MSNA burst freq.						
Positive responders	18 ± 3	19 ± 5	10 ± 2	12 ± 3	13 ± 3	14 ± 3
Negative responders	31 ± 7	14 ± 2	13 ± 2	11 ± 3	17 ± 2	11 ± 3
Grouped via total MSNA						
Positive responders	15 ± 4	12 ± 4	11 ± 3	9 ± 3	11 ± 3	10 ± 3
Negative responders	29 ± 6	21 ± 3 ✗	13 ± 2	13 ± 2	17 ± 3	15 ± 3 ✗
Stroop test						
Grouped via MSNA burst freq.						
Positive responders	17 ± 4	7.6 ± 2 *	10 ± 2	4 ± 1 *	13 ± 3	6 ± 1 *
Negative responders	33 ± 11	15 ± 3 ✗	23 ± 6	11 ± 2 ✗	22 ± 6	12 ± 2 ✗
Grouped via total MSNA						
Positive responders	24 ± 12	8 ± 1	13 ± 5	5 ± 1	15 ± 7	6 ± 1
Negative responders	23 ± 3	16 ± 3	16 ± 4	10 ± 2	17 ± 4	11 ± 3

* Significantly different from males and females ($P<0.05$); SBP, systolic blood pressure, DBP, diastolic blood pressure, MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

✗Significantly different from positive responders ($P<0.05$); SBP, systolic blood pressure, DBP, diastolic blood pressure, MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

Table 4.3 (b): Peak change, time of peak, and rate of change in blood pressure in positive and negative responders to mental stressor tasks for the males (n=21) and females (n=18).

	Time of peak (s)					
	SBP		DBP		MAP	
	Males	Females	Males	Females	Males	Females
Mental arithmetic						
Grouped via MSNA burst freq.						
Positive responders	15 ± 3	36 ± 5	42 ± 6	43 ± 5	43 ± 5	41 ± 4
Negative responders	36 ± 8	24 ± 7	27 ± 8	29 ± 8 ✗	23 ± 7	24 ± 8 ✗
Grouped via total MSNA						
Positive responders	38 ± 7	31 ± 7	52 ± 2	45 ± 3	45 ± 6	39 ± 5
Negative responders	31 ± 7	31 ± 5	22 ± 7	30 ± 8 ✗	27 ± 6	29 ± 7 ✗
Stroop test						
Grouped via MSNA burst freq.						
Positive responders	39 ± 6	23 ± 7	35 ± 6	14 ± 7	32 ± 6	18 ± 7
Negative responders	39 ± 7	38 ± 7	29 ± 8	33 ± 8	29 ± 8	35 ± 7
Grouped via total MSNA						
Positive responders	47 ± 5	26 ± 6	44 ± 5	18 ± 7	48 ± 4	21 ± 6
Negative responders	34 ± 6	36 ± 8	26 ± 6	31 ± 8	20 ± 6	33 ± 8

* Significantly different from males and females ($P<0.05$); SBP, systolic blood pressure, DBP, diastolic blood pressure, MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

✗Significantly different from positive responders ($P<0.05$); SBP, systolic blood pressure, DBP, diastolic blood pressure, MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

Table 4.3 (c): Peak change, time of peak, and rate of change in blood pressure in positive and negative responders to mental stressor tasks for the males (n=21) and females (n=18).

	Rate of change (mmHg/s)					
	SBP		DBP		MAP	
	Males	Females	Males	Females	Males	Females
Mental arithmetic						
Grouped via MSNA burst freq.						
Positive responders	0.7 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	0.9 ± 0.7	0.7 ± 0.3	0.5 ± 0.2
Negative responders	2.2 ± 1.0	2.2 ± 1.6 ✗	1.3 ± 0.5	0.5 ± 0.2	1.9 ± 0.7	1.0 ± 0.3 ✗
Grouped via total MSNA						
Positive responders	0.7 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.7 ± 0.4	0.3 ± 0.1
Negative responders	1.7 ± 0.7	2.1 ± 1.4	1.0 ± 0.3	1.1 ± 0.6 ✗	1.7 ± 0.5	1.0 ± 0.3 ✗
Stroop test						
Grouped via MSNA burst freq.						
Positive responders	0.9 ± 0.3	1 ± 0.4	0.5 ± 0.2	1.4 ± 0.5	0.9 ± 0.3	1.1 ± 0.5
Negative responders	1.2 ± 0.4	0.8 ± 0.4	1.4 ± 0.4	0.9 ± 0.4	1.3 ± 0.4	0.6 ± 0.3
Grouped via total MSNA						
Positive responders	0.5 ± 0.2	1.0 ± 0.3	0.3 ± 0.1	1.3 ± 0.4	0.3 ± 0.1	1.0 ± 0.5
Negative responders	1.3 ± 0.3	0.8 ± 0.5	1.2 ± 0.3	0.9 ± 0.5	1.5 ± 0.4	0.6 ± 0.3

* Significantly different from males and females ($P<0.05$); SBP, systolic blood pressure, DBP, diastolic blood pressure, MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

✗Significantly different from positive responders ($P<0.05$); SBP, systolic blood pressure, DBP, diastolic blood pressure, MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity

Table 4.4: Mean changes in sympathetic and cardiovascular variables during physical stressor tasks for the males (n=21) females (n=19).

Variable	Cold pressor		Handgrip		Ischaemia	
	Males	Females	Males	Females	Males	Females
Systolic BP (mmHg):	18 ± 4	19 ± 3*	13 ± 3	13 ± 2*	14 ± 3	13 ± 4*
MAP (mmHg):	14 ± 3	19 ± 2*	19 ± 2	11 ± 2‡*	11 ± 2	13 ± 3*
Diastolic BP (mmHg):	11 ± 2	15 ± 2*	11 ± 1	10 ± 2*‡	9 ± 2	10 ± 3*
Heart rate (beats/min):	6 ± 2	9 ± 2*	11 ± 2	4 ± 1*†‡	-0.05 ± 1	-2 ± 1*
Total MSNA (%):	62 ± 11	107 ± 24*	34 ± 11	32 ± 17*	42 ± 12	56 ± 16*
MSNA burst ampl. (%):	30 ± 6	55 ± 15*	21 ± 8	19 ± 9*	24 ± 6	54 ± 20*
MSNA burst freq. (bursts min-1):	3 ± 1	4 ± 1*	3 ± 1	4 ± 1*	4 ± 2	3 ± 1*

BP = blood pressure; MAP = mean arterial pressure; MSNA = muscle sympathetic nerve activity.

*Significant main effect of time (P<0.05). †Significant effect of sex (P<0.05). ‡Significant interaction (P<0.05).

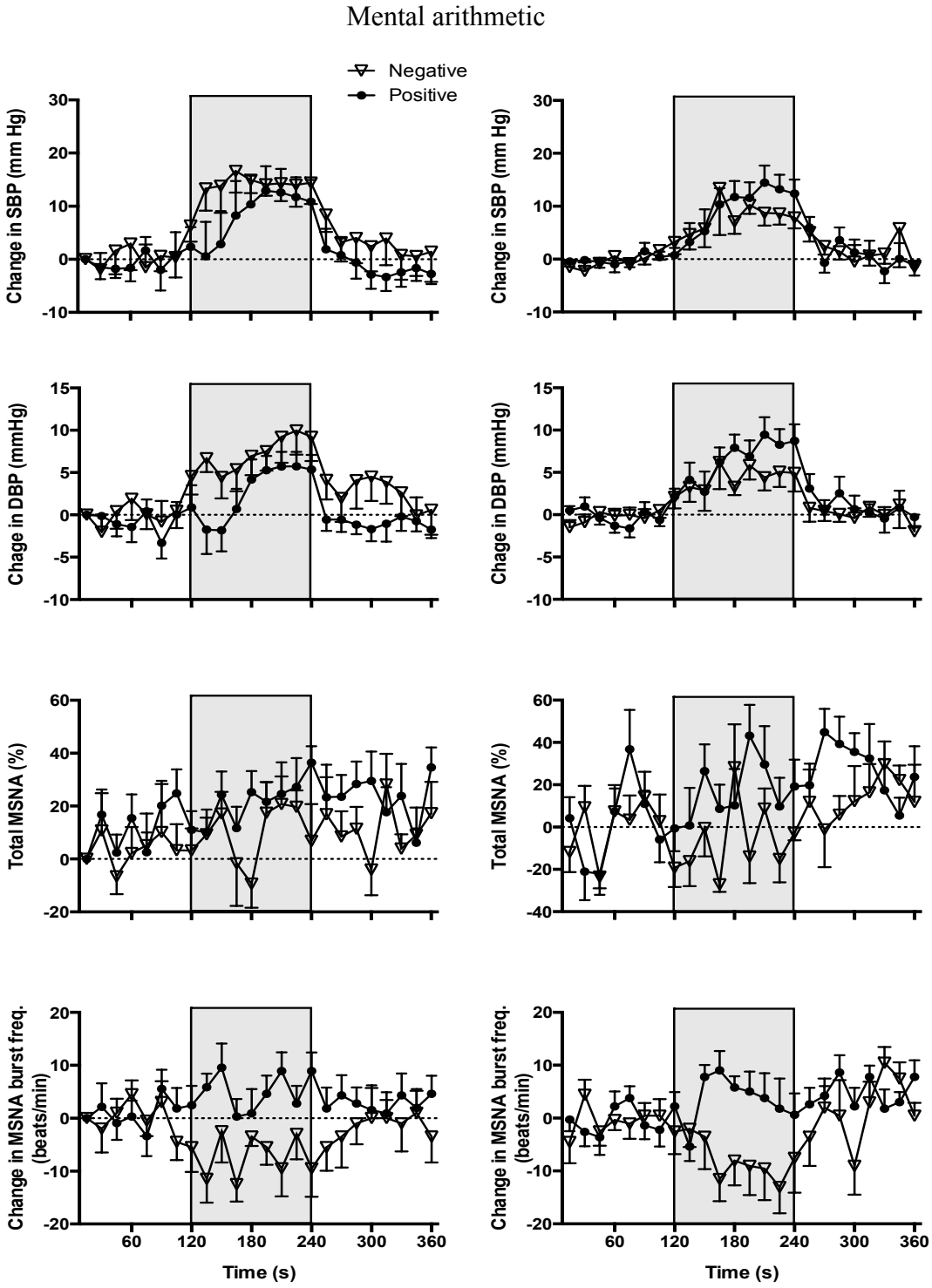

Figure 4.1: Time course of the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), total MSNA and burst frequency in positive and negative responders (grouped according to burst frequency) in both males and females during the mental arithmetic task.

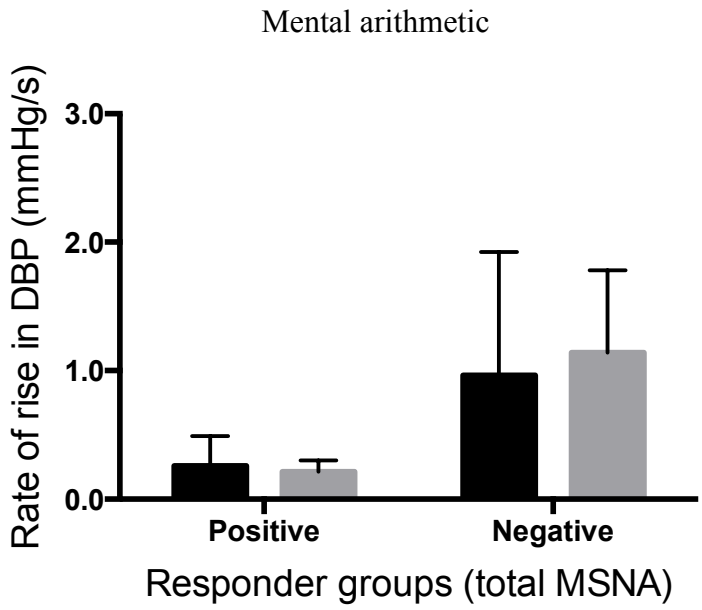

Figure 4.2: Rate of rise in diastolic blood pressure (DBP) in positive and negative responders to mental arithmetic task in both males (black panels) and females (grey panels). Responders are classified according to total MSNA.

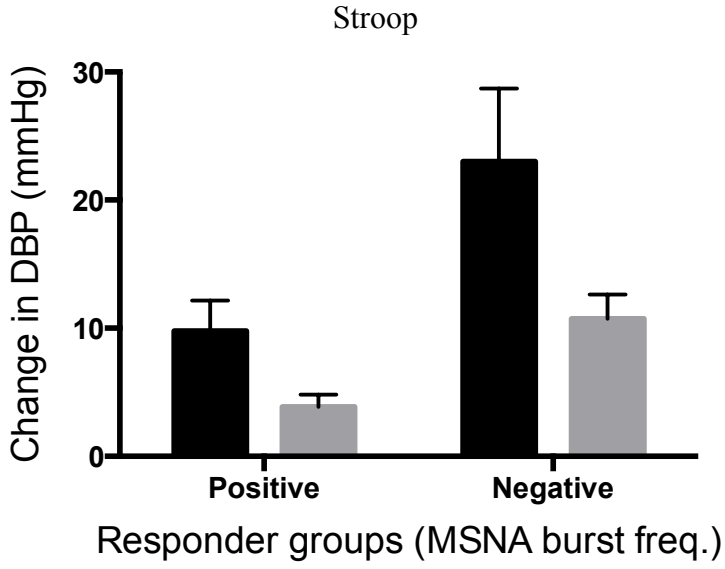

Figure 4.3: Change in diastolic blood pressure (DBP) in positive and negative responders to the Stroop test in both males (black panels) and females (grey panels). Responders are classified according to MSNA burst frequency.

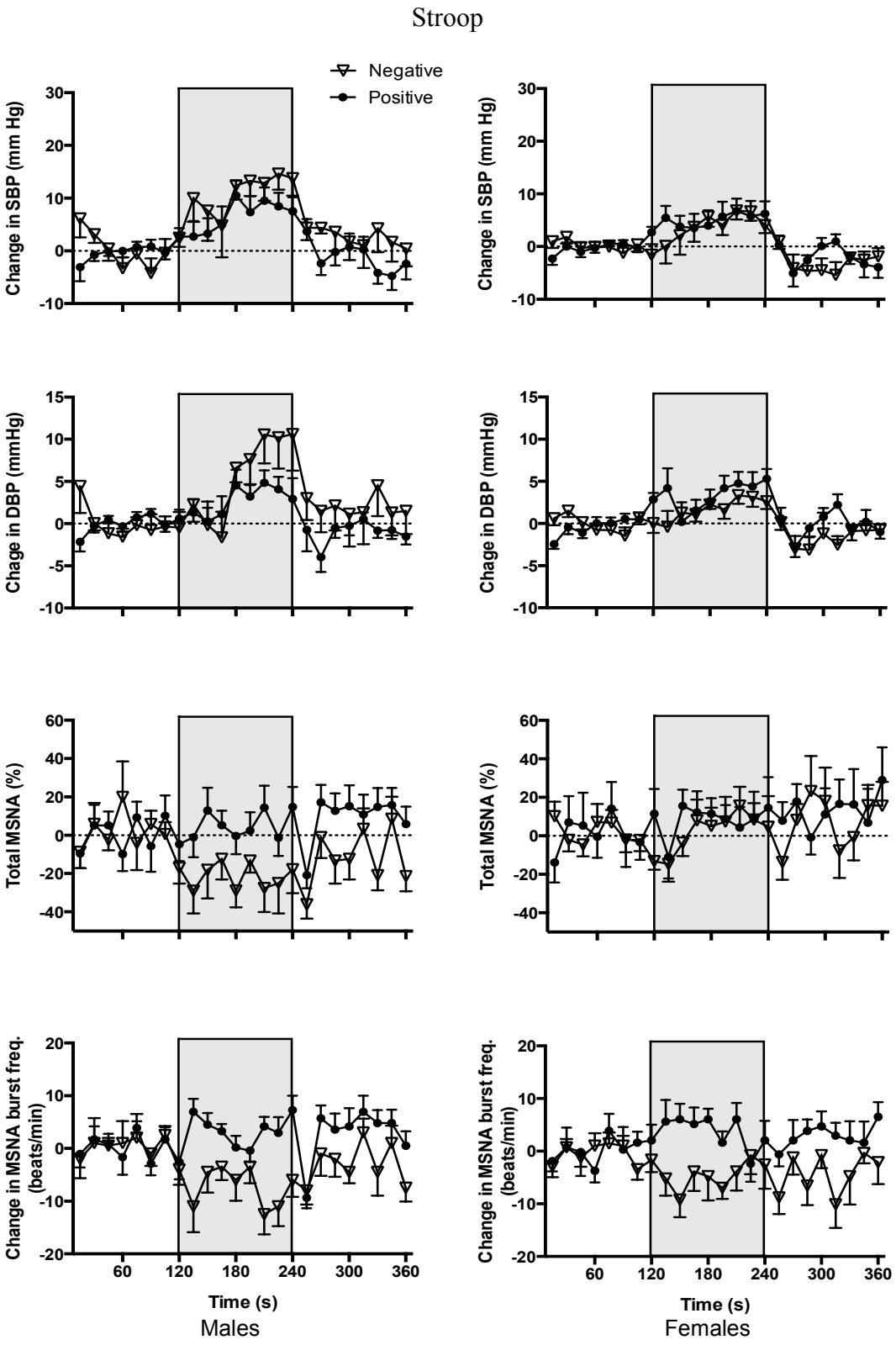

Figure 4.4: Time course of the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), total MSNA and burst frequency in positive and negative responders (grouped according to burst frequency) in both males and females during the Stroop test.

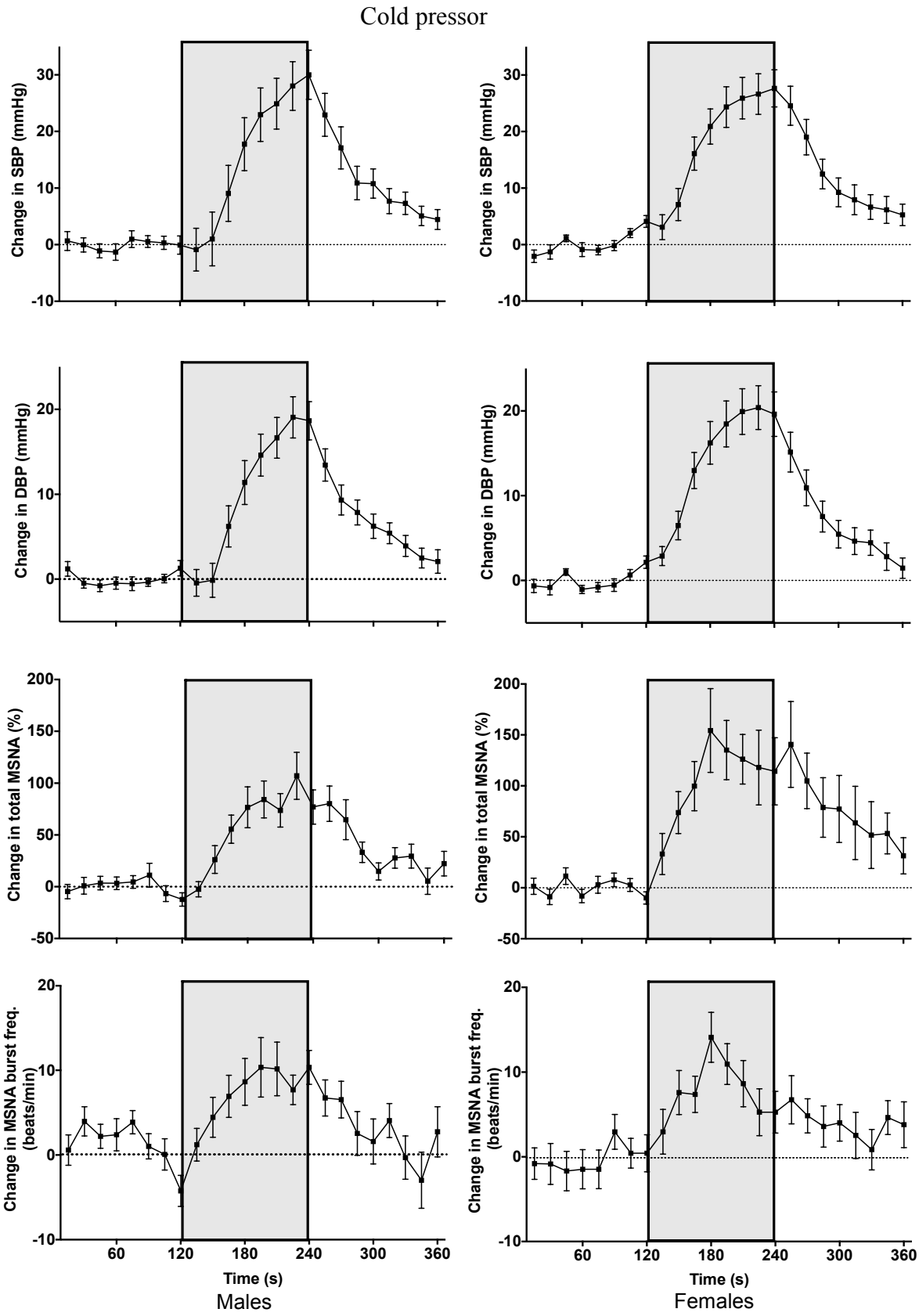

Figure 4.5: Time course of the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), total MSNA and burst MSNA frequency during the cold pressor task. The grey rectangles indicate the 2-min cold pressor task.

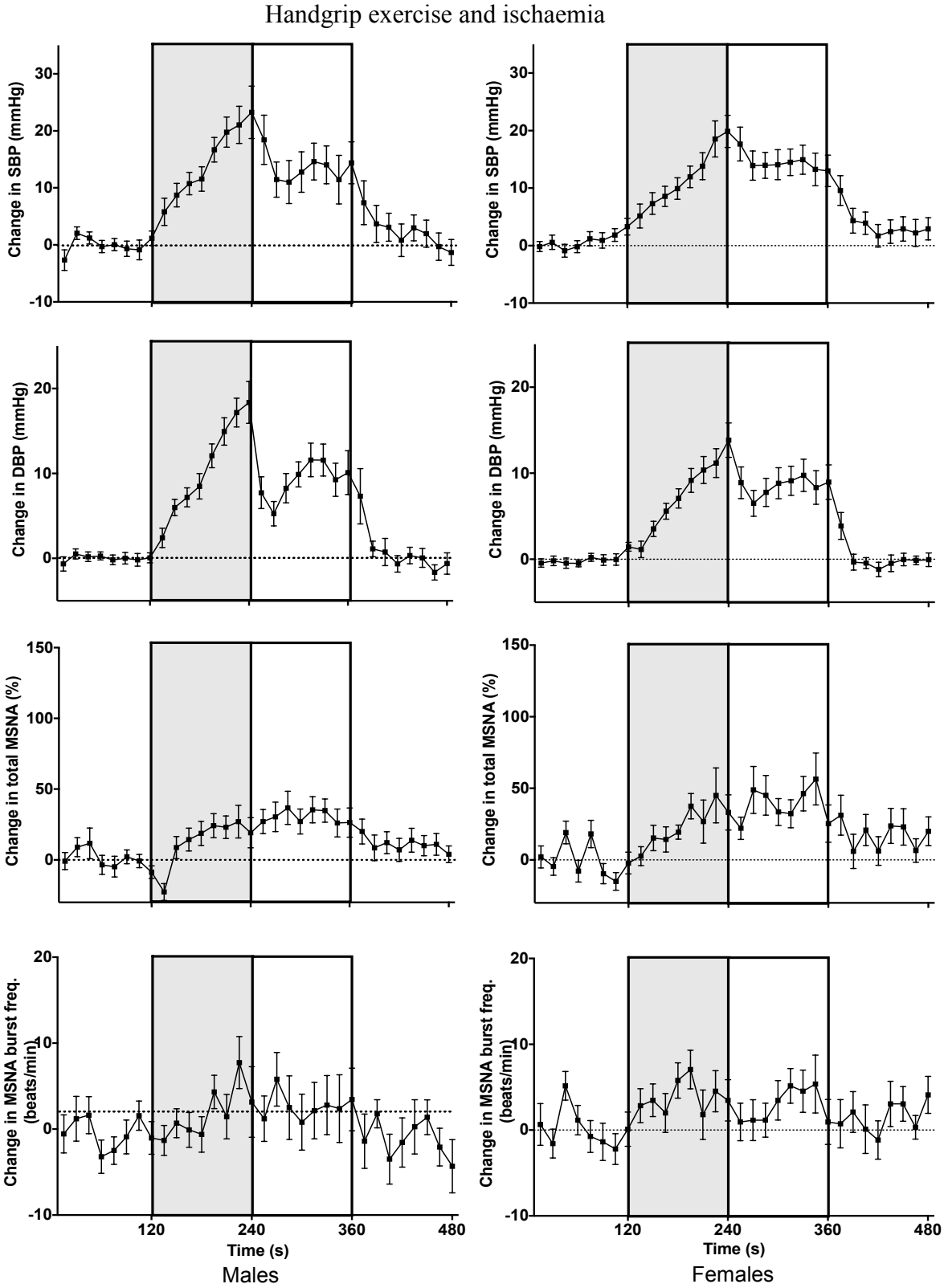

Figure 4.6: Time course of the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), total MSNA and burst MSNA frequency during the handgrip exercise/ischaemia task. The grey rectangles indicate the 2-min handgrip task; the white rectangle indicates the 2-min period of post-exercise ischaemia.

Chapter 5: Study 3

Effects of the menstrual cycle and changes in muscle sympathetic nerve activity during physical and mental stressors

5.1 ABSTRACT

Aim: The influence of the menstrual cycle on MSNA remains controversial. Some studies report elevated resting MSNA during the HH phase compared with the LH phase, whereas other studies do not. Moreover, some studies have shown increases in MSNA responsiveness to stressors and others have not. The current study aimed to examine the effects of the menstrual cycle on cardiovascular and sympathetic responses to stressors in young, healthy females.

Methods: The time course of MSNA, BP and HR responses to mental stressors (Stroop colour-word test and mental arithmetic) and physical stressors (CPT, static handgrip exercise, and post-exercise ischemia) were recorded in 10 healthy young (19-29-yr old) females.

Results: Resting MSNA burst frequency was significantly lower during the LH phase (34±1 bursts/min) compared with the HH phase (39±1; $P<0.05$). The LH phase was associated with greater increases in MSNA burst frequency during the cold pressor (9±2 bursts/min) compared with the HH phase (5±3; $P=0.014$). There was a significant effect of menstrual phase and interaction for SBP, with larger increases during the HH phase (20±5 mmHg) compared with the LH phase (17±4; $P=0.014$). The remaining stressor tasks caused inconsistent results across the two phases of the menstrual cycle.

Conclusions: We conclude that the CPT elicits greater sympathetic responsiveness during the LH phase compared with the HH phase, but this was not associated with larger elevations in BP. The findings highlight the influence of the menstrual cycle on cardiovascular control and the importance of considering menstrual phase when examining sympathetic responses to stressors.

5.2 INTRODUCTION

During the LH phase oestrogen and progesterone are low and during the HH phase oestrogen and progesterone are high.

The focus of this study was to determine how the low and high hormone phases of the menstrual cycle influence the time course of BP and sympathetic responsiveness to mental and physical stress in healthy, premenopausal females. It has been suggested that the different phases of the menstrual cycle not only influence resting sympathetic outflow, but BP and sympathetic responsiveness to stress. However, the effects of stress on MSNA are reported to be highly variable (Carter and Goldstein, 2015). The time course of BP, MSNA and HR responses were examined during the Stroop colour-word test, mental arithmetic, the CPT, static handgrip exercise and post-exercise ischaemia. Consistent with previous research, it is hypothesized that resting MSNA is higher in the HH phase compared with the LH phase (Usselman et al. 2014). It is reasoned that lower resting MSNA will provide greater capacity for an increase in sympathetic activity, and therefore it is hypothesised that increases in MSNA and BP are greater in the LH phase than HH phase during physical and mental stressors. Therefore, the aim of this study was to investigate what drives the increases in BP and MSNA in the LH phase by examining the time course of BP, MSNA and HR responses during a series of mental and physical stressor tasks.

5.3 METHODS

5.3.1. Participants

Ten healthy female participants aged between 18 and 29 with no history of cardiovascular disease, were recruited for the study. Participants attended the laboratory on two occasions: during the LH phase (between days 1-7) of their menstrual cycle, and during the HH phase (between days 19-23) of their menstrual cycle. Out of the 10 subjects, 5 were taking OCs pills (were not excluded).

Participants were also tested during the same time of day, for both the LH and HH phases, in order to prevent any effects of time of day due to the interactions of circadian rhythm on BRS (Taylor et al. 2011).

Refer to methods section 2.1 for further detail.

5.3.2. Measurements

Refer to section 2.3.

5.3.3. Experimental procedures

Refer to section 2.5.

5.3.4. Data analysis

Two-way repeated measures ANOVAs were performed to determine the main effect of time and menstrual phase for each variable. Post-hoc multiple comparisons were made to determine which time points were significantly different from rest. Mean changes in each variable are reported for each stressor task. Mean changes from rest were quantified by comparing to the average of the 2-min rest period prior to the stressor.

5.4 RESULTS

Ten females completed the study, with a mean age of 23 ± 1 year and BMI of 25.7 ± 2 kg/m^2. Although BMI falls into the overweight category (≥25 kg/m^2), fat-free mass was 46.5 ± 2 kg. This value is above the average for healthy, young individuals (Kyle et al. 2001), which can be explained by the physical activity levels in the cohort. According to self-reports, the participants exercised regularly (≥2 days per week), with some participating in resistance training. There was a significant difference in the 10-min baseline MSNA burst frequency between the LH and HH phases, with greater resting values in the HH phase (P=0.0094), this could be due to the higher HR and MSNA burst incidence values in the HH hormone phase. This difference in resting MSNA was not significant when reported as MSNA burst incidence (P=0.36).

Furthermore, the 2-min rest periods before each stressor tasks were compared between the two phases, table 5.1, shows that resting variables were consistent prior to each stressor task. There were significant differences between the LH and HH phases in DBP and MAP across all tasks (P<0.0005). In addition, there were significant differences in MSNA burst frequency (P<0.005) and MSNA burst incidence (P<0.05) during the rest period of the mental arithmetic task between the two phases. Blood pressure and HR were not significantly different at rest prior to each stressor task (P>0.05). However, during the LH phase MSNA burst frequency and incidence were significantly different, with higher resting values prior to the Stroop test compared with the cold pressor (P=0.03) and mental arithmetic compared with the handgrip task (P=0.006) respectively. During the HH phase resting MSNA was consistent during the rest periods prior to the stressor tasks (P>0.05).

All ten females completed each of the stressor tasks. However, in one individual the MSNA recording deteriorated during mental arithmetic and the Stroop test and therefore responses to mental stressors are reported for nine participants.

5.4.1. High vs low hormone phases

Mental stressor tasks

The mental arithmetic task was associated with significant increases in BP and HR (table 5.2; $P<0.05$). There was a significant effect of time on total MSNA ($P=0.02$), although the time course of the response suggests that increases occurred during recovery from the task. There was no significant effect of time for MSNA burst frequency ($P=0.20$) or MSNA burst amplitude ($P=0.26$). Figures 5.1 and 5.2 illustrate the time course of the responses to the mental arithmetic task during the LH and HH phases.

There was a significant interaction between time and menstrual phase for SBP ($P=0.04$), but no significant effects of menstrual phase or interactions for DBP, MAP, HR or MSNA ($P<0.05$). For both phases of the menstrual cycle the increases in BP occurred after the peak in HR, as shown in figure 5.1. MSNA responses to mental stress were highly variable between individuals. This variability is illustrated in figure 5.3.

The Stroop test was associated with significant increases in BP and HR over time ($P<0.05$), but - surprisingly - there were no significant changes in MSNA ($P>0.05$). This is shown in figures 5.4 and 5.5. In both phases of the menstrual cycle HR peaked before the increases in BP occurred. There were no significant effects of menstrual phase or interactions between time and menstrual phase for any of the variables ($P>0.05$).

Physical stressor tasks

The CPT elicited significant increases in BP, HR and MSNA over time (table 5.3; $P<0.05$). Figures 5.6 and 5.7 illustrate the time course of the responses to the CPT during the LH and HH phases of menstrual cycle. For both menstrual phases, there were gradual and concurrent increases in BP and MSNA during the 2-min CPT. There was a significant effect of menstrual phase ($P=0.014$) and a significant interaction ($P<0.0001$) for SBP, with larger increases in BP during the HH phase. There was a significant effect of menstrual phase on MSNA burst frequency ($P=0.014$), with greater increases in the LH phase. There was a significant interaction between time and menstrual phase ($P=0.01$) for total MSNA: increases were greater during the LH phase compared with the HH phase.

The average pain score associated with the CPT during the LH phase was 8.3 ± 0.6 out of 10. Linear regression analysis revealed no significant relationship between pain score and the mean change in SBP ($r^2=0.15$; $P=0.27$). The average pain score during the HH phase was 8.05 ± 0.6. Linear regression analysis revealed no significant relationship between max pain score and the mean change in SBP ($r^2=0.38$; $P=0.059$).

There were no significant differences in max pain scores between the LH and HH phases (P=0.56).

As expected, static handgrip exercise generated significant increases in BP, HR and MSNA over time (table 5.3; P<0.05). Figures 5.8 and 5.9 illustrate the time course of the responses to the static handgrip task and post-exercise ischaemia between the LH and HH phases. As shown in figure 5.8 there was a drop in BP and HR when the exercise ceased but BP still remained significantly elevated above baseline during ischaemia for both the LH and HH phases. Post-exercise ischaemia was associated with significant increases in BP and MSNA over time (table 5.3; P<0.05). For the handgrip task and post-exercise ischaemia there were no significant effects of menstrual phase or interactions for any of the variables (P>0.05).

5.5 DISCUSSION

The present study examined the effects of the menstrual cycle on BP and sympathetic responsiveness to physical and mental stress. The results indicate that the CPT elicited greater increases in MSNA during the LH phase, when resting MSNA is lower, compared to the HH phase of the menstrual cycle. This finding, however, was not consistent across other stressors, with no significant differences between menstrual cycle phases for handgrip or ischaemia. Effects of mental stress on MSNA were extremely modest and not consistent between individuals, suggesting considerable inter-individual variability and no clear effect of menstrual cycle phase.

The significance of these findings will be explored in the General Discussion.

Table 5.1: 2-min rest stressor periods for sympathetic and cardiovascular variables (mean ± SE) in the low hormone (LH) and high hormone (HH) phases of the menstrual cycle (n=10).

	Cold pressor		Handgrip		Mental Arithmetic		Stroop	
Variable	LH	HH	LH	HH	LH	HH	LH	HH
Systolic BP (mmHg)	136 ± 7	127 ± 6	131 ± 8	128 ± 5	129 ± 8	124 ± 5	134 ± 7	123 ± 5
Diastolic BP (mmHg)	72 ± 3	55 ± 3***	71 ± 3	52 ± 4***	71 ± 3	53 ± 3***	71 ± 3	53 ± 3***
MAP (mmHg)	91 ± 4	75 ± 3***	89 ± 4	72 ± 4***	90 ± 4	73 ± 3***	90 ± 4	73 ± 3***
Heart rate (beats min^{-1})	78 ± 3	78 ± 3	75 ± 2	75 ± 2	80 ± 3	75 ± 3	76 ± 2	76 ± 3
MSNA burst frequency (bursts/min)	32 ± 2	36 ± 1	36 ± 2	38 ± 1	32 ± 1	39 ± 2**	39 ± 2	35 ± 2
MSNA burst incidence (bursts/100 heartbeats)	42 ± 3	48 ± 3	53 ± 4	51 ± 2	41 ± 2	53 ± 4*	47 ± 3	51 ± 3

*Significant difference between the low hormone and high hormone phases ($P<0.05$). ** Significant difference between the low hormone and high hormone phases ($P<0.005$). ***Significant difference between the low hormone and high hormone phases ($P<0.0005$).

BP = blood pressure; MAP = mean arterial pressure; MSNA = muscle sympathetic nerve activity; LH = low hormone phase; HH = high hormone phase.

Table 5.2: Mean changes in sympathetic and cardiovascular variables during mental stressor tasks for the LH and HH phases of the menstrual cycle (n=9).

Variable	Mental arithmetic		Stroop test	
	LH	HH	LH	HH
Systolic BP (mmHg)	7 ± 3*	4 ± 3‡	2 ± 3	3 ± 3*
MAP (mmHg)	6 ± 2*	3 ± 1*	2 ± 1*	1 ± 2
Diastolic BP (mmHg)	5 ± 2*	2 ± 1*	2 ± 1	0 ± 1*
Heart rate (beats min^{-1})	4 ± 2	7 ± 2*	3 ± 2	2 ± 2
Total MSNA (%)	6 ± 8	-11 ± 12	10 ± 14	0 ± 9
MSNA burst ampl. (%)	10 ± 8	24 ± 21	6 ± 9	1 ± 8
MSNA burst freq. (bursts min-1)	0.05 ± 2	-2 ± 2	1 ± 2	-1 ± 2

BP = blood pressure; MAP = mean arterial pressure; MSNA = muscle sympathetic nerve activity; LH = low hormone phase; HH = high hormone phase

*Significant main effect of time ($P<0.05$). ‡Significant interaction between time and menstrual phase ($P<0.05$).

Table 5.3: Mean changes in sympathetic and cardiovascular variables during physical stressor tasks for the low hormone (LH) and high hormone (HH) phases of the menstrual cycle (n=10).

Variable	Cold pressor		Handgrip Exercise		Post-Exercise Ischaemia	
	LH	HH	LH	HH	LH	HH
Systolic BP (mmHg):	17 ± 4*	20 ± 5*† ‡	11 ± 2*	11 ± 2*	16 ± 3*	13 ± 4*
MAP (mmHg):	16 ± 3*	18 ± 4*	10 ± 2*	11 ± 2*	12 ± 3*	13 ± 3*
Diastolic BP (mmHg):	13 ± 3*	14 ± 3*	9 ± 2*	9 ± 1*	9 ± 3*	10 ± 3*
Heart rate (beats/min):	5 ± 1*	5 ± 2*	5 ± 1*	2 ± 1*	-1 ± 1	-2 ± 1
Total MSNA (%):	133 ± 39*	47 ± 18‡	29 ± 31	40 ± 14	35 ± 20	56 ± 16*
MSNA burst ampl. (%):	67 ± 21*	33 ± 17	22 ± 15	35 ± 13	27 ± 10	54 ± 20
MSNA burst freq. (bursts min-1):	9 ± 2	5 ± 3†	4 ± 2	2 ± 2	1 ± 2	2 ± 2

BP = blood pressure; MAP = mean arterial pressure; MSNA = muscle sympathetic nerve activity; LH = low hormone phase; HH = high hormone phase

*Significant main effect of time ($P<0.05$). †Significant effect of menstrual phase ($P<0.05$). ‡Significant interaction between time and menstrual phase ($P<0.05$).

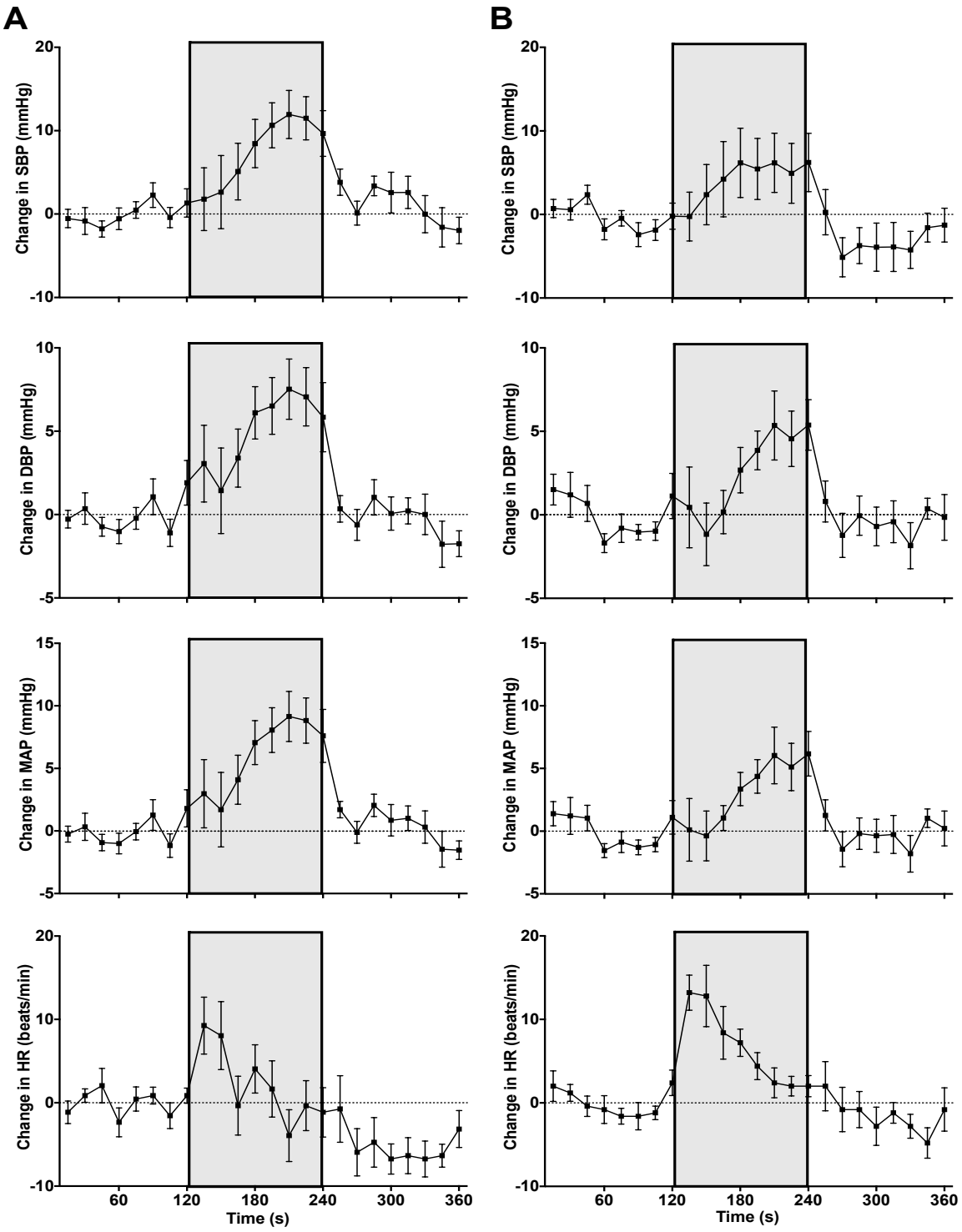

Figure 5.1: Cardiovascular responses to the mental arithmetic task during (A) the low hormone phase, and (B) the high hormone phase. The grey rectangles indicate the 2-min stressor task.

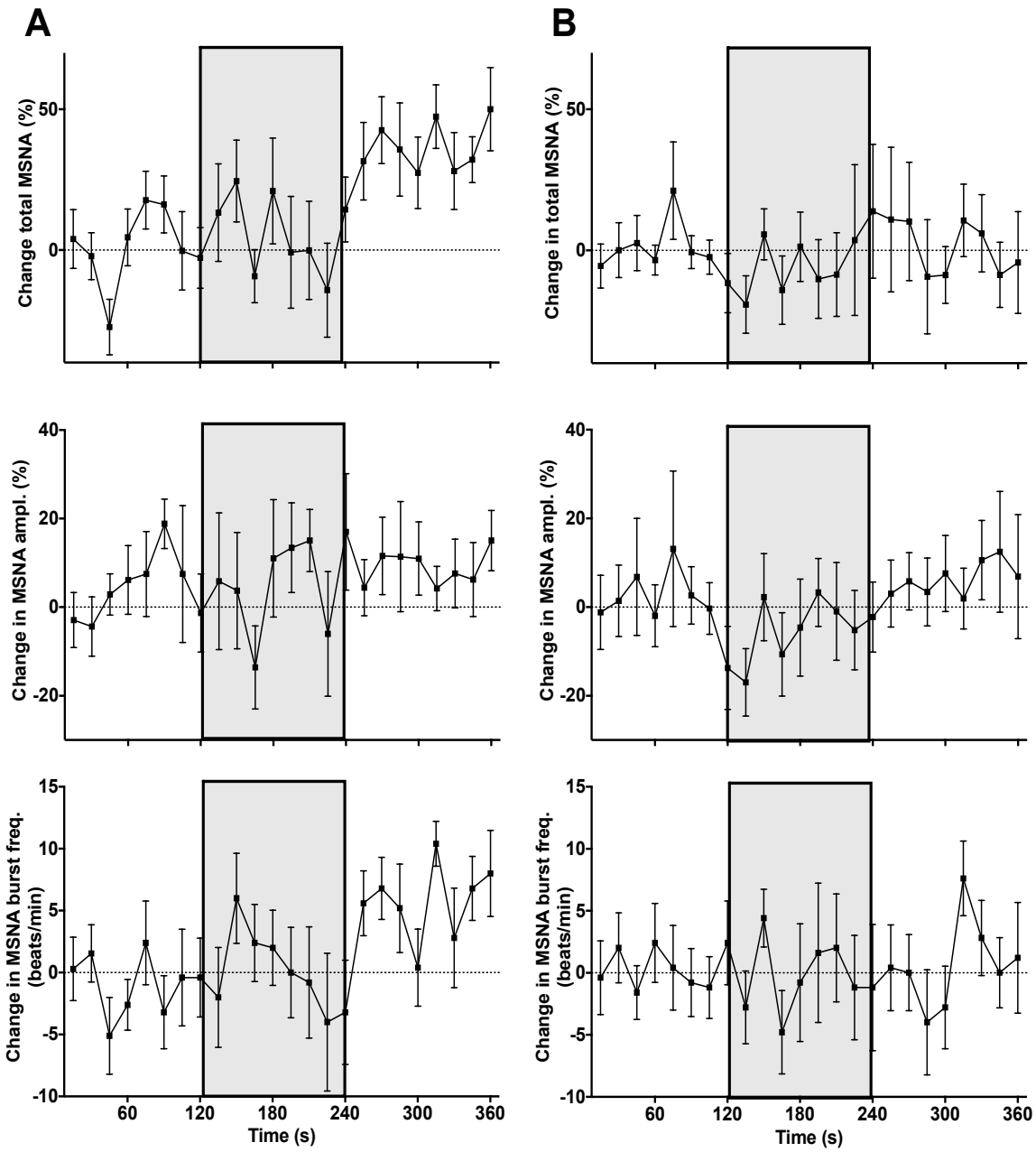

Figure 5.2: Sympathetic responses to the mental arithmetic task during (A) the low hormone phase, and (B) the high hormone phase. The grey rectangles indicate the 2-min stressor task.

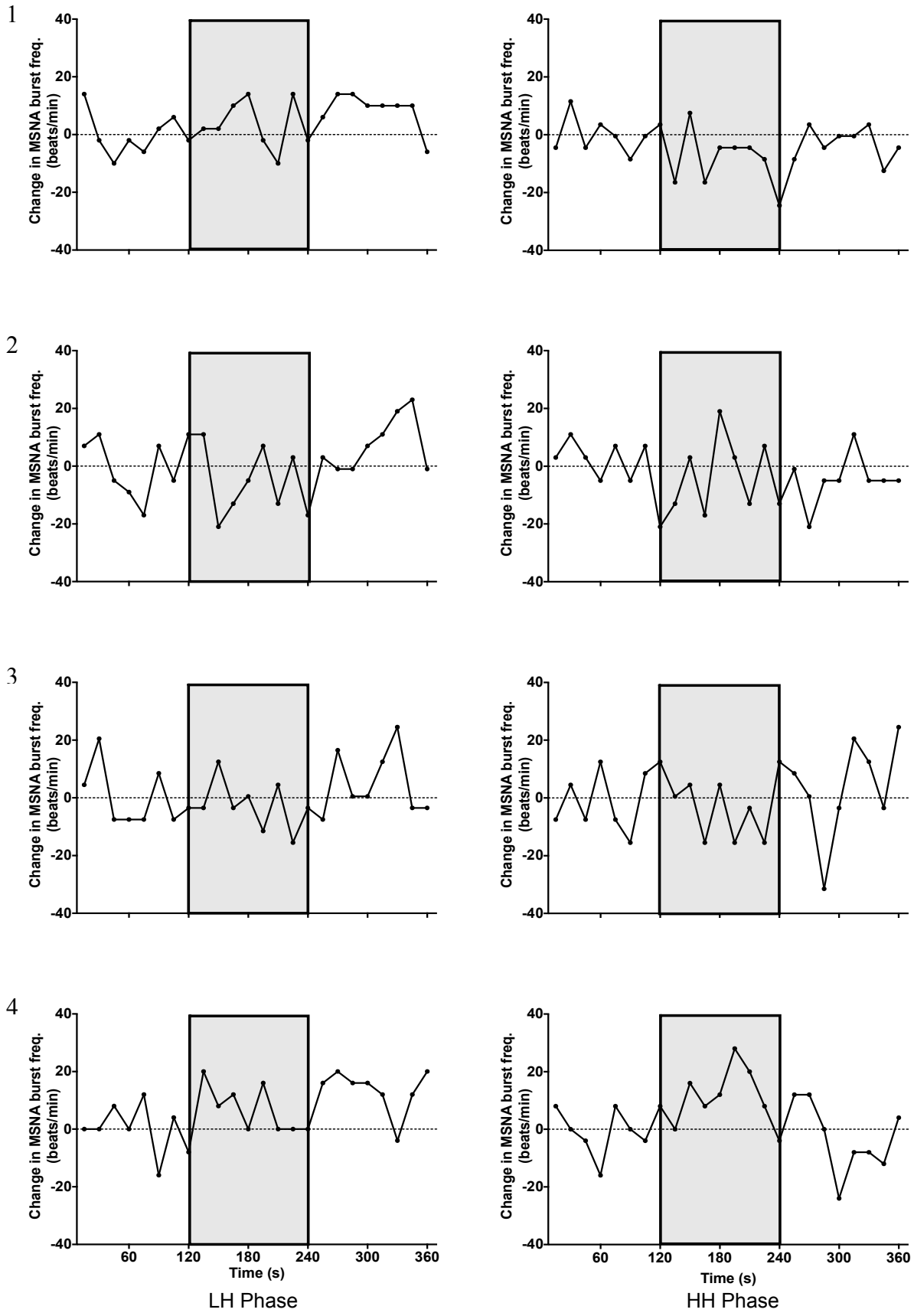

Figure 5.3: Sympathetic responses to the mental arithmetic task in 4 different individuals.

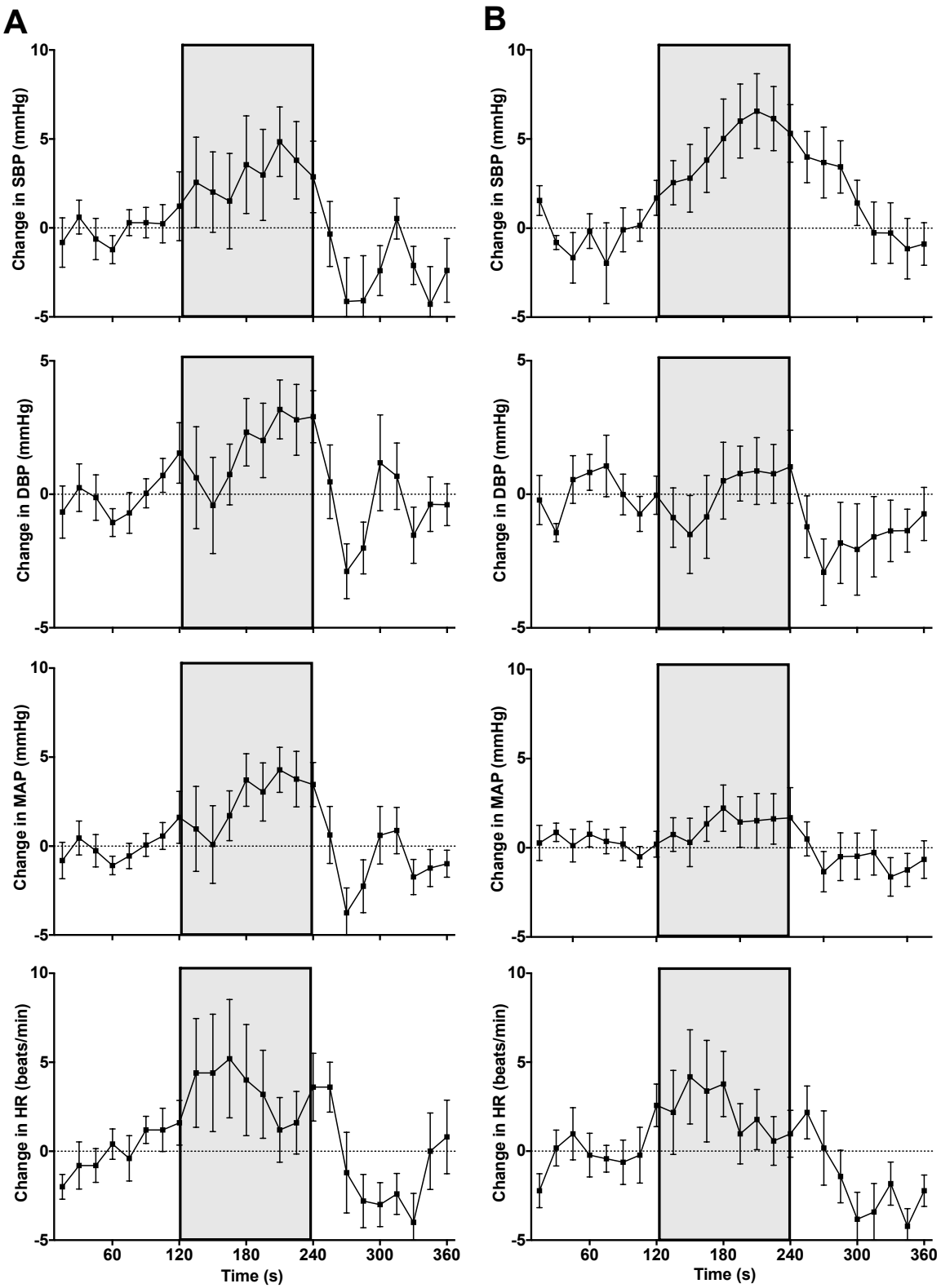

Figure 5.4: Cardiovascular to the Stroop test during (A) the low hormone phase, and (B) the high hormone phase. The grey rectangles indicate the 2-min stressor task.

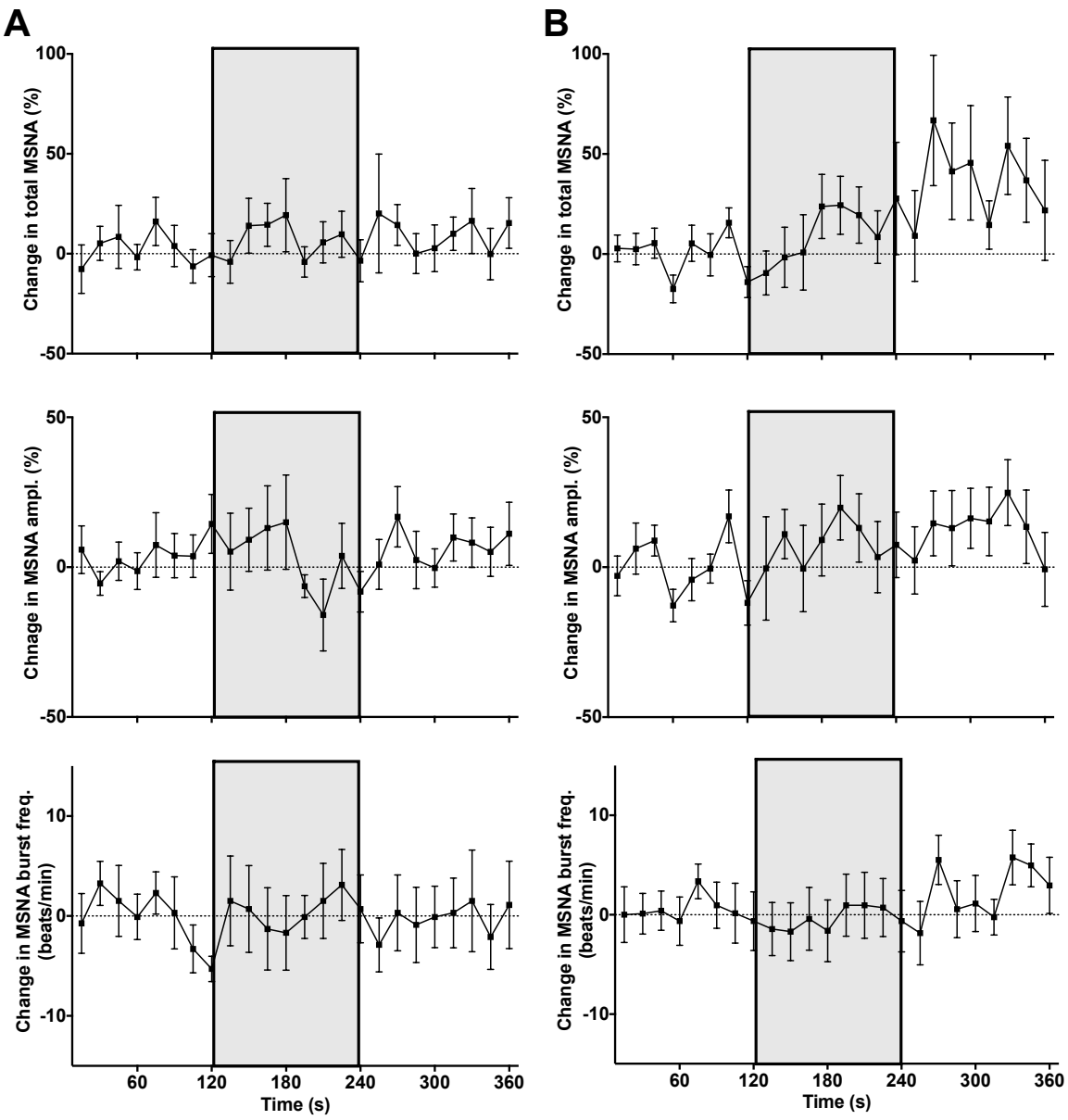

Figure 5.5: Sympathetic responses to the Stroop test during (A) the low hormone phase, and (B) the high hormone phase. The grey rectangles indicate the 2-min stressor task.

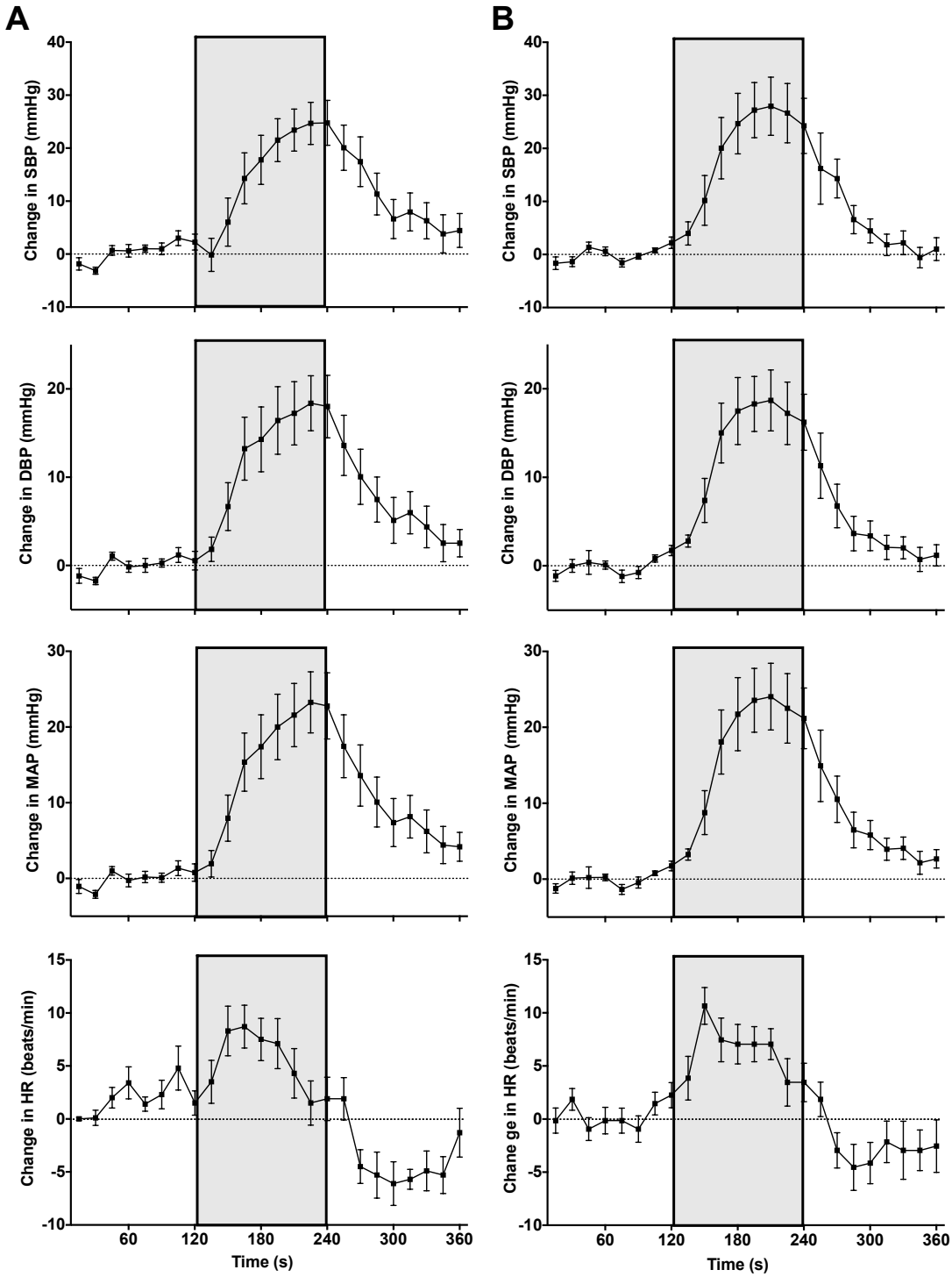

Figure 5.6: Cardiovascular responses to the cold pressor test during (A) the low hormone phase, and (B) the high hormone phase. The grey rectangles indicate the 2-min stressor task.

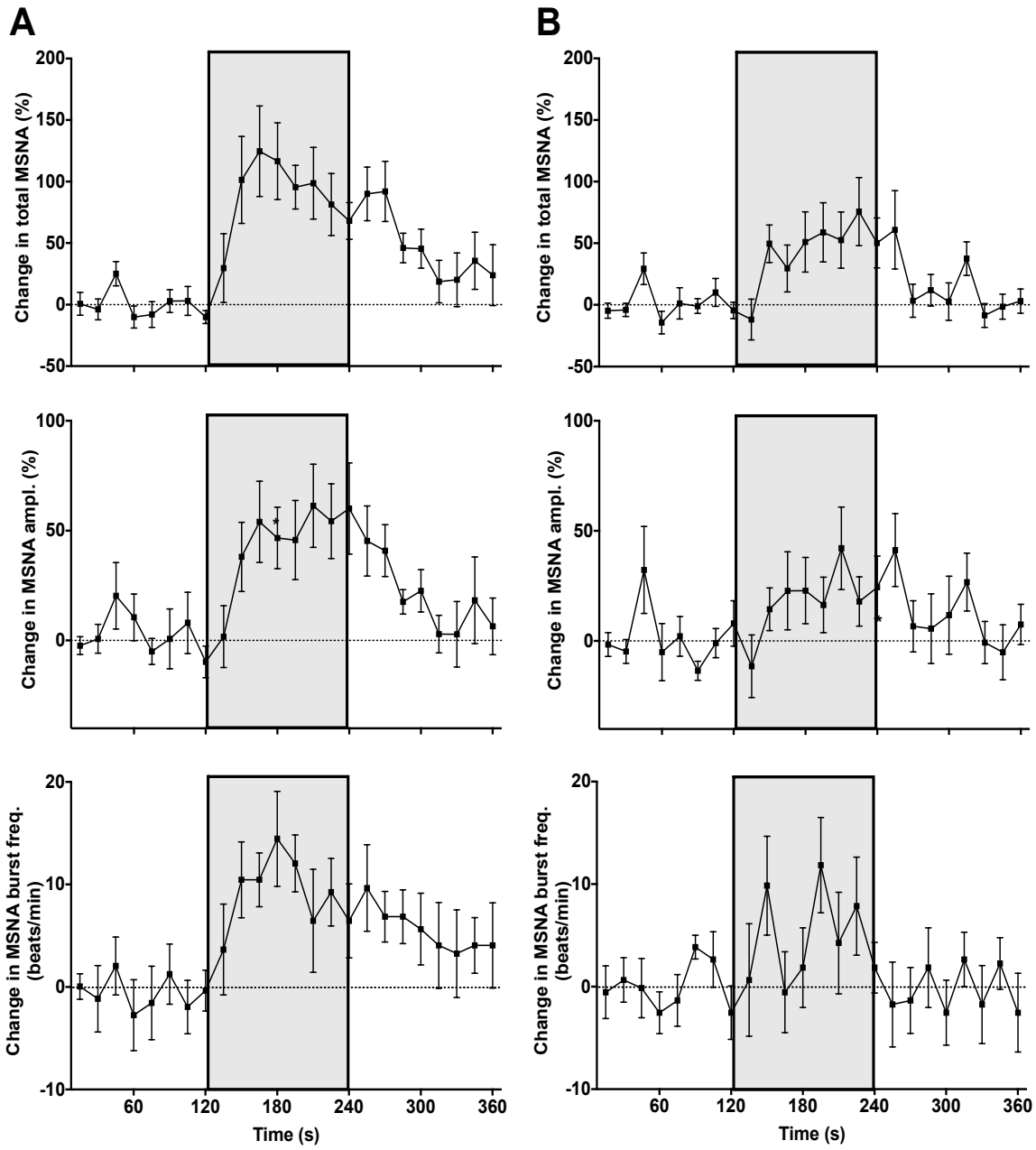

Figure 5.7: Sympathetic responses to the cold pressor test during (A) the low hormone phase, and (B) the high hormone phase. The grey rectangles indicate the 2-min stressor task.

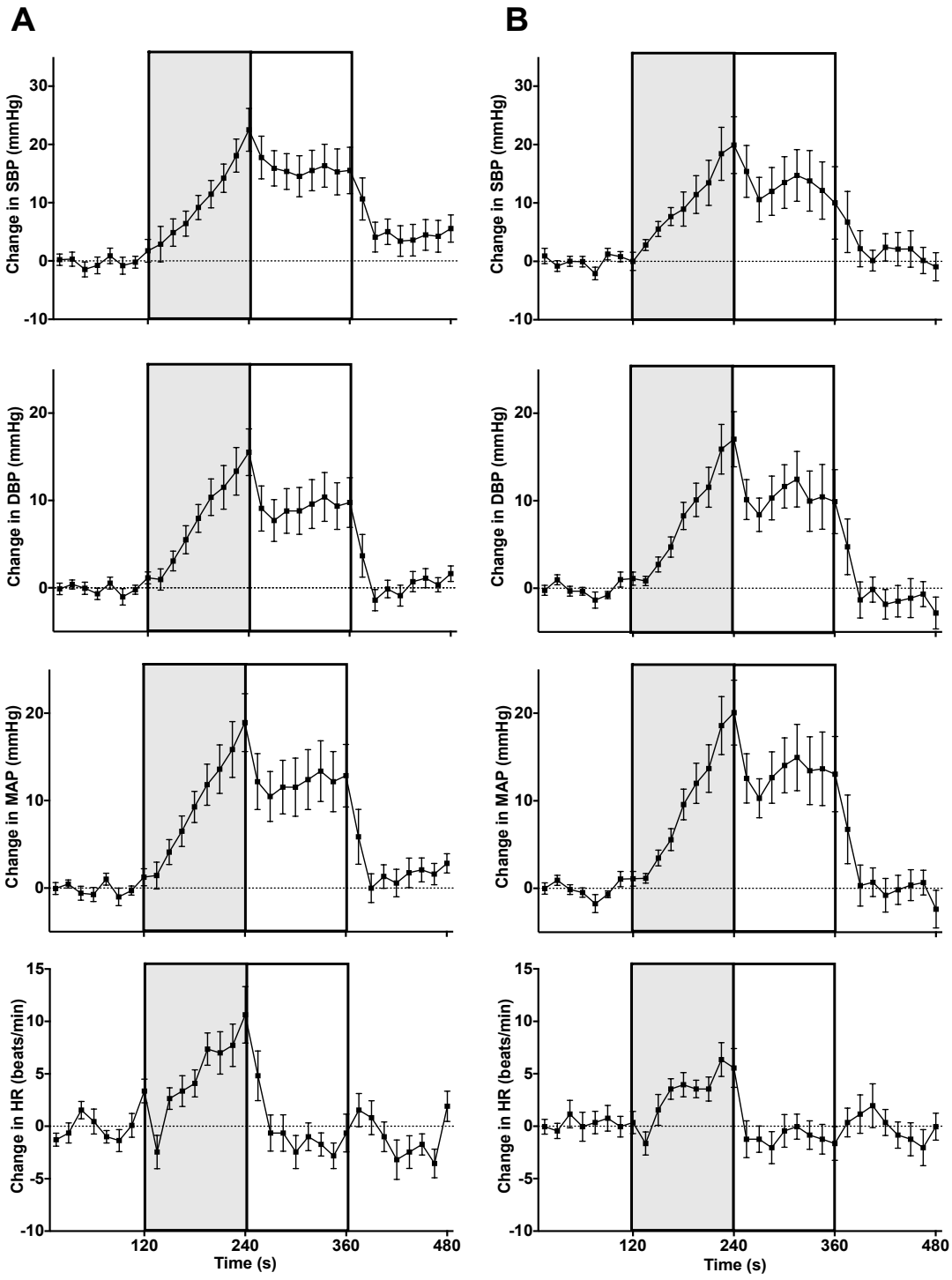

Figure 5.8: Cardiovascular responses to the handgrip and post-exercise ischaemia task during (A) the low hormone phase, and (B) the high hormone phase. The grey rectangles indicate the 2-min stressor task; the white rectangles indicate the 2-min period of post-exercise ischaemia.

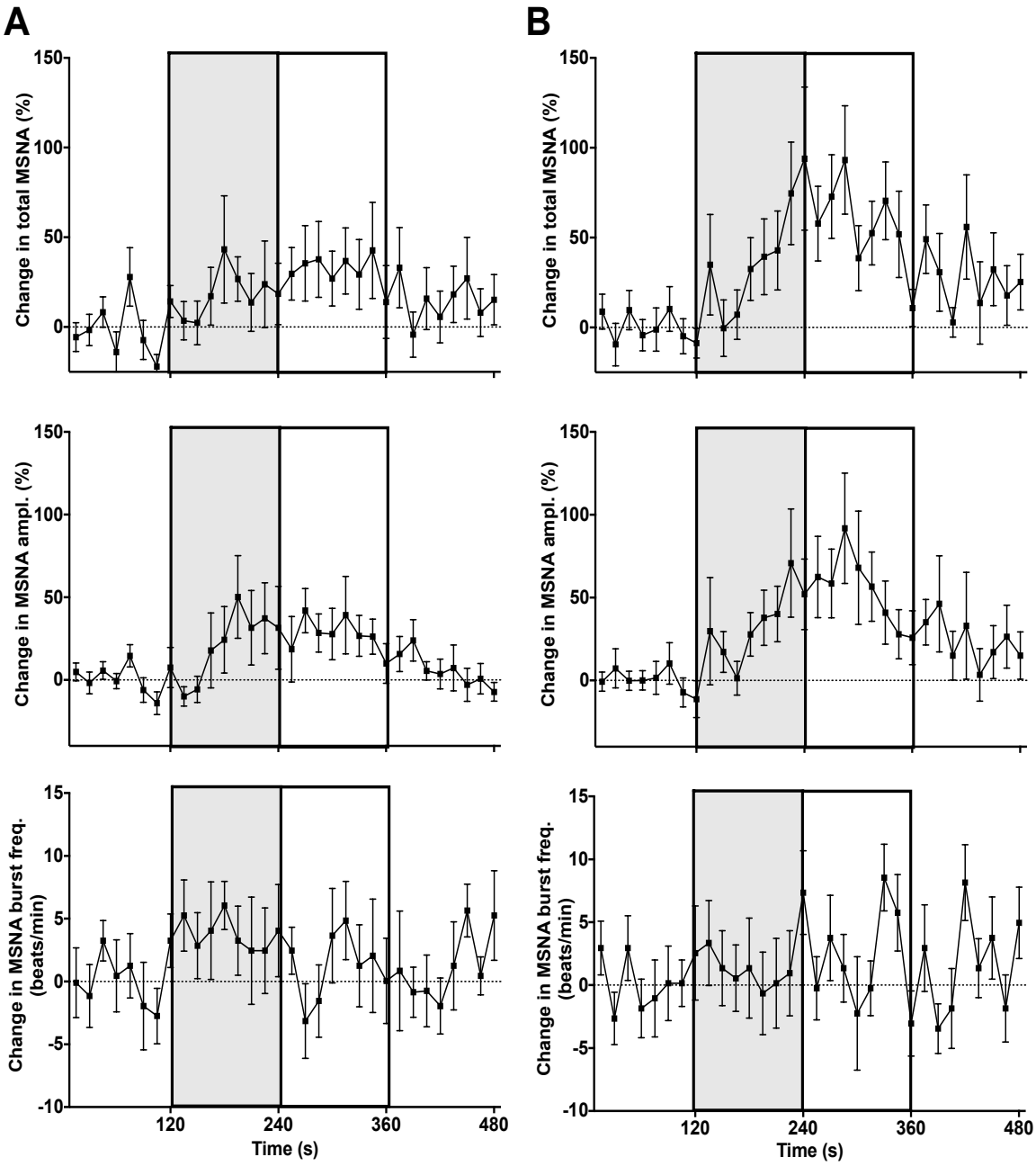

Figure 5.9: Sympathetic responses to the handgrip and post-exercise ischaemia task during (A) the low hormone phase, and (B) the high hormone phase. The grey rectangles indicate the 2-min stressor task; the white rectangles indicate the 2-min period of post-exercise ischaemia.

Chapter 6

General Discussion

The chapters of this thesis cover the research that was undertaken to investigate the influence of the effects of time course of the BP response on MSNA during stress. Sex differences in cardiovascular and sympathetic responsiveness to physical and mental stressors were examined, and the effects of the menstrual cycle on changes in MSNA during physical and mental stressors were investigated. The main findings of this thesis are that during the physical stressors, significant increases in MSNA are associated with parallel increases in BP, but mental stress is associated with a highly variable MSNA response, despite consistent increases in BP. The direction of the change in MSNA during mental stress is influenced by the rate of the rise in DBP early on in the stressor task. There were no major sex differences in MSNA or cardiovascular responses to physical or mental stress. However, the cold pressor elicits significantly larger increases in MSNA during the LH phase of the menstrual cycle than the HH phase. This, however, was not consistent across the other stressors.

6.1 TIME COURSE OF MSNA AND BLOOD PRESSURE RESPONSES TO MENTAL AND PHYSICAL STRESS

6.1.1. Inter-individual differences in MSNA responses to mental stress

Consistent with previous studies (Carter & Ray, 2009; Yang et al. 2013; Dunn & Taylor, 2014), in ***Studies 1 - 3*** mental stress was associated with significant increases in BP and HR. However, in contrast to physical stressors, there was considerable inter-individual variability in MSNA responses to mental stressors. In ***Study 1***, for the first time, the time course of MSNA and BP responses to mental stress were characterised in healthy young males, taking into account the direction of the change

in MSNA in each individual. When responses for the 21 males were pooled, mental arithmetic was only associated with a significant change in MSNA during the last 15s of the task and during recovery, and the Stroop test was not associated with significant changes in MSNA at all. The lack of significant response may be due to the similar numbers of positive and negative responders within the group. This inter-individual variability in MSNA responses during mental stress seen in ***Studies 1 and 2*** can be explained by the baroreflex.

Although sympathetic BRS did not differ between positive and negative responders, the stimulus the baroreflex received in the early stages of the stressor tasks did. It is clear from the current study and previous research that individuals may experience a rise or fall in MSNA. The current findings suggest that the direction of the MSNA response is associated with the rate at which BP rises during stress. Specifically, the rate of rise in DBP is greater in those individuals who demonstrate a reduction in MSNA. The sympathetic baroreflex serves to regulate BP by adjusting MSNA in response to acute changes in DBP. During mental stress the sympathetic baroreflex is reset and BRS is also lower in the first two minutes of the task (Durocher et al. 2011). Under these conditions, the lag in the rise in BP in positive responders would allow MSNA to rise due to less baroreflex inhibition. In contrast, a brisk rise in BP in negative responders caused by non-MSNA mechanisms may occur concurrently with baroreflex resetting and thus the baroreflex suppresses MSNA. These mechanisms cause BP to remain elevated above baseline in these individuals despite the suppression of MSNA.

Classic studies of the haemodynamic responses to stressors such as handgrip exercise have demonstrated that a pressor response consistently occurs regardless of the mechanism behind it; if one element of the ANS is unavailable then it is compensated for by another (Martin et al. 1973). The rise in BP, deemed an appropriate physiological response to handgrip exercise, may be driven by increases in MSNA, elevated CO, reduced BRS and/or resetting, or a combination of these factors. If the same applies to mental stress then an increase in BP will occur regardless of whether it is driven by MSNA or whether MSNA is suppressed and another mechanism takes over. ***Study 1*** suggests that the role of MSNA is dependent upon the early response in blood pressure. Negative MSNA responses to mental stress are associated with more rapid increases in DBP, suggesting that the baroreflex suppresses MSNA and an alternate driving factor is responsible for the rise in BP. If the BP response at the onset of mental stress is sluggish then MSNA increases (*i.e*. a positive response) and contributes to the rise in pressure.

6.1.2. Rate of rise analysis

The aim of the rate-of-rise analytical approach is to determine the peak in BP and when it occurs, and to detract from these the rate of the rise during stress. In ***Studies 1 and 2,*** the influence of the rate of rise in pressure and the differences between positive and negative responders are clearest and most consistent for the mental arithmetic task. Mental arithmetic was associated with greater anxiety levels and greater elevations in BP than the Stroop test, and may explain the ability to detect more distinct differences between groups. The use of extreme stress has ethical implications in human research and thus appropriate limits must be adhered to. It may be

speculated that tasks that are deemed to be more stressful provide a greater stimulus and may well evoke larger and potentially more robust responses between individuals. Whilst other tasks, such as making a speech, have also been used in mental stress research (Lipman et al. 2002) and may be considered more stressful, the most prominent stressors in the MSNA literature are mental arithmetic and the Stroop test (Carter & Goldstein, 2015).

Given previous research (Dunn & Taylor, 2014) and observations in our lab regarding the timing of the plateau in the BP response to mental stress, the primary rate of rise analyses were performed using the first minute of the mental stressor tasks. This also appeared to be suitable for the current data in which BP tends to peak in the first minute. However, diastolic BP in negative responders (which turned out to be key to ***studies 1 and 2***) did continue to rise throughout the 2min of mental arithmetic. In order to provide a more comprehensive review of this new approach, analyses were therefore performed using the first 30s and the full 2min stressor task in ***Study 1***.

In ***Study 1*** the 1-min analysis the rate of rise was the clearest and most consistent difference between the positive and negative responders, *i.e*. significant for both stressors and for both classifications of responders (MSNA burst frequency and total MSNA), bar the Stroop test with total MSNA which neared significance (P=0.06). With the 30s analysis, the difference in the rate of rise in DBP between groups did not reach statistical significance, although there was evidence to suggest larger increases in BP in negative responders in this time period. For the 2-min analysis, the rate of rise in SBP during mental arithmetic was significantly greater in negative responders, likely due to the lateness of the peak in SBP in the positive responders which, on

average, occurred in the second minute. Trends for a greater rate of rise in DBP did not reach significance. These differences may be explained by the timing of the peaks in BP and how adjusting the time period can affect which peak is captured by the analysis. During the first 30s BP was still in its primary rise for many individuals. Conversely, over the full 2-min task there can be more than one peak. For instance, if a sharp rise in BP is followed by further fluctuations in pressure in which the final peak is eventually reached, selecting the largest BP and determining the rate of the rise it can give the impression that the rise is slow when in fact the early response was rapid. The selection of the time period should reflect the research question. The primary aims of ***Studies 1 and 2*** were to examine the influence of the early peak in BP, hence focusing the analysis on the first minute of stress.

The data from ***Studies 1 and 2*** do suggest that there is considerable variability in the timing of the BP peak during mental stress. In support of this, there is evidence in the literature indicating that BP may continue to rise for three minutes of mental stress or beyond (Anderson et al. 1991; Wallin et al. 1992; Middlekauff et al. 2001). This makes defining the most appropriate time period difficult and therefore future work with this approach might involve longer periods of stress in which specific criteria are developed for the identification of the peak or early BP response, depending on what is of interest.

6.1.3. Positive and negative responders to mental stress

Carter and Ray (2009) used the changes in MSNA burst frequency during mental stress to divide participants into groups of positive responders (≥Δ3 bursts/min), negative responders (≤Δ-3 bursts/min) and non-responders. The study of 82 healthy

males and females suggests that the typical distribution of MSNA responses in healthy, young populations may include large proportions of positive responders (n=40, 49%) and non-responders (n=33, 40%), with smaller numbers of negative responders (n=9, 11%). This may be expected given that the majority of previous studies indicate either a significant mean increase (Carter et al. 2013; Yang et al. 2013; Schwartz et al. 2011; Scalco et al. 2009; Hering et al. 2013; Heindl et al. 2006; Kuniyoshi et al. 2003) or no change in MSNA with mental stress (Kuipers et al. 2008; Carter et al. 2008; Wasmund et al. 2002; Wilkinson et al. 1998; Jones et al. 1996), suggesting that these groups contained predominantly positive responders and/or non-responders.

If the MSNA burst frequency thresholds used by Carter & Ray (2009) were applied to the data in ***Study 1***, the sample would consist of six positive responders (29%), seven negative responders (33%) and seven non-responders (33%) to mental arithmetic. For the Stroop test, there would be nine positive responders (43%), seven negative responders (33%) and five non-responders (24%). The sample (n=21), albeit smaller than that of Carter and Ray (2009), therefore suggests a more even split into positive-, negative- and non-responders. When our analyses between positive and negative responders were repeated using the extremes, *i.e*. increases/decreases in MSNA burst frequency of ≥ 3 bursts/min (Carter & Ray, 2009), the rate of rise in DBP remained greater in negative responders but did not reach statistical significance (mental arithmetic, P=0.09; Stroop, P=0.08). Since dividing the current samples, from ***Studies 1 and 2***, into three groups would have limited the statistical power for detecting significant differences between groups, the participants were classified as either positive or negative responders according to the direction of the change in MSNA.

This meant that the two groups contained some individuals with changes of <3bursts/min. If our approach for examining MSNA and BP responses to mental stress were applied to a larger group of participants then positive, negative and non-responder classifications may shed further light on the effects of the rate of rise in BP on MSNA during mental stress. The use of different methods of classifying responses may also be useful within the literature, and in ***Studies 1 and 2,*** we have added to this with the use of the total MSNA the for grouping of participants. This ensures that changes in MSNA burst amplitude are also taken into account.

6.1.4. Driving factors that regulate blood pressure responses to mental stress

The driving factors behind the increases in BP during mental stress remains unclear; however, it is reported in ***Studies 1 - 3*** that HR consistently peaks before the increases in BP, in males and females and independent of the menstrual cycle phases. Measures such as cortisol levels, and SV during mental and physical stressors may provide further insight into what drives BP reactivity and what causes some individuals to experience exaggerated responses.

The current data indicate that healthy young individuals experience increases or decreases in MSNA during mental stress despite consistent elevations in BP, suggesting that the relationship between MSNA and BP is not consistent between individuals during mental stress. The data in ***Studies 1 and 2*** indicate that the rate of the rise in DBP at the onset of mental stress influences the direction of the change in MSNA.

6.1.5. Parallel increases in blood pressure and MSNA during physical stressors

During the CPT ***(Studies 1 – 3)*** HR increases rapidly, while the slower elevations in MSNA during the task parallel those of BP. Likewise, during static handgrip exercise the increase in BP occurs in parallel with the increase in both HR and MSNA, whilst during post-exercise ischaemia the increases in BP are driven by increases in MSNA ***(Studies 1 – 3)***. These responses have been well described previously (Victor et al. 1987a; Victor et al. 1987b; Fagius et al. 1989; Sander et al. 2010), and the current findings suggest that they are robust and consistent amongst healthy young males. An increase in BP and MSNA (and a resetting of the baroreflex) represents an appropriate response of the ANS to physiological challenges, such as exercise, ensuring adequate BP and flow to the relevant regions (Mark et al. 1985; Boulton et al. 2014).

6.1.6. Reproducibility

In ***Study 1***, it is reported that the sympathetic and cardiovascular responsiveness to mental and physical stressor tasks are repeatable on the same day (in one laboratory visit). In support of these findings, Fonkoue and Carter (2015) have shown that MSNA and cardiovascular responsiveness to mental arithmetic and CPT were repeatable on the same day. They also demonstrated that these responses are reproducible on different days (second laboratory visit).

6.2 INFLUENCE OF SEX ON MSNA AND BLOOD PRESSURE RESPONSES TO PHYSICAL AND MENTAL STRESS

6.2.1. Sympathetic control during mental tasks

Sex plays an important role in health and disease. Males and females respond differently to mental stressors (Satish et al. 2015). In ***Study 2***, sex differences were reported in total MSNA during the mental arithmetic task and in MSNA burst amplitude during the Stroop test. In contrast to these findings, Kajantie et al. (2006) have shown that females had lower SBP responses and Carter and Ray (2009) reported that females had lower MAP responses to mental stress compared with the males. However, this trend was not seen in MSNA, as there were no sex differences observed during mental stress in either study. The present evidence confirms this, and also indicates that the increase in MSNA was only significantly different from rest during the recovery from mental arithmetic.

In ***Study 2***, there were no sex differences in BP responses to mental stress. However, in contrast to these findings, Traustadóttir et al. (2003), showed significant sex differences in the DBP response in men compared to females during psychological stress, which included the Stroop test. In support of this study but inconsistent with our finding, Satish et al. (2015) have shown that during the Stroop test, males exhibited significantly greater increases in DBP compared to females. It is suggested that the greater DBP response in the males is due to greater sympathetic arousal or alternatively, greater parasympathetic withdrawal. This is due to the influence of the

male hormones on vagal activity (Montano & Porta, 2009).

In addition, Matthews et al. (2001) and Traustadóttir et al. (2003) have shown that BP is greater in males compared to females during mental stress and could be due to the fact that males exhibit greater HPAA and sympathetic responses to laboratory mental stressors (Kudielka et al. 2005; Kajantie et al. 2006; Montano & Porta, 2009). Moreover, Heponiemi et al. (2004) and Kudielka et al. (2004) have shown that females had a greater increase in HR compared with males during psychological stress such as the Trier Social Stress Test. The current results were in contrast to these findings, with no significant sex differences in HR or other cardiovascular variables during the stressors. However, in support of the results, Jones et al. (1996) concluded that during mental arithmetic task, there are no sex differences in BP, HR and MSNA. Furthermore, the current results show that the BP and HR responses to mental stress are not paralleled by increases in MSNA and are also independent of sex. The discrepancies in the findings of these studies could be due to the ages of the participants. Some studies recruited children with mean ages of 12 years old, 23 years old and 67 years old.

6.2.2. Recovery between males and females

Studies have shown that MSNA increases after the mental task ceases when compared to baseline levels (Carter & Lawrence 2007; Carter et al. 2004; Carter et al. 2005; Dishman, 2013; Ellenbogen et al. 1997; Fonkoue & Carter, 2015). Callister et al. (1992) found that both HR and arterial pressure decreased immediately at task cessation, whereas MSNA increased. This MSNA response to mental stress varies

between males and females, and in ***Study 2*** it is reported that females had greater MSNA increases in the recovery period compared with the males. This could be due the influence of the hormones involved in the phases of the menstrual cycle. Carter & Lawrence (2007), found that MSNA remained elevated during the initial 5-min of recovery in both the LH and HH phases, which is consistent with our findings, although our recovery period lasted for only 2-min. However, they concluded that in the final 5-min, of the total 10-min recovery period, MSNA only remained elevated during the HH phase, which is believed to be due to the increased sympathetic BRS (Minson et al. 2000).

In some individuals there is an overshoot in MSNA during recovery to above resting levels. Elevated MSNA in the recovery following mental stress has been demonstrated in several studies (Anderson et al. 1987; Callister et al. 1992; Carter et al. 2004; Carter et al. 2005; Carter et al. 2007; Kamiya et al. 2000), may be explained by a baroreflex-driven increase in MSNA in response to the fall in pressure following completion of the mental stressor task. The MSNA response is exaggerated because the baroreflex was reset to higher pressures during stress (Durocher et al. 2011; Fonkoue & Carter, 2015).

6.2.3. Sympathetic control during physical tasks

The physical stressors examined in ***Study 2*** did not reveal significant effects of sex in BP or sympathetic reactivity. The only significant sex difference we found was the effect of handgrip on HR, with greater responses in males. In support of our study Jones et al. (1996) reported that during the CPT, there were no differences between males and females in MSNA, BP and HR. However, the same group also found that

during handgrip exercise MSNA reactivity in males was greater than in females. This finding was in contrast with our results, as we found no significant sex differences in MSNA during the handgrip task. However, Matthews et al. (2004) have shown that only DBP changes were significantly greater in males than in females during this task. In another study Jarvis et al. (2011) reported that HR and MSNA responses were similar between sexes, but noted that males had higher SBP and DBP responses than females. The increases in MAP were also found to be similar in both sexes during the CPT (Shoemaker et al. 2001).

Furthermore, Ettinger et al. (1996) reported that during the static handgrip exercise, women had lower cardiovascular and sympathetic responses compared to men. This finding, however, was in contrast to the present results where HR increased more significantly in males than in the females. In addition, Jarvis et al. (2011), have shown that increases in HR from baseline to fatigue were greater in men than in women. Males also had higher SBP and DBP compared with women during both the static handgrip exercise and post-exercise ischaemia. In addition to lower baseline values, females also had smaller increases in MSNA burst incidence, total MSNA and MSNA burst frequency compared with the males.

The conflicting findings within and across studies could be due to some individuals finding one task more difficult, for example the handgrip test, or more painful, for example the CPT. Furthermore, some participants have different tolerance to pain such as athletes who are trained to immerse themselves into ice cold baths and thus be more acclimatized to the test as opposed to participants who are not trained for these

conditions. Other reasons include not controlling for menstrual cycle, OCs and family history of hypertension. Participants with a family history of hypertension have been found to possess a heightened cardiovascular response stressor tasks (Menkes at al. 1989).

6.3 STRESSOR RESPONSES AND THE MENSTRUAL CYCLE

6.3.1. Lower resting MSNA during low hormone phase

In ***Study 3***, MSNA burst frequency was significantly lower in the LH phase compared with the HH phase of the menstrual cycle. This supports previous research by Minson et al. (2000a), who reported that in healthy eumenorrheic women, MSNA and plasma NE are lower during the LH phase compared with the HH phase. The LH phase is associated with lower resting MSNA due to low concentrations of progesterone, which is believed to be sympathoexcitatory (Carter et al. 2013). In contrast to progesterone, oestrogen has inhibitory effects on MSNA. During the HH phase both female sex hormones are elevated (Carter et al. 2013). The high MSNA during this phase of the menstrual cycle suggests that the excitatory effects of progesterone dominate over the inhibitory effects of oestrogen.

Other studies have shown no differences in resting MSNA (Ettinger et al. 1998; Carter & Lawrence, 2007) and plasma NE concentration (Mills et al. 1996) between the LH and HH phases of the menstrual cycle. Subsequent studies also support (Middlekauff et al. 2012; Park & Middlekauff, 2008) and refute (Carter et al. 2009;

Fu et al. 2009; Lawrence et al. 2010; Lawrence et al. 2008) the concept that resting MSNA is elevated during the HH phase. Lower resting MSNA levels during the LH phase may explain the greater increases in MSNA burst frequency during the CPT during this phase of the menstrual cycle.

6.3.2. Mental stress during the high and low hormone phases of the menstrual cycle

It was hypothesised that both phases of the menstrual cycle would be associated with similar sympathetic responsiveness during mental stress. A study by Carter and Lawence (2007) found that that mental stress (such as mental arithmetic) increases MSNA, MAP and HR similarly during the LH and HH phases of the menstrual cycle. The present results suggest significant increases in SBP, DBP, MAP and HR for both mental stressors during both menstrual phases which is supported by the study by Carter and Lawence (2007), but no significant changes in MSNA during the mental tasks. This may be due to the group containing a mixture of positive, negative and non-responders. The data also suggest that the direction of change in MSNA is not consistent within subjects across the menstrual cycle. ***Study 3*** has shown that during the mental tasks considerable inter-individual variability exists, especially with regards to sympathetic and BP responses. Evidence suggests that MSNA reactivity to mental stress demonstrates high inter-individual variability (Carter & Ray, 2009; Carter & Goldstein, 2015) although a recent study indicates that individual responses are repeatable (Fonkoue & Carter 2015). Fonkoue and Carter (2015) have shown that MSNA reactivity to mental stress is consistent within a single laboratory visit and across laboratory sessions conducted on separate days during the LH phase of the

menstrual cycle. As the current results illustrate, the timing of HR and BP peaks are not aligned, suggesting that HR is not a major factor in driving the BP response. Also, the changes in BP were not aligned with the changes in MSNA, suggesting that the relationship between BP and MSNA during mental stress is more complex than it is at rest.

Study 3 was sufficiently powered to detect differences in MSNA burst frequency and BP responses to the cold pressor between the low and high hormone phases of the menstrual cycle. However, variability in MSNA and BP responses was greater during the other stressor tasks, particularly the mental stressors. Retrospective sample size calculations reveal that in order to detect similar differences in MSNA burst frequency (≥4 bursts/min) between menstrual cycle phases, 38 participants would have been required for the Stroop test and 57 would have been required for the mental arithmetic task. This is based on standard deviations of the difference of 8.5 and 10.5, respectively. As it was, the study was powered to detect differences in MSNA burst frequency of 8.4 bursts/min for the Stroop test and 31 bursts/min for mental arithmetic. The study was therefore not sufficiently powered given the large variability in MSNA responses to mental stress. However, it was sufficiently powered to detect meaningful differences between menstrual phases in MSNA during the CPT. Furthermore, based on standard deviations of the difference in SBP of 4.7 mmHg it was sufficiently powered to detect differences of ≥5 mmHg for the Stroop test. To detect similar differences for mental arithmetic, 11 participants would have been required. Whilst we were able to identify significant effects of menstrual cycle phase on cardiovascular and sympathetic responses to the CPT, considerably more

participants will be required in future research to explore the effects during mental stress.

6.3.3. Physical stress during the high and low hormone phases of the menstrual cycle

It was hypothesized that the lower resting MSNA in the LH phase would provide greater capacity for an increase in sympathetic activity during the physical tasks. This was indeed seen in the results, as MSNA during the CPT increased significantly more in the LH phase, compared with the HH phase. Whilst these findings oppose the negative findings of Middlekauff et al. (2012) and Jarvis et al. (2011), other studies involving different physical tasks have produced similar results. For example, Usselman et al. (2014) have shown that during chemoreflex activation (end-expiratory apnoea) increases in MSNA are larger in the LH phase than HH phase. The authors also found that baseline MSNA burst frequency, burst incidence, and total MSNA were greater in the HH than the LH phase of the menstrual cycle.

In ***Study 3*** it was reported that the static handgrip task was associated with no significant differences in the magnitude of the increase in BP or MSNA between the LH and HH phases of the menstrual cycle. These results are consistent with those of Jarvis et al. (2011) in which MSNA was measured in 11 females during handgrip exercise at 40% MVC until fatigue. Although Ettinger et al. (1998) reported a significant effect of the menstrual cycle on MSNA responses during handgrip exercise, the phases used for comparison were the early follicular (low hormone) and late follicular (high oestrogen). The mid-luteal phase represents the phase when both

oestrogen and progesterone are high, and therefore the differences between studies suggest that the balance of oestrogen and progesterone may influence the responses to exercise. Future studies comparing the early follicular, late follicular and ML phases may help to distinguish the influences of elevated oestrogen and elevated progesterone.

6.4 LIMITATIONS

A general limitation (for ***Studies 1 - 3***) involves the exclusion criteria, as participants who may have a family history of hypertension were not controlled for. Studies have shown that individuals with a family history of hypertension show significant increases in MSNA whereas individuals with no family history of hypertension show no change in MSNA during mental stress (Noll et al. 1996; Fonkoue et al. 2016). Due to the small number of participants in the current cohort with a family history of hypertension (n=9) there was insufficient statistical power to examine the effects of family history on MSNA reactivity to mental stress.

For each study post-exercise ischaemia was used as a physical stressor. However, levels of pain experienced during this task were not recorded. The occlusion of blood flow following exercise can cause pain in some individuals, but potential correlations between BP reactivity to post-exercise ischemia and pain were not assessed. However, Mark et al. (1985) and Victor et al. (1987) report that pain during post-exercise ischemia is not a contributor to the elevations in MSNA and BP. According to Kregel et al. (1992), sympathoexcitation during the CPT occurs only when skin temperature falls to levels that produce a sensation of intense pain. However, some

individuals reported no pain during the CPT but experienced large increases in BP. In support of this, Nilsen et al. (2014) reported that pain has no effect on the elevations in BP during the CPT. Similarly, we found that pain during the CPT was not related to BP or MSNA reactivity. For some individuals it took several minutes to reach resting BP and MSNA values and for pain to subside following the CPT, and therefore in future studies it may be worth placing the CPT at the end of the experimental protocol.

A comparable number of males (n=21) and females (n=19) were involved in ***Study 2*** and the same females (n=10) for the LH and HH phases were involved in ***Study 3***. One limitation is that females who were taking OCs were not excluded, which may have affected the current results. Minson et al. (2000b) have shown that resting MSNA and sympathetic and cardiovagal BRS are greater in women taking exogenous estrogen and progestin than women who do not take OCs. Nevertheless, each of these female participants served as their own control in ***Study 3***, and OC use was not discontinued. In addition, during ***Study 3***, the female reproductive hormones were not measured to confirm differences between the two phases; rather the subject's knowledge of their menstrual cycles was used to know whether they were in the low (days 1-7) or high (days 19-23) hormone phases. Furthermore, the separate effects of progesterone and estrogen were not investigated by examining responses during the late follicular phase (high estrogen, low progesterone). However, Carter et al. (2013) have shown that changes in estradiol from the low to HH phase were negatively correlated with changes in resting MSNA. Those individuals with a greater surge in estradiol experienced smaller increases in MSNA. The changes in progesterone were not significantly correlated with MSNA, although there was a trend for a positive

correlation. These findings are consistent with the prevailing concept that oestrogen is sympathoinhibitory (He et al. 1998; Vongpatanasin et al. 2001), whereas progesterone is sympathoexcitatory (Minson et al. 2000). The measurement of sex hormones may help to explain some of the variability in MSNA between individuals, especially in response to mental stressors.

Another area that could have been investigated (in ***Studies 1-3***) is vascular transduction. Vasoconstrictor responses and changes in vascular resistance in response to changes in MSNA may have shed light on the differences between males and females and during the difference phases of the menstrual cycle. Yang et al. (2013), found that vascular transduction of MSNA into vascular tone is different between sexes during mental stress *i.e*. men are more sensitive to the vasoconstricting action of MSNA during mental stress. In addition, Lawrence et al. (2008) and Lawrence et al. (2010) found that vascular transduction is increased during the HH phase when compared with the LH phase of the menstrual cycle.

6.5 CONCLUSIONS AND IMPLICATIONS

This thesis, for the first time, demonstrates the time course of individual BP responses to mental stress and how this can influence changes in MSNA. The relationship between MSNA and BP is more complex during mental stress than during physical challenges such as exercise, ischaemia and the CPT, during which parallel increases in MSNA and BP are consistently observed across all three studies. The current findings suggest that healthy young individuals may experience increases or decreases in MSNA during mental stress despite consistent elevations in BP. The data (from

Studies 1 and 2) indicate that in healthy males and females the rate of the rise in DBP at the onset of mental stress influences the direction of the change in MSNA. Negative responders to mental stress exhibit more rapid increases in DBP at the onset of the stressor, leading to reductions in MSNA that are likely due to baroreflex suppression of nerve activity. In positive responders the rise in BP during mental stress is sluggish and appears to be MSNA-driven. These findings suggest that whether MSNA has a role in the pressor response is dependent upon the reactivity of BP early in the task. The findings from ***Study 3*** indicate that the menstrual cycle can also influence the changes in MSNA during stress. It was found that the CPT elicited significantly larger increases in MSNA during the LH phase than the HH phase.

6.6 FUTURE WORK

Future work should include measuring pain scores during post-exercise ischaemia (discussed in section 6.4), control of cortisol levels, SV and BRS during mental and physical stressors. Another important measure, which should be considered, is the test for plasma catecholamines. It measures the amount of the hormones epinephrine, NE, and dopamine in the blood. For example, in a study by Menkes at el. (1989) reported that the mental arithmetic test elicited higher levels of plasma epinephrine than did the CPT whereas the CPT elicited higher levels of plasma norepinephrine. In addition, when studying sex differences, controlling for OCs is required and while examining the effects of the menstrual cycle it is required to test within-participant repeatability during different phases of the menstrual cycle. Furthermore, increasing the sample size in future studies would allow further examination of MSNA and BP responses to

mental stress so that positive, negative and non-responder classifications may be investigated. This may provide further insight into understanding of the effects of the rate of change in BP on MSNA during mental stress. Vascular transduction is another aspect that needs to be addressed during mental stress, in order to gain insight into the role of mental stress in the development of hypertension. Furthermore, another aspect that warrants further investigation is how the timing of HR and BP peaks are not aligned. This will help us understand what drives BP during mental stress.

While all participants were young, a factor worth addressing in the future is ageing; knowledge of the relationship between MSNA and BP as we age is important for understanding long-term BP regulation and reducing the risk of cardiovascular events. As men and women age a positive relationship between MSNA and BP becomes apparent: older men and women with higher MSNA have higher BP (Hart et al. 2011). This correlation between blood pressure and MSNA was found to be stronger in older females than in older males. Okada et al. (2012) have shown that sympathetic activity increases with age, whereas the increment is greater in women, especially after menopause, compared with men. Whilst the prevalence of hypertension increases with age in both sexes, postmenopausal women are at the greatest risk (Vianna et al. 2012). The application of the methods used in ***Study 1*** may provide useful insights into muscle sympathetic and cardiovascular responses to stress in this population.

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Appendix

Relationship between spontaneous sympathetic baroreflex sensitivity and cardiac baroreflex sensitivity in healthy young individuals.

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RELATIONSHIP BETWEEN SPONTANEOUS SYMPATHETIC BAROREFLEX SENSITIVITY AND CARDIAC BAROREFLEX SENSITIVITY IN HEALTHY YOUNG INDIVIDUALS

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Abstract

Low baroreflex sensitivity (BRS) is associated with elevated cardiovascular risk. However, the evidence is based primarily on measurements of cardiac BRS. It cannot be assumed that cardiac or sympathetic BRS alone represent a true reflection of baroreflex control of blood pressure. The aim of this study was to examine the relationship between spontaneous sympathetic and cardiac BRS in healthy, young individuals. Continuous measurements of blood pressure, heart rate and muscle sympathetic nerve activity (MSNA) were made under resting conditions in 50 healthy individuals (18-28yrs). Sympathetic BRS was quantified by plotting MSNA burst incidence against diastolic pressure (sympathetic BRS_{inc}), and by plotting total MSNA against diastolic pressure (sympathetic BRS_{total}). Cardiac BRS was quantified by plotting R-R interval against systolic pressure using the sequence method. Significant sympathetic BRS_{inc} and cardiac BRS slopes were obtained for 42 participants. A significant positive correlation was found between sympathetic BRS_{inc} and cardiac BRS (r=0.31, *P*=0.049). Amongst this group, significant sympathetic baroreflex slopes were obtained for 39 participants when plotting total MSNA against diastolic pressure. In this subset, a significant positive correlation was observed between sympathetic BRS_{total} and cardiac BRS (r=0.40, *P*=0.012). When males and females were assessed separately, these modest relationships only remained significant in females. Analysis by gender revealed correlations in the females between sympathetic BRS_{inc} and cardiac BRS (r=0.49, P=0.062), and between sympathetic BRS_{total} and cardiac BRS (r=0.57, *P*=0.025). These findings suggest that gender interactions exist in baroreflex control of blood pressure, and that cardiac BRS is not appropriate for estimating overall baroreflex function in healthy, young populations. This relationship warrants investigation in ageing and clinical populations.

Key words: blood pressure, heart rate, muscle sympathetic nerve activity, sequence method

Introduction

The baroreflex acts to regulate blood pressure, primarily through the modulation of heart rate and sympathetic outflow to the vasculature. The two arms of the baroreflex, cardiac and sympathetic, share the same afferent pathway, in which baroreceptors in the carotid sinuses and aortic arch detect pressure-driven increases in radial distension. Baroreceptor afferents project via the glossopharyngeal and vagus nerves to the nucleus tractus solitarius (NTS) within the medulla, from which excitatory projections synapse within the caudal ventrolateral medulla (CVLM), nucleus ambiguous (NA) and the dorsal motor nucleus of the vagus (DMX) (1). The excitatory sign of the baroreceptor afferents is reversed at the level of the rostral ventrolateral medulla (RVLM), the primary output nucleus for muscle sympathetic nerve activity (MSNA) (4, 24), to which inhibitory projections from the CVLM project and lead to withdrawal of sympathetic outflow to the muscle vascular bed. Reversal of the sign of the baroreceptor afferent input also occurs at the level of the sinoatrial node, via the release of the acetylcholine from terminals of the cardiac vagus nerve (2). As such, the baroreflex plays a critically important role in regulating blood pressure constant, though its sensitivity can be adjusted to suit current physiological needs. The sensitivity of this negative feedback loop system can be assessed by quantifying the relationship between systolic blood pressure and heart rate (or R-R interval) (16, 30), and between diastolic blood pressure and MSNA (19, 31).

Low baroreflex sensitivity (BRS) is associated with elevated cardiovascular risk (3, 8, 17, 22, 23). However, the evidence is based primarily on measurements of cardiac BRS alone. It cannot be assumed that cardiac or sympathetic BRS on their own represent a true reflection of baroreflex control of blood pressure. Ageing has been associated with a fall in cardiac BRS due to arterial stiffness and therefore a reduced capacity for the baroreceptors to encode changes in arterial pressure (26). In theory, arterial stiffness with ageing ought to affect sympathetic BRS for the same reasons. However, Studinger et al. (31) reported that sympathetic BRS is maintained in older individuals due to a compensatory increase in the 'neural component' of the baroreflex response. While it is not clear which part of the neural response the increase can be attributed to (afferent, central and/or efferent), the finding does suggest that poor baroreflex control of heart rate does not necessarily imply poor baroreflex control of MSNA.

To our knowledge, there have been only two studies to date in which cardiac and sympathetic BRS have been directly compared. Both Rudas et al. (29) and Dutoit et al. (6) reported that there is no correlation between the two in healthy individuals. The authors of these two studies employed the modified Oxford method for the assessment of cardiac and sympathetic BRS. Although Rudas et al. (29) also included spontaneous methods of assessing BRS, significant sympathetic baroreflex slopes were reported in only five of the 18 participants, thus limiting the capacity to examine the relationship with spontaneous cardiac BRS. The modified Oxford method is a pharmacological technique, which involves administering bolus injections of sodium nitroprusside and phenylephrine in order to cause blood pressure to fall and subsequently rise. The beat-to-beat heart rate and MSNA responses to this active

perturbation of blood pressure allow BRS to be quantified. The modified Oxford method is typically referred to as the gold standard approach for the assessment of cardiac BRS because it allows baroreflex responses to be quantified during rapid changes in arterial pressure (5). However, this approach can have limitations when applied to sympathetic BRS testing because of fewer data points available to produce a baroreflex slope, particularly in response to rising pressures. In contrast to the assessment of cardiac BRS, where each cardiac cycle is associated with an R-R interval, the assessment of sympathetic BRS relies upon the occurrence of bursts of MSNA, which do not occur with every cardiac cycle and which vary in their incidence across individuals. This issue is particularly troublesome at higher pressures when there is significant inhibition of sympathetic bursts (6). The alternative is to use spontaneous techniques, and these are frequently used for the assessment of sympathetic BRS (12, 14, 18, 19). Spontaneous fluctuations in diastolic pressure and MSNA at rest are used to quantify sympathetic BRS using a significantly larger number of cardiac cycles with which to construct the baroreflex slope. When examining the relationship between cardiac and sympathetic BRS, it is logical that the same type of approach be used to assess the two arms of the baroreflex. To the best of our knowledge there has yet to be a study in which this relationship has been investigated with the use of spontaneous baroreflex techniques.

In the current study we have used two methods to assess spontaneous sympathetic BRS; one approach involves the use of MSNA burst incidence and the other total MSNA. Previous research indicates that plotting MSNA burst incidence against diastolic pressure results in a greater number of significant baroreflex slopes, compared with plotting MSNA burst amplitude or area against diastolic pressure (19).

However, using total MSNA allows both MSNA burst amplitude and MSNA burst incidence to be taken into account in the assessment of BRS (18). Therefore, we will use the total MSNA method as well as the more common MSNA burst incidence method to examine the relationship with cardiac BRS. It is hypothesized that spontaneous sympathetic BRS is correlated with spontaneous cardiac BRS in healthy, young individuals.

Methods

Participants

Fifty healthy young males (n=31) and females (n=19) aged 18-28 years were recruited for the study. Exclusion criteria included diagnosed cardiovascular, respiratory or endocrine disease and those who smoked or took regular medication. Participants were instructed to abstain from alcohol or vigorous exercise 24 hours prior and to not consume any caffeine on the day of the experiment. All experiments took place in the morning, beginning between 0800 and 0900 h, as we have previously demonstrated that diurnal variation exists in cardiac BRS (32). The changes in hormone levels during the menstrual cycle have been shown to affect MSNA and sympathetic BRS (25). Accordingly, females were tested in the low hormone (early follicular) phase of their menstrual cycle to minimize the effects of sex hormones on BRS. Written informed consent was obtained from all participants prior to conducting the experiment, who were reminded that they could withdraw at any time. The study was conducted with the approval of the Human Research Ethics committee, University of Western Sydney, and satisfied the Declaration of Helsinki.

Measurements and experimental protocol

Participants were studied in an upright-seated position in a comfortable chair, with the legs supported in the extended position. Continuous MSNA recordings were made from muscle fascicles of the common peroneal nerve through tungsten microelectrodes (FHC, Bowdoinham, ME, USA) inserted percutaneously at the level of the fibular head. Multi-unit neural activity was amplified (gain 20,000, bandpass 0.3–5.0 kHz) using an isolated amplifier (Neuroamp EX, ADInstruments, Sydney, Australia) and stored on computer (10 kHz sampling rate) using a computer-based data acquisition and analysis system (Powerlab 16SP hardware and LabChart 7 software; ADInstruments, Sydney, Australia). A root-mean-square (RMS) processed version of this signal was computed, with a moving average of 200 ms. Blood pressure was recorded non-invasively via a finger cuff (Finometer; Finapres Medical System, Amsterdam, the Netherlands). Heart rate was recorded via electrocardiogram (0.3-1.0kHz, Ag-AgCl surface electrodes, sampled at 2kHz). Respiration was measured via a strain-gauge transducer (Pneumotrace, UFI, Morro Bay CA, USA) wrapped around the chest. A minimum of 10 minutes of resting data was recorded in order to examine spontaneous fluctuations in blood pressure and the corresponding changes in R-R interval and MSNA (Fig 1). Participants were asked to breathe normally throughout.

Data analysis

Beat-to-beat values were extracted from LabChart (ADInstruments, Sydney, Australia) for systolic blood pressure, diastolic blood pressure, R-R interval, and MSNA. A custom-written program, developed in LabView software (National Instruments, USA), was used to detect and measure the area of individual bursts of

MSNA. The numbers of bursts per minute (MSNA burst frequency) and per 100 heartbeats (MSNA burst incidence) were determined for each individual. Total integrated MSNA was determined using a segregated signal averaging approach described by Halliwill (10), whereby the largest MSNA burst during the 10-min rest period was assigned a value of 1000 and a prolonged section without bursts was assigned a value of zero. The remaining MSNA bursts were calibrated against this to allow measures of MSNA to be normalized to individual resting values. The measurement of total MSNA allows both MSNA burst incidence and MSNA burst amplitude to be taken into account when quantifying MSNA for a given diastolic pressure bin.

Sympathetic baroreflex sensitivity: burst incidence method

Sympathetic BRS was quantified using methods previously described by Kienbaum et al. (19). For all methods of assessing sympathetic BRS, the nerve trace was shifted to account for the sympathetic baroreflex conduction delay, and this was adjusted for each participant to account for inter-individual differences in burst latency. The average shift applied was 1.28 ± 0.01s, relative to the R-wave to which the sympathetic burst was aligned. For each participant, the diastolic pressure values for each cardiac cycle throughout the 10-min rest period were assigned to 3 mmHg bins to reduce the influence of respiratory-related oscillations (7, 35). For each bin the corresponding MSNA burst incidence (number of bursts per 100 cardiac cycles) was determined. Sympathetic BRS was quantified by plotting MSNA burst incidence against the mean diastolic blood pressure for each bin. Each data point was weighted according to the number of cardiac cycles because the bins at the highest and lowest diastolic pressures contain fewer cardiac cycles (19). Baroreflex slopes were

determined using linear regression with the acceptance level set at r >0.5 (14). The value of the slope provided the sympathetic BRS for the individual, which will be referred to as 'sympathetic BRS_{inc}' in order to differentiate from other methods of determining sympathetic BRS.

Sympathetic baroreflex sensitivity: total MSNA method

The relationship between diastolic blood pressure and total MSNA was assessed using 3 mmHg bins. Since all cardiac cycles are incorporated in this analysis, including those not associated with MSNA bursts, this measure of total MSNA takes into account both MSNA burst incidence and MSNA burst amplitude. Fig. 2A. illustrates the mean MSNA burst amplitudes for each diastolic pressure bin for one individual. The lowest diastolic pressure bins are associated with the largest MSNA bursts, with the average burst amplitude becoming progressively smaller with high diastolic pressures. Total integrated MSNA was determined for each bin using segregated signal averaging approach (10) and expressed as arbitrary units (AU) per beat. Linear regression was used to determine the relationship between total MSNA and diastolic blood pressure with the application of the weighting procedure described above and an acceptance level of r >0.5. Fig. 2B. illustrates the baroreflex slope for one individual. These baroreflex values will be referred to as 'sympathetic BRS_{total}' in order to differentiate from the MSNA burst incidence method for assessing sympathetic BRS.

Cardiac baroreflex sensitivity: sequence method

Cardiac BRS was assessed using the sequence method in which 'up' and 'down' sequences are identified. Up sequences consist of three or more consecutive cardiac

cycles for which there is a sequential rise in both systolic pressure and R-R interval. Down sequences consist of three of more cardiac cycles for which there is a sequential fall in systolic pressure and R-R interval. The threshold for changes in systolic BP was set at 1 mmHg and the threshold for changes in R-R interval was set at 6 ms (27). Sequences containing changes smaller than these thresholds were not used in the assessment of cardiac BRS. Baroreflex sensitivity was quantified by plotting R-R interval against systolic pressure for each sequence ($r \geq 0.8$ acceptance level) and taking the average slope value for up and down sequences combined. Values of cardiac BRS were accepted when the number of sequences was ≥3 for both up and down sequences.

Statistical analysis

Linear regression analysis was used to examine the relationships between sympathetic BRS and cardiac BRS. Subgroup analyses were performed to assess these relationships for males and females separately. All statistical analyses were performed using SPSS v22. For all statistical tests, a probability level of $P<0.05$ was regarded as significant. Values are presented as mean ± SE.

Results

Participants

Recordings of MSNA were successfully obtained in all 50 participants. Sympathetic baroreflex slopes ($r >0.5$) were successfully obtained for 48 participants using the burst incidence method. For six participants the number of cardiac BRS sequences was <3 for up and/or down sequences, and thus data for these participants were removed from the analysis, leaving a total of 42 (27 males). For these participants, the

mean number of cardiac BRS sequences was 29 ± 3. Resting cardiovascular and sympathetic variables for these 42 participants are presented in Table 1. There were no significant differences between males and females except for resting MSNA, which was significantly higher in males when expressed as both MSNA burst frequency and MSNA burst incidence (P<0.01). Mean body mass index (BMI) was above 25 kg/m^2, and thus in the overweight category. However, fat free mass was 67.4 ± 9.1 kg for males and 49.8 ± 5.8 kg for females. These values are above average for healthy, young individuals (21), which can be explained by the physical activity levels of the sample. Subjects exercised regularly (≥ 2 x per week), with several partaking in resistance exercise. Spontaneous cardiac BRS and sympathetic BRS values are presented in Table 2. There were no significant differences between males and females (P>0.05).

Relationship between sympathetic and cardiac baroreflex sensitivity

A significant positive correlation was found between cardiac BRS and sympathetic BRS_{inc} (r=0.31, P=0.049; Fig 3A). In 39 participants (24 males) significant sympathetic baroreflex slopes were obtained when using the total MSNA method. In this subset, correlations were observed between sympathetic BRS_{total} and cardiac BRS (r=0.40, P=0.012; Fig 3B).

Gender interactions

When the relationship between cardiac BRS and sympathetic BRS_{inc} was assessed separately for males and females, there was no significant correlation for males (r=0.11, P=0.585; Fig 4A). Conversely, for females there was a positive relationship between cardiac BRS and sympathetic BRS_{inc} (r=0.49; Fig 4B), although this failed to

reach statistical significance (P=0.062). In the subset of 39 participants who exhibited significant sympathetic baroreflex slopes, calculated from total MSNA, there was a significant correlation between sympathetic BRS_{total} and cardiac BRS for females (r=0.57, P=0.025; Fig 2D) but not males (r=0.20, P=0.345; Fig 2C).

Discussion

We have examined, for the first time, the relationship between spontaneous cardiac and sympathetic baroreflex sensitivity. Whilst the initial results indicate a relationship between cardiac and sympathetic BRS in young individuals, when assessed according to gender this modest relationship is evident only in females. Assessment of sympathetic BRS using both MSNA burst incidence and total MSNA yielded similar results. The findings suggest that cardiac BRS may only predict a small portion of the variance in sympathetic BRS in this group. This study indicates that gender interactions exist in baroreflex control of blood pressure, and that cardiac BRS is not appropriate for estimating overall baroreflex function in healthy, young populations.

Relationship between cardiac and sympathetic baroreflex sensitivities

The cardiac and sympathetic baroreflexes share the same afferent pathway. It therefore seems logical that an individual would be effective in regulating blood pressure with both arms of the baroreflex, or be less effective with both. The current study findings suggest that in young females there is a relationship between cardiac and sympathetic BRS, and this could be attributed to the common afferent pathway. However, a considerable portion of the variance in BRS remains unexplained, and thus it appears there are other factors influencing the central integration of the baroreceptor input and the efferent pathways that lead to differences in cardiac and

sympathetic BRS within individuals. It is also unclear as to why no relationship appears to exist between the two arms of the baroreflex in young males. The ability to regulate BP through the modulation of heart rate and MSNA appear to be quite separate, and the hypothesis that high cardiac BRS is indicative of high sympathetic BRS is therefore rejected.

Gender Interactions

Dutoit et al. (6) reported no direct relationship between cardiac and sympathetic BRS in young individuals when both genders were investigated as one group. It is possible that the methods used may explain the discrepancy with the current findings; in the study by Dutoit et al. (6) participants lay in the supine position, whereas in the current study participants were in an upright-seated position. We have previously shown that posture significantly affects cardiac BRS (33). Furthermore, resting MSNA is lower in the supine position (28), which may reduce the number of MSNA bursts with which to produce a sympathetic baroreflex slope. Despite this, in both studies, gender-based differences were apparent when separate analyses were performed for males and females. Consistent with the findings of Dutoit et al. (6), the current study indicates that there is a positive relationship between cardiac BRS and sympathetic BRS in young females; a relationship that was not found in young males in either study.

There is evidence to suggest that cardiovascular control, particular at the level of the vasculature, differs between young males and females. Hart et al., (11) reported that MSNA is correlated with total peripheral resistance in males but not females. Later, the same group demonstrated that β-adrenergic vasodilation blunts sympathetic

vasoconstriction in young females (13), thus providing an explanation for the lack of correlation between MSNA and total peripheral resistance. The authors reported that this mechanism was not apparent in young men or postmenopausal women. This infers that in young females with a high sympathetic BRS, baroreflex control of blood pressure via MSNA may not necessarily be more effective. An increase in sympathetic outflow to the vasculature is more likely to be counteracted by local vasodilator mechanisms than it would in their male counterparts.

Vascular transduction is a key step in the baroreflex response that is not taken into account with conventional methods of assessing sympathetic BRS. The inclusion of ultrasound measurements of vessel diameter and blood flow to determine the direct effects of MSNA on peripheral resistance (i.e. end-organ responsiveness), may help to explain gender-based differences in the relationship between cardiac and sympathetic BRS. In the current study, young males had significantly higher levels of resting MSNA than young females, as has been reported previously (15). This may highlight the reliance on local vasodilator mechanisms in females for adjusting vascular tone under resting conditions.

Whilst the mechanisms surrounding gender differences remain somewhat speculative, the current findings suggest that potential gender interactions ought not to be ignored when investigating blood pressure regulation.

Methodological Considerations

The purpose of the current study was to use spontaneous techniques to assess the relationship between cardiac and sympathetic BRS. Spontaneous techniques specifically target the regulation of blood pressure under normal resting conditions. In contrast, the modified Oxford method involves bolus injections of sodium

nitroprusside followed 60 s later by phenylephrine, generally producing a fall in arterial pressure of ~15 mmHg and a subsequent rise of ~15 mmHg above baseline. The modified Oxford method therefore offers more rapid changes in blood pressure typically over a wider range. This approach has been questioned on the basis of direct effects on the vessels (20). As we have discussed previously in detail, there are distinct advantages and disadvantages to both methods with the potential for confounding factors with either approach (34). Whilst the modified Oxford method is considered the gold standard approach for assessing cardiac BRS, it has some disadvantages for the assessment of sympathetic BRS. The process of quantifying sympathetic BRS relies upon the occurrence of MSNA bursts, which do not occur with every cardiac cycle. This severely limits the number of data points with which to plot a baroreflex slope. During the rise in pressure following the bolus injection of phenylephrine MSNA bursts can be inhibited altogether, which means that values of sympathetic BRS will often be determined mostly from the fall in pressure, following the sodium nitroprusside bolus (6). The use of spontaneous techniques in the current study allows these issues to be overcome as well as an opportunity to investigate the findings of Dutoit et al. (6) using alternative approaches. Although the capacity of spontaneous baroreflex techniques to eliminate non-baroreflex stimuli has been questioned, it is suggested that they hold predictive power (5), thus providing useful information about baroreflex function as an alternative to methods where blood pressure changes are driven externally.

Interestingly, out of the six participants whose data were excluded due to a lack of cardiac BRS sequences, five had below-average values for sympathetic BRS_{inc} and all six had below average values for sympathetic BRS_{total}. The lack of cardiac baroreflex

sequences itself may be interpreted as a sign of poor cardiac BRS and, consistent with the current findings, these individuals also exhibited low sympathetic BRS. Alternatively, the failure to obtain significant cardiac and sympathetic baroreflex slopes may be due to the existence of a non-linear relationship between blood pressure and RR interval or MSNA. Whilst the example in Fig 2 illustrates a significant relationship between diastolic pressure and MSNA, the bin representing the lowest diastolic pressure does not follow the linear trend, with MSNA bursts being much larger than those in the higher pressure bins. The process of removing data sets due to a lack of significant baroreflex slopes is common practice and, to our knowledge, has not been questioned. Eliminating the results entirely from the investigation, based on insignificant linear regression slopes, could mean that useful information about blood pressure regulation in those individuals is ignored. Alternative methods for dealing with non-linear relationships may be an important analytical problem worth investigating in baroreflex research. Previous studies indicate that MSNA burst incidence is closely related to diastolic BP, and is therefore more successful than MSNA burst area (12, 19). The total MSNA method for quantifying sympathetic BRS has been associated with both low (12) and high success rates (18). In the current study, the total MSNA method was only marginally less successful (39 successful baroreflex slopes) than the MSNA burst incidence method (42 successful baroreflex slopes). The sympathetic BRS $_{total}$ method incorporates both MSNA burst amplitude, unlike the burst incidence method, and therefore it could be argued that it provides a better overall indication of sympathetic BRS than using MSNA burst incidence alone. Furthermore, Fairfax et al. (9) recently demonstrated that MSNA burst amplitude has more influence than MSNA burst frequency on vascular conductance. In other words, clusters of bursts with higher

amplitudes lead to greater reductions in blood vessel diameter than clusters of smaller but more numerous bursts (when total MSNA remains the same). Given its influence on the vasculature it therefore seems logical to incorporate MSNA burst amplitude in the quantification of sympathetic BRS.

Conclusions

In healthy, young females there is a correlation between cardiac and sympathetic baroreflex sensitivity. In this group, cardiac BRS may predict a small portion of the variance in baroreflex modulation of MSNA burst incidence and total MSNA. In contrast, this relationship appears not to be present in young males. We therefore conclude that cardiac BRS is not appropriate for estimating overall baroreflex function in healthy, young individuals. This relationship warrants further investigation, particularly in clinical and ageing populations.

Disclosures

The authors declare no conflict of interest

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Table 1. Resting cardiovascular and sympathetic variables

Variable	All participants (n=42)	Males (n=27)	Females (n=15)	*P*
Age (yrs)	22 ± 0.5	22 ± 0.4	23 ± 0.9	0.30
BMI (kg/m^2)	25.6 ± 0.8	25.1 ± 0.6	26.6 ± 2.0	0.48
Systolic BP (mmHg)	121 ± 4	121 ± 4	121 ± 7	1.0
Diastolic BP (mmHg)	76 ± 2	76 ± 3	77 ± 4	0.74
Heart rate (beats/min)	69 ± 1	67 ± 2	71 ± 3	0.13
MSNA burst frequency (bursts/min)	37 ± 2	40 ± 2	33 ± 2	0.009*
MSNA burst incidence (bursts/100heartbeats)	55 ± 2	60 ± 2	45 ± 4	0.002*

BMI indicates body mass index; BP indicates blood pressure; MSNA indicates muscle sympathetic nerve activity

*Significant difference between males and females ($p<0.05$).

Table 2. Cardiac and sympathetic baroreflex sensitivities

Baroreflex sensitivity	All participants (n=42)	Males (n=27)	Females (n=15)	*P*
Cardiac BRS (ms/mmHg)	14.6 ± 0.9	14.0 ± 1.0	15.7 ± 1.7	0.33
Sympathetic BRS_{inc} (bursts/100heartbeats/mmHg)	-1.94 ± 0.21	-1.70 ± 0.24	-2.38 ± 0.38	0.12
Sympathetic BRS_{total} (AU/beat/mmHg)	-2.45 ± 0.22*	-2.32 ± 0.26*	-2.65 ± 0.39	0.47

BRS indicates baroreflex sensitivity; AU indicates arbitrary units

*Sample size is 39 (all), 24 (males)

Figure legends

Figure 1. Experimental records from a 22-year old male at rest. The nerve signal has been shifted by 1.2 s to account for sympathetic baroreflex conduction delay. The baroreflex drives a shortening of RR intervals (increase in heart rate) and increase in MSNA burst incidence in response to falling systolic and diastolic pressures (A). A lengthening of R-R intervals (decrease in heart rate) and inhibition of MSNA bursts occurs in response to rising systolic and diastolic pressures (B). MSNA burst incidence increases in response to falling diastolic pressures despite maintained systolic pressure (C), demonstrating that MSNA is mostly strongly related to diastolic pressure.

Figure 2. Sympathetic baroreflex assessment in a 21-year old male using the segregated signal averaging approach. MSNA bursts are normalised to the burst with the largest amplitude and entered into diastolic pressure bins of 3 mmHg (A). Total MSNA per beat is determined for each bin and plotted against diastolic pressure (B).

Figure 3. Relationship between cardiac and sympathetic baroreflex sensitivities (BRS) when using the MSNA burst incidence method (A) and the total MSNA method (B) for assessing sympathetic BRS for all male and female participants

Figure 4. Relationship between cardiac and sympathetic baroreflex sensitivities (BRS) in males (A, C) and females (B, D) when using the MSNA burst incidence method and the total MSNA method for assessing sympathetic BRS

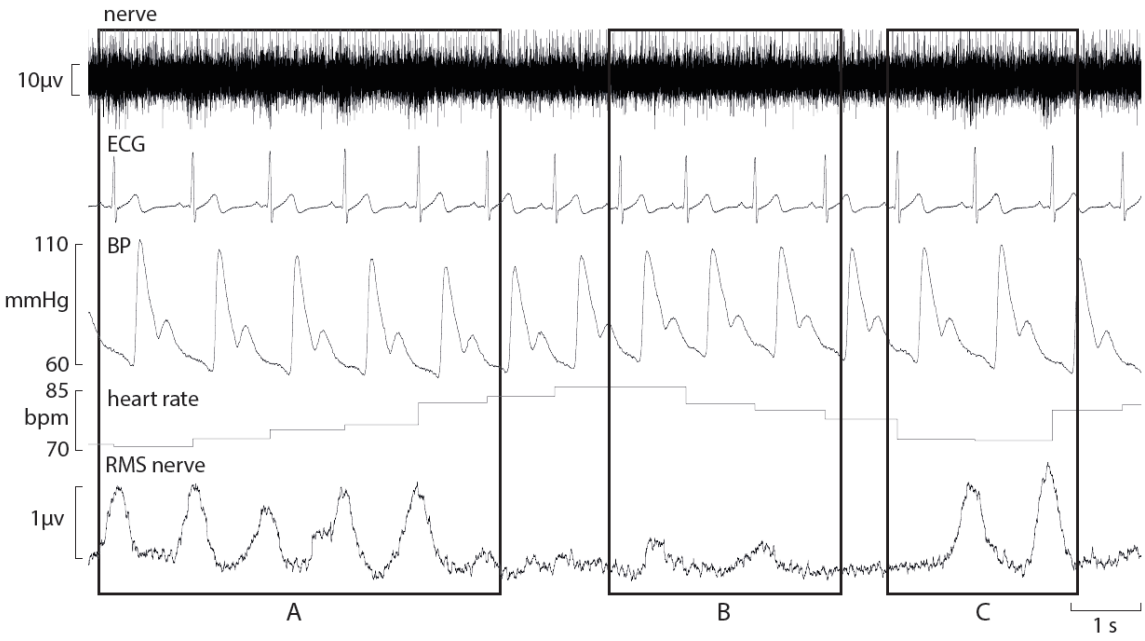

Figure 1

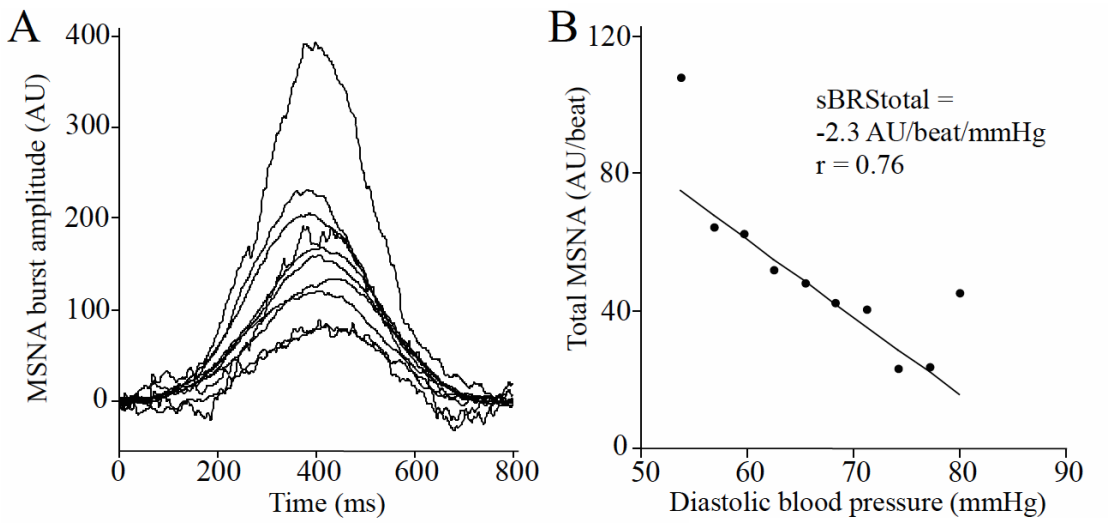

Figure 2

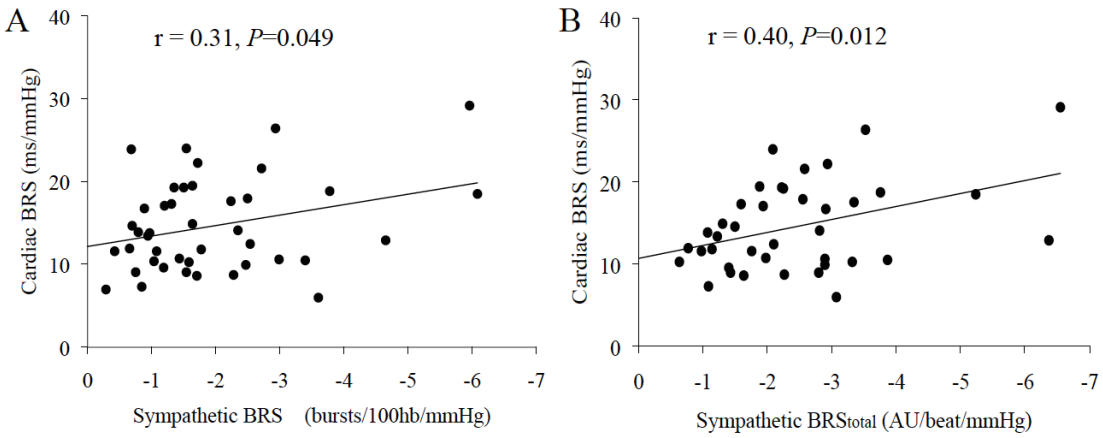

Figure 3

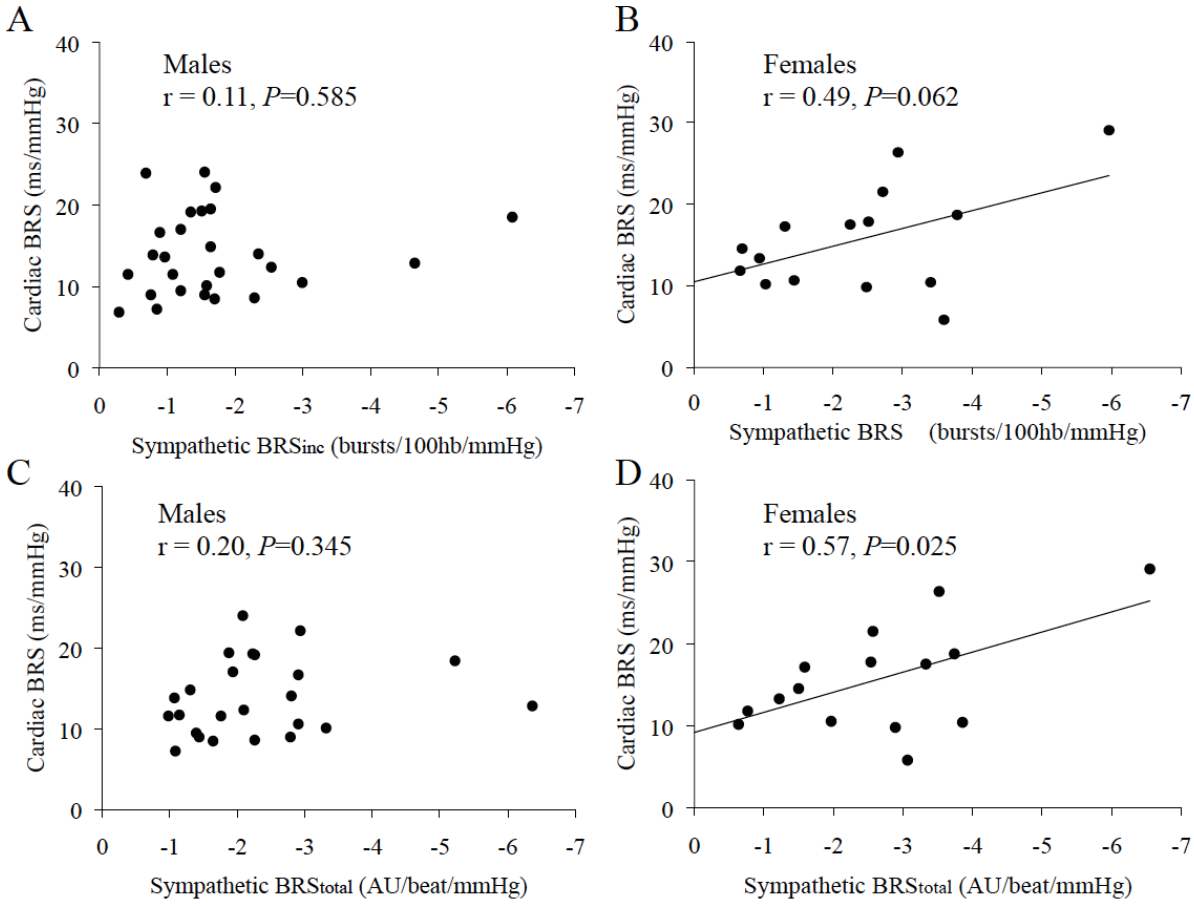

Figure 4