Cardiac cycle-related modulation of electrocutaneous pain and tactile stimuli

by

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ABSTRACT

Research suggests hypertension is associated with reduced somatosensory perception. Further, natural fluctuations in blood pressure (BP) across the cardiac cycle have been shown to modulate nociceptive responding, pain and tactile sensitivity, suggesting that arterial baroreceptors may be important moderators of somatosensation. This thesis further examined the influence of natural fluctuations in BP, and thus baroreceptor activity, across the cardiac cycle on electrocutaneous pain and tactile sensory thresholds and pain-related evoked potentials (PREPs) in normotensive individuals. Study 1 found pain thresholds were higher, i.e. pain was reduced, during systole compared to diastole. Further analysis revealed only participants with low-normal systolic BP displayed this cardiac cycle modulation, suggesting tonic BP may moderate cardiac cycle-related pain modulation. In the second study, tactile sensory thresholds did not vary across the cardiac cycle. However, when participants were split into high-normal and low-normal BP groups, interactions between BP and tactile sensory thresholds across the cardiac cycle were revealed. This finding suggests tonic BP may be an important factor determining the cardiac cycle modulation of tactile sensation. Study 3 found no variation in the N2 or P2 peak amplitudes, or N2-P2 peak-to-peak amplitudes across the cardiac cycle at scalp recording sites Cz, C3, or C4. Furthermore, BP median split analyses revealed no BP Group or interaction effect. As previous work reported a systolic dampening of PREPs, these data suggest the cardiac cycle-related modulation of PREPs may not be as robust as other measures of pain such as the nociceptive flexion reflex. Study 4 reported, in line with Study 3, no cardiac cycle related modulation of PREPs following stimulation of the right and left hands. However, a Hand × Scalp Electrode Site × Interval interaction was revealed for N2 peak amplitudes. These data suggest that the combination of side of stimulation and scalp recording site may be important in determining the patterning of PREPs across the cardiac cycle. Taken together, the findings of these studies suggest that pain perception, and to a lesser extent tactile sensation, are influenced by natural variations in BP across the cardiac cycle. However, modulation appears dependent on tonic BP. Conversely, pain-related brain activity across the cardiac cycle was not affected by tonic BP, but may be influenced by the combination of stimulation and recording sites.

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PUBLICATIONS AND PRESENTATIONS

This thesis includes the following paper, corresponding to the first study in this thesis (Chapter 2):

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Additionally, the following abstracts refer to presentations of material from this thesis:

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Wilkinson, M., McIntyre, D., & Edwards, L. (2011). Electrocutaneous pain thresholds are higher during systole than diastole. *Psychophysiology, 48*, S21-S119. doi:10.1111/j.1469-8986.2011.01259.x

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Wilkinson, M., McIntyre, D., & Edwards, L. (2011). *The effects of natural variation in BP across the cardiac cycle on pain thresholds.* Oral presentation at the Health and Life Sciences Research Student Conference, Loughborough, UK.

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ONE

General Introduction

At some point in life everyone experiences pain, but what exactly is pain and what factors can influence it?

Nociception is the neural process of encoding noxious stimuli (The International Association of the Study of Pain, 2012). Whereas, pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Merskey & Bogduk, 1994). Thus, the pain we feel is more complex than simply a physical sensation resulting from the processing of noxious stimuli. It also has significant psychological, cognitive and environmental components and therefore further research is needed to understand its multidimensional nature.

Ancient civilisations associated pain with evil, magic, and demons, but as early as Greek and Roman times, theories were being developed that pain was more than this introducing the theory of sensation and the idea that the brain and nervous system were involved in the perception of pain (NINDS, 2001). Over the centuries that followed, the intrigue with pain continued and in 1644 French philosopher René Descartes described what we would now regard as a "pain pathway" when describing the pain sensation associated with the transmission of fire touching the foot travelling to the brain as similar to the ringing of a bell (Descartes, 1644). Subsequently, pain research has become even more extensive and has sought to unravel the processing of pain and the mechanisms underlying it, but because of the complex, multidimensional nature of pain there are still many unanswered questions (Brooks & Tracey, 2005).

Pain is generally perceived negatively and chronic pain can certainly be debilitating, but pain is important, it is a way of your body telling you something is, or could potentially be wrong. So when conditions such as high blood pressure (BP) are associated with a reduced sensitivity to pain (see Ghione, 1996 for review), it is important to try and understand what causes this and subsequently how best to try and treat it. The aim of this thesis was to add to the body of research and understanding regarding the possible role of baroreceptors in the modulation of pain and other sensations. The studies employed a cardiac cycle paradigm (explained on

page 28 in the natural baroreceptor stimulation across the cardiac cycle studies section of this introduction) with participants whose resting BP was within the normal range (normotensive). The aim was to investigate the effects of natural fluctuations in BP, and consequently baroreceptor activation, across the cardiac cycle on various sensory indices in a controlled, within-subjects approach.

This chapter describes: (a) a brief overview of hypertension and the phenomenon of hypertensive hypoalgesia; (b) the processing of arterial baroreceptor afferents and pain signals, and evidence for a possible convergence; (c) the methodological techniques used to study baroreceptor influences on pain with an emphasis on the cardiac cycle paradigm; d) a brief introduction to the possible lateralisation of pain and baroreceptor processing in the brain and; (d) an outline of the studies in the present thesis.

1.1 Hypertension

Hypertension is a chronic medical condition associated with elevated BP in the arteries (Chobanian et al., 2003). Blood pressure is measured with two values; systolic and diastolic. Systolic BP (SBP) equates to a period of high pressure when the ventricles of the heart contract forcing the blood out of the heart and into peripheral circulation. Diastolic BP (DBP) corresponds to a period of low pressure when the ventricles relax to allow blood to flow in to them for the next contraction, and thus give a maximum and minimum BP respectively (Mackenzie & Brown, 2009).

The continuous nature of BP, and specifically the continuous relationship between BP and health risks make the identification of cut-off points for the definition between normal BP and high BP (hypertension) difficult (Mancia et al., 2013). However, it is necessary to provide guidelines for practitioners to identify and initiate treatment for hypertension. The current BP classifications proposed by The Task Force for the management of arterial hypertension of the European Society of Hypertension and of the European Society of Cardiology (Mancia et al., 2013) are outlined in Table 1. When SBP and DBP fall into different categories, the highest category is used, although it should also be noted that in addition to the 6 groupings

outlined in Table 1, a further classification relates to isolated systolic hypertension (SBP above 140 mmHg when DBP is less than 90 mmHg), is graded as 1, 2, or 3, according to the SBP level. These guidelines are consistent with the 2011 Hypertension Guidelines produced in a collaboration between the British Hypertension Society (BHS) and National Institute of Health and Clinical Excellence (NICE) guidelines (NICE, 2011) and those of The World Health Organization (WHO)/International Society of Hypertension (ISH) (World Health Organisation, International Society of Hypertension Writing Group, 2003).

Table 1. European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) guidelines for the management of arterial hypertension - Definitions and classification of blood pressure levels (Mancia et al., 2013)

Category	Systolic BP (mmHg)		Diastolic BP (mmHg)
Optimal	<120	and	<80
Normal	120-129	and/or	80-84
High Normal	130-139	and/or	85-89
Grade 1 Hypertension	140-159	and/or	90-99
Grade 2 Hypertension	160-179	and/or	100-109
Grade 3 Hypertension	≥180	and/or	≥110

1.1.1 Prevalence of hypertension, its association with cardiovascular disease and increased risk of mortality

Hypertension is a growing concern in the developed world. According to the 2011 Health Survey for England 31% of adult men and 28% of women have been diagnosed as hypertensive in England (Knott & Mindell, 2011), with similar reports in America (Yoon, Burt, Louis, & Carroll, 2012), several European countries (Germany, Finland, Sweden, England, Spain, Italy) and Canada (Wolf-Maier et al., 2003).

Raised BP is the single most important cause of death worldwide (Lopez, Mathers, Ezzati, Jamison, & Murray, 2006; World Health Organisation, 2009), according to the Global Health Risks Report by the WHO High BP accounts for 13%

of deaths globally (World Health Organisation, 2009). Specifically, hypertension is a highly prevalent risk factor for cardiovascular disease (CVD) throughout the developed world. An increase in SBP of 2mmHg has been associated with a 7% increased risk of ischaemic heart disease mortality and a 10% increased risk of mortality from stroke (Lewington, Clarke, Qizilbash, Peto, & Collins, 2002). Cardiovascular disease has been the most common cause of death in England and Wales for nearly a century in both males and females; according to the National Statistics Death Registrations Summary Statistics for England and Wales in 2011, circulatory diseases, such as heart disease and strokes accounted for 29% of all deaths (Office for National Statistics, 2011) and the WHO estimates that 51% of stroke (cerebrovascular disease), 45% of ischemic heart disease deaths and 45% of CVD deaths among those older than 30 years are attributable to high BP (World Health Organisation, 2009). Importantly, the leading cause of CVD death is elevated BP, with high BP causing between 37% of CVD deaths in the South-East Asia Region to 54% of CVD deaths in middle-income European countries (World Health Organisation, 2009). Elevated BP increases the risk of CVD due to the effect high BP has on the arteries supplying blood to the brain, heart and kidneys which significantly increases the risk of stroke and ischaemic heart disease leading to heart attacks, as well as contributing to the development of chronic kidney disease and cognitive decline (Lewington et al., 2002; Rothwell, 2011).

With these worrying statistics it is clear to see that hypertension is a major health problem and therefore an important area of research. Effective hypertension treatment can reduce the risk of stroke, heart attack and congestive heart failure, hypertensive retinopathy and nephropathy (Sawicka et al, 2011) therefore it is vital to maximise our understanding of the condition so that strategies to reduce its impact can be developed.

1.2 Hypertensive hypoalgesia

Hypertension is predominantly asymptomatic, except at extremely elevated levels, and as such many sufferers are unaware that anything is wrong (Kannel, Dannenberg, & Abbott, 1985; Yurenev, DeQuattro, & Devereux, 1990). Significantly this may interfere with early detection of cardiac disease (France, 1999). It is

because of the lack of symptoms and the increased risk of premature death associated with hypertension outlined above that hypertension is sometimes referred to as the silent killer (France, 1999; France & Ditto, 1996). Interestingly, the lack of symptoms associated with hypertension may actually be a symptom. It is now well established that patients with hypertension exhibit reduced sensitivity to pain (Ghione, 1996) and this could be considered a symptom.

1.2.1 Animal research evidencing hypertensive hypoalgesia

The first reports of an association between hypertension and reduced pain perception (hypoalgesia) were in 1979 in experimentally-induced hypertensive rats (Dworkin, Filewich, Miller, Craigmyle, & Pickering, 1979; Zamir & Segal, 1979). Initially, Zamir and Segal (1979) reported a delayed paw-licking response to noxious thermal hot-plate stimulation following experimentally induced hypertension by renal artery clipping, compared to pre renal artery clipping. Soon after, Dworkin and colleagues (1979) repeated the findings in rats with acutely elevated BP following infusion of the drug phenylephrine, which exhibited slower escape-avoidance running responses to noxious trigeminal nucleus stimulation compared to saline infusion. These findings were repeated in rats in response to the tail flick test (Saavedra, 1981), the hot-plate test (Maixner, Touw, Brody, Gebhart, & Long, 1982; Sitsen & de Jong, 1983), and an electric foot shock test (Sitsen & de Jong, 1983).

1.2.2 Hypertensive hypoalgesia in humans and the relationship with tonic blood pressure

The hypertensive hypoalgesia phenomenon was first identified in humans in 1980 by Zamir and Shuber (1980) who reported that individuals with unmedicated hypertension had higher pain thresholds in response to electrical tooth pulp stimulation compared to individuals with normal BP. The relationship between BP and perceived pain in humans has been found to follow an inverse linear pattern throughout the range of tonic BP from low BP (hypotension), through the normotensive range, into borderline hypertension and hypertension. Specifically, it has been repeatedly reported that in hypertensive populations, as BP increases, pain sensitivity reduces in a linear manner (Bruehl, Carlson, & McCubbin, 1992; Bruehl, Chung, Ward, Johnson, & McCubbin, 2002; McCubbin & Bruehl, 1994;

Ghione, Rosa, Mezzasalma, & Panattoni, 1988; Guasti et al., 1995; Guasti et al., 1996; Guasti, Gaudio et al., 1999; Guasti, Zanotta et al., 1999; Guasti et al., 2002; Rosa, Vignocchi, Panattoni, Rossi, & Ghione, 1994; Sheffield et al., 1997; Sheps et al., 1992; Zamir & Shuber, 1980). Individuals with borderline hypertension have been shown to have a reduced pain perception compared to normotensive controls (Rau et al., 1994; Rosa, Ghione, Panattoni, Mezzasalma, & Giuliano, 1986; Schobel et al., 1996; Schobel et al., 1998). In normotensive samples it has been well established that as BP increases, pain is reduced (al'Absi, Petersen, Wittmers, 2000; Bruehl et al., 1992; Campbell & Ditto, 2002; D'Antono, Ditto, Rios, & Moskowitz, 1999; Ditto, Seguin, Boulerice, Pihl, & Tremblay, 1998; Fillingim, & Maixner, 1996; Fillingim, Maixner, Bunting, & Silva, 1998; France, 1999; McCubbin & Bruehl, 1994; Myers, Robinson, Riley, & Sheffield, 2001; Sheffield, Biles, Orom, Maixner, & Sheps, 2000). However, the linear relationship between tonic BP and pain and normotensive samples is not always evident (Bruehl, Chung, Diedrich, Diedrich, & Robertson, 2008; Bruehl et al., 2010; Edwards, McIntyre, Carroll, Ring, & Martin, 2002; Mechlin et al., 2011; Stewart & France, 1996). Finally, in hypotensive participants (systolic BP <100 mmHg) have been shown to present an increase in pain compared to normotensives (Duschek & Schandry, 2006). Additionally, there is good evidence that individuals with a genetic risk of hypertension (i.e. a parental history of hypertension) show reduced pain than individuals without a parental history of hypertension (al'Absi, Buchanan, & Lovallo, 1996; Campbell & Ditto, 2002; France, 1999; France & Stewart, 1995; Stewart & France, 1996). However, this reduced pain sensitivity in individuals with a parental history of hypertension is not always evident (al'Absi et al., 2000; Ghione et al., 1988). Taken together these data provide strong evidence that hypoalgesia is related to risk for hypertension rather than hypertension per se and that the degree of pain reduction increases with increasing BP (France, 1999).

1.2.3 Implications of hypertensive hypoalgesia

Hypertensive hypoalgesia has severe, acute clinical implications; hypertension is associated with an increased prevalence of silent myocardial ischemia and unrecognized myocardial infarction (Valensi, Lorgis & Cottin, 2011). For example analysis of data from the extensive Framingham Heart Study reported that 45% of women and 35% of men with hypertension had suffered an asymptomatic

myocardial infarction (Kannel, Dannenberg & Abbott, 1985). Subsequent experimental data has supported these findings; an inverse relationship between BP and levels of chest pain during exercise tolerance tests screening for myocardial ischemia has been found (Ditto, D'Antono, Dupuis, & Burelle, 2007) and participants with elevated BP have shown a delayed onset of angina pain during episodes of exercise-induced myocardial ischemia (Krittayaphong & Sheps, 1996; Sheps et al., 1989). Additionally, during 24-hr Holter monitoring recordings, as BP increased, so did the prevalence of silent myocardial ischemia (Siegel, Cheitlin, Seeley, Black, & Hulley, 1992). This data provides evidence that hypertensive hypoalgesia has significant negative consequences for health and thus it is important to try and further the understanding of the phenomenon.

1.2.4 Mechanisms for hypertensive hypoalgesia

The hypertensive hypoalgesia phenomenon has been well studied. However the mechanisms underlying it are yet to be fully established. France and Ditto (1996) proposed three mechanisms to explain hypertensive hypoalgesia; a) elevated central and peripheral endogenous opioid levels, b) increased descending supraspinal pain inhibition, and c) increased baroreceptor stimulation. This thesis was designed to examine the third of these potential mechanisms; the role of arterial baroreceptors in pain perception.

1.3 Neural pathways for arterial baroreceptors and nociception

Maintaining cardiovascular homeostasis requires tight regulation of arterial BP within a narrow mean arterial pressure range of 85 and 100 mmHg (Klabunde, 2011). This regulation depends on a balance of feed-forward or central command, and feedback or reflex mechanisms operating in response to deviations from a state of homeostasis (Benarroch, 2008). Baroreceptors are central to the feedback mechanism controlling short-term regulation of BP via a fast acting reflex response called the baroreflex (Stanfield & Germann, 2008).

Arterial baroreceptors are mechanoreceptors located in the walls of the aortic arch and carotid sinuses (at the bifurcation of the external and internal carotids) (Bell, 2009). Baroreceptors are stimulated by stretch of the vessel walls and are

sensitive to both absolute pressure and the degree of stretch within the structures (Angell James, 1971; Dembrowsky & Seller, 1995). At rest, arterial baroreceptor firing is maximal during the systolic upstroke when the pulse pressure wave of blood surging through the vessels stretches the walls of the aortic arch and carotid sinus and is minimal during diastole when the bolus of blood has passed the baroreceptors and entered peripheral circulation (Angell James, 1971; Mancia & Mark, 1983). Thus, there is natural phasic baroreceptor stimulation during each cardiac cycle (Angell James & Lumley, 1974).

1.3.1 The arterial baroreflex

The arterial baroreflex is a negative feedback mechanism buffering acute changes in BP (Benarroch, 2008); an increase in the mean arterial pressure increases the tension in the vessel, stimulating the baroreceptors and increasing their afferent output. The carotid sinus baroreceptors are innervated by the sinus nerve of Hering, a branch of the glossopharyngeal nerve. The aortic arch baroreceptors are innervated by the aortic nerve which joins the vagus nerve. Both the glossopharyngeal and vagus nerve project to the nucleus tractus solitarius (NTS) in the medulla of the brainstem (Eckberg & Sleight, 1992; Benarroch, 2008; Bell, 2009; Klabunde, 2011). Sympathetic and parasympathetic neuron activity in the medulla is modulated by the NTS (Klabunde, 2011). An increase in baroreceptor afferent input to the NTS excites the nucleus ambiguous neurons and inhibits the rostral ventrolateral medulla (RVLM) neurons leading to a reduction in BP via an increase in parasympathetic nervous system activity and a reduction in sympathetic nervous system activity to return the body to a state of homeostasis (Bell, 2009).

1.3.2 Transmission of baroreceptor afferent information to the brain

Afferent baroreceptor information is transmitted from the baroreceptors locations in the aortic arch and the carotid sinuses via myelinated A-δ and unmyelinated C-fibres (Coleridge, Coleridge & Schultz, 1987). A-δ have higher conduction velocities, and lower firing thresholds than C-fibres (Kunze & Andresen, 1991), but there is no difference between the neural pathways, reflex regulation effects or central nervous system effects of the two fibre types. Therefore, the main difference between the

fibre types appears to be the range of BP in which the operate (Dembrowsky & Seller, 1995).

1.3.3 Baroreceptor afferent transmission within the brain

Baroreceptor afferents reach the first synapse in NTS, which is a major brainstem centre for relaying visceral-afferent signals. Baroreceptor stimulation results in the parasympathetic efferent branch of the arterial baroreflex exciting the preganglionic cardiomotor neurons in the nucleus ambiguus which directly influences the cardiac pacemaker to decrease heart rate (Jänig, 2006). Similarly, baroreceptor stimulation leads to the sympathetic branch of the arterial baroreflex projecting excitory fibres to the caudal ventrolateral medulla (CVLM) which to turn send inhibitory fibres to the rostral ventrolateral medulla (RVLM), which subsequently inhibits the autonomic nervous system decreasing heart rate and BP (Jänig, 2006). Beyond these brain stem reflexes baroreceptor inputs are projected to higher brain centres. Baroreceptor afferent information is transmitted both directly, and indirectly (via the lateral parabrachial nucleus) to the limbic structures (Dembrowsky & Seller, 1995), and the thalamus via the reticular formation (Rau & Elbert, 2001), which both subsequently project onto the insular cortex (Dembrowsky & Seller, 1995). Specifically, the insular cortex (Butcher & Cechetto, 1995; Zhang, Dougherty, & Oppenheimer, 1999), Anterior cingulate cortex (ACC) (Terreberry & Neafsey, 1987; Verberne & Owens, 1998), amygdala (Cechetto & Calaresu, 1983, 1984, 1985; Gelsema, Agarwal, & Calaresu, 1989) and the cerebellum (Bradley, Paton, & Spyer, 1987; Nisimaru, Okahara, Yanai, 1998) are all higher brain areas identified as being associated with baroreceptor-related cardiovascular function. There is also good recent evidence in humans from functional magnetic resonance imaging (fMRI) studies indicating changes in autonomic function during baroreceptor unloading activity in the insular cortex, ACC, medial prefrontal cortex (MPFC), cerebellum, and amygdala (Kimmerly, O'Leary, Menon, Gati, Shoemaker, 2005).

1.3.4 Transmission of nociception in the peripheral nerves

Nociceptive information is transmitted from peripheral nociceptive receptors to a first synaptic relay in the spinal cord via A-δ fibres and C-fibres. The myelinated A-δ afferents conduction velocity is approximately 15 m/s (Meyer, Walker, &

Mountcastle, 1976), with high firing rates up to 30 Hz and reflect the fast “first pain” sensation being pinprick like (Arendt-Nielsen & Chen, 2003). Whereas the unmyelinated C fibres have slower conduction velocities approximately 0.86–1.25 m/s (Gybels, Handwerker, & Van Hees, 1979), have slower firing rates (15-20Hz) and reflect the “second pain” described as slow burning (Arendt-Nielsen & Chen, 2003).

1.3.5 Cerebral representation of pain

For many years, researchers have sought to map the areas of the brain involved in the processing of pain. As a result, studies utilising neuroimaging techniques including magnetoencephalography (MEG), functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) several different structures have been identified as contributing to this process of pain perception. The areas identified as being activated following painful stimulation include the insular cortex, the ACC, the amygdala, the thalamic nuclei, the hippocampus, the primary (SI) and secondary (SII) somatosensory cortices, the primary and secondary motor cortices, the prefrontal and posterior parietal cortices, the basal ganglia, the periaqueductal grey matter, the cerebellum and the posterior parietal cortex (Bushnell & Apkarian, 2006; Coghill, et al., 1994; Craig, 2002; Davis, Pope, Crawley, & Mikulis, 2002; Kakigi, Inui, & Tamura, 2005; Ogino et al., 2007; Peyron, Garcia-Larrea et al., 2000; Qiu et al., 2006; Rainville, 2002).The SII, insular regions, and the ACC have been found to be most consistently activated following painful stimulation with slightly less consistency in the contralateral thalamus and SI (Peyron, Garcia-Larrea et al., 2000; Apkarian, Bushnell, Treede, & Zubieta, 2005). With so many different brain areas identified as contributing to pain processing, it is safe to suggest that there isn’t a single “pain centre” in the brain (Tracey, 2005). On the contrary, it appears that pain processing relies on several different brain structures, which collectively are often referred to as the ‘pain matrix’ (Melzack, 1999) i.e., a network of cortical areas through which pain is generated from nociception (Ingvar, 1999; Peyron, Laurent et al., 2000; Porro, 2003; Rainville, 2002; Tracey & Mantyh, 2007). Thus it appears that pain perception is dependent on the interaction of several different cerebral structures, and that it is the pattern and degree of activation of these structures, together with the integration of other factors influencing pain perception (e.g., cognition, mood, injury, and so

forth) that determine how an individual experiences pain (e.g., Tracey & Mantyh, 2007).

1.3.6 The convergence of baroreceptor afferent information and pain in the brain

There is substantial support for a baroreceptor role in the modulation of pain (Ghione, 1996). With regards to the modulating effects of baroreceptors on pain, many of the same areas of the brain identified as contributing to the perception of pain have also been identified as being activated by baroreceptor afferent inputs. Firstly, direct stimulation of the NTS, which as discussed above is central to the baroreflex, has been shown to induce antinociception (Aicher & Randich, 1990). Secondly, there is evidence, in monkeys, that somatosensory and baroreceptor inputs converge on the same neurons within the insular cortex (Zhang et al., 1999) and the insular cortex and thalamus in rats (Hanamori, Kunitake, Kato & Kannan, 1998; Zhang & Oppenheimer, 1997). Additionally, baroreceptor activity across the cardiac cycle has been shown to influence the cortical processing of somatosensory stimuli, with an integration of somatosensory stimuli presented either before or during early cardiac systole in humans being identified in the insula, amygdala, and brain stem nuclei (Gray et al., 2009). Third, several studies have shown significant overlap between brain areas involved in baroreflex control and pain modulation. For example when the periaqueductal grey matter is stimulated it induces analgesia (Bandler, Carrive, & Zhang, 1991), the periaqueductal grey matter is also important in arterial baroreflex modulation (Inui, Murase, & Nosaki, 1994; Nosaka, Murata, Inui, & Murase, 1993). Additionally, the nucleus raphe magnus in the RVLM plays a central role in pain modulation and contains neurons whose activity fluctuate spontaneously with both natural and experimentally-induced BP changes (Thurston & Randich, 1992; 1995). Taken together these data suggest there is a significant overlap in the brain areas regulating BP and processing pain which is important from the point of view of identifying a possible baroreceptor role in the modulation of pain.

1.4 Pain stimulation modalities

Various pain modalities have been used to study pain including noxious electrical tooth pulp, thermal, mechanical, intracutaneous, electrocutaneous and laser

stimulation. In relation to this thesis the electrocutaneous modality will be the focus of this introduction following a brief discussion of laser stimulation.

1.4.1 Noxious laser stimulation in pain study

Noxious laser stimulation has been used in the study of pain since 1970 (Mor & Carmon, 1975). Since its introduction, laser stimulation has been used extensively to elicit pain-related evoked potentials (PREPs) to try and identify the cortical brain areas involved in pain processing (Carmon, Mor, & Goldberg, 1976; Carmon, Dotan, & Sarne, 1978; Bromm & Treede, 1987; Treede, Kief, Holzer, & Bromm, 1988; Kakigi, Shibasaki, & Ikeda, 1989; Miyazaki et al., 1994; Xu et al., 1995; Kanda et al., 1996, 1999; Edwards, Inui, Ring, Wang, & Kakigi, 2008). Laser stimulation is regarded as the most reliable assessment tool for selectively stimulating A-δ nociceptive fibres and thus studying pain in healthy subjects and patients (Treede, Lorenz, & Baumgartner, 2003; Cruccu & García-Larrea, 2004). Laser stimulation has a rapid onset and excites a limited number of primary afferent fibres, primarily thin myelinated A-δ- and unmyelinated C-fibres which are pain fibres known to respond to thermal stimulation (Meyer, Ringkamp, Campbell, & Raja, 2006). However the equipment required for laser stimulation is bulky and expensive. The nature of laser stimulation also leads to superficial burns, although these are less intense when employing thulium lasers (2.03 μm wavelength) compared to carbon dioxide lasers (10.6 μm wavelength), due to differences temperature distribution in the skin (Spiegel, Hansen, & Treede, 2000). To try and counter these limitations researchers have sought to utilise alternative methods to elicit pain, electrocutaneous stimulation has been developed as a suitable alternative.

1.4.2 Electrocutaneous stimulus in pain study

Commonly used electrocutaneous stimulation electrodes, such as the bar electrode, is known to stimulate larger A-β tactile fibres in addition to A-δ nociceptive fibres (Katsarava et al., 2006; Kaube, Katsarava, Kaufer, Diener, Ellrich, 2000). This is due to the lower electrical thresholds of A-β tactile fibres compared to nociceptive A-δ fibres. The stimulation of the powerful A-β fibres is felt like an aversive stab or vibration without actually being felt as pain (Gracely, 2006). As such the pain response may be contaminated and thus the responses may not be truly nociceptive

(Katsarava et al., 2006; Kaube et al., 2000). Indeed, studies have confirmed this showing that increasing electrical stimulation intensity produces cortical responses that, even at noxious levels, are more related to stimulation of non-pain than noxious afferents (De Broucker & Willer, 1985; Dowman, 1994).

The pain studies outlined in the current thesis delivered electrocutaneous stimulations via a concentric planar electrode (Kaube et al., 2000) designed to more selectively stimulate A-δ nociceptive fibres compared to the bar electrode electrocutaneous stimulations methods (Katsarava et al., 2006; Kaube et al., 2000). The small cathode-anode distance of the concentric planar electrode produces a highly superficial current field to more selectively stimulate nociceptive A-δ fibres which are closer to the surface of the skin (Katsarava et al., 2006; Kaube et al., 2000). The sensation associated with the concentric planar electrode is a sharp pinprick-like pain, and has been shown to elicit PREPs and nociceptive blink reflexes (Katsarava et al., 2006; Kaube et al., 2000; Serrao et al., 2010). Thus, it has been proposed that the concentric planar electrode is an efficient method for evaluating the nociceptive system. Indeed, PREPs using the concentric planar electrode have revealed group abnormalities in HIV and diabetic neuropathic patients (Mueller et al., 2010; Obermann et al., 2008). These findings indicate the efficiency of the concentric electrode in identifying abnormalities in pain processing.

Therefore, it is reasonable to assume, and should be taken into account that electrocutaneous stimulation may differentially activate nociceptive and non-nociceptive fibres compared to laser stimulation (e.g. Lefaucheur et al., 2012; Perchet et al., 2012). Indeed, two recent studies (de Tommaso et al., 2011; Lefaucheur et al., 2012), reported differences in the latency of the N2 and P2 components between PREP responses evoked by laser stimuli and concentric planar electrode, with laser stimulation demonstrating later peak amplitudes than electrical stimulation. The latency differences could be attributed to differences in axon activation between the two stimulation modalities. Electrical stimulation directly activates peripheral afferents (Perchet et al., 2012), whereas laser stimulation incurs a peripheral delay usually about 40 ms, although considerably less (approx.10-20 ms) when brief duration laser stimulations are used (Iannetti et al., 2004). This

difference is due to signal transduction between thermoreceptor heating and action potential generation in A-δ nociceptive fibres following laser stimulation (Bromm & Treede, 1991; Plaghki & Mouraux, 2003).

1.5 Methods of pain assessment

Pain is subjective and multidimensional (Clark, Yang, Tsui, Ng, Bennett, & Clark 2002; Kumar, Tandon, & Mathur, 2002). In terms of research this is a problem as it restricts the ability to accurately quantitatively assess it. Commonly used subjective self-report measures of pain such as the Visual Analogue Scale (VAS), numerical rating scales e.g. 0-10 or 0-20, and category rating scales based on verbal descriptions (e.g. nil, mild, moderate, severe, very severe) are limited by their measurement of a single aspect of pain i.e. intensity, considerable within-subject and between-subject variability (Gracely, 1995) and various kinds of bias such as memory bias (Magnusson, List, & Helkimo, 1995) and experimenter bias (Branch, Carlson, & Okeson, 2000). Although such self-report measures have limitations they still provide important information and their value should not be dismissed. Indeed some measures, such as the McGill Pain Questionnaire (Melzack, 1975) permit the scaling of multiple dimensions of subjective experience which allows a deeper appreciation of the nature of pain experience.

1.5.1 The nociceptive flexion reflex as a method to study pain

To try and address the issues with subjective measures of pain researchers have sought alternative more objective methods for studying pain. The nociceptive flexion reflex (NFR) is a polysynaptic spinal reflex that facilitates withdrawal from noxious stimuli to avoid tissue injury (Sandrini et al., 2005), and the threshold for which serves as a physiological correlate of pain (Hugon, 1973; Willer, 1977). The NFR is typically elicited via stimulation of either the cutaneous superficial branch of the radial nerve at the wrist or the sural nerve at the ankle. The subsequent withdrawal motor response is recorded at the flexor muscle associated with the nerve (Garcia-Larrea, 2012) e.g. in response to sural nerve stimulation this is the electromyographic activity in the biceps femoris is recorded and this is assumed to indicate the level of nociceptive responding (Hugon, 1973; Willer, 1977). Studies

utilising this methodology to study the hypertensive hypoalgesia phenomenon are discussed in detail in section 8.6 of this introduction.

1.5.2 Pain-related evoked potentials as a method for studying pain

In a further development in the study of pain, researchers have examined the time-locked derivative of the electroencephalogram (EEG) termed the event-related potential (ERP) which in response to painful stimuli is termed the pain-related evoked potential (PREP). Electroencephalography represents the electrical activity of the brain measured at scalp level (Niedermeyer & da Silva, 2004) and PREPs elicited by noxious stimuli are thought to represent the central processing of nociception and as such, are seen as an objective measure of pain (Miltner, Larbig, & Braun, 1987; Granovsky, Granot, Nir, & Yarnitsky, 2008).

Two components of PREP waveforms have been extensively studied in relation to pain; the second negative (N2) and positive (P2) peaks (Kanda et al., 1996; Garcia-Larrea, Peyron, Laurent, & Mauguiere, 1997; Fila & Bogucki, 2009). The N2 occurs approximately 130–240 ms post stimulus and P2 approximately 230–390 ms post stimulus (Bromm, 1984; Zaslansky et al., 1996) and have been found to be maximal at the midline central area, specifically Cz electrode following hand stimulation (Carmon et al., 1976, 1978; Bromm & Treede, 1987; Treede et al., 1988; Kakigi et al., 1989; Miyazaki et al., 1994; Xu et al., 1995; Kanda et al., 1996, 1999). Evidence suggests the origin of the N2 and P2 components is mainly the ACC, whilst SII and insula cortex, bilaterally, also contribute to the N2 component (Bromm & Chen, 1995; Tarkka & Treede, 1993; Valeriani, Rambaud, & Mauguiere, 1996). These data further suggest, as discussed, above that PREPs are generated in the brain areas involved in pain processing and that these same areas are also involved in the processing baroreceptor afferents.

Harkins and Chapman (1978) was one of the first studies to report a significant relationship between ERP components and subjective pain ratings in response to electrical dental pain. These investigators reported that an increase in all major ERP components was accompanied by an increase in subjective ratings of pain using a VAS (r = 0.67-0.77). These findings were further supported by Chen and colleagues

(Chen, Chapman, & Harkins, 1979) who reported that peak-to-peak amplitudes between 175 and 260 ms (i.e. N2-P2 peak-to-peak amplitude) in response to painful dental stimulation had a strong linear correlation (r = 0.67 and r = 0.41 respectively) with subject ratings of painfulness. Further research has added support indicating that the amplitude of the N2 and P2 PREP components correlate with the intensity of pain stimulus (Becker, Haley, Urena, & Yingling, 2000; Bromm, 1984; Stowell, 1977; Zaslansky et al., 1996), as well as with subjective ratings of pain (Kanda et al., 2002). However it should be acknowledged that this is not always the case. Research investigating the habituation of painful stimuli reported that despite a decrease in the PREP N150-P360 peak-to-peak amplitudes across trials, there was no accompanying reduction in pain ratings (Miltner et al., 1987).

The PREP represents the averaged cortical processing of the nociceptive stimulus and thus reflects the cortical activity that is involved in the response to a pain stimulus (Iannetti, Hughes, Lee, & Mouraux, 2008). Therefore, one limitation of the PREP methodology is its relatively poor spatial resolution due to the electrical responses of the PREP being spread over the scalp, compared to more recent brain imaging techniques such as PET or fMRI which have a better spatial resolution. This makes source localisation of the site of the PREP generation difficult (Devinsky & D'Esposito, 2004). The poor spatial resolution of PREPs is being addressed by the use of concurrent fMRI and PREPs. However, PREPs make up for their relatively poor spatial resolution with exceptional temporal resolution, processes activated within milliseconds of the pain stimulus can be measured extremely accurately and this makes PREPs the a very direct measure of neuronal activity (Kupers & Kehlet, 2006). This is vitally important for cardiac cycle studies were stimulus-response timings are in milliseconds.

1.6 The role of baroreceptor activation in hypertensive hypoalgesia

1.6.1 Baroreceptor influence on cortical activity

In addition to regulating short term BP, there is also considerable evidence that baroreceptor stimulation generates a widespread inhibition of the central nervous system (Rau, Pauli, Brody, Elbert, & Birbaumer, 1993). As early as 1932, Koch

reported that prolonged mechanical stimulation of arterial baroreceptors in dogs induced lethargy. At a similar time the first reports of baroreceptor effects beyond BP control were reported in humans. Weiss and Baker (1933) highlighted a loss of consciousness following mechanical stimulation of the carotid baroreceptors, without any simultaneous BP changes or cerebral ischemia. Subsequent animal studies supported these initial findings reporting that arterial baroreceptor stimulation in decerebrate cats increased EEG synchronisation indicating a decrease in cortical arousal (Bonvallet, Dell, & Hiebel, 1954; Nakao, Ballim, Gellhorn, & Gellhorn, 1956) and inhibited attacks of sham rage (Bartorelli, Bizzi, Libretti & Zanchetti, 1960). Taken together these early findings suggested that stimulation of the baroreceptors could lead to reduced cortical activity and arousal and this eventually lead to the development of the Laceys' Visceral Afferent Feedback (VAF) hypothesis (Lacey & Lacey, 1974), which proposed that increased afferent baroreceptor neural feedback integrated into the medullary and cortical structures causes interference with other processes within these structures. As a consequence sensory and motor function is reduced. Conversely, when baroreceptor stimulation is reduced, (i.e. lower BP) sensory and motor functions would be facilitated (Lacey, 1967). Based on these findings it may be concluded that the effects of baroreceptor stimulation may help to explain the hypertensive hypoalgesia phenomenon and thus researchers have sought to investigate this hypothesis.

In order to study the potential role of baroreceptors in hypertensive hypoalgesia three main approaches have been employed; a) pharmacologically, or b) mechanically stimulating the baroreceptors and c) utilising the natural fluctuations in BP, and thus baroreceptor activation, across the cardiac cycle. The studies included in this thesis employed cardiac cycle methodology.

1.6.2 Pharmacological baroreceptor stimulation studies

In order to investigate a possible baroreceptor influence on cortical activity, researchers studied the effects of administrating either a BP enhancer (Norfenefrin–HCl2), or a placebo on event-related EEG activity (Larbig, Elbert, Rockstroh, Lutzenberger & Birbaumer, 1985). The results indicated that event-related EEG activity was significantly reduced during administration of the Norfenefrin–HCl2

compared to the placebo measured in terms of CNV-like brain potential amplitude (Larbig et al., 1985). These findings were supported when the neck cuff approach was used, with the CNV-like brain potential amplitudes generated under control conditions being found to be significantly reduced during baroreceptor stimulation (Elbert et al., 1988; Rau et al., 1988; Elbert & Rau, 1995; Rau, Pauli et al., 1993). These findings suggest that baroreceptor activation appears to influence cortical activity.

Several animal studies administrating arterial pressure increasing agents (initially angiotensin II and subsequently phenylephrine hydrochloride) to rats have reported hypoalgesia (Dworkin et al., 1979; Randich & Hartunian, 1983; Randich & Maixner, 1984; 1986; Watkins, Thursten, & Fleshner, 1990). Studies employing pharmacological manipulation of BP in humans are less common due to ethical limitations. However, a few studies have intravenously injected BP raising agents to investigate the effects of baroreceptor stimulation on pain. Experimentally elevated BP via injection of the pressor drug norfenefrin increased pain tolerance in borderline hypertensive participants but reduced pain tolerance in normotensive participants compared to a saline placebo (Larbig et al., 1985). A further study in a purely normotensive sample, which elevated BP via phenylephrine injection, found pain thresholds for electrical tooth pulp stimulation were reduced compared to a placebo but this effect was less prominent the higher the tonic BP of the participant (Rockstroh et al., 1988). These studies indicate the inconclusive findings regarding pharmacological manipulation of the BP. One of the major concerns regarding the findings from studies employing pharmacological elevation of BP is a possible direct effect of the pressor agents on the central nervous system (CNS) and thus pain processing not directly associated with baroreceptor stimulation (Eckberg & Sleight, 1992; Imaizumi, Brunk, Gupta, & Thames, 1984).

1.6.3 Mechanical baroreceptor stimulation studies

In order to more directly test the baroreceptor influence on cortical function, early studies employed artificial manipulation of baroreceptor afferent output via the application of constant neck suction over the carotid sinus for several seconds to manipulate the transmural pressure (Eckberg, Cavanaugh, Mark, & Abboud, 1975).

Because arterial baroreceptors respond to distension of the arterial wall, lowering the pressure in the surrounding tissue via the application of negative pressure, i.e. suction to the area of the skin over the carotid sinus in the neck, will stretch the artery walls and thus increase baroreceptor firing (Eckberg & Sleight, 1992; Mancia & Mark, 1983). On the other hand, when positive pressure is applied to the same area the artery walls are compressed and thus baroreceptor firing is reduced (Eckberg & Sleight, 1992; Mancia & Mark, 1983). The findings from studies employing the constant cuff technique to study hypertensive hypoalgesia were mixed, with reports of reduced electrocutaneous pain in borderline hypertensive's (Elbert et al., 1988) whilst electrocutaneous pain was increased (Elbert et al., 1988) and ischemic pain unaffected (France, Ditto, & Adler, 1991) in normotensive participants.

Advancements in methodology lead to the development initially of the phasic pressure technique (Strange, Rowell, Christensen, & Saltin, 1990) whereby brief applications of negative pressure were applied to the neck based on the R-wave (i.e. the initial upward deflection of the QRS complex in the normal electrocardiogram (ECG), representing early depolarization of the ventricles – Dorlan, 2011) of the ECG for part or all of the cardiac cycle. This approach was further developed into the phase related external suction (PRES) technique (Rau, Elbert, Geiger, & Lutzenberger, 1992) which involved precisely timed bursts of negative and positive pressure being applied to the neck via the external cuff to coincide with specific phases of the cardiac cycle, and thus enhance or counter natural baroreceptor stimulation. Maximal baroreceptor stimulation occurred when negative pressure was applied during systole and baroreceptor activation was minimised when positive pressure was applied during diastole. The PRES technique also benefited from the inclusion of a control conditions i.e. when positive pressure is applied during systole and negative pressure is applied during diastole the natural baroreceptor activity is reduced (Elbert et al., 1992). Studies employing phasic baroreceptor stimulation have also produced inconsistent findings. In some studies neck suction during systole (baroreceptor stimulation) reduced mechanical (Rau et al., 1994) and electrocutaneous pain in normotensives and hypertensives (Al'Absi et al., 2005; Angrilli et al., 1997; Brody & Rau, 1994; Droste et al., 1994; Dworkin et al., 1994;

Kardos et al., 1994; Mini, Rau, Montoya, Palomba, & Birbaumer, 1995; Rau, Schweizer, et al., 1993). However, other studies found that neither thermal pain in normotensives or hypertensives (Rau et al., 1994), nor electrocutaneous pain in normotensives (Rau, Elbert, & Birbaumber, 1995; Rau, Schweizer, et al., 1993) or hypotensives, (Angrilli et al., 1997) was modulated by baroreceptor stimulation.

To conclude artificial baroreceptor manipulation via external neck cuffs produced inconclusive evidence for baroreceptor influences on pain, which is possibly due to the limitations of artificial baroreceptor stimulation (Rau & Elbert, 2001). For example, the constant cuff method typically involved application of neck suction for several seconds whilst responses were recorded. While initial stimulation of the baroreceptors was effective, rapidly increasing their firing rate in response to the increased pressure, after approximately 500 ms the baroreceptors adapt (Eckberg, 1977) and firing declines over the subsequent few seconds (Bell, 2009) thus the effects of increased afferent output on responses beyond the initial application of pressure would be questionable. Due to the shorter periods of pressure application in the PRES technique, there would be less habituation effect (Kardos, Rau, Greenlee, Droste, & Roskamm, 1995). However, the alternating pressures exerted during the neck suction and compression may still have made the procedure aversive and distracting to participants (Rau & Elbert, 2001) and this may influence the results. Specifically, distraction has been known to reduce the contingent negative variation (CNV) amplitude (Rockstroh, Elbert, Canavan, Lutzenberger, & Birbaumer, 1989). Additionally, only the baroreceptors located in the carotid sinus in the neck are stimulated using the non-invasive neck cuff approach and thus the integrated effects of the aortic and carotid baroreceptors cannot be determined. As the aortic arch baroreceptor afferent output would continue at its natural level, this afferent activity may counter the experimental manipulations applied to the carotid sinus baroreceptors. Specifically, the application of positive pressure during systole to the carotid sinus baroreceptors, which is designed to reduce baroreceptor firing, would coincide with stimulation of the aortic arch baroreceptors which would still fire and consequently baroreceptor afferent information would still be travelling to the NTS.

1.6.4 Natural baroreceptor stimulation across the cardiac cycle studies

Within a single cardiac cycle, there is a natural fluctuation in BP, which subsequently stimulates the baroreceptors in a cyclic manner due to the systolic pressure wave, following ventricular contraction, distending the arterial walls and stimulating the baroreceptors maximally (e.g. Rowell, 1993). Psychophysiologists have taken advantage of this cyclic change in baroreceptor stimulation during the cardiac cycle to investigate the influence of arterial baroreceptors on various indices of pain. Cardiac cycle studies deliver stimuli to coincide with systole, when BP and baroreceptor activation is highest, and diastole, when BP and baroreceptor stimulation is lowest, and compare the respective responses. One significant advantage of the cardiac cycle paradigm is that due to the intrinsic nature of the paradigm the cardiac cycle allows the integrated effect of both the aortic and carotid baroreceptors to be studied. A second advantage of the cardiac cycle paradigm for studying baroreceptor influences is that the level of baroreceptor stimulation is at natural levels, as opposed to artificially elevated stimulation associated with mechanical stimulation methods. This means the findings are more applicable to the real world. Additionally, as there is no external cuff applied or pharmacological agent administered the methodology is; a) less aversive and distracting for participants, and; b) the participants have no idea which condition is being applied at which time, thus reducing any possible participant influence on the results.

1.7 The cardiac cycle paradigm for investigating baroreceptor influences

Below is a summary of the development of cardiac cycle studies. The discussion starts with studies indicating a general cortical interference associated with the cardiac cycle and progresses through studies investigating the cardiac cycle-related modulation of simple reaction times, sensory perceptions (auditory, visual & tactile), the NFR, PREPs and pain.

1.7.1 The influence of the cardiac cycle on cortical activity

To better understand how BP effects sensorimotor functioning, cardiac cycle studies sought to generate neurophysiological evidence for baroreceptor-related cortical

interference. With regards to this, natural fluctuations in BP across the cardiac cycle have been shown to inhibit CNS function. Electroencephalographic studies have reported inhibited cortical activity (Koriath & Lindholm, 1986; Koriath, Lindholm, & Landers, 1987) and lower frequency EEG oscillations measured in the alpha band (Walker & Walker, 1983).

1.7.2 Cardiac cycle-related influence on reaction time

Early studies employing the cardiac cycle paradigm to investigate the influence of natural fluctuations in BP on sensorimotor function looked at reaction time responses across the cardiac cycle. The findings from these reaction time studies were mixed (Carroll & Anastasiades, 1978). The first such reports were by Birren and colleagues (Birren, Phillips, & Cardon, 1963) who reported that reaction times were shorter during the P-wave (approx. R-wave + 700ms to R-wave + 810ms) in response to auditory stimuli compared to the other intervals investigated (QRS, T and T-P cardiac wave intervals). These preliminary findings provided tentative support for baroreceptor activity influencing sensorimotor function and thus support the VAF hypothesis, and were interpreted as interference by afferent baroreceptor inputs integrated in the medullary and cortical structures (Lacey & Lacey, 1974). In contrast, later studies found that reaction times did not vary during different phases of the cardiac cycle in response to auditory (Thompson & Botwinick, 1970; Salzman & Jaques, 1976; Jennings & Wood, 1977) stimuli.

Similar conflicting findings were reported for reaction times in response to visual stimuli. Initial reports suggested that reaction times in response to visual stimuli were slowest when presented early in the cardiac cycle (Callaway & Layne, 1964). However, a subsequent study failed to find any effect of cardiac cycle phase on reaction times to visual stimuli (Coles, Pellegrini, & Wilson, 1982).

1.7.3 Cardiac cycle-related influence on auditory and visual perception

As with reaction times, cardiac cycle signal detection studies also produced mixed results (Carroll & Anastasiades, 1978). Further support for a baroreceptor influence on sensory function was demonstrated using signal detection methods. Saxon (1970) reported that auditory stimuli were detected more accurately when presented

during the P-wave of the cardiac cycle of the ECG than during the QRS complex (approx. R-wave – 40ms to R-wave + 40ms). Supporting these provisional findings, Cohen and colleagues (Cohen, Lieb, & Rist, 1980) found that supra-threshold tones presented during systole were perceived as quieter than those presented during diastole. Additionally, Sandman (1984) reported that auditory evoked potentials were reduced during systole compared to diastole. However, some studies found that timing of stimulus presentation within the cardiac cycle had no effect on supra-threshold auditory (Delfini & Campos, 1972; Velden & Juris, 1975) sensitivity.

Similar results have been reported for visual acuity with some researchers reporting that visual stimuli were detected more accurately when presented during the P-wave of the cardiac cycle of the ECG than during the QRS complex (Requin & Brouchon, 1964; Sandman, Mccanne, Kaiser, & Diamond, 1977). Similar to auditory evoked potentials, visual evoked potentials have been reported to be reduced during systole compared to diastole (Walker & Sandman, 1982; 1979). However, continuing the inconsistent theme, other researchers failed to find any effect of the cardiac cycle on visual sensitivity (Elliott & Graf, 1972).

Although the reason for the inconclusive findings has not been fully explained (Carroll & Anastasiades, 1978), it now seems reasonable to assume that because the studies were conducted several decades ago the methods employed may not withstand current investigation standards. Specifically, small sample sizes, insufficient sampling across the cardiac cycle and primitive equipment (Carroll & Anastasiades, 1978) may explain the inconsistent findings. In response to the potential methodology limitations of early cardiac cycle studies, the influence of BP variation across the cardiac cycle received renewed interest in the 2000's when more robust methodological approaches were introduced. Recent, larger studies employing more advanced methodology have repeatedly reported that simple reaction times in responses to visual, auditory, electrocutaneous and vibrotactile stimuli are slowest for stimuli presented early in the cardiac cycle (Edwards et al., 2007; McIntyre, Ring, Hamer, & Carroll, 2007; McIntyre, Ring, Edwards, & Carroll, 2008b) and decrease in a linear manner with increasing time after the R-wave.

However, this is not always the case as Stewart and colleagues (Stewart, France, & Suhr, 2006) reported slower reaction times during systole compared to diastole.

1.7.4 Cardiac cycle effects on the nociceptive flexion reflex

Taking this research approach forward, researchers have sought to determine if the NFR is modulated by natural fluctuations in BP across the cardiac cycle as a method to study the potential role of a baroreceptor mechanism explaining the hypertensive hypoalgesia phenomena. The first study to investigate the cardiac cycle-related modulation of the NFR was Edwards and colleagues (Edwards, Ring, McIntyre, & Carroll, 2001) who reported a reduction in the NFR between 200 and 400 ms after the R-wave of the ECG, which coincided with the systolic phase of the cardiac cycle. These timings were suggested to correspond to the maximal baroreceptor input to the pain related areas of the brain (see Chapter two for a detailed discussion).

Subsequently, the NFR has been repeatedly found to be reduced, or its threshold higher during the systolic phase of the cardiac cycle (al'Absi et al., 2005; Edwards et al., 2001, 2002, 2003; McIntyre, Edwards, Ring, Parvin, & Carroll, 2006; McIntyre, Kavussanu, & Ring, 2008a) compared to diastole. These results suggest that nociceptive responding may be dampened when arterial baroreceptor activity is maximal and thus provides support for a baroreceptor mechanism modulating nociception.

1.7.5 PREPs across the cardiac cycle

Furthering the cardiac cycle-related modulation of pain indices, only one study has utilised the natural variations in BP across the cardiac cycle to investigate the cortical processing of noxious thulium-evoked laser stimuli (Edwards et al., 2008). N2 amplitudes and N2-P2 peak-to-peak amplitudes were attenuated mid cardiac cycle, corresponding to maximal baroreceptor activation, compared to early and late cardiac cycle when baroreceptor activation is lowest (Edwards et al., 2008). These results concurred with several previous artificial baroreceptor stimulation studies investigating PREP responses to noxious stimulation (Angrilli, et al., 1997; Mini et al., 1995), and to the previous cardiac cycle studies investigating NFR responses to noxious stimulation (Edwards et al., 2001; 2002; 2003; McIntyre et al., 2006; 2008a)

discussed above. Taken together, these studies suggest that stimulation of the arterial baroreceptors modulates cortical processing of noxious stimuli. However, as with previous reaction time and visual/auditory sensitivity studies not all studies have reported a cardiac cycle-related modulation of PREPs. For example, when studying the influence of the cardiac cycle on the PREP potentials associated with cued and un-cued pain stimuli, Gray and colleagues (Gray, Minati, Paoletti, Critchley, 2010) reported that following un-cued pain stimuli there was no cardiac cycle influence on PREPs. However, they did find a cardiac cycle modulation of the P2 component of the PREP following cued pain stimuli. Cued stimuli were associated with larger P2 amplitudes than un-cued stimuli, but this effect was abolished when cued stimuli were presented during baroreceptor activation (Gray et al., 2010). The authors suggest that these findings related to differences in the subjective experiences of pain for cued and un-cued stimuli, as the P2 component of the PREP is known to correlate with subjective ratings of pain intensity (Gray et al., 2010).

1.7.6 Cardiac cycle effects on pain perception

Despite the NFR (e.g. Willer, 1977) and PREPs (Miltner et al, 1987; Granovsky et al, 2008) being considered as a correlate of pain, the aforementioned studies that measured pain perception concurrently with the NFR (Edwards et al., 2001, 2002, 2003) or PREPs (Edwards et al., 2008) found pain ratings were not modulated across the cardiac cycle. However these studies were not specifically designed to investigate pain perception (Edwards et al., 2001, 2002, 2003, 2008). A recent study specifically designed to study the cardiac cycle effects on pain perception reported that pain was increased during systole (Martins, Ring, McIntyre, Edwards, & Martin, 2009). This finding contradicts with previous studies showing null effects on the modulation of pain across the cardiac cycle and with reports of systolic dampening of the NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006; 2008a) and PREPs (Edwards et al., 2008). However, differences in methodological design may help explain these unexpected results. Specifically, the bar electrode used by Martins et al., (2009) may not have selectively stimulated A-δ nociceptive fibres. As a consequence, the pattern of modulation may not reflect a specific nociceptive effect (Martins et al., 2009). Indeed, a recent study found that cutaneous sensory thresholds were lower during systole than diastole (Edwards, Ring, McIntyre, Winer,

& Martin, 2009). Second, Martins et al. (2009) included higher stimulation intensities (i.e., up to pain tolerance) than previous studies (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006; 2008a), this may have induced physiological arousal which has been shown to influence cardiac cycle-related NFR modulation (McIntyre et al., 2006). Notwithstanding the unexpected pattern of modulation, the findings of Martins et al. (2009) suggest pain perception may be modulated across the cardiac cycle.

1.7.7 Baroreceptor influence on tactile perception

The discussions above provide strong evidence for a widespread cardiac cycle-related modulation of various different sensory processes, and thus it would be reasonable to assume that tactile somatosensory processing may also be modulated across the cardiac cycle. The hypothesised global baroreceptor modulation of sensation is further supported by the early animal neurophysiological research discussed earlier, that reported a sedative effect of baroreceptor stimulation in dogs (Koch, 1932), and an inhibition of cortical activity when baroreceptor afferents, which project to the nucleus tractus solitarius, were stimulated (e.g., Bonvallet & Allen, 1963; Bonvallet & Bloch, 1961; Bonvallet et al., 1954).

In relation to tactile somatosensory sensitivity recent work by Edwards and colleagues (2009) reported a reduction in cutaneous sensory thresholds during systole compared to diastole, indicating that in contrast to pain, cutaneous sensitivity was heightened during baroreceptor activation. These findings provide further evidence of a global baroreceptor influence on the modulation of sensations, but that the pattern of modulation may be specific to each sensory modality, rather than baroreceptor activation inducing a global diminution of sensations. However, as this is the only study specifically investigating the cardiac cycle-related modulation of tactile somatosensory processing further studies are required to substantiate the findings.

1.7.8 The influence of tonic blood pressure on the cardiac cycle of pain

In specific relation to the cardiac cycle modulation of pain, according to Elbert et al. (1988) one of the most influential variables determining the degree of pain inhibition through baroreceptor activation is tonic BP. In PRES studies, during mechanical

baroreceptor stimulation, participants with BP towards the higher end of the normotensive range have demonstrated reduced pain sensitivity (Angrilli et al., 1997; Elbert et al., 1988) and PREPs (Angrilli et al., 1997; Brody et al., 1997), whereas individuals with lower normal BP showed no such effects (Angrilli et al., 1997). Furthermore, Edwards et al., (2009) reported that individuals with higher diastolic BP had larger reductions in tactile sensory thresholds during systole compared to diastole than those with lower BP. When taken together with the hypertension hypoalgesia studies discussed earlier, the finding that tonic BP influences the cardiac cycle-related modulation of pain and tactile sensation adds further support to a role of baroreceptors in explaining the hypertensive hypoalgesia phenomenon and that baroreceptor influences extend to a range of sensory modalities.

To conclude, there is good evidence to suggest a cardiac cycle-related modulation of sensorimotor function, sensory perception and neurophysiological functions, indicating a strong baroreceptor influence on cortical processing (e.g. Rau & Elbert, 2001). These findings provide support for the VAF hypothesis (Lacey & Lacey, 1974), whereby the cardiovascular information transmission appears to interfere with cortical processing. It appears that this influence is evident in a range of sensory modalities i.e. auditory, visual, pain and tactile sensations, with the pattern of modulation varying with sensory modality, presumably indicating differences in the different processing pathways.

1.8 Pain & baroreceptor lateralisation

As discussed in the cerebral representation of pain section above (pages 11-12), there is good evidence that the cardiac cycle-related modulation of pain is more than a low-level gating phenomenon, with several cerebral structures having been identified as being sites of overlap for baroreceptor and pain processing. These areas include the anterior insular and ACC (Craig, 2002; Critchley, 2005; Gianaros, Jennings, Sheu, Derbyshire, & Matthews, 2007), the periaqueductal grey (PAG) matter, amygdala and insula (Gray et al., 2009). However, there is evidence to suggest that both pain (e.g. Symonds et al., 2006) and baroreceptor (Critchley, Corfield, Chandler, Mathias, & Dolan, 2000; Henderson et al., 2004; Weisz et al., 2001) processing may be lateralised in the brain with a right hemisphere dominance

proposed. Perhaps surprisingly, there has been very little research investigating the possibility of lateralisation in specific relation to the cardiac cycle-related modulation of pain.

1.8.1 Lateralisation of baroreceptor afferent information

Research indicates that a major location for integrating baroreceptor information in the brain is the insular cortex (Zhang, Dougherty, & Oppenheimer, 1998; Saleh & Connell, 1998; Oppenheimer, 2001). There is strong evidence to suggest that baroreceptor processing has a right hemispheric bias. In rats, microelectrode sampling indicates a significantly greater number of baroreceptor sensitive cells in the right posterior insula than other insula areas (Zhang & Oppenheimer, 1997; Zhang, Tang, Yuan, & Jia, 1997). Similarly, in monkeys, greater numbers of baroreceptor units were identified in the right anterior and mid-insula (Zhang et al., 1998). Studies employing fMRI have provided further evidence for a right lateralisation of baroreceptor processing with greater activity being found in the right side of the insula cortex compared to the left. In cats, Henderson and colleagues highlighted a right-hemispheric dominance of baroreceptor processing in the insula (Henderson et al., 2004). In humans, positron emission tomography (PET) studies have also indicated greater activity in the right anterior cingulate and right insula in response to changes in BP via exercise and mental arithmetic tasks (Critchley et al., 2000). Similarly, Weisz and colleagues (2001) reported increased regional cerebral blood flow in the right anterior-inferior prefrontal cortex following external neck suction to stimulate the carotid sinus baroreceptors. Although the majority of findings indicate a predominately right sided baroreceptor processing, it should be noted that other studies have suggested a left hemisphere dominance in relation to baroreflex sensitivity (Hilz et al., 2001; Sykora, Diedler, Rupp, Turcani, Steiner, 2009).

1.8.2 Lateralisation of pain processing

It has traditionally been thought that processing of somatosensory stimulation is processed contralateral to the side of stimulation i.e. stimulation on one side of the body is largely processed in brain areas in the opposite side (Willis & Westlund, 1997). For example a recent PET study further supports this idea, with reports that cerebral blood flow was increased following painful contact thermal stimulation of

both the left and right arms in contralateral regions of the SI, SII, insular cortex and bilateral regions of the cerebellum, putamen, thalamus, ACC, and frontal operculum regardless of side of stimulation (Coghill, Gilron, & Iadarola, 2001). Additionally, several further studies in primates and humans have identified the SI, SII ACC and insula to be activated contralateral to the side of pain stimulation (see Chapter 5 for further details & Peyron, Laurent et al., 2000 for review).

As with baroreceptor processing, there is evidence suggesting that pain processing may also be lateralisated with a right hemisphere dominance. In specific relation to the lateralisation of electrical pain stimulation, Symonds and colleagues (Symonds, Nakia, Bixby, & Mande, 2006) utilised fMRI to investigate lateralisation effects following electrocutaneous stimulation of the right and left index fingers. They reported that the SII and posterior insula were activated contralateral to the pain stimuli, whereas the mid/posterior insula, anterior insula, and posterior cingulate were activated bilaterally. Additionally, the middle frontal gyrus, anterior cingulate, inferior frontal gyrus, medial/superior frontal gyri, and inferior parietal lobule showed either an exclusive or strong lateralisation to the right hemisphere (Symonds et al., 2006). In addition, Symonds et al. (2006) also reported that activity in the right somatosensory cortex, during left hand stimulation was greater than activity seen in the left hemisphere during right hand stimulation. Similarly, right anterior cingulate activity during left hand stimulation was significantly greater than right hand stimulation. Furthermore, Brooks and colleagues (Brooks, Nurmikko, Bimson, Singh, & Roberts, 2002) reported a right lateralisation in the anterior insula when pain was attended to, and in the ACC regardless of attentional focus, whereas activity in the posterior insula was found to be contralateral to the stimulus and no significant activation in response to painful stimulation was detected in SI or the thalamus following painful thermal stimulation of both the right and left hands.

As evidenced by the Symonds et al (2006) and Brooks et al. (2002) studies, a right sided processing bias is not evident in all pain processing areas and several further studies have reported no lateralisation effects for certain pain processing areas. Utilising fMRI, studies have reported bilateral responses within the SI, SII and insula but with a significantly greater contralateral response in SI and the thalamus in

response to painful laser stimulation applied to the right and left hands (Bingel et al., 2003) and right and left lower legs (Youell et al., 2004). Furthermore, Youell and colleagues (2004) reported an increase in left insula activity following lower leg stimulation.

1.8.3 Lateralisation of cardiac cycle-related modulation

Based on the proposed lateralised processing of baroreceptor afferents there have been a few studies that have sought to determine if cardiac cycle modulation is also lateralisated. Visual evoked potentials recorded in the right hemisphere were found to be largest during the diastolic phase of the cardiac cycle, whereas the visual evoked potentials recorded from the left hemisphere were unaffected by the phase of the cardiac cycle (Walker & Sandman, 1982). This suggests that baroreceptor activation (systole) appears to impact the processing of visual input in the right hemisphere to a greater extent than the left (Walker & Sandman, 1982). Reporting similar findings, Schulz and colleagues (2009) found that the systolic dampening of startle eye blink was only present following left ear presentation. This result suggests that the cardiac cycle modulation of startle eye blink maybe right hemispheric dominant reflecting the right brain advantage in relaying viscero afferent and baroreceptor afferent information. In contrast, utilising a visual reaction time task, Weisz and Adam (1996) found that when right stimuli were presented or responses were made with the right hand, reaction time was marginally longer during systole than diastole, whereas there was no difference for central and left stimuli or for left hand responses. These findings suggest that, sensorimotor functions processed in the left cerebral hemisphere may be influenced more by cardiac cycle changes than those of the right hemisphere (Weisz & Adam, 1996).

Taken together the discussions regarding lateralisation indicate that baroreceptor processing appears to be right-hemispherically biased and certain pain processing areas, such as the ACC, SII and insula tend to show contralateral or right hemisphere dominance. Thus, it may be suggested that maximal convergence of the baroreceptor and pain processing input would occur following left hand stimulation, when nociceptive afferent information would activate pain areas including the ACC, SII and insula with greater activation evident in the right hemisphere. Whereas right

hand noxious stimulation would lead to greatest activity in the left hemisphere for brain areas that are stimulated contralaterally (e.g. SII) and reduced activity in the right ACC thus convergence with the baroreceptor input would be reduced.

1.9 Overview of thesis

The four studies presented in this thesis were designed to further study the cardiac cycle modulation of pain and tactile stimuli. The first study aimed to further investigate the proposed cardiac cycle-related modulation of pain perception. Specifically the first two studies (Chapters 2 & 3) investigated the cardiac cycle-related modulation of pain and tactile sensory thresholds.

Study one (Chapter 2) re-investigated the findings of Martins et al. (2009) who were the first researchers to specifically study the cardiac cycle modulation of pain perception and reported, unexpectedly, that pain ratings were elevated during systole compared to diastole which was contra to the majority of previous research indicating a systolic dampening of the NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006; 2008a) and laser evoked PREPs (Edwards et al., 2008). Study one employed different methodology to address several potential reasons for the unexpected findings.

The second study (Chapter 3) sought to further the findings of Edwards et al. (2009) who were the first to report a cardiac cycle-related modulation of cutaneous sensory thresholds, indicating heighted tactile sensitivity during baroreceptor activation. Specifically, study two aimed to determine if this pattern of modulation was evident in a sample of normotensives as the sample studied by Edwards et al. (2009) included newly diagnosed, unmedicated hypertensives who may have biased the results. The study also sought to increase the resolution of the temporal patterning of the cardiac cycle modulation of tactile sensory thresholds by presenting stimuli at seven intervals across the cardiac cycle, rather than the three that Edwards et al. (2009) used.

The final two studies in the thesis (Chapters 4 & 5) built on the results of the first study and sought to further the understanding the mechanisms underlying the

cardiac cycle-related modulation of pain. Both studies employed EEG methodology to study the influence of the cardiac cycle on electrocutaneous PREPs.

Study three (Chapter 4) was designed to investigate if electrocutaneous PREPs were modulated across the cardiac cycle. Previous studies have indicated that laser evoked N2 and N2-P2 peak-to-peak amplitudes were attenuated during systole compared to diastole (Edwards et al., 2008) as was P2 following cued electrocutaneous stimulation (Gray et al., 2010). The study sought to increase the resolution of the cardiac cycle related modulation of electrocutaneous PREPs reported by Gray et al. (2009) by delivering stimuli at 7 intervals across the cardiac cycle compared to just 2 studied by Gray et al. (2009). The study also sought to determine if the cardiac cycle-related modulation identified by Edwards et al. (2008) at a single midline scalp electrode (Cz) extended beyond this region into brain areas identified by fMRI and PET imaging studies as potential sites of interaction for the baroreceptor and pain afferents. Specifically, we used a multi-channel EEG system with a focus on analysis of data obtained from scalp electrode sites Cz, C3 and C4 which were identified as overlaying the brain areas associated with pain perception and baroreceptor processing i.e. the ACC, SII, insular cortex bilaterally (Bentley, Derbyshire, Youell, & Jones, 2003; Bromm & Chen, 1995; Garcia-Larrea, Frot, & Valeriani, 2003; Ohara, Crone, Weiss, & Lenz, 2006; Tarkka & Treede, 1993; Treede, Lorenz, & Baumgartner, 2003; Valeriani et al., 1996) and the contralateral SI (Kakigi et al., 2005) and were also the same electrode sites studied by Gray et al. (2009) therefore improving the ability to compare findings between the two studies.

Study four (Chapter 5) was designed to investigate if the cardiac cycle modulation of PREPs may be a lateralised phenomenon. The study rationale was based on the discussion above regarding a right hemispheric dominance for baroreceptor processing and a right or contralateral bias for pain processing. If we accept a potentially greater cardiac cycle modulation in the right hemisphere, right hand pain stimulation may activate the right ACC to a lesser extent and a contralateral activation of SII would be predominately in the left hemisphere. Therefore, there would be less convergence of the pain and baroreceptor inputs in the right hemisphere and thus, it is hypothesised that cardiac cycle related

modulation of PREPs would be less evident following painful stimulation delivered to the right hand than the left hand. Although Edwards et al. (2008) reported modulation of laser evoked potentials elicited from the right hand, as did Gray et al. (2009) following electrocutaneous stimulation, but using a different type of electrode to the concentric planar electrode used in the studies in this thesis, we propose that differing stimulation modalities may also be an important consideration.

REFERENCES

Aicher, S. A., & Randich, A. (1990). Antinociception and cardiovascular responses produced by electrical stimulation in the nucleus tractus solitarius, nucleus reticularis ventralis, and the caudal medulla. *Pain, 42*(1), 103-119.

Al'Absi, M., France, C. R., Ring, C., France, J., Harju, A., McIntyre, D., & Wittmers, L. E. (2005). Nociception and baroreceptor stimulation in hypertension-prone men and women. *Psychophysiology, 42*(1), 83-91. doi:10.1111/j.1469-8986.2005.00257.x ER

al'Absi, M., Buchanan, T., & Lovallo, W. R. (1996). Pain perception and cardiovascular responses in men with positive parental history for hypertension. *Psychophysiology, 33*(6), 655-661.

al'Absi, M., Petersen, K. L., & Wittmers, L. E. (2000). Blood pressure but not parental history for hypertension predicts pain perception in women. *Pain, 88*(1), 61-68.

Almeida, T. F., Roizenblatt, S., & Tufik, S. (2004). Afferent pain pathways: A neuroanatomical review. *Brain Research, 1000*(1–2), 40-56. doi:10.1016/j.brainres.2003.10.073

Angell James, J. E. (1971). Effects of altering mean pressure, pulse pressure and pulse frequency on impulse activity in baroreceptor fibres from aortic arch and right subclavian artery in rabbit. *Journal of Physiology-London, 214*(1), 65-88.

Angell James, J. E., & Lumley, J. S. P. (1974). Effects of carotid endarterectomy on mechanical-properties of carotid-sinus and carotid-sinus nerve activity in atherosclerotic patients. *British Journal of Surgery, 61*(10), 805-810.

Angrilli, A., Mini, A., Mucha, R. F., & Rau, H. (1997). The influence of low blood pressure and baroreceptor activity on pain responses. *Physiology & Behaviour, 62*(2), 391-397.

Apkarian, A. V., Bushnell, M. C., Treede, R. D., & Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain (London, England), 9*(4), 463-484. doi:10.1016/j.ejpain.2004.11.001

Arendt-Nielsen, L., & Chen, A. C. (2003). Lasers and other thermal stimulators for activation of skin nociceptors in humans. *Clinical Neurophysiology, 33*(6), 259-268.

Bandler, R., Carrive, P., & Zhang, S. P. (1991). Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: Viscerotopic, somatotopic and functional organization. *Progress in Brain Research, 87*, 269-305.

Bartorelli, C., Bizzi, E., Libretti, A., & Zanchelti, A. (1960). Inhibitory control of sinocarotid pressoceptive afferents on hypothalamic autonomic activity and sham rage behaviour. *Archives Italiennes de Biologie,* 98, 308-326.

Becker, D. E., Haley, D. W., Urena, V. M., & Yingling, C. D. (2000). Pain measurement with evoked potentials: Combination of subjective ratings, randomized intensities, and long interstimulus intervals produces a P300-like confound. *Pain, 84*(1), 37-47.

Bell, D. R. (2009). Control Mechanisms in Circulatory Function. In R. A. Rhoades & D. R. Bell D.R. (Eds.), *Medical Physiology: Principles for clinical medicine 3rd Edition.* Philadelphia: Lippincott Williams & Wilkins.

Benarroch, E. E. (2008). The arterial baroreflex: Functional organization and involvement in neurologic disease. *Neurology, 71*(21), 1733-1738. doi:10.1212/01.wnl.0000335246.93495.92

Bentley, D. E., Derbyshire, S. W. G., Youell, P. D., & Jones, A. K. P. (2003). Caudal cingulate cortex involvement in pain processing: An inter-individual laser evoked

potential source localisation study using realistic head models. *Pain, 102*(3), 265-271. doi:10.1016/S0304-3959(02)00405-0 ER

Bingel, U., Quante, M., Knab, R., Bromm, B., Weiller, C., & Buchel, C. (2003). Single trial fMRI reveals significant contralateral bias in responses to laser pain within thalamus and somatosensory cortices. *Neuroimage, 18*(3), 740-748.

Birren, J. E., Phillips, S. L., & Cardon, P. V. (1963). Reaction time as a function of cardiac cycle in young adults. *Science, 140*(356), 195-196.

Bonvallet, M., Dell, P., & Hiebel, G. (1954). Tonus sympathique et activite electrique corticale. *Electroencephalography and Clinical Neurophysiology, 6*(1), 119-144.

Bonvallet, M., & Allen, M. B., Jr. (1963). Prolonged spontaneous and evoked reticular activation following discrete bulbar lesions. *Electroencephalography and Clinical Neurophysiology, 15*, 969-988.

Bonvallet, M., & Bloch, V. (1961). Bulbar control of cortical arousal. *Science (New York, N.Y.), 133*(3459), 1133-1134. doi:10.1126/science.133.3459.1133

Bradley, D. J., Paton, J. F., & Spyer, K. M. (1987). Cardiovascular responses evoked from the fastigial region of the cerebellum in anaesthetized and decerebrate rabbits. *The Journal of Physiology, 392*, 475-491.

Branch, M. A., Carlson, C. R., & Okeson, J. P. (2000). Influence of biased clinician statements on patient report of referred pain. *Journal of Orofacial Pain, 14*(2), 120-127.

Brody, S., Angrilli, A., Weiss, U., Birbaumer, N., Mini, A., Veit, R., & Rau, H. (1997). Somatotosensory evoked potentials during baroreceptor stimulation in chronic low back pain patients and normal controls. *International Journal of Psychophysiology, 25*(3), 201-210.

Brody, S., & Rau, H. (1994). Behavioral and psychophysiological predictors of self-monitored 19 month blood pressure change in normotensives. *Journal of Psychosomatic Research, 38*(8), 885-891.

Bromm, B. (1984). Pain-related components in the cerebral potential: experimental and multivariate statistical approaches. In B. Bromm (Ed.), *Pain measurement in man: Neurophysiological correlates of pain,* (pp. 257-290). Elsevier: Amsterdam.

Bromm, B., & Chen, A. C. (1995). Brain electrical source analysis of laser evoked potentials in response to painful trigeminal nerve stimulation. *Electroencephalography and Clinical Neurophysiology, 95*(1), 14-26.

Bromm, B., & Treede, R. D. (1987). Human cerebral potentials evoked by CO2 laser stimuli causing pain. *Experimental Brain Research, 67*(1), 153-162.

Bromm, B., & Treede, R. D. (1991). Laser-evoked cerebral potentials in the assessment of cutaneous pain sensitivity in normal subjects and patients. *Revue Neurologique, 147*(10), 625-643.

Brooks, J., & Tracey, I. (2005). From nociception to pain perception: Imaging the spinal and supraspinal pathways. *Journal of Anatomy, 207*(1), 19-33. doi:10.1111/j.1469-7580.2005.00428.x

Brooks, J. C., Nurmikko, T. J., Bimson, W. E., Singh, K. D., & Roberts, N. (2002). fMRI of thermal pain: Effects of stimulus laterality and attention. *Neuroimage, 15*(2), 293-301. doi:10.1006/nimg.2001.0974

Bruehl, S., Burns, J. W., Chung, O. Y., Magid, E., Chont, M., Gilliam, W., . . . Cairl, H. (2010). Hypoalgesia associated with elevated resting blood pressure: Evidence for endogenous opioid involvement. *Journal of Behavioral Medicine, 33*(2), 168-176. doi:10.1007/s10865-009-9241-4

Bruehl, S., Carlson, C. R., & Mccubbin, J. A. (1992). The relationship between pain sensitivity and blood-pressure in normotensives. *Pain, 48*(3), 463-467.

Bruehl, S., Chung, O. Y., Diedrich, L., Diedrich, A., & Robertson, D. (2008). The relationship between resting blood pressure and acute pain sensitivity: Effects of chronic pain and alpha-2 adrenergic blockade. *Journal of Behavioral Medicine, 31*(1), 71-80. doi:10.1007/s10865-007-9133-4

Bruehl, S., Chung, O. Y., Ward, P., Johnson, B., & McCubbin, J. A. (2002). The relationship between resting blood pressure and acute pain sensitivity in healthy normotensives and chronic back pain sufferers: The effects of opioid blockade. *Pain, 100*(1-2), 191-201.

Bushnell M, C., & Apkarian, A, V. (2006). Representation of pain in the brain. In S. McMahon B, & M. Koltzenburg (Eds.), (5th ed., pp. 107-124). Philadelphia: Elsevier.

Butcher, K. S., & Cechetto, D. F. (1995). Autonomic responses of the insular cortex in hypertensive and normotensive rats. *The American Journal of Physiology, 268*(1 Pt 2), R214-22.

Callaway, E., & Layne, R. S. (1964). Interaction between visual evoked response & 2 spontaneous biological rhythms - EEG alpha cycle & cardiac arousal cycle. *Annals of the New York Academy of Sciences, 112*(A1), 421-431.

Campbell, T. S., & Ditto, B. (2002). Exaggeration of blood pressure-related hypoalgesia and reduction of blood pressure with low frequency transcutaneous electrical nerve stimulation. *Psychophysiology, 39*(4), 473-481. doi:10.1017.S0048577202000847

Carmon, A., Dotan, Y., & Sarne, Y. (1978). Correlation of subjective pain experience with cerebral evoked responses to noxious thermal stimulations. *Experimental Brain Research, 33*(3-4), 445-453.

Carmon, A., Mor, J., & Goldberg, J. (1976). Evoked cerebral responses to noxious thermal stimuli in humans. *Experimental Brain Research, 25*(1), 103-107.

Carroll, D., & Anastasiades, P. (1978). Behavioral significance of heart-rate - laceys hypothesis. *Biological Psychology, 7*(4), 249-275.

Cechetto, D. F., & Calaresu, F. R. (1983). Response of single units in the amygdala to stimulation of buffer nerves in cat. *The American Journal of Physiology, 244*(5), R646-51.

Cechetto, D. F., & Calaresu, F. R. (1984). Units in the amygdala responding to activation of carotid baro- and chemoreceptors. *The American Journal of Physiology, 246*(5 Pt 2), R832-6.

Cechetto, D. F., & Calaresu, F. R. (1985). Central pathways relaying cardiovascular afferent information to amygdala. *The American Journal of Physiology, 248*(1 Pt 2), R38-45.

Chen, A. C., Chapman, C. R., & Harkins, S. W. (1979). Brain evoked potentials are functional correlates of induced pain in man. *Pain, 6*(3), 365-374.

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., Jr, . . . National High Blood Pressure Education Program Coordinating Committee. (2003). Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension, 42*(6), 1206-1252. doi:10.1161/01.HYP.0000107251.49515.c2

Clark, W. C., Yang, J. C., Tsui, S. L., Ng, K. F., & Bennett Clark, S. (2002). Unidimensional pain rating scales: A multidimensional affect and pain survey (MAPS) analysis of what they really measure. *Pain, 98*(3), 241-247.

Coghill, R. C., Gilron, I., & Iadarola, M. J. (2001). Hemispheric lateralization of somatosensory processing. Journal of Neurophysiology, 85(6), 2602-2612.

Coghill, R. C., Talbot, J. D., Evans, A. C., Meyer, E., Gjedde, A., Bushnell, M. C., & Duncan, G. H. (1994). Distributed processing of pain and vibration by the human brain. *The Journal of Neuroscience, 14*(7), 4095-4108.

Cohen, R., Lieb, H., & Rist, F. (1980). Loudness judgments, evoked potentials, and reaction time to acoustic stimuli early and late in the cardiac cycle in chronic schizophrenics. *Psychiatry Research, 3*(1), 23-29.

Coleridge, H. M., Coleridge, J. C. G., & Schultz, H. D. (1987). Characteristics of C-fiber baroreceptors in the carotid-sinus of dogs. *Journal of Physiology-London, 394*, 291-313.

Coles, M. G. H., Pellegrini, A. M., & Wilson, G. V. (1982). The cardiac cycle time effect - influence of respiration phase and information-processing requirements. *Psychophysiology, 19*(6), 648-657.

Craig, A. D. (2002). Pain mechanisms: Labeled lines versus convergence in central processing. *Annual Review of Neuroscience, 26*, 1-30.

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *The Journal of Comparative Neurology, 493*(1), 154-166. doi:10.1002/cne.20749

Critchley, H. D., Corfield, D. R., Chandler, M. P., Mathias, C. J., & Dolan, R. J. (2000). Cerebral correlates of autonomic cardiovascular arousal: A functional

neuroimaging investigation in humans. *The Journal of Physiology, 523 Pt 1*, 259-270.

Cruccu, G., & Garcia-Larrea, L. (2004). Clinical utility of pain-laser evoked potentials. *Supplements to Clinical Neurophysiology, 57*, 101-110.

D'Antono, B., Ditto, B., Rios, N., & Moskowitz, D. S. (1999). Risk for hypertension and diminished pain sensitivity in women: Autonomic and daily correlates. *International Journal of Psychophysiology, 31*(2), 175-187.

Davis, K. D., Pope, G. E., Crawley, A. P., & Mikulis, D. J. (2002). Neural correlates of prickle sensation: A percept-related fMRI study. *Nature Neuroscience, 5*(11), 1121-1122. doi:10.1038/nn955

De Broucker, T., & Willer, J. C. (1985). Comparative study of the nociceptive reflex and late components of the evoked somatosensory potential during stimulation of the sural nerve in healthy subjects. *Revue d'Electroencephalographie Et De Neurophysiologie Clinique, 15*(2), 149-153.

de Tommaso, M., Santostasi, R., Devitofrancesco, V., Franco, G., Vecchio, E., Delussi, M., . . . Katzarava, Z. (2011). A comparative study of cortical responses evoked by transcutaneous electrical vs CO(2) laser stimulation. *Clinical Neurophysiology, 122*(12), 2482-2487. doi:10.1016/j.clinph.2011.05.006

Delfini, L. F., & Campos, J. J. (1972). Signal detection and cardiac arousal cycle. *Psychophysiology, 9*(5), 484-491.

Dembrowsky, K., & Seller, H. (1995). Arterial baroreceptor reflexes. In D. Vaitl, & R. Schandry (Eds.), *From the heart to the brain: The psychophysiology of circulation brain interaction* (pp. 35--60). Frankfurt: Peter Lang.

Descartes, R. (1644). Lectures on the History of Physiology During the 16th, 17th and 18th Centuries. Cambridge: Cambridge University Press: L'Homme.

Devinsky, O, & D'Esposito, M. (2004). *Neurology of Cognitive and Behavioral Disorders*. Oxford University Press: New York.

Ditto, B., D'Antono, B., Dupuis, G., & Burelle, D. (2007). Chest pain is inversely associated with blood pressure during exercise among individuals being assessed for coronary heart disease. *Psychophysiology, 44*(2), 183-188. doi:10.1111/j.1469-8986.2007.00494.x

Ditto, B., Seguin, J. R., Boulerice, B., Pihl, R. O., & Tremblay, R. E. (1998). Risk for hypertension and pain sensitivity in adolescent boys. *Health Psychology, 17*(3), 249-254.

Dowman, R. (1994). SEP topographies elicited by innocuous and noxious sural nerve stimulation. I. identification of stable periods and individual differences. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section, 92*(4), 291-302. doi:10.1016/0168-5597(94)90097-3

Droste, C., Kardos, A., Brody, S., Greenlee, M. W., Roskamm, H., & Rau, H. (1994). Baroreceptor stimulation - pain perception and sensory thresholds. *Biological Psychology, 37*(2), 101-113.

Duschek, S., & Schandry, R. (2006). Reduced drive and impaired cerebral performance: A new perspective on chronically low blood pressure. *Deutsche Medizinische Wochenschrift (1946), 131*(6), 272-277. doi:10.1055/s-2006-924962

Dworkin, B. R., Elbert, T., Rau, H., Birbaumer, N., Pauli, P., Droste, C., & Brunia, C. H. M. (1994). Central effects of baroreceptor activation in humans - attenuation

of skeletal reflexes and pain perception. *Proceedings of the National Academy of Sciences of the United States of America, 91*(14), 6329-6333.

Dworkin, B. R., Filewich, R. J., Miller, N. E., Craigmyle, N., & Pickering, T. G. (1979). Baroreceptor activation reduces reactivity to noxious-stimulation - implications for hypertension. *Science, 205*(4412), 1299-1301.

Eckberg, D. L., & Sleight, P. (1992). *Human baroreflexes in health and disease*. Oxford: Clarendon Press.

Eckberg, D. L. (1977). Baroreflex inhibition of the human sinus node: Importance of stimulus intensity, duration, and rate of pressure change. *The Journal of Physiology, 269*(3), 561-577.

Eckberg, D. L., Cavanaugh, M. S., Mark, A. L., & Abboud, F. M. (1975). A simplified neck suction device for activation of carotid baroreceptors. *The Journal of Laboratory and Clinical Medicine, 85*(1), 167-173.

Edwards, L., Inui, K., Ring, C., Wang, X. H., & Kakigi, R. (2008). Pain-related evoked potentials are modulated across the cardiac cycle. *Pain, 137*(3), 488-494. doi:10.1016/j.pain.2007.10.010 ER

Edwards, L., McIntyre, D., Carroll, D., Ring, C., France, C. R., & Martin, U. (2003). Effects of artificial and natural baroreceptor stimulation on nociceptive responding and pain. *Psychophysiology, 40*(5), 762-769.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology, 39*(5), 678-681. doi:10.1017/S0048577202011770 ER

Edwards, L., Ring, C., McIntyre, D., & Carroll, D. (2001). Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology, 38*(4), 712-718.

Edwards, L., Ring, C., McIntyre, D., Carroll, D., & Martin, U. (2007). Psychomotor speed in hypertension: Effects of reaction time components, stimulus modality, and phase of the cardiac cycle. *Psychophysiology, 44*(3), 459-468. doi:10.1111/j.1469-8986.2007.00521.x ER

Edwards, L., Ring, C., McIntyre, D., Winer, J. B., & Martin, U. (2009). Sensory detection thresholds are modulated across the cardiac cycle: Evidence that cutaneous sensibility is greatest for systolic stimulation. *Psychophysiology, 46*(2), 252-256. doi:10.1111/j.1469-8986.2008.00769.x ER

Elbert, T., & Rau, H. (1995). What goes up (from heart to brain) must come down (from brain to heart)! studies on the interaction between baroreceptor activity and cortical excitability. In D. Vaitl, & R. Schandry (Eds.), *From the heart to the brain: The psychophysiology of circulation-brain interaction* (pp. 133--150). Frankfurt: Peter Lang.

Elbert, T., Rockstroh, B., Lutzenberger, W., Kessler, M., Pietrowsky, R., & Birbaumer, N. (1988). Baroreceptor stimulation alters pain sensation depending on tonic blood-pressure. *Psychophysiology, 25*(1), 25-29.

Elliott, R., & Graf, V. (1972). Visual sensitivity as a function of phase of cardiac cycle. *Psychophysiology, 9*(3), 357-361.

Fila, M., & Bogucki, A. (2009). Laser evoked potentials as a method of evaluating the function of small fibres - application technique and clinical implications. *Neurologia i Neurochirurgia Polska, 43*(4), 368-381.

Fillingim, R. B., & Maixner, W. (1996). The influence of resting blood pressure and gender on pain responses. *Psychosomatic Medicine, 58*(4), 326-332.

Fillingim, R. B., Maixner, W., Bunting, S., & Silva, S. (1998). Resting blood pressure and thermal pain responses among females: Effects on pain unpleasantness but not pain intensity. *International Journal of Psychophysiology, 30*(3), 313-318.

France, C., Ditto, B., & Adler, P. (1991). Pain sensitivity in offspring of hypertensives at rest and during baroreflex stimulation. *Journal of Behavioral Medicine, 14*(5), 513-525.

France, C. R. (1999). Decreased pain perception and risk for hypertension: Considering a common physiological mechanism. *Psychophysiology, 36*(6), 683-692.

France, C. R., & Ditto, B. (1996). Risk for high blood pressure and decreased pain perception. *Current Directions in Psychological Science, 5*(4), 120-125.

France, C. R., & Stewart, K. M. (1995). Parental history of hypertension and enhanced cardiovascular reactivity are associated with decreased pain ratings. *Psychophysiology, 32*(6), 571-578.

Garcia-Larrea, L. (2012). Objective pain diagnostics: Clinical neurophysiology. *Clinical Neurophysiology, 42*(4), 187-197. doi:10.1016/j.neucli.2012.03.001

Garcia-Larrea, L., Frot, M., & Valeriani, M. (2003). Brain generators of laser-evoked potentials: From dipoles to functional significance. *Clinical Neurophysiology, 33*(6), 279-292.

Garcia-Larrea, L., Peyron, R., Laurent, B., & Mauguiere, F. (1997). Association and dissociation between laser-evoked potentials and pain perception. *Neuroreport, 8*(17), 3785-3789.

Gelsema, A. J., Agarwal, S. K., & Calaresu, F. R. (1989). Cardiovascular responses and changes in neural activity in the rostral ventrolateral medulla elicited by electrical stimulation of the amygdala of the rat. *Journal of the Autonomic Nervous System, 27*(2), 91-99. doi:10.1016/0165-1838(89)90091-X

Ghione, S. (1996). Hypertension-associated hypoalgesia - evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension, 28*(3), 494-504.

Ghione, S., Rosa, C., Mezzasalma, L., & Panattoni, E. (1988). Arterial hypertension is associated with hypoalgesia in humans. *Hypertension, 12*(5), 491-497.

Gianaros, P. J., Jennings, J. R., Sheu, L. K., Derbyshire, S. W., & Matthews, K. A. (2007). Heightened functional neural activation to psychological stress covaries with exaggerated blood pressure reactivity. *Hypertension, 49*(1), 134-140. doi:10.1161/01.HYP.0000250984.14992.64

Gracely, R. H. (2006). Studies of pain in human subjects. In S. McMahon B, & M. Koltzenburg (Eds.), *Wall and Melzack's textbook of pain* (5th ed., pp. 267-290). Philadelphia: Elsevier.

Granovsky, Y., Granot, M., Nir, R., & Yarnitsky, D. (2008). Objective correlate of subjective pain perception by contact heat-evoked potentials. *The Journal of Pain, 9*(1), 53-63. doi:10.1016/j.jpain.2007.08.010

Gray, M. A., Minati, L., Paoletti, G., & Critchley, H. D. (2010). Baroreceptor activation attenuates attentional effects on pain-evoked potentials. *Pain, 151*(3), 853-861. doi:10.1016/j.pain.2010.09.028

Gray, M. A., Rylander, K., Harrison, N. A., Wallin, B. G., & Critchley, H. D. (2009). Following one's heart: Cardiac rhythms gate central initiation of sympathetic

reflexes. *The Journal of, 29*(6), 1817-1825. doi:10.1523/JNEUROSCI.3363-08.2009

Greenspan, J. D., & Winfield, J. A. (1992). Reversible pain and tactile deficits associated with a cerebral tumor compressing the posterior insula and parietal operculum. *Pain, 50*(1), 29-39.

Guasti, L., Cattaneo, R., Daneri, A., Bianchi, L., Gaudio, G., Regazzi, M. B., . . . Venco, A. (1996). Endogenous beta-endorphins in hypertension: Correlation with 24-hour ambulatory blood pressure. *Journal of the American College of Cardiology, 28*(5), 1243-1248. doi:10.1016/S0735-1097(96)00312-9

Guasti, L., Cattaneo, R., Rinaldi, O., Rossi, M. G., Bianchi, L., Gaudio, G., . . . Venco, A. (1995). Twenty-four-hour noninvasive blood pressure monitoring and pain perception. *Hypertension, 25*(6), 1301-1305.

Guasti, L., Gaudio, G., Zanotta, D., Grimoldi, P., Petrozzino, M. R., Tanzi, F., . . . Venco, A. (1999). Relationship between a genetic predisposition to hypertension, blood pressure levels and pain sensitivity. *Pain, 82*(3), 311-317.

Guasti, L., Zanotta, D., Mainardi, L. T., Petrozzino, M. R., Grimoldi, P., Garganico, D., . . . Cerutti, S. (2002). Hypertension-related hypoalgesia, autonomic function and spontaneous baroreflex sensitivity. *Autonomic Neuroscience : Basic & Clinical, 99*(2), 127-133.

Guasti, L., Zanotta, D., Petrozzino, M. R., Grimoldi, P., Diolisi, A., Garganico, D., . . . Venco, A. (1999). Relationship between dental pain perception and 24 hour ambulatory blood pressure: A study on 181 subjects. *Journal of Hypertension, 17*(12 Pt 2), 1799-1804.

Gybels, J., Handwerker, H. O., & Van Hees, J. (1979). A comparison between the discharges of human nociceptive nerve fibres and the subject's ratings of his sensations. *The Journal of Physiology, 292*, 193-206.

Hanamori, T., Kunitake, T., Kato, K., & Kannan, H. (1998). Neurons in the posterior insular cortex are responsive to gustatory stimulation of the pharyngolarynx, baroreceptor and chemoreceptor stimulation, and tail pinch in rats. *Brain Research, 785*(1), 97-106.

Harkins, S. W., & Chapman, C. R. (1978). Cerebral evoked potentials to noxious dental stimulation: Relationship to subjective pain report. *Psychophysiology, 15*(3), 248-252.

Head, H., & Holmes, G. (1911). Sensory disturbances from cerebral lesions. *Brain*, 34, 102–254.

Henderson, L. A., Richard, C. A., Macey, P. M., Runquist, M. L., Yu, P. L., Galons, J. P., & Harper, R. M. (2004). Functional magnetic resonance signal changes in neural structures to baroreceptor reflex activation. *Journal of Applied Physiology, 96*(2), 693-703. doi:10.1152/japplphysiol.00852.2003

Hilz, M. J., Dutsch, M., Perrine, K., Nelson, P. K., Rauhut, U., & Devinsky, O. (2001). Hemispheric influence on autonomic modulation and baroreflex sensitivity. *Annals of Neurology, 49*(5), 575-584.

Hugon, M. (1973). Exteroreceptive reflexes to stimulation of the sural nerve in normal man. In J. E. Desmedt (Ed.), *New developments in electromyography and clinical neurophysiology* (pp. 713--729). Basel: Karger.

Iannetti, G. D., Hughes, N. P., Lee, M. C., & Mouraux, A. (2008). Determinants of laser-evoked EEG responses: Pain perception or stimulus saliency? *Journal of Neurophysiology, 100*(2), 815-828. doi:10.1152/jn.00097.2008

Iannetti, G. D., Leandri, M., Truini, A., Zambreanu, L., Cruccu, G., & Tracey, I. (2004). A-delta nociceptor response to laser stimuli: Selective effect of stimulus duration on skin temperature, brain potentials and pain perception. *Clinical Neurophysiology, 115*(11), 2629-2637. doi:10.1016/j.clinph.2004.05.023

Imaizumi, T., Brunk, S. D., Gupta, B. N., & Thames, M. D. (1984). Central effect of intravenous phenylephrine on baroreflex control of renal nerves. *Hypertension, 6*(6 Pt 1), 906-914.

Ingvar, M. (1999). Pain and functional imaging. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 354*(1387), 1347-1358. doi:10.1098/rstb.1999.0483

Inui, K., Murase, S., & Nosaka, S. (1994). Facilitation of the arterial baroreflex by the ventrolateral part of the midbrain periaqueductal grey matter in rats. *The Journal of Physiology, 477 (Pt 1)*(Pt 1), 89-101.

Janig, W. (2006). *The integrative action of the autonomic nervous system: neurobiology of homeostasis.* Cambridge, UK: Cambridge UP.

Jennings, J. R., & Wood, C. C. (1977). Cardiac cycle time effects on performance, phasic cardiac responses, and their intercorrelation in choice reaction-time. *Psychophysiology, 14*(3), 297-307.

Kakigi, R., Inui, K., & Tamura, Y. (2005). Electrophysiological studies on human pain perception. *Clinical Neurophysiology, 116*(4), 743-763. doi:10.1016/j.clinph.2004.11.016

Kakigi, R., Shibasaki, H., & Ikeda, A. (1989). Pain-related somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology, 74*(2), 139-146.

Kanda, M., Fujiwara, N., Xu, X., Shindo, K., Nagamine, T., Ikeda, A., & Shibasaki, H. (1996). Pain-related and cognitive components of somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology, 100*(2), 105-114.

Kanda, M., Matsuhashi, M., Sawamoto, N., Oga, T., Mima, T., Nagamine, T., & Shibasaki, H. (2002). Cortical potentials related to assessment of pain intensity with visual analogue scale (VAS). *Clinical Neurophysiology, 113*(7), 1013-1024.

Kanda, M., Shindo, K., Xu, X., Fujiwara, N., Ikeda, A., Nagamine, T., & Shibasaki, H. (1999). Cortical mechanisms underlying point localization of pain spot as studied by event-related potentials following CO2 laser stimulation in man. *Experimental Brain Research, 127*(2), 131-140.

Kannel, W. B., Dannenberg, A. L., & Abbott, R. D. (1985). Unrecognized myocardial infarction and hypertension: The framingham study. *American Heart Journal, 109*(3 Pt 1), 581-585.

Kardos, A., Rau, H., Greenlee, M. W., Droste, C., Brody, S., & Roskamm, H. (1994). Reduced pain during baroreceptor stimulation in patients with symptomatic and silent myocardial ischaemia. *Cardiovascular Research, 28*(4), 515-518.

Kardos, A., Rau, H., Greenlee, M. W., Droste, C., & Roskamm, H. (1995). Comparison of two mechanical carotid baroreceptor stimulation techniques. *Acta Physiologica Hungarica, 83*(2), 121-133.

Katsarava, Z., Ayzenberg, I., Sack, F., Limmroth, V., Diener, H. C., & Kaube, H. (2006). A novel method of eliciting pain-related potentials by transcutaneous electrical stimulation. *Headache, 46*(10), 1511-1517. doi:10.1111/j.1526-4610.2006.00446.x ER

Kaube, H., Katsarava, Z., Kaufer, T., Diener, H. C., & Ellrich, J. (2000). A new method to increase nociception specificity of the human blink reflex. *Clinical Neurophysiology, 111*(3), 413-416.

Kimmerly, D. S., O'Leary, D. D., Menon, R. S., Gati, J. S., & Shoemaker, J. K. (2005). Cortical regions associated with autonomic cardiovascular regulation during lower body negative pressure in humans. *The Journal of Physiology, 569*(Pt 1), 331-345. doi:10.1113/jphysiol.2005.091637

Klabunde, R. E. (2011). *Cardiovascular Physiology Concepts. Second Edition.* Philadelphia, PA: Lippincott Williams & Wilkins.

Knott, C., & Mindell, J. (2011). *Health Survey for England 2011: Hypertension*. England: The Health and Social Care Information Centre.

Koch, E. B. (1932). Die Irradiation der pressorezeptorischen Kreislaufreflexe. *Klinische Wochenschrift*, 2, 225-227.

Koriath, J. J., & Lindholm, E. (1986). Cardiac-related cortical inhibition during a fixed foreperiod reaction time task. *International Journal of Psychophysiology, 4*(3), 183-195.

Koriath, J. J., Lindholm, E., & Landers, D. M. (1987). Cardiac-related cortical activity during variations in mean heart rate. *International Journal of Psychophysiology, 5*(4), 289-299.

Krittayaphong, R., & Sheps, D. S. (1996). Relation between blood pressure at rest and perception of angina pectoris during exercise testing. *The American Journal of Cardiology, 77*(14), 1224-1226.

Kumar, S., Tandon, O. P., & Mathur, R. (2002). Pain measurement: A formidable task. *Indian Journal of Physiology and Pharmacology, 46*(4), 396-406.

Kupers, R., & Kehlet, H. (2006). Brain imaging of clinical pain states: A critical review and strategies for future studies. *Lancet Neurology, 5*(12), 1033-1044. doi:10.1016/S1474-4422(06)70624-X

Lacey, J. I. (1967). Somatic response patterning and stress: Some revisions of activation theory. In M. H. Appley, & R. Trunbull (Eds.), *Psychological stress: Issues in research* (). New York: Appleton-Century-Crofts.

Lacey, J. I., & Lacey, B. C. (1974). Studies of heart rate and other bodily processes in sensorimotor behaviour. In P. A. Obrist, A. H. Black, J. Brener & L. V. Di Cara (Eds.), *Cardiovascular psychophysiology: Current issues in response mechanism, biofeedback and methodology.* Chicago: Aldine.

Larbig, W., Elbert, T., Rockstroh, B., Lutzenberger, W., & Birbaumer, N. (1985). Elevated blood pressure and reduction of pain sensitivity. In J. Orlebeke, G. Mulder, & L. van Doornen (Eds.), *Psychophysiology of Cardiovascular Control* (pp. 113-122). New York: Plenum Press.

Lefaucheur, J. P., Ahdab, R., Ayache, S. S., Lefaucheur-Menard, I., Rouie, D., Tebbal, D., . . . Ciampi de Andrade, D. (2012). Pain-related evoked potentials: A comparative study between electrical stimulation using a concentric planar electrode and laser stimulation using a CO2 laser. *Clinical Neurophysiology, 42*(4), 199-206. doi:10.1016/j.neucli.2011.12.003

Lewington, S., Clarke, R., Qizilbash, N., Peto, R., Collins, R., & Prospective Studies Collaboration. (2002). Age-specific relevance of usual blood pressure to vascular mortality: A meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet, 360*(9349), 1903-1913.

Liang, X., Guo, Y., Figg, W. D., Fojo, A. T., Mueller, M. D., & Yu, J. J. (2011). The role of wild-type p53 in cisplatin-induced Chk2 phosphorylation and the inhibition of platinum resistance with a Chk2 inhibitor. *Chemotherapy Research and Practice, 2011*, 715469. doi:10.1155/2011/715469

Lopez, A. D., Mathers, C. D., Ezzati, M., Jamison, D. T., & Murray, C. J. L. (2006). Measuring the global burden of disease and risk factors, 1990-2001. In A. D. Lopez, C. D. Mathers, M. Ezzati, D. T. Jamison & C. J. L. Murray (Eds.), *Global burden of disease and risk factors* (). Washington (DC): The International Bank for Reconstruction and Development/The World Bank Group.

Mackenzie, I. S., & Brown, M. J. (2009). Molecular and clinical investigations in patients with low-renin hypertension. *Clinical and Experimental Nephrology, 13*(1), 1-8. doi:10.1007/s10157-008-0071-4

Magnusson, T., List, T., & Helkimo, M. (1995). Self-assessment of pain and discomfort in patients with temporomandibular disorders: A comparison of five different scales with respect to their precision and sensitivity as well as their capacity to register memory of pain and discomfort. *Journal of Oral Rehabilitation, 22*(8), 549-556.

Maixner, W., Touw, K. B., Brody, M. J., Gebhart, G. F., & Long, J. P. (1982). Factors influencing the altered pain perception in the spontaneously hypertensive rat. *Brain Research, 237*(1), 137-145.

Mancia, G., & Mark, A. L. (1983). Arterial baroreflexes in humans. In J. T. Shepherd, & F. M. Abboud (Eds.), *Handbook of physiology: The cardiovascular system* (pp. 755--793). Bethesda, Maryland: American Physiological Society.

Mancia, G., Fagard, R., Narkiewicz, K., Redon, J., Zanchetti, A., Bohm, M., . . . Task Force Members. (2013). 2013 ESH/ESC guidelines for the management of arterial hypertension: The task force for the management of arterial hypertension of the european society of hypertension (ESH) and of the european society of cardiology (ESC). *Journal of Hypertension, 31*(7), 1281-1357. doi:10.1097/01.hjh.0000431740.32696.cc

Martins, A., Q, Ring, C., McIntyre, D., Edwards, L., & Martin, U. (2009). Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. *Pain, 147*(1-3), 84-90. doi:10.1016/j.pain.2009.08.016

McCubbin, J. A., & Bruehl, S. (1994). Do endogenous opioids mediate the relationship between blood pressure and pain sensitivity in normotensives? *Pain, 57*(1), 63-67.

McIntyre, D., Edwards, L., Ring, C., Parvin, B., & Carroll, D. (2006). Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology, 43*(3), 314-319. doi:10.1111/j.1469-8986.2006.00407.x ER

McIntyre, D., Kavussanu, M., & Ring, C. (2008a). Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. *Pain, 137*(3), 550-555. doi:10.1016/j.pain.2007.10.018 ER

McIntyre, D., Ring, C., Edwards, L., & Carroll, D. (2008b). Simple reaction time as a function of the phase of the cardiac cycle in young adults at risk for hypertension. *Psychophysiology, 45*(2), 333-336. doi:10.1111/j.1469-8986.2007.00619.x ER

McIntyre, D., Ring, C., Hamer, M., & Carroll, D. (2007). Effects of arterial and cardiopulmonary baroreceptor activation on simple and choice reaction times. *Psychophysiology, 44*(6), 874-879. doi:10.1111/j.1469-8986.2007.00547.x

Mechlin, B., Heymen, S., Edwards, C. L., & Girdler, S. S. (2011). Ethnic differences in cardiovascular-somatosensory interactions and in the central processing of noxious stimuli. *Psychophysiology, 48*(6), 762-773. doi:10.1111/j.1469-8986.2010.01140.x

Melzack, R. (1975). The McGill pain questionnaire: Major properties and scoring methods. *Pain, 1*(3), 277-299.

Melzack, R. (1999). From the gate to the neuromatrix. *Pain, Suppl 6*, S121-6.

Merskey, H., & Bogduk, N. (1994). Part III: Pain terms, A current list with definitions and notes on usage. In H. Merskey, & N. Bogduk (Eds.), *Classification of chronic pain, IASP task force on taxonomy* (2nd ed., pp. 209-214). Seattle: WA: IASP.

Meyer, R. A., Ringkamp, M., Campbell, J. N., & Raja, S. N. (2006). Peripheral mechanisms of cutaneous nociception. In S. B. McMahon, & M. Koltzenburg (Eds.), *Wall and Melzack's textbook of pain* (5th ed., pp. 3-34). Philadelphia: Elsevier.

Meyer, R. A., Walker, R. E., & Mountcastle, V. B., Jr. (1976). A laser stimulator for the study of cutaneous thermal and pain sensations. *IEEE Transactions on Bio-Medical Engineering, 23*(1), 54-60.

Miltner, W., Larbig, W., & Braun, C. (1987). Habituation of subjective rating and event related potentials to painful intracutaneous electrical stimulation of the skin. *Journal of Psychophysiology*, **1**, 221-228.

Mini, A., Rau, H., Montoya, P., Palomba, D., & Birbaumer, N. (1995). Baroreceptor cortical effects, emotions and pain. *International Journal of Psychophysiology, 19*(1), 67-77.

Miyazaki, M., Shibasaki, H., Kanda, M., Xu, X., Shindo, K., Honda, M., . . . Kimura, J. (1994). Generator mechanism of pain-related evoked potentials following CO2 laser stimulation of the hand: Scalp topography and effect of predictive warning signal. *Journal of Clinical Neurophysiology, 11*(2), 242-254.

Mor, J., & Carmon, A. (1975). Laser emitted radiant heat for pain research. *Pain, 1*(3), 233-237.

Myers, C. D., Robinson, M. E., Riley, J. L.,3rd, & Sheffield, D. (2001). Sex, gender, and blood pressure: Contributions to experimental pain report. *Psychosomatic Medicine, 63*(4), 545-550.

Nakao, H., Ballim, H. M., Gellhorn, B. A., & Gellhorn, E. (1956). The role of the sino-aortic receptors in the action of adrenaline, nor-adrenaline and acetylcholine on the cerebral cortex. *Electroencephalography and Clinical Neurophysiology, 8*(3), 413-420. doi:10.1016/0013-4694(56)90006-2

Niedermeyer, E., & Lopes da Silva, F.H. (2004). *Electroencephalography - Basic Principles, Clinical Applications and Related Fields (fifth edition)*. Lippincott Williams and Wilkins: Philadelphia.

National Institute of Neurological Disorders and Stroke (NINDS). (2001, December). Pain: hope through research. *Ninds.nih.gov.* Retrieved June, 2013, from http://www.ninds.nih.gov.

Nisimaru, N., Okahara, K., & Yanai, S. (1998). Cerebellar control of the cardiovascular responses during postural changes in conscious rabbits. *Neuroscience Research, 32*(3), 267-271. doi:10.1016/S0168-0102(98)00094-7

Nosaka, S., Murata, K., Inui, K., & Murase, S. (1993). Arterial baroreflex inhibition by midbrain periaqueductal grey in anaesthetized rats. *European Journal of Physiology, 424*(3-4), 266-275.

Obermann, M., Katsarava, Z., Esser, S., Sommer, C., He, L., Selter, L., . . . Maschke, M. (2008). Correlation of epidermal nerve fiber density with pain-related evoked potentials in HIV neuropathy. *Pain, 138*(1), 79-86. doi:10.1016/j.pain.2007.11.009

Ogino, Y., Nemoto, H., Inui, K., Saito, S., Kakigi, R., & Goto, F. (2007). Inner experience of pain: Imagination of pain while viewing images showing painful events forms subjective pain representation in human brain. *Cerebral Cortex, 17*(5), 1139-1146. doi:10.1093/cercor/bhl023

Ohara, S., Crone, N. E., Weiss, N., & Lenz, F. A. (2006). Analysis of synchrony demonstrates 'pain networks' defined by rapidly switching, task-specific, functional connectivity between pain-related cortical structures. *Pain, 123*(3), 244-253. doi:10.1016/j.pain.2006.02.012

Oppenheimer, S. (2001). Forebrain lateralization of cardiovascular function: Physiology and clinical correlates. *Annals of Neurology*, 49(5), 555–556. doi: 10.1002/ana.1038

Perchet, C., Frot, M., Charmarty, A., Flores, C., Mazza, S., Magnin, M., & Garcia-Larrea, L. (2012). Do we activate specifically somatosensory thin fibres with the concentric planar electrode? A scalp and intracranial EEG study. *Pain, 153*(6), 1244-1252. doi:10.1016/j.pain.2012.03.004

Peyron, R., Garcia-Larrea, L., Gregoire, M. C., Convers, P., Richard, A., Lavenne, F., . . . Laurent, B. (2000). Parietal and cingulate processes in central pain. A combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) study of an unusual case. *Pain, 84*(1), 77-87.

Peyron, R., Laurent, B. & Garcia-Larrea, L. (2000). Functional imaging of brain responses to pain. A review and meta-analysis. Journal of Clinical Neurophysiology, 30, 263-288.

Plaghki, L., & Mouraux, A. (2003). How do we selectively activate skin nociceptors with a high power infrared laser? physiology and biophysics of laser stimulation. *Clinical Neurophysiology, 33*(6), 269-277.

Porro, C. A. (2003). Functional imaging and pain: behavior, perception, and modulation. *Neuroscientist*, 9, 354–369.

Qiu, Y., Noguchi, Y., Honda, M., Nakata, H., Tamura, Y., Tanaka, S., . . . Kakigi, R. (2006). Brain processing of the signals ascending through unmyelinated C fibers in humans: An event-related functional magnetic resonance imaging study. *Cerebral Cortex), 16*(9), 1289-1295. doi:10.1093/cercor/bhj071

Rainville, P. (2002). Brain mechanisms of pain affect and pain modulation. *Current Opinion in Neurobiology, 12*(2), 195-204.

Randich, A,. & Hartunian, C. (1983). Activation of the sinoaortic baroreceptor reflex arc induces analgesia: Interactions between cardiovascular and endogenous pain inhibitory systems. *Physiological Psychology*, 11, 214-220.

Randich, A., & Maixner, W. (1984). Interactions between cardiovascular and pain regulatory systems. *Neuroscience and Biobehavioral Reviews, 8*(3), 343-367.

Randich, A., & Maixner, W. (1986). The role of sinoaortic and cardiopulmonary baroreceptor reflex arcs in nociception and stress-induced analgesia. *Annals of the New York Academy of Sciences, 467*, 385-401.

Rau, H., Brody, S., Larbig, W., Pauli, P., Vohringer, M., Harsch, B., . . . Birbaumer, N. (1994). Effects of pres baroreceptor stimulation on thermal and mechanical pain threshold in borderline hypertensives and normotensives. *Psychophysiology, 31*(5), 480-485.

Rau, H., & Elbert, T. (2001). Psychophysiology of arterial baroreceptors and the etiology of hypertension. *Biological Psychology, 57*(1-3), 179-201.

Rau, H., Elbert, T., & Birbaumer, N. (1995). Baroreceptor activity and nociception. In D. Vaitl, & R. Schandry (Eds.), *From the heart to the brain: The psychophysiology of circulation brain interaction.* (pp. 151-168). Frankfurt: Peter Lang.

Rau, H., Pauli, P., Brody, S., Elbert, T., & Birbaumer, N. (1993). Baroreceptor stimulation alters cortical activity. *Psychophysiology, 30*(3), 322-325.

Rau, H., Elbert, T., Geiger, B., & Lutzenberger, W. (1992). PRES: The controlled noninvasive stimulation of the carotid baroreceptors in humans. *Psychophysiology, 29*(2), 165-172.

Rau, H., Elbert, T., Lutzenberger, W., Eves, F., Rockstroh, B., Larbig, W., & Birbaumer, N. (1988). Pavlovian conditioning of peripheral and central components of the baroreceptor reflex. *Journal of Psychophysiology*, 2, 119-127.

Rau, H., Schweizer, R., Zhuang, P., Pauli, P., Brody, S., Larbig, W., . . . Dworkin, B. (1993). Cigarette smoking, blood lipids, and baroreceptor-modulated nociception. *Psychopharmacology, 110*(3), 337-341.

Requin, J., & Brouchon, M. (1964). Demonstration in man of a fluctuation of visual perceptive thresholds in the cardiac period. *Comptes Rendus Des Seances De La Societe De Biologie Et De Ses Filiales, 158*, 1891-1894.

Rockstroh, B., Dworkin, B., Lutzenberger, W., Larbig, W., Ernst, M., Elbert, T., & Birbaumer, N. (1988). The influence of baroreceptor activity on pain perception. In T. Elbert, W. Langosch, A. Steptoe, & D. Vaitl (Eds.), *Behavioral Medicine in Cardiovascular Disorders* (pp. 49-60). John Wiley & Sons Ltd.

Rockstroh, B., Elbert, T, Canavan, A., Lutzenberger, W., & Birbaumer, N. (1989). *Slow Cortical Potentials and behaviour*. Urban and Schwarzenberg: Munich.

Rosa, C., Ghione, S., Panattoni, E., Mezzasalma, L., & Giuliano, G. (1986). Comparison of pain perception in normotensives and borderline hypertensives by means of a tooth pulp-stimulation test. *Journal of Cardiovascular Pharmacology, 8 (Suppl 5)*, S125-7.

Rosa, C., Vignocchi, G., Panattoni, E., Rossi, B., & Ghione, S. (1994). Relationship between increased blood pressure and hypoalgesia: Additional evidence for the existence of an abnormality of pain perception in arterial hypertension in humans. *Journal of Human Hypertension, 8*(2), 119-126.

Rothwell, P. M. (2011). Does blood pressure variability modulate cardiovascular risk? *Current Hypertension Reports, 13*(3), 177-186. doi:10.1007/s11906-011-0201-3

Rowell, L. B. (1993). *Human cardiovascular control*. Oxford: Oxford University Press.

Saavedra, J. M. (1981). Naloxone reversible decrease in pain sensitivity in young and adult spontaneously hypertensive rats. *Brain Research, 209*(1), 245-249.

Saleh, T. M., & Connell, B. J. (1998). Role of the insular cortex in the modulation of baroreflex sensitivity. The American Journal of Physiology, 274(5 Pt 2), R1417-24.

Salzman, L. F., & Jaques, N. (1976). Heart-rate and cardiac cycle effects on reaction-time. *Perceptual and Motor Skills, 42*(3), 1315-1321.

Sandman, C. A., Mccanne, T. R., Kaiser, D. N., & Diamond, B. (1977). Heart-rate and cardiac phase influences on visual-perception. *Journal of Comparative and Physiological Psychology, 91*(1), 189-202.

Sandman, C. A. (1984). Augmentation of the auditory event related potentials of the brain during diastole. *International Journal of Psychophysiology, 2*(2), 111-119.

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., & Willer, J. C. (2005). The lower limb flexion reflex in humans. *Progress in Neurobiology, 77*(6), 353-395. doi:10.1016/j.pneurobio.2005.11.003 ER

Sawicka, K., Szczyrek, M., Jastrzębska, I., Prasał, M., Zwolak, A., & Daniluk, J. (2011). Hypertension – The Silent Killer. *Journal of Pre-Clinical and Clinical Research,* 5(2), 43-46.

Saxon, S. A. (1970). Detection of near threshold signals during 4 phases of cardiac cycle. *Alabama Journal of Medical Sciences, 7*(4), 427-430.

Schobel, H. P., Handwerker, H. O., Schmieder, R. E., Heusser, K., Dominiak, P., & Luft, F. C. (1998). Effects of naloxone on hemodynamic and sympathetic nerve responses to pain in normotensive vs. borderline hypertensive men. *Journal of the Autonomic Nervous System, 69*(1), 49-55.

Schobel, H. P., Langenfeld, M., Gatzka, C., & Schmieder, R. E. (1996). Treatment and post-treatment effects of alpha- versus beta-receptor blockers on left ventricular structure and function in essential hypertension. *American Heart Journal, 132*(5), 1004-1009.

Schobel, H. P., Ringkamp, M., Behrmann, A., Forster, C., Schmieder, R. E., & Handwerker, H. O. (1996). Hemodynamic and sympathetic nerve responses to painful stimuli in normotensive and borderline hypertensive subjects. *Pain, 66*(2-3), 117-124.

Schulz, A., Reichert, C. F., Richter, S., Lass-Hennemann, J., Blumenthal, T. D., & Schaechinger, H. (2009). Cardiac modulation of startle: Effects on eye blink and

higher cognitive processing. *Brain and Cognition, 71*(3), 265-271. doi:10.1016/j.bandc.2009.08.002

Serrao, M., Coppola, G., Di Lorenzo, C., Di Fabio, R., Padua, L., Sandrini, G., & Pierelli, F. (2010). Nociceptive trigeminocervical reflexes in healthy subjects. *Clinical Neurophysiology, 121*(9), 1563-1568. doi:10.1016/j.clinph.2010.03.031

Sheffield, D., Biles, P. L., Orom, H., Maixner, W., & Sheps, D. S. (2000). Race and sex differences in cutaneous pain perception. *Psychosomatic Medicine, 62*(4), 517-523.

Sheffield, D., Krittayaphong, R., Go, B. M., Christy, C. G., Biles, P. L., & Sheps, D. S. (1997). The relationship between resting systolic blood pressure and cutaneous pain perception in cardiac patients with angina pectoris and controls. *Pain, 71*(3), 249-255.

Sheps, D. S., Bragdon, E. E., Gray, T. F.,3rd, Ballenger, M., Usedom, J. E., & Maixner, W. (1992). Relation between systemic hypertension and pain perception. *The American Journal of Cardiology, 70*(16), 3F-5F.

Sheps, D. S., Maixner, W., Hinderliter, A. L., Herbst, M. C., Bragdon, E. E., Herdt, J., . . . Ekelund, L. G. (1989). Relationship between systolic blood pressure, ventricular volume and ischemic pain perception in patients with angina pectoris: A potential role for baroreceptors. *Israel Journal of Medical Sciences, 25*(9), 482-487.

Siegel, D., Cheitlin, M. D., Seeley, D. G., Black, D. M., & Hulley, S. B. (1992). Silent myocardial ischemia in men with systemic hypertension and without clinical evidence of coronary artery disease. *The American Journal of Cardiology, 70*(1), 86-90.

Sitsen, J. M., & de Jong, W. (1983). Hypoalgesia in genetically hypertensive rats (SHR) is absent in rats with experimental hypertension. *Hypertension, 5*(2), 185-190.

Spiegel, J., Hansen, C., & Treede, R. D. (2000). Clinical evaluation criteria for the assessment of impaired pain sensitivity by thulium-laser evoked potentials. *Clinical Neurophysiology, 111*(4), 725-735.

Stanfield, C. L., & Germann, W. J. (2008). *Principles of Human Physiology*. 3rd ed. Pearson: Benjamin Cummings.

Stewart, J. C., France, C. R., & Suhr, J. A. (2006). The effect of cardiac cycle phase on reaction time among individuals at varying risk for hypertension. *Journal of Psychophysiology, 20*(1), 1-8. doi:10.1027/0269-8803.20.1.1 ER

Stewart, K. M., & France, C. R. (1996). Resting systolic blood pressure, parental history of hypertension, and sensitivity to noxious stimuli. *Pain, 68*(2-3), 369-374.

Stowell, H. (1977). Cerebral slow waves related to the perception of pain in man. *Brain Research Bulletin, 2*(1), 23-30.

Strange, S., Rowell, L. B., Christensen, N. J., & Saltin, B. (1990). Cardiovascular responses to carotid sinus baroreceptor stimulation during moderate to severe exercise in man. *Acta Physiologica Scandinavica, 138*(2), 145-153. doi:10.1111/j.1748-1716.1990.tb08826.x

Sykora, M., Diedler, J., Rupp, A., Turcani, P., & Steiner, T. (2009). Impaired baroreceptor reflex sensitivity in acute stroke is associated with insular involvement, but not with carotid atherosclerosis. *Stroke; a Journal of Cerebral Circulation, 40*(3), 737-742. doi:10.1161/STROKEAHA.108.519967

Symonds, L. L., Gordon, N. S., Bixby, J. C., & Mande, M. M. (2006). Right-lateralized pain processing in the human cortex: An FMRI study. *Journal of Neurophysiology, 95*(6), 3823-3830. doi:10.1152/jn.01162.2005

Tarkka, I. M., & Treede, R. D. (1993). Equivalent electrical source analysis of pain-related somatosensory evoked potentials elicited by a CO2 laser. *Journal of Clinical Neurophysiology, 10*(4), 513-519.

Terreberry, R. R., & Neafsey, E. J. (1987). The rat medial frontal cortex projects directly to autonomic regions of the brainstem. *Brain Research Bulletin, 19*(6), 639-649.

Thompson, L. W., & Botwinick, J. (1970). Stimulation in different phases of cardiac cycle and reaction time. *Psychophysiology, 7*(1), 57-65.

Thurston, C. L., & Randich, A. (1992). Effects of vagal afferent stimulation on ON and OFF cells in the rostroventral medulla: Relationships to nociception and arterial blood pressure. *Journal of Neurophysiology, 67*(1), 180-196.

Thurston, C. L., & Randich, A. (1995). Responses of on and off cells in the rostral ventral medulla to stimulation of vagal afferents and changes in mean arterial blood pressure in intact and cardiopulmonary deafferented rats. *Pain, 62*(1), 19-38.

Tracey, I. (2005). Nociceptive processing in the human brain. *Current Opinion in Neurobiology, 15*(4), 478-487. doi:10.1016/j.conb.2005.06.010

Tracey, I., & Mantyh, P. W. (2007). The cerebral signature for pain perception and its modulation. *Neuron, 55*(3), 377-391. doi:10.1016/j.neuron.2007.07.012

Treede, R. D., Kief, S., Holzer, T., & Bromm, B. (1988). Late somatosensory evoked cerebral potentials in response to cutaneous heat stimuli. *Electroencephalography and Clinical Neurophysiology, 70*(5), 429-441.

Treede, R. D., Lorenz, J., & Baumgartner, U. (2003). Clinical usefulness of laser-evoked potentials. *Clinical Neurophysiology, 33*(6), 303-314.

Valensi, P., Lorgis, L., & Cottin, Y. (2011). Prevalence, incidence, predictive factors and prognosis of silent myocardial infarction: A review of the literature. *Archives of Cardiovascular Diseases, 104*(3), 178-188. doi:10.1016/j.acvd.2010.11.013

Valeriani, M., Rambaud, L., & Mauguiere, F. (1996). Scalp topography and dipolar source modelling of potentials evoked by CO2 laser stimulation of the hand. *Electroencephalography and Clinical Neurophysiology, 100*(4), 343-353.

Velden, M., & Juris, M. (1975). Perceptual performance as a function of intra-cycle cardiac activity. *Psychophysiology, 12*(6), 685-692.

Verberne, A. J., & Owens, N. C. (1998). Cortical modulation of the cardiovascular system. *Progress in Neurobiology, 54*(2), 149-168.

Walker, B. B., & Sandman, C. A. (1982). Visual evoked-potentials change as heart-rate and carotid pressure change. *Psychophysiology, 19*(5), 520-527.

Walker, B. B., & Sandman, C. A. (1979). Human visual evoked responses are related to heart rate. *Journal of Comparative and Physiological Psychology, 93*(4), 18-25.

Walker, B. B., & Walker, J. M. (1983). Phase relations between carotid pressure and ongoing electrocortical activity. *International Journal of Psychophysiology, 1*(1), 65-73.

Watkins, L. R., Thurston, C. L., & Fleshner, M. (1990). Phenylephrine-induced antinociception: Investigations of potential neural and endocrine bases. *Brain Research, 528*(2), 273-284.

Weisz, J., & Adam, G. (1996). The influence of cardiac phase on reaction time depending on heart period length and on stimulus and response laterality. *Psychobiology, 24*(2), 169-175.

Weiss, S, & Baker, J. R. (1933). The carotid sinus reflex in health and disease. *Medicine*, 12, 297–351.

Weisz, J., Emri, M., Fent, J., Lengyel, Z., Marian, T., Horvath, G., . . . Adam, G. (2001). Right prefrontal activation produced by arterial baroreceptor stimulation: A PET study. *Neuroreport, 12*(15), 3233-3238.

Willer, J. C. (1977). Comparative study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

Willis, W, D., & Westlund, K, N. (1997). Neuroanatomy of the pain system and of the pathways that modulate pain. Journal of Clinical Neurophysiology, 14, 2-31.

Wolf-Maier, K., Cooper, R. S., Banegas, J. R., Giampaoli, S., Hense, H. W., Joffres, M., . . . Vescio, F. (2003). Hypertension prevalence and blood pressure levels in 6 european countries, canada, and the united states. *JAMA : The Journal of the American Medical Association, 289*(18), 2363-2369. doi:10.1001/jama.289.18.2363

Xu, X., Kanda, M., Shindo, K., Fujiwara, N., Nagamine, T., Ikeda, A., . . . Shibasaki, H. (1995). Pain-related somatosensory evoked potentials following CO2 laser stimulation of foot in man. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section, 96*(1), 12-23. doi:10.1016/0013-4694(94)00223-8

Yoon, S. S., Burt, V., Louis, T., & Carroll, M. D. (2012). Hypertension among adults in the United States, 2009-2010. *NCHS Data Brief,* (107), 1-8.

Youell, P. D., Wise, R. G., Bentley, D. E., Dickinson, M. R., King, T. A., Tracey, I., & Jones, A. K. (2004). Lateralisation of nociceptive processing in the human brain: A functional magnetic resonance imaging study. *Neuroimage, 23*(3), 1068-1077. doi:10.1016/j.neuroimage.2004.07.004

Yurenev, A. P., DeQuattro, V., & Devereux, R. B. (1990). Hypertensive heart disease: Relationship of silent ischemia to coronary artery disease and left ventricular hypertrophy. *American Heart Journal, 120*(4), 928-933. doi:10.1016/0002-8703(90)90212-G

Zamir, N., & Segal, M. (1979). Hypertension-induced analgesia: Changes in pain sensitivity in experimental hypertensive rats. *Brain Research, 160*(1), 170-173.

Zamir, N., & Shuber, E. (1980). Altered pain perception in hypertensive humans. *Brain Research, 201*(2), 471-474.

Zaslansky, R., Sprecher, E., Katz, Y., Rozenberg, B., Hemli, J. A., & Yarnitsky, D. (1996). Pain-evoked potentials: What do they really measure? *Evoked Potentials-Electroencephalography and Clinical Neurophysiology, 100*(5), 384-391.

Zhang, Y. Q., Tang, J. S., Yuan, B., & Jia, H. (1997). Inhibitory effects of electrically evoked activation of ventrolateral orbital cortex on the tail-flick reflex are mediated by periaqueductal gray in rats. *Pain, 72*(1-2), 127-135.

Zhang, Z., & Oppenheimer, S. M. (1997). Characterization, distribution and lateralization of baroreceptor-related neurons in the rat insular cortex. *Brain Research, 760*(1-2), 243-250.

Zhang, Z. H., Dougherty, P. M., & Oppenheimer, S. M. (1998). Characterization of baroreceptor-related neurons in the monkey insular cortex. *Brain Research, 796*(1-2), 303-306.

Zhang, Z. H., Dougherty, P. M., & Oppenheimer, S. M. (1999). Monkey insular cortex neurons respond to baroreceptive and somatosensory convergent inputs. *Neuroscience, 94*(2), 351-360.

TWO

Electrocutaneous pain thresholds are higher during systole than diastole

Reference

Wilkinson, M., McIntyre, D., & Edwards, L. (2013). Electrocutaneous pain thresholds are higher during systole than diastole. *Biological Psychology, 94*(1), 71-73. doi:10.1016/j.biopsycho.2013.05.002

2.1 Abstract

Arterial baroreceptors may modulate pain. Evidence suggests that the neurophysiological correlates of pain are dampened during systole, when baroreceptors are naturally stimulated, compared to diastole, when baroreceptor stimulation is minimal. However, the influence of the cardiac cycle on perception of pain remains unclear. This study examined pain thresholds in 49 healthy adults at seven intervals after the *R*-wave of the electrocardiogram, using an interleaved up-down staircase procedure. Electrocutaneous stimuli were delivered to the hand and participants indicated the presence or absence of pain. Pain thresholds were higher mid-cycle, indicative of pain attenuation during systole compared to diastole. Analysis using blood pressure median splits revealed that only participants with low systolic blood pressure displayed this cardiac cycle modulation of pain, suggesting that tonic blood pressure may moderate cardiac cycle-related pain modulation. These findings suggest fluctuations in arterial baroreceptor activity across the cardiac cycle may influence pain in normotensive individuals.

Descriptors: Arterial baroreceptors; Blood pressure; Cardiac cycle; Electrocutaneous; Pain threshold

2.1 Introduction

A baroreceptor mechanism may account for the reduced pain perception reported in hypertensive patients (France & Ditto, 1996). Arterial baroreceptors, stretch receptors located in the aortic arch and carotid sinus, are stimulated during the systolic phase of the cardiac cycle by the arrival of the pressure pulse wave (Eckberg & Sleight, 1992; Mancia & Mark, 1983). At rest, when mean arterial pressure (MAP) is low, arterial baroreceptors are stimulated during the systolic upstroke and show a pulsatile discharge (Angell James, 1971; Coleridge, Coleridge, & Schultz, 1987). Several studies have examined the influence of the cardiac cycle on neurophysiological correlates of pain (Edwards, Ring, McIntyre, & Carroll, 2001; Edwards, McIntyre, Carroll, Ring, & Martin, 2002; Edwards et al., 2003; Edwards, Inui, Ring, Wang, & Kakigi, 2008; McIntyre, Edwards, Ring, Parvin, & Carroll, 2006; McIntyre, Kavussanu, & Ring, 2008a). The majority of these studies examined the nociceptive flexion reflex (NFR), a polysynaptic spinal reflex sub-serving withdrawal from noxious stimuli (Sandrini et al., 2005), the threshold for which serves as a physiological correlate of pain (Hugon, 1973; Willer, 1977). These studies reported the NFR to be attenuated during systole compared to diastole (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006, 2008a) suggesting nociceptive responding may be dampened when arterial baroreceptor activity is maximal. Additionally, pain-related evoked brain potential (PREP) amplitudes were shown to be reduced for stimuli delivered during systole compared to diastole (Edwards et al., 2008), providing further evidence of a cardiac cycle-related pain modulation.

Pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Merskey & Bogduk, 1994). As such, the subjective nature of pain is inherently different to its neurophysiological correlates (Chen, Arendt-Nielsen, & Plafhki, 1998; Iannetti, Hughes, Lee, & Mouraux, 2008; Sandrini et al., 2005) described in previous cardiac cycle time studies (Edwards et al., 2001, 2002, 2003, 2008; McIntyre et al., 2006, 2008a). Therefore, it is important to establish if perception of pain may also be modulated across the cardiac cycle. This is particularly relevant in the context of a baroreceptor hypothesis to explain hypertensive hypoalgesia. However, those studies that measured pain perception concurrently to the NFR or PREPs found pain ratings were not modulated

across the cardiac cycle (Edwards et al., 2001, 2002, 2003, 2008). These findings are counter to prior reports that pain is attenuated when the carotid baroreceptors were artificially stimulated (Rau & Elbert, 2001). Importantly, the studies by Edwards and colleagues (2001, 2002, 2003, 2008) were not specifically designed to investigate perception of pain across the cardiac cycle. For example, those studies examining cardiac cycle-related NFR modulation presented stimuli at intensities relative to the NFR threshold, and consequentially the stimuli were not always perceived as painful (Edwards et al., 2001, 2002, 2003). Thus, the absence of pain modulation in these studies may be, in part, attributable to stimulus intensities that were insufficient to induce pain. Another study employing painful stimuli reported no cardiac cycle-related pain modulation (Edwards et al., 2008). However, this finding may be explained, at least in part, by the unvarying stimulus intensities, possibly resulting in some participant disengagement with the pain rating task.

A recent study (Martins, Ring, McIntyre, Edwards, & Martin, 2009) sought to address the limitations of previous pain-related cardiac cycle studies by assessing pain ratings at a range of pseudorandomly presented stimulus intensities. Pain was modulated across the cardiac cycle, with increased pain during systole (Martins et al., 2009). This finding contradicts previous reports of systolic dampening of the NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006, 2008a) and PREPs (Edwards et al., 2008). However, differences in methodological design may help explain these unexpected results. First, the electrode used by Martins et al. may not have selectively stimulated A-δ nociceptive fibres, thus the pattern of modulation may not reflect a specific nociceptive effect (Martins et al., 2009). Indeed, a recent study found cutaneous sensory thresholds were lower during systole than diastole (Edwards, Ring, McIntyre, Winer, & Martin, 2009). Second, Martins et al. (2009) included higher stimulation intensities (i.e., to pain tolerance) than previous studies (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006, 2008a). The higher stimulation intensities utilised by Martins et al. may have induced physiological arousal which has previously been shown to influence cardiac cycle-related NFR modulation (McIntyre et al., 2006). Notwithstanding the unexpected pattern of modulation, the findings of Martins et al. suggest pain perception may be modulated across the cardiac cycle.

The current study examined pain thresholds at seven intervals within the cardiac cycle and included several methodological features. First, stimuli were delivered using an electrode considered to more selectively stimulate A-δ nociceptive fibres (Katsarava et al., 2006; Kaube, Katsarava, Kaufer, Diener, & Ellrich, 2000). Second, stimulus intensities oscillated around pain threshold levels to examine cardiac cycle-related pain modulation at lower pain intensities than Martins et al., thereby more closely emulating the stimulus intensities used in prior cycle time studies which reported NFR modulation (e.g., Edwards et al., 2001, 2002, 2003) and minimising the possible influence of physiological arousal. Third, stimulus intensities were variable, reducing risk of participant disengagement with the pain task. Last, pain assessment at seven cardiac cycle intervals provided greater resolution of cardiac cycle effects compared to Martins et al. (2009). Based on the majority of prior findings (Edwards et al., 2001, 2002, 2003, 2008; McIntyre et al., 2006, 2008a) it was hypothesised that pain thresholds would be higher during systole than diastole (cf. Martins et al., 2009).

2.3 Methods

2.3.1 Participants

Fifty participants were recruited from the university campus and local community to participate in the study. One participant, identified as an extreme outlier (pain threshold >3 *SD*s above the mean), was subsequently removed from the analyses. Therefore, the final sample included 49 healthy normotensive adults (10 men, 39 women) with a mean (*SD*) age of 27.98 (11.6) years and body mass index (BMI) of 21.98 (2.73) kg/m^2. Mean (*SD*) resting systolic blood pressure (SBP) was 116.2 (11.3) mmHg, diastolic blood pressure (DBP) was 70.6 (11.2) mmHg, and resting heart rate (HR) was 71.2 (11.8) bpm. Individuals were excluded if they had any known health problems including chronic pain disorders, cerebrovascular, cardiovascular or neurological diseases, had a cardiac pacemaker, history of a major psychiatric disorder, were pregnant or had missed their last menstrual cycle, were taking routine prescription medicine except for birth control or were currently using any narcotic substances. Participants were asked to refrain from analgesic medication for 24 hrs and caffeine, nicotine and vigorous exercise for 2 hrs prior to

testing. The Loughborough University Ethical Advisory Committee approved the study, and all participants provided written informed consent.

2.3.2 Apparatus and measurements

Resting blood pressure (BP) and HR were obtained using an automated oscillometric sphygmomanometer (705-IT, Omron Healthcare Europe) and a brachial cuff attached around the upper non-dominant arm. An electrocardiogram (ECG) was recorded continuously at 2500 Hz using three disposable spot electrodes (Cleartrace, ConMed) placed in a modified chest configuration and connected to an AC amplifier (LP511, Grass). The two active electrodes were placed on the right clavicle and a rib below the heart on the left side of the torso; the ground electrode was placed on the left clavicle. Stimuli presented for pain threshold assessment (triple 0.5 ms monopolar square wave pulse with 5 ms inter-pulse interval at 200Hz) were delivered electrocutaneously by a constant current stimulator (DS7A, Digitimer) via a concentric planar electrode (Kaube et al., 2000). The concentric planar electrode was secured with tape (Transpore, 3M) to the dorsal surface of the right hand between the metacarpals of the index and middle fingers. The electrode sites were prepared by exfoliating (Nuprep, D.O. Weaver & Co) and degreasing the skin using isopropyl alcohol swabs (Sterets, Medlock Medical Ltd.) to reduce impedance. Participants sat upright and supported their right forearm on a table while their hand rested on a response box. Mounted on the response box (16 cm $\times$ 16 cm $\times$ 3 cm) were a red light emitting diode (top left), a green light emitting diode (top right), and buttons marked “Yes” and “No” (centre left and right, respectively). A computer was programmed with Spike2 (CED) to record responses and present stimuli using a Micro1401 II (CED).

2.3.3 Procedure

Participants were tested in a single 1.5 hr session. At the start of the session participants sat quietly whilst completing the following questionnaires: Demographics questionnaire containing questions about age, sex, health habits, education, Spielberger State and Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970), a 40-item inventory which assesses levels of state and trait anxiety and the Center for Epidemiologic Studies Depression (CES-D) Scale (Radloff, 1977), a 20-

item scale which is designed to measure depressive symptomatology in the general population (10 min). Next, participants rested quietly while BP and HR were measured at 60, 180, and 300 s (6 min). If a participant's HR exceeded 100 bpm, they were to be excluded from the study; however, none were excluded. Following instrumentation (15 min) participants undertook two threshold determination procedures. First, they completed a cutaneous sensory detection threshold assessment (15 min; data not reported here). Next, participants rested for 5 min, while the concentric stimulating electrode was fixed in place. Participants then completed the pain threshold assessment (20 min).

The pain threshold assessment determined seven pain thresholds at seven intervals after the *R*-wave of the ECG (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms). The pain thresholds were determined concurrently by interleaving seven up-down staircases (Levitt, 1971). For each trial, the start was signified by a green warning light illuminated for 1000 ms followed by a red light (variable duration; the light remained illuminated until the participant made a response, up to a maximum of 7500 ms) indicating the end of each trial. Following illumination of the green light, a 1s delay occurred after which the computer program initiated a search for the *R*-wave of the ECG. The participants hand was then stimulated at one of the seven *R*-wave intervals (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms). The *R*-wave interval used in each trial was selected pseudorandomly such that each post *R*-wave interval was stimulated once within seven trials. Participants were informed that the stimulus could occur at any time between the illumination of the green and red lights. Once the red light was illuminated participants pressed the "*Yes*" button if they perceived the stimulation as painful or the "*No*" button if they did not perceive it as painful. The next trial commenced following the participants response. For each of the seven interleaved staircases, stimulation intensity was increased from 0 mA in 1 mA steps until the participant first reported a painful sensation (first reversal). The stimulus intensity then decreased in 0.4 mA steps until the stimulus was no longer reported as painful (second reversal). Each staircase then continued in 0.1 mA steps until the seven staircases had completed two further ascending and descending series (i.e. four more reversals). The pain threshold (mA) was defined as the average of the peaks during the second and third series (i.e. the third and fifth

reversal points) of each staircase. The maximum allowable stimulus intensity was 30 mA; however, this stimulus intensity was never reached. The mean (*SD*) number of trials required to determine all seven pain thresholds was 75.10 (17.7).

2.3.4 Data reduction and analyses

Blood pressure and HR readings were averaged to provide measures of resting SBP, DBP and HR. Repeated measures analysis of variance (ANOVA) with *R*-wave to stimulation interval (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) as a within subjects factor were performed on pain thresholds.

To examine the effect of tonic BP on pain thresholds across the cardiac cycle, participants were classified as having relatively low and high BP based on SBP and DBP median splits. ANOVA revealed differences in BMI between the low SBP (Mean = 21.23, *SD* = 2.80 kg/m^2) and high SBP (Mean = 22.77, *SD* = 2.47 kg/m^2) groups, $F(1, 47) = 4.17$, $p = .05$, $\eta_p^2 = .081$ and between the low DBP (Mean = 20.73, *SD* = 2.20 kg/m^2) and high DBP (Mean = 23.28, *SD* = 2.66 kg/m^2) groups, $F(1, 47) = 13.42$, $p = .001$, $\eta_p^2 = .222$. There were no BP group differences in age or sex. Separate 2 BP Group (low, high) $\times$ 2 Sex (male, female) $\times$ 7 Interval (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) repeated measures ANOVAs were performed on pain thresholds for SBP and DBP groups, with Group and Sex as between-subjects factors and Interval as the within-subjects factor. Sex was used as a between-subjects factor because men typically have higher BPs and there is good evidence that pain sensitivity is greater in women (Fillingim et al., 2009). Although BMI did not correlate with pain thresholds averaged across intervals ($r(49) = –.20$, $p = .17$), the analysis was repeated with BMI as a covariate.

ANOVAs were corrected for the assumption of independence of data points using Huynh-Feldt correction (ε). Significant results were followed by Newman-Keuls post hoc comparisons (all possible pairwise comparisons were computed). Planned orthogonal comparisons were conducted to further examine the patterning of pain thresholds across the intervals of the cardiac cycle. Partial eta-squared (η_p^2), a measure of effect size, is reported. A significance level of .05 was adopted. Data were analysed using SPSS 16.0 and Statistica Version 10.

2.4 Results

2.4.1 Pain threshold across the cardiac cycle

A 7 Interval (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) repeated measures ANOVA revealed significant variation in pain thresholds across the cardiac cycle, ε = .90, $F(5.40, 259.21) = 3.74$, $p = .002$, $\eta_p^2 = .072$. Newman-Keuls post hoc comparisons confirmed pain thresholds were higher at *R*+200 and *R*+300 ms than at *R*+100 and *R*+500 ms and pain thresholds at *R*+300 ms were higher than at *R*+600 ms (see Figure 1). In addition, planned orthogonal comparisons revealed significant quartic, $F(1, 48) = 12.95$, $p = .001$, $\eta_p^2 = .212$, and quadratic, $F(1, 48) = 5.19$, $p = .027$, $\eta_p^2 = .097$, trends. The mean (*SD*) pain threshold across all cardiac cycle intervals was 2.25 (1.62) mA.

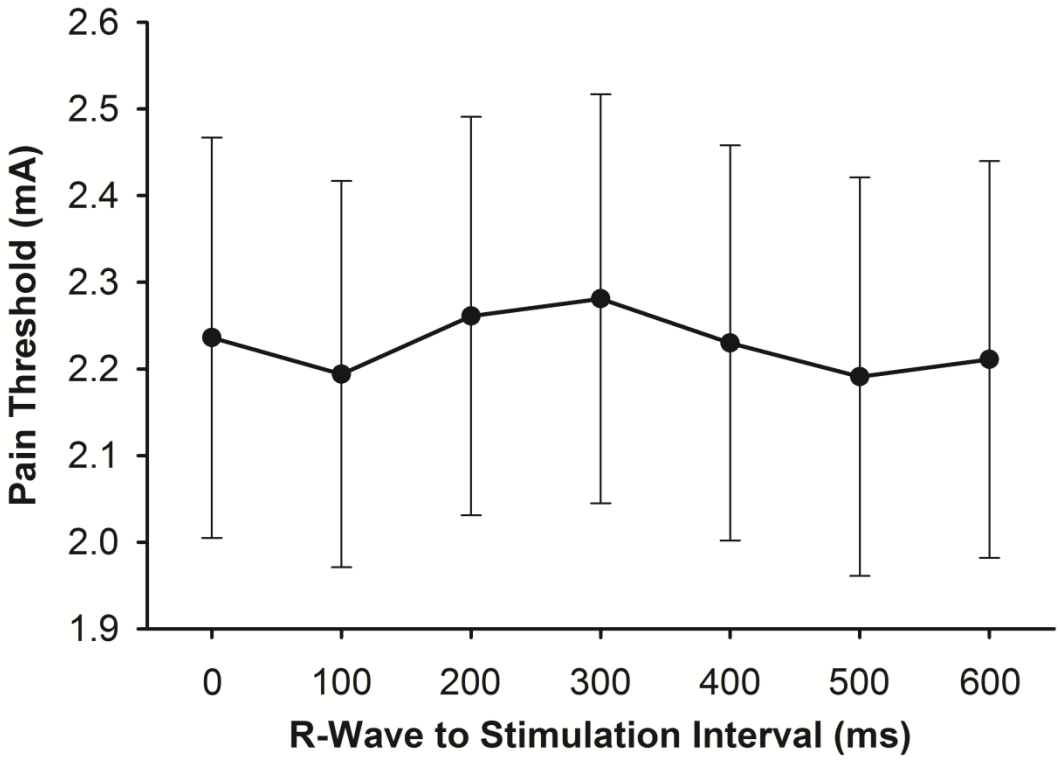

Figure 1. *Mean (SE) electrocutaneous pain thresholds as a function of phase of the cardiac cycle.*

2.4.2 Tonic blood pressure and pain thresholds

The effect of tonic BP on pain thresholds across the cardiac cycle was investigated by splitting the participants into low-normal and high-normal SBP and DBP groups. The median SBP was 116.00 mmHg; thus the low-SBP group comprised 25 participants (Mean = 108.03, *SD* = 5.67 mmHg) and the high-SBP group comprised 24 participants (Mean = 124.72, *SD* = 9.15 mmHg). Regarding DBP, the median DBP was 69.67 mmHg; thus the low-DBP group comprised 25 participants (Mean = 62.40, *SD* = 5.14 mmHg) and the high-DBP group consisted of 24 participants (Mean = 78.63, *SD* = 7.32 mmHg).

As illustrated in Figure 2a, for SBP, a 2 Group (low, high) × 2 Sex (male, female) × 7 Interval (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) ANOVA revealed no Group effect, $F(1, 45) = 0.43$, $p = .51$, $\eta_p^2 = .010$. Mean (*SD*) pain thresholds were 2.11 (1.47) mA and 2.40 (1.77) mA in the low and high SBP groups, respectively. However, a Group × Interval interaction was found, $\varepsilon = .946$, $F(5.67, 255.34) = 2.23$, $p = .04$, $\eta_p^2 = .047$. Analysis also revealed a main effect for Sex, $F(1, 45) = 5.86$, $p = .02$, $\eta_p^2 = .115$. Mean (*SD*) pain thresholds were 3.32 (2.50) and 1.98 (1.20) mA for men and women, respectively. There was no Sex × Interval, $\varepsilon = .946$, $F(5.67, 255.34) = 1.57$, $p = .16$, $\eta_p^2 = .034$, or Group × Sex × Interval, $\varepsilon = .946$, $F(5.67, 255.34) = 1.57$, $p = .16$, $\eta_p^2 = .034$ effects. Similar analysis for DBP revealed a main effect for Sex, $F(1, 45) = 4.97$, $p = .03$, $\eta_p^2 = .099$. Mean (*SD*) pain thresholds were 3.32 (2.50) and 1.98 (1.20) mA for men and women, respectively. No other significant effects were found (see Figure 2b). These analyses were repeated with potential confounding variable BMI entered as a covariate. The results of these analyses were the same as those yielded originally.

To further investigate the differing pattern of cardiac cycle modulation between SBP groups, 7 Interval (*R*+ 0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) repeated measures ANOVAs were conducted separately for the low and high groups. Pain thresholds varied across the cardiac cycle in the low SBP group, $\varepsilon = .77$, $F(4.59, 110.26) = 4.40$, $p = .002$, $\eta_p^2 = .155$, but not in the high SBP group, $\varepsilon = .72$, $F(4.30, 98.94) = 0.67$, $p = .62$, $\eta_p^2 = .028$. Post hoc analysis for the low SBP group revealed pain thresholds were higher at *R*+300 ms than *R*+100, 500 and 600

ms and pain thresholds at *R*+200 ms were higher than *R*+100 and *R*+500 ms. Planned comparisons indicated that the cardiac cycle modulation of pain thresholds in the low SBP group was characterised by quadratic, $F(1, 24) = 6.11$, $p = .02$, $\eta_p^2 = .203$ and quartic, $F(1, 24) = 21.51$, $p = .0001$, $\eta_p^2 = .473$, terms.

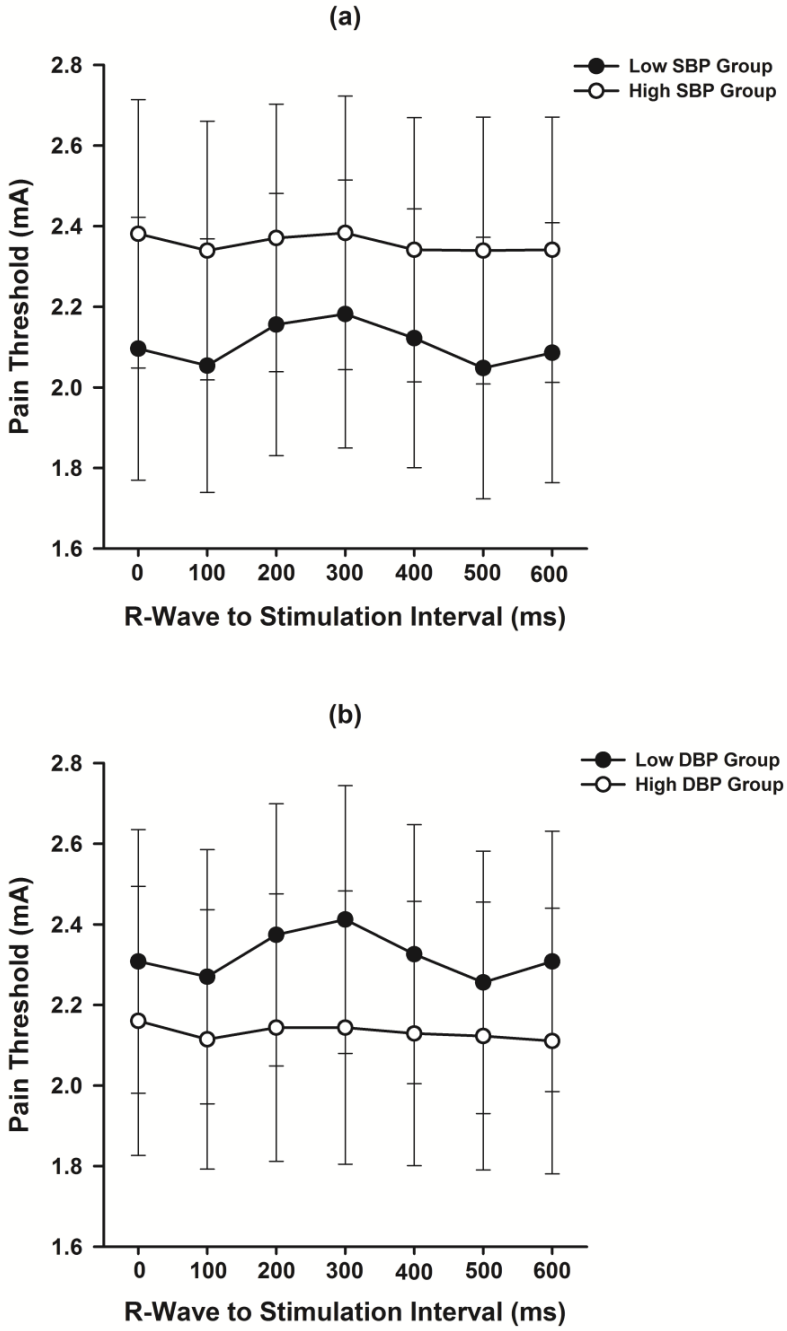

Figure 2. *Mean (SE) electrocutaneous pain thresholds at seven intervals across the cardiac cycle as a function of (a) systolic blood pressure (SBP) and (b) diastolic blood pressure (DBP).*

2.5 Discussion

Pain thresholds were higher, indicating pain perception was reduced, when electrocutaneous stimuli were delivered during systole compared to diastole. The current study is the first to demonstrate that pain perception at pain threshold levels is modulated across the cardiac cycle.

The present findings are in line with previous cardiac cycle studies reporting dampening of the NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006, 2008a) and PREP amplitudes (Edwards et al., 2008) during systole compared to diastole. However, the current findings conflict with the only previous study specifically designed to investigate the effect of natural variation in BP across the cardiac cycle on pain (Martins et al., 2009). This prior study reported increased pain intensity and unpleasantness midcycle, indicating heightened pain during systole. Methodological differences may, in part, explain this discrepancy. First, whilst both studies used variable stimulus intensities to minimise participant disengagement with the pain assessment procedure, stimuli in the current study oscillated around pain threshold levels. Conversely, Martins et al. (2009) combined pain ratings elicited by stimuli at intensities equal to pain threshold, midway between pain threshold and tolerance, and pain tolerance. The differing pain intensities employed by these two studies make comparison of the findings difficult as it is unclear how higher pain intensities may affect pain modulation. For example, it is possible that stimulus intensities approaching pain tolerance may induce physiological arousal which has been shown to change pain perception and moderate the midcycle dampening of the NFR, probably through reduced transmission of baroreceptor afferents (McIntyre et al., 2006). Indeed, elevated heart rates indicative of increased arousal were reported by Martins et al. However, further studies are needed to investigate cardiac cycle-related pain modulation as stimulation intensities increase.

Second, prior to assessing pain across the cardiac cycle, Martins et al. (2009) determined pain threshold and tolerance using a single ascending method of limits, whereas the current study used a more sophisticated adaptive up-down staircase procedure (Levitt, 1971) incorporating three ascending and descending series. Further, Martins et al. did not time stimuli in the pain threshold and tolerance

assessment task with specific intervals within the cardiac cycle, unlike the present study. Thus, in this prior study, stimuli during the pain threshold and tolerance determination may not have been equally distributed across the cardiac cycle (Martins et al., 2009), potentially influencing pain assessment. Therefore, the present study may provide a more precise assessment of pain thresholds. Regardless, the use of different pain assessment procedures makes comparison of results difficult.

Finally, pain was elicited using different stimulating electrodes. The present study used a concentric planar electrode designed to more selectively stimulate A-δ nociceptive fibres (Katsarava et al., 2006; Kaube et al., 2000). Martins and colleagues (2009) used a bar electrode to elicit pain. As acknowledged by Martins et al. bar electrodes are more likely to stimulate A-β tactile fibres, in addition to A-δ nociceptive fibres (Katsarava et al., 2006; Kaube et al., 2000). Possible stimulation of tactile fibres may have affected the pattern of cardiac cycle-related pain modulation in this prior study (Martins et al., 2009). Indeed, Edwards et al. (2009) found cutaneous tactile sensory thresholds were lower during systole compared to diastole, indicative of greater sensitivity to tactile stimulation during systole. This pattern of modulation is similar to that reported by Martins et al. (2009). Accordingly the current study may reflect a cardiac cycle modulation pattern more specific to pain.

Although the precise mechanisms underlying the systolic dampening of pain have yet to be established, the temporal patterning of systolic pain dampening is consistent with the hypothesis that pain is reduced during systole due to arterial baroreceptor activation of pain inhibition pathways (Ghione, 1996). The baroreceptors are stimulated during systole when the pulse pressure wave stretches the walls of the aortic arch and carotid sinus (Angell James, 1971; Mancia & Mark, 1983). Increased baroreceptor activation during systole has been reported to result in cortical inhibition (e.g. Rau, Elbert, & Birbaumer, 1995) and there is strong evidence for an overlap in brain areas involved in cardiovascular control and pain modulation, suggesting interactions between cardiovascular and pain systems are possible (Ghione, 1996). Indeed, the timing of pain attenuation (*R*+200 and *R*+300 ms) in the current study coincides with the arrival of the afferent signal relayed from the aortic and carotid baroreceptors reaching the brainstem areas involved in

descending nociceptive inhibition (Dembrowsky & Seller, 1995). Specifically, following onset of left ventricular contraction, blood is forced from the heart approximately 74 ± 14 ms after the *R*-wave (Kelsey & Guethlein, 1990; Kroeker & Wood, 1955). The ensuing systolic pulse wave stimulates aortic baroreceptors 10-15 ms later and carotid baroreceptors 40-65 ms later (Rushmer, 1976). Transmission of baroreceptor afferents from the aortic arch and carotid sinus to the nucleus tractus solitarius takes approximately 10-15 ms and projection from the nucleus tractus solitarius to brainstem areas involved in the descending inhibition of pain, including the rostral ventrolateral medulla, takes approximately 100-150 ms (Dembrowsky & Seller, 1995). Further, blood is ejected from the heart for a duration of approximately 250 ms (Ring, Lui, & Brener, 1994; Stafford, Harris, & Weissler, 1970), with peak baroreceptor firing corresponding to peak pulse pressure at around 100 ms (Angell James & Lumley, 1974). Therefore, the aortic baroreceptors are potentially active 70-353 ms after the *R*-wave and the carotid baroreceptors 100-403 ms after the *R*-wave. Thus, it is estimated that the arterial baroreceptors activate pain inhibiting brain areas between 180 ms and 568 ms after the *R*-wave, with maximal inhibition approximately 280-418 ms after the *R*-wave, compatible with the pattern of modulation shown in the current study.

To further support the hypothesis of baroreceptor mediated pain modulation, we must consider the conduction time for the nociceptive signal to reach the brainstem. The conduction time from the hand to the spinal cord (C7) can be estimated at 53 ms, based on a hand to C7 peripheral conduction velocity from noxious electrical stimulation of A-δ nociceptive fibres of 15.1 m/s (Inui, Tran, Hoshiyama, & Kakigi, 2002) and a distance of approximately 80.2 cm (Tran, Inui, Hoshiyama, Lam, Kakigi, 2002). Further, the conduction time from C7 to C1 can be estimated at 7 ms, based on a spinothalamic tract conduction velocity following noxious laser stimulation of 16.8 m/s for signals conveyed through a pathway to the primary somatosensory cortex (SI) (Tsuji, Inui, Kojima, & Kakigi, 2006) and a 12.1 cm distance from C7 to C1 (Smahel & Skvarilova, 1993). Thus, the shortest conduction time to brainstem can be estimated at 60ms. Additionally, following noxious electrical stimulation of the hand, the onset latency of cortical activity in SI has been recorded at 80 ms (Inui et al., 2003). Therefore, it can be estimated that

the nociceptive signal should reach the brainstem 60-80 ms following stimulation. Given pain modulation begins at *R*+200 ms and peaks at *R*+300 ms in the current study, these timings fit with a baroreceptor modulation of pain across the cardiac cycle. Specifically, the nociceptive signal presented at *R*+200 ms is anticipated to reach brainstem areas between *R*+260 ms and *R*+280 ms, which converges with baroreceptor activation of brainstem sites (i.e., *R*+180 to *R*+568 ms). Moreover, a stimulus at *R*+300 ms will reach the brainstem between *R*+360 ms and *R*+380 ms, coinciding with peak baroreceptor input to the brainstem (i.e., *R*+280 to *R*+418 ms). The current finding that pain thresholds for stimuli presented at *R*+100 and *R*+500 ms were lower than those at *R*+200 and *R*+300 ms fit with these calculations regarding the overlapping of pain and afferent baroreceptor activity in the above mentioned brainstem sites. Stimuli presented at *R*+100 ms would reach these brainstem sites between *R*+160 and *R*+180 ms and stimuli elicited at *R*+500 ms would reach these same brainstem sites at *R*+560 to *R*+580 ms, providing minimal overlap with baroreceptor activity within these brainstem sites.

As tonic BP is known to influence pain (e.g. Droste et al., 1994, France, 1999; Ghione, 1996), the present study investigated the influence of tonic BP on cardiac cycle-related pain modulation. No BP group differences in pain threshold were found, counter to several studies that have reported an inverse relationship between BP and pain within the normotensive range (for review see France, 1999). However, this relationship is not always evident (e.g., Bruehl, Chung, Diedrich, Diedrich, & Robertson, 2008; Bruehl et al., 2010; Edwards et al., 2002; France, 1999; Mechlin, Heymen, Edwards, & Girdler, 2011; Stewart & France, 1996). Importantly, the current study revealed differences in pain threshold modulation across the cardiac cycle between the SBP groups. The patterning of modulation illustrated in Figure 2a tentatively suggests that cardiac cycle effects on pain thresholds may be reduced at higher-normal SBPs. This interpretation is in line with a report of an abolition of systolic NFR dampening during a stress task that increased physiological arousal (McIntyre et al., 2006).

Although the mechanism for a difference in cardiac-cycle time modulation of pain with SBP group is unclear, one possibility may be due to BP group differences

in baroreceptor firing patterns. Indeed, acute increases in BP may lead to changes in baroreceptor output, with baroreceptor saturation increasing during systole and baroreceptor discharge increasing during diastole (Angell James, 1971; Coleridge et al., 1987). Consequentially, at higher BPs there may be less difference in baroreceptor activity between systole and diastole, potentially resulting in less pain modulation across the cardiac cycle. Further, baroreceptors may not completely reset when BP is chronically elevated (Thrasher, 2004). However, given that our participants' BP was within the normotensive range, it seems unlikely that BP levels would be high enough to result in baroreceptor saturation (Eckberg, 1977). Alternatively, the difference in cardiac cycle-related pain modulation between groups may be accounted for, in part, by the amount of baroreceptor afferent activity reaching the pain inhibition pathways. In line with this hypothesis, an inverse relationship between BP and both baroreceptor sensitivity (Sleight, Robinson, Brooks, & Read, 1977) and baroreflex sensitivity (Bristow, Honour, Pickering, Sleight, & Smyth, 1969; Gribbin, Pickering, Sleight, & Peto, 1971) has been reported. Indeed, additional analyses of current data revealed pulse pressures were greater in the high (Mean = 48.51, *SD* = 8.49 mmHg) than low (Mean = 43.31, *SD* = 7.11 mmHg) SBP group, $F(1, 47) = 5.44$, $p = .02$, $\eta_p^2 = .104$. If we accept that a baroreceptor mechanism is responsible for the cardiac-cycle related pain modulation and we assume comparable baroreceptor sensitivities between groups, then higher pulse pressures in the high SBP group would lead to more exaggerated baroreceptor activity in systole (Angell James, 1971) compared to the low SBP group. Such augmented baroreceptor activity might be expected to result in greater cardiac cycle-related pain modulation in the high SBP group. However, the present findings indicate the opposite pattern, suggestive of reduced transmission in baroreceptor afferents. Regardless of the mechanism, the current data provide preliminary evidence that tonic BP may influence the cardiac cycle-related pain modulation within the normotensive range. However, further studies are required to investigate this relationship.

The current study has many strengths, including the use of a concentric planar electrode to deliver nociceptive-specific stimuli, a study design delivering stimulus intensities relative to pain thresholds rather than related to neurophysiological

correlates of pain (e.g., NFR threshold), variable stimulus intensities, pain thresholds determined with a relatively high temporal resolution across the cardiac cycle, and the use of an adaptive up-down staircase procedure to determine pain thresholds. Nonetheless, some limitations should be acknowledged. Firstly, the participants were predominately female. Consistent with previous literature (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009), women were more sensitive to pain than men in the current study. However the present findings suggest sex does not influence the modulation of pain across the cardiac cycle. This is in line with previous studies that have not found sex differences in the cardiac cycle modulation of pain ratings (Martins et al., 2009), nociceptive responding (Edwards et al., 2001; Martins et al., 2009) or reaction times (Birren, Phillips, & Cardon, 1963; Edwards, Ring, McIntyre, Carroll, & Martin, 2007; McIntyre, Ring, Hamer, & Carroll, 2007; McIntyre, Ring, Edwards, & Carroll, 2008b). Secondly, parental history of hypertension was not assessed, which has been found to influence pain perception (France, 1999) and baroreflex sensitivity (Parmer, Cervenka, & Stone, 1992). However, prior studies suggest that parental history may not influence the cardiac cycle modulation of reaction times (McIntyre et al., 2008b; Stewart, France, & Suhr, 2006). Accordingly, future studies would be necessary to examine the influence of parental history of hypertension on pain modulation across the cardiac cycle.

In summary, the finding of pain dampening during systole compared to diastole provides further support for the theory that natural variation in arterial baroreceptor activation across the cardiac cycle modulates pain. Further, it appears tonic SBP within the normotensive range may also influence cardiac cycle-related pain modulation.

REFERENCES

Angell James, J. E. (1971). Effects of altering mean pressure, pulse pressure and pulse frequency on impulse activity in baroreceptor fibres from aortic arch and right subclavian artery in rabbit. *Journal of Physiology-London, 214*(1), 65-88.

Angell James, J. E., & Lumley, J. S. P. (1974). Effects of carotid endarterectomy on mechanical-properties of carotid-sinus and carotid-sinus nerve activity in atherosclerotic patients. *British Journal of Surgery, 61*(10), 805-810.

Birren, J. E., Phillips, S. L., & Cardon, P. V. (1963). Reaction time as a function of cardiac cycle in young adults. *Science, 140*(356), 195-196.

Bristow, J. D., Honour, A. J., Pickerin, G. W., Sleight, P., & Smyth, H. S. (1969). Diminished baroreflex sensitivity in high blood pressure. *Circulation, 39*(1), 48-&.

Bruehl, S., Burns, J. W., Chung, O. Y., Magid, E., Chont, M., Gilliam, W., . . . Cairl, H. (2010). Hypoalgesia associated with elevated resting blood pressure: Evidence for endogenous opioid involvement. *Journal of Behavioral Medicine, 33*(2), 168-176. doi:10.1007/s10865-009-9241-4

Bruehl, S., Chung, O. Y., Diedrich, L., Diedrich, A., & Robertson, D. (2008). The relationship between resting blood pressure and acute pain sensitivity: Effects of chronic pain and alpha-2 adrenergic blockade. *Journal of Behavioral Medicine, 31*(1), 71-80. doi:10.1007/s10865-007-9133-4

Chen, A. C. N., Arendt-Nielsen, L., & Plaghki, L. (1998). Laser-evoked potentials in human pain: I. use and possible misuse. *Pain Forum, 7*(4), 174-184. doi:10.1016/S1082-3174(98)70001-7

Coleridge, H. M., Coleridge, J. C. G., & Schultz, H. D. (1987). Characteristics of C-fiber baroreceptors in the carotid-sinus of dogs. *Journal of Physiology-London, 394*, 291-313.

Dembrowsky, K., & Seller, H. (1995). Arterial baroreceptor reflexes. In D. Vaitl, & R. Schandry (Eds.), *From the heart to the brain: The psychophysiology of circulation brain interaction* (pp. 35--60). Frankfurt: Peter Lang.

Droste, C., Kardos, A., Brody, S., Greenlee, M. W., Roskamm, H., & Rau, H. (1994). Baroreceptor stimulation - pain perception and sensory thresholds. *Biological Psychology, 37*(2), 101-113.

Eckberg, D. L., & Sleight, P. (1992). *Human baroreflexes in health and disease*. Oxford: Clarendon Press.

Eckberg, D. L. (1977). Baroreflex inhibition of the human sinus node: Importance of stimulus intensity, duration, and rate of pressure change. *The Journal of Physiology, 269*(3), 561-577.

Edwards, L., Inui, K., Ring, C., Wang, X. H., & Kakigi, R. (2008). Pain-related evoked potentials are modulated across the cardiac cycle. *Pain, 137*(3), 488-494. doi:10.1016/j.pain.2007.10.010 ER

Edwards, L., McIntyre, D., Carroll, D., Ring, C., France, C. R., & Martin, U. (2003). Effects of artificial and natural baroreceptor stimulation on nociceptive responding and pain. *Psychophysiology, 40*(5), 762-769.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology, 39*(5), 678-681. doi:10.1017/S0048577202011770 ER

Edwards, L., Ring, C., McIntyre, D., & Carroll, D. (2001). Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology, 38*(4), 712-718.

Edwards, L., Ring, C., McIntyre, D., Carroll, D., & Martin, U. (2007). Psychomotor speed in hypertension: Effects of reaction time components, stimulus modality, and phase of the cardiac cycle. *Psychophysiology, 44*(3), 459-468. doi:10.1111/j.1469-8986.2007.00521.x ER

Edwards, L., Ring, C., McIntyre, D., Winer, J. B., & Martin, U. (2009). Sensory detection thresholds are modulated across the cardiac cycle: Evidence that cutaneous sensibility is greatest for systolic stimulation. *Psychophysiology, 46*(2), 252-256. doi:10.1111/j.1469-8986.2008.00769.x ER

Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B., & Riley, J. L. (2009). Sex, gender, and pain: A review of recent clinical and experimental findings. *Journal of Pain, 10*(5), 447-485. doi:10.1016/j.jpain.2008.12.001 ER

France, C. R. (1999). Decreased pain perception and risk for hypertension: Considering a common physiological mechanism. *Psychophysiology, 36*(6), 683-692.

France, C. R., & Ditto, B. (1996). Risk for high blood pressure and decreased pain perception. *Current Directions in Psychological Science, 5*(4), 120-125.

Ghione, S. (1996). Hypertension-associated hypoalgesia - evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension, 28*(3), 494-504.

Gribbin, B., Pickerin, T. G., Sleight, P., & Peto, R. (1971). Effect of age and high blood pressure on baroreflex sensitivity in man. *Circulation Research, 29*(4), 424-&.

Hugon, M. (1973). Exteroreceptive reflexes to stimulation of the sural nerve in normal man. In J. E. Desmedt (Ed.), *New developments in electromyography and clinical neurophysiology* (pp. 713--729). Basel: Karger.

Iannetti, G. D., Hughes, N. P., Lee, M. C., & Mouraux, A. (2008). Determinants of laser-evoked EEG responses: Pain perception or stimulus saliency? *Journal of Neurophysiology, 100*(2), 815-828. doi:10.1152/jn.00097.2008

Inui, K., Tran, T. D., Hoshiyama, M., & Kakigi, R. (2002). Preferential stimulation of A delta fibers by intra-epidermal needle electrode in humans. *Pain, 96*(3), 247-252.

Inui, K., Wang, X. H., Qiu, Y. H., Nguyen, B. T., Ojima, S., Tamura, Y., . . . Kakigi, R. (2003). Pain processing within the primary somatosensory cortex in humans. *European Journal of Neuroscience, 18*(10), 2859-2866. doi:10.1046/j.1460-9568.2003.02995.x ER

Katsarava, Z., Ayzenberg, I., Sack, F., Limmroth, V., Diener, H. C., & Kaube, H. (2006). A novel method of eliciting pain-related potentials by transcutaneous electrical stimulation. *Headache, 46*(10), 1511-1517. doi:10.1111/j.1526-4610.2006.00446.x ER

Kaube, H., Katsarava, Z., Kaufer, T., Diener, H. C., & Ellrich, J. (2000). A new method to increase nociception specificity of the human blink reflex. *Clinical Neurophysiology, 111*(3), 413-416.

Kelsey, R. M., & Guethlein, W. (1990). An evaluation of the ensemble averaged impedance cardiogram. *Psychophysiology, 27*(1), 24-33.

Kroeker, E. J., & Wood, E. H. (1955). Comparison of simultaneously recorded central and peripheral arterial pressure pulses during rest, exercise and tilted position in man. *Circulation Research, 3*(6), 623-632.

Levitt, H. (1971). Transformed up-down methods in psychoacoustics. *Journal of the Acoustical Society of America, 49*(2), 467-&.

Mancia, G., & Mark, A. L. (1983). Arterial baroreflexes in humans. In J. T. Shepherd, & F. M. Abboud (Eds.), *Handbook of physiology: The cardiovascular system* (pp. 755--793). Bethesda, Maryland: American Physiological Society.

Martins, A., Q, Ring, C., McIntyre, D., Edwards, L., & Martin, U. (2009). Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. *Pain, 147*(1-3), 84-90. doi:10.1016/j.pain.2009.08.016

McIntyre, D., Edwards, L., Ring, C., Parvin, B., & Carroll, D. (2006). Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology, 43*(3), 314-319. doi:10.1111/j.1469-8986.2006.00407.x ER

McIntyre, D., Kavussanu, M., & Ring, C. (2008). Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. *Pain, 137*(3), 550-555. doi:10.1016/j.pain.2007.10.018 ER

McIntyre, D., Ring, C., Edwards, L., & Carroll, D. (2008). Simple reaction time as a function of the phase of the cardiac cycle in young adults at risk for hypertension. *Psychophysiology, 45*(2), 333-336. doi:10.1111/j.1469-8986.2007.00619.x ER

McIntyre, D., Ring, C., Hamer, M., & Carroll, D. (2007). Effects of arterial and cardiopulmonary baroreceptor activation on simple and choice reaction times. *Psychophysiology, 44*(6), 874-879. doi:10.1111/j.1469-8986.2007.00547.x

Mechlin, B., Heymen, S., Edwards, C. L., & Girdler, S. S. (2011). Ethnic differences in cardiovascular-somatosensory interactions and in the central processing of noxious stimuli. *Psychophysiology, 48*(6), 762-773. doi:10.1111/j.1469-8986.2010.01140.x

Merskey, H., & Bogduk, N. (1994). Part III: Pain terms, A current list with definitions and notes on usage. In H. Merskey, & N. Bogduk (Eds.), *Classification of chronic pain, IASP task force on taxonomy* (2nd ed., pp. 209-214). Seattle: WA: IASP.

Parmer, R. J., Cervenka, J. H., & Stone, R. A. (1992). Baroreflex sensitivity and heredity in essential hypertension. *Circulation, 85*(2), 497-503.

Radloff, L. S. (1977). The center for epidemiologic studies depression scale a self report depression scale for research in the general population. Applied Psychological Measurement, 1(3), 385-401.

Rau, H., & Elbert, T. (2001). Psychophysiology of arterial baroreceptors and the etiology of hypertension. *Biological Psychology, 57*(1-3), 179-201.

Rau, H., Elbert, T., & Birbaumer, N. (1995). Baroreceptor activity and nociception. In D. Vaitl, & R. Schandry (Eds.), *From the heart to the brain: The psychophysiology of circulation brain interaction.* (pp. 151--168). Frankfurt: Peter Lang.

Ring, C., Liu, X. Q., & Brener, J. (1994). Cardiac stimulus-intensity and heartbeat detection - effects of tilt-induced changes in stroke volume. *Psychophysiology, 31*(6), 553-564.

Rushmer, R. F. (1976). *Cardiovascular dynamics* (4th ed.). London: W.B. Saunders.

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., & Willer, J. C. (2005). The lower limb flexion reflex in humans. *Progress in Neurobiology, 77*(6), 353-395. doi:10.1016/j.pneurobio.2005.11.003 ER

Sleight, P., Robinson, J. L., Brooks, D. E., & Rees, P. M. (1977). Characteristics of single carotid sinus baroreceptor fibers and whole nerve activity in the normotensive and the renal hypertensive dog. *Circulation Research, 41*(6), 750-758.

Smahel, Z., & Skvarilova, B. (1993). Length of the cervical-spine as a factor in the etiology of cleft-palate. *Cleft Palate-Craniofacial Journal, 30*(3), 274-278.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). Manual for the state-trait anxiety inventory. Palo Alto, CA: Consulting Psychologists Press, 1970.

Stafford, R. W., Harris, W. S., & Weissler, A. M. (1970). Left ventricular systolic time intervals as indices of postural circulatory stress in man. *Circulation, 41*(3), 485-492.

Stewart, J. C., France, C. R., & Suhr, J. A. (2006). The effect of cardiac cycle phase on reaction time among individuals at varying risk for hypertension. *Journal of Psychophysiology, 20*(1), 1-8. doi:10.1027/0269-8803.20.1.1 ER

Stewart, K. M., & France, C. R. (1996). Resting systolic blood pressure, parental history of hypertension, and sensitivity to noxious stimuli. *Pain, 68*(2-3), 369-374.

Thrasher, T. N. (2004). Baroreceptors and the long-term control of blood pressure. *Experimental Physiology, 89*(4), 331-335. doi:10.1113/expphysiol.2004.027441

Tran, T. D., Inui, K., Hoshiyama, M., Lam, K., & Kakigi, R. (2002). Conduction velocity of the spinothalamic tract following CO2 laser stimulation of C-fibers in humans. *Pain, 95*(1-2), 125-131.

Tsuji, T., Inui, K., Kojima, S., & Kakigi, R. (2006). Multiple pathways for noxious information in the human spinal cord. *Pain, 123*(3), 322-331. doi:10.1016/j.pain.2006.03.009 ER

Willer, J. C. (1977). Comparative-study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

THREE

Effects of blood pressure across the cardiac cycle on tactile detection thresholds in normotensives

3.1 Abstract

Research suggests that hypertensive individuals have reduced sensory perception. Further, natural fluctuations in blood pressure (BP) over the cardiac cycle have been shown to influence sensory perception in normotensive individuals. A recent study comprising individuals with hypertensive and normotensive BP found tactile sensory thresholds were reduced during systole, when BP is highest, compared to diastole. In this previous study, the magnitude of cardiac cycle modulation was found to increase with diastolic BP. The current study examined the influence of the cardiac cycle on tactile sensory thresholds in an exclusively normotensive sample and with greater temporal resolution. Tactile detection thresholds were determined concurrently at 7 intervals (0, 100, 200, 300, 400, 500 and 600 ms) after the *R*-wave of the electrocardiogram in 49 normotensive adults, using an interleaved up-down staircase procedure. Tactile sensory thresholds were defined as the average of the final four reversals in each staircase. Electrocutaneous stimuli were delivered to the dorsal surface of the right index finger and participants indicated the presence or absence of sensation using a response box. Tactile sensory thresholds did not vary across the cardiac cycle ($p > .05$). However, when participants were split into high-normal and low-normal BP groups, significant interactions emerged between BP and tactile sensory thresholds across the cardiac cycle. These findings suggest tonic BP has an important influence on the cardiac cycle modulation of tactile sensibility.

Descriptors: Arterial baroreceptors; Blood pressure; Cardiac cycle; Electrocutaneous; Tactile sensory threshold

3.2 Introduction

It is well established that hypertension is characterised by a reduced sensitivity to pain (Ghione, 1996). There is also evidence that hypertension affects other sensory perceptions, for example reduced visual perception has been reported in individuals with hypertension (Mazzucchi et al., 1986; Shapiro, Miller, King, Ginchereau, & Fitzgibbon, 1982) suggesting that elevated blood pressure (BP) may affect the visual system. Zamir and Shuber (1980) found that sensory, as well as pain thresholds, were elevated in hypertensive compared to normotensive subjects in response to graded electrical tooth pulp stimulation. Furthermore, higher sensory detection thresholds were reported in unmedicated hypertensives compared to normotensives following electrocutaneous stimulation of the hand (Edwards, Ring, France, McIntyre, & Martin, 2008) and Rosa and colleagues (Rosa, Vignocchi, Panattoni, Rossi, & Ghione, 1994) reported that cutaneous perception thresholds and the R2 component of the blink reflex were increased in hypertension.

It has been suggested that a baroreceptor mechanism may account for the reduced pain perception reported in hypertensive patients (France, 1999; France & Ditto, 1996; Ghione, 1996). Arterial baroreceptors are stretch receptors located in the aortic arch and carotid sinus and are responsible for monitoring BP and maintaining cardiovascular homeostasis (Persson & Kirchheim, 1991), thus providing a link between the cardiovascular system and the central nervous system. At rest, when mean arterial pressure (MAP) is low baroreceptors are stimulated during the systolic phase of the cardiac cycle by the arrival of the pulse pressure wave (Eckberg & Sleight, 1992; Mancia & Mark, 1983) and show decreased activity during diastole (Angell-James & Lumley, 1974) resulting in a pulsatile discharge (Angell James, 1971; Coleridge, Coleridge, & Schultz, 1987).

It is less clear if baroreceptor effects are specific to pain or if they influence sensation generally. A more global baroreceptor modulation of sensation may be hypothesised based on early animal neurophysiological research that reported a sedative effect of baroreceptor stimulation in dogs (Koch, 1932), and an inhibition of cortical activity when baroreceptor afferents, which project to the nucleus tractus

solitarius, were stimulated (e.g., Bonvallet & Allen, 1963; Bonvallet & Bloch, 1961; Bonvallet, Dell, & Hiebel, 1954).

The aforementioned animal studies (Koch, 1932; Bonvallet & Allen, 1963; Bonvallet & Bloch, 1961; Bonvallet et al., 1954) form the neurophysiological foundations for the visceral afferent feedback hypothesis to explain the antinociceptive effect associated with baroreceptor activation. The visceral afferent feedback hypothesis proposes that natural variations in baroreceptor activation across the cardiac cycle lead to changes in cortical inhibition and consequently differences in sensorimotor performance across the cardiac cycle (e.g. Lacey & Lacey, 1967).

In humans, cardiac cycle time studies have investigated the visceral afferent feedback hypothesis. Cardiac cycle time studies deliver stimuli at various points across the cardiac cycle and compare the responses between stimulations presented during systole, which occurs approximately 50 ms to 300 ms after the *R*-wave, when the arrival of the pulse pressure wave stimulates the baroreceptors in the aortic arch and carotid sinus, distending the vessel walls and resulting in maximal baroreceptor afferent firing, to those presented during diastole, which occurs less than 50 ms and greater than 300 ms after the *R*-wave, when baroreceptor activation is lowest (see Eckberg & Sleight, 1992 for review). The cardiac cycle provides an ethical paradigm via which to investigate the effects of baroreceptors as it takes advantage of a natural fluctuation in BP and is totally non-invasive. Earlier studies investigating baroreceptor effects in humans involved using phenylephrine and nitroprusside to raise or lower the BP pharmacologically, a procedure known as the Oxford technique (Raven, Fadel, & Ogoh, 2006). The major limitations of such approaches are the ethical constrictions associated with using pharmacological interventions and also the possibility of the drugs influencing factors other than BP that may influence the outcome of the study. In response to these limitations, investigators introduced constant (e.g., Eckberg, Cavanaugh, Mark, & Abboud, 1975) or variable (e.g., Brody & Rau, 1994) external suction and compression of the neck to directly manipulate the carotid sinus, the main limitations of the external suction/compression method are that they only target the carotid and

not aortic baroreceptors and therefore the integrated baroreceptor effect is not known and they are also distractive to the participants which may also influence the individuals BP.

Cardiac cycle time studies have reported attenuated nociception (Edwards, Ring, McIntyre, & Carroll, 2001; Edwards, McIntyre, Carroll, Ring, & Martin, 2002; Edwards et al., 2003; McIntyre, Edwards, Ring, Parvin, & Carroll, 2006; McIntyre, Kavussanu, & Ring, 2008), pain-related evoked potentials (PREPs) (Edwards, Inui, Ring, Wang, & Kakigi, 2008) and pain (Wilkinson, McIntyre, & Edwards, 2013), during systole, when baroreceptor activation is greatest, compared to diastole, when baroreceptor activation is lowest. Additional evidence from cardiac cycle studies further support a hypothesis that baroreceptors may modulate other sensory functions, although the findings are mixed; Saxon (1970) reported a reduced ability to detect near threshold auditory stimuli during the QRS complex (approx. *R*-wave – 40ms to *R*-wave + 40ms) compared to during the P-wave (approx. *R*-wave + 700ms to *R*-wave + 810ms) of the electrocardiogram (ECG). Additionally, supra-threshold tones presented during systole were perceived as quieter than those presented during diastole (Cohen, Lieb & Rist, 1980), whereas others have found no cardiac cycle-related modulation for supra-threshold auditory stimuli detection (Delfini & Campos, 1972; Velden & Juris, 1975). Similarly, in relation to visual stimuli, recognition was increased during the *P*-wave compared to the *R*-wave and *T*-wave of the ECG (Sandman, Mccanne, Kaiser, & Diamond, 1977), whereas Elliott and Graf (1972) found visual sensitivity was not influenced by phase of the cardiac cycle. Additionally, cardiac cycle studies employing electroencephalographic measures have reported that amplitudes of auditory (Sandman, Walker & Berka, 1982) and visual (Walker & Sandman, 1979) evoked potentials were reduced during systole compared to diastole. Although mixed, these findings suggest that baroreceptor effects are not limited to pain but influence other sensations too.

Despite a significant number of studies investigating the cardiac cycle modulation of pain, very little research has been conducted on the tactile somatosensory modality. Recent work by Edwards and colleagues (2009) reported a reduction in cutaneous sensory thresholds during systole compared to diastole,

indicating that in contrast to pain, cutaneous sensitivity was heightened during baroreceptor activation. The study also reported that individuals with higher diastolic BP had larger reductions in sensory threshold during systole compared to diastole suggesting that the baroreceptor influence on cutaneous sensibility becomes greater as tonic BP increases. These findings provide further evidence that baroreceptors influence other somatosensory systems, but that the pattern of modulation may be specific to each sensory modality, rather than baroreceptor activation inducing a global diminution of sensations.

The study by Edwards and colleagues (2009) was the first study to indicate a modulation of tactile sensation by natural variations in BP across the cardiac cycle, but only included three intervals within the cardiac cycle and included participants newly diagnosed and untreated hypertensive patients. As hypertensives may present altered baroreceptor function compared to normotensives (e.g. Bristow, Honour, Pickering, Sleight, & Smyth, 1969; Gribbin, Pickering, Sleight, & Peto, 1971; Simon, Kiowski, & Julius, 1977; Goldstein, 1983), the current study aimed to investigate cardiac cycle-related modulation of tactile sensation in a completely normotensive group to explore if modulation is evident in normotension, similar to other modalities, including nociception (Edwards et al., 2001; 2002), PREPs (Edwards, Inui et al., 2008) and pain (Martins, Ring, McIntyre, Edwards, & Martin, 2009; Wilkinson et al., 2013). Moreover, the study will investigate in more detail the temporal pattern of cardiac cycle modulation by presenting stimuli at seven intervals across the cardiac cycle.

3.3 Methods

3.3.1 Participants

Fifty (10 men, 40 women) normotensive adults were recruited from the university campus and local community to participate in the study. One participant, identified as an extreme outlier (tactile sensory threshold >3 *SD*s above the mean), was subsequently removed from the analyses. Therefore, the final sample included 49 healthy normotensive adults (9 men, 40 women) with a mean (*SD*) age of 28.14 (11.7) years and body mass index (BMI) of 22 (2.7) kg/m^2. Mean (*SD*) resting systolic BP (SBP) was 116.02 (10.9) mmHg, diastolic BP (DBP) was 70.5 (10.2) mmHg, and

resting heart rate (HR) was 72.0 (11.7) bpm. Individuals were excluded if they had any known health problems including chronic pain disorders, cerebrovascular, cardiovascular or neurological diseases, had a cardiac pacemaker, history of major psychiatric disorders, were pregnant or had missed their last menstrual cycle, were taking routine prescription medicine except for birth control, were currently using any narcotic substances or had an alcohol intake greater than 28 units per week for men and 21 units per week for women. Participants were asked to refrain from analgesic medication for 24 hrs and caffeine, nicotine and vigorous exercise for 2 hrs prior to testing. The Loughborough University Ethical Advisory Committee approved the study, and all participants provided written informed consent.

3.3.2 Apparatus and measurements

Resting BP (mmHg) and HR (bpm) were obtained using an automated oscillometeric sphygmomanometer (Omron 705-IT, Omron Healthcare Europe) and a brachial cuff attached around the upper non-dominant arm. An ECG was recorded continuously at 2500 Hz using three disposable spot electrodes (Cleartrace, ConMed) placed in a modified chest configuration and connected to an AC amplifier (LP511, Grass). The two active electrodes were placed on the right clavicle and a rib below the heart on the left side of the torso; the ground electrode was placed on the left clavicle. Stimuli for tactile sensory threshold assessment (1 ms square wave pulses at 250 Hz for 60 ms) were delivered electrocutaneously by a constant current stimulator (DS7A, Digitimer) via a bar electrode (Nicolet) with 9 mm diameter contacts and a 22 mm inter-contact spacing secured to the dorsal surface of the intermediate and proximal phalanges of the right index finger with tape (Transpore, 3M). The electrode sites were prepared by exfoliating (Nuprep, D.O. Weaver & Co) and degreasing the skin using isopropyl alcohol swabs (Sterets, Medlock Medical Ltd.) to ensure impedance was <10kΩ (Checktrode, UFI). Participants sat upright and supported their dominant forearm on a table while their hand rested on the response box. Mounted on the response box (16 cm × 16 cm × 3 cm) were a piezo-oscillator (top middle), a red light emitting diode (top left), a green light emitting diode (top right), and buttons marked “Yes” and “No” (centre left and right, respectively). A computer was programmed with Spike2 (CED) to record responses and present stimuli using a Micro II 1401 (Cambridge Electronic Design).

3.3.3 Procedure

Participants were tested in a single 1.5-hr session. At the start of the session participants sat quietly whilst completing the following questionnaires: Demographics questionnaire containing questions about age, sex, health habits, education, Spielberger State and Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970), a 40-item inventory which assesses levels of state and trait anxiety and the Center for Epidemiologic Studies Depression (CES-D) Scale (Radloff, 1977), a 20-item scale which is designed to measure depressive symptomatology in the general population (10 min). Next, participants rested quietly while baseline BP and heart rate were measured at 60, 180, and 300 s (6 min). If a participant's heart rate exceeded 100 bpm, they were excluded from the study; however, none were excluded. Following instrumentation and instruction (15 min) participants undertook two threshold determination procedures. First, they completed a tactile sensory thresholds assessment (15 min). Next, participants rested for 5 min, after which a pain threshold determination task was completed (15 min, data not reported here).

The tactile sensory threshold assessment concurrently determined seven tactile sensory thresholds by interleaving seven up-down staircases (Levitt, 1971). Each staircase assessed a tactile sensory threshold at one of seven intervals after the R-wave of the ECG (*R*+0 ms, *R*+100 ms, *R*+200 ms, *R*+300 ms, *R*+400 ms, *R*+500 ms, *R*+600 ms). A green warning light (1000 ms duration) illuminated to signify the start of each trial and a red light (variable duration; remaining illuminated until the participant made a response up to a maximum of 7500 ms) indicated the end of each trial. Following illumination of the green light, a 1-s delay occurred after which the computer program initiated a search for the *R*-wave of the ECG. The participants finger was then stimulated at one of seven *R*-wave intervals, selected pseudorandomly. Participants were informed that the stimulus could occur at anytime between the illumination of the green and red lights. Once the red light was illuminated participants pressed the "Yes" button if they perceived the stimulation or the "No" button if they did not perceive it. On the first trial of each staircase the stimulus intensity was 0 mA and subsequently increased in 1 mA steps until the participant first detected a sensation (first reversal), and then decreased in 0.4 mA steps until the participant no longer detected a sensation (second reversal). Each

staircase then continued in 0.1 mA steps until all seven staircases had completed two further ascending and descending series (i.e. four more reversals). The 50% tactile detection threshold (mA) was defined as the average of the peaks and troughs during the second and third series (i.e. the third, fourth, fifth and sixth reversal points) of each staircase. The maximum stimulus intensity was 5 mA. However, this was not reached. The mean (*SD*) number of trials required to determine all 7 tactile sensory thresholds was 51.06 (12.9).

3.3.4 Data reduction and analyses

The BP and heart rate readings were averaged to provide measures of resting SBP, DBP and HR. Repeated measures analysis of variance (ANOVAs) with *R*-wave to stimulation interval (i.e., *R*+0 ms, *R*+100 ms, *R*+200 ms, *R*+300 ms, *R*+400 ms, *R*+500 ms, *R*+600 ms) as a within subjects factor were performed on tactile sensory thresholds.

To examine the effect of tonic BP on tactile sensory thresholds across the cardiac cycle, participants were classified as having relatively low and high BP based on a median split of SBP and DBP. Median SBP was 116.00 mmHg; the SBP_{low} group comprised 25 participants (Mean = 108.03, *SD* = 5.68 mmHg) and the SBP_{high} group comprised 24 participants (Mean = 124.07, *SD* = 8.03 mmHg). Chi-squared analysis confirmed there were no significant differences in the distribution of males and females between the SBP_{low} (21 female, 4 male) and SBP_{high} (19 female, 5 male) groups (χ^2 = .191, df = 1, *p* = .662). For DBP the median was 69.67 mmHg; the DBP_{low} group comprised 25 participants (Mean = 62.40, *SD* = 5.14 mmHg) and the DBP_{high} group comprised 24 participants (Mean = 78.15, *SD* = 5.40 mmHg). Chi-squared analysis again confirmed there were no significant differences in the distribution of males and females between the DBP_{low} (20 female, 5 male) and DBP_{high} (20 female, 4 male) groups (χ^2 = .091, df = 1, *p* = .763). ANOVA revealed differences in BMI between the DBP_{low} (Mean = 20.73, *SD* = 2.20 kg/m^2) and DBP_{high} (Mean = 23.25, SD = 2.65 kg/m^2) groups, *F*(1, 47) = 13.10, *p* = .001, η_p^2 = .218, but not between the SBP_{low} (Mean = 21.23, *SD* = 2.80 kg/m^2) and SBP_{high} (Mean = 22.74, *SD* = 2.46 kg/m^2) groups, *F*(1, 47) = 4.02, *p* = .05, η_p^2 = .079. There were no BP group differences in age. A 2 BP Group (low, high) × 2 Sex (male, female) × 7

Interval (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) repeated measures ANOVA, with Group and Sex as between-subjects factors and Interval as the within-subjects factor, were performed on tactile sensory thresholds. Sex was used as a between-subjects factor because men typically have higher BPs and there is evidence that sensory thresholds are lower in women than men (e.g. Takekuma, Ando, Niino, & Shimokata, 2000; Leong, Lauschke, Rutowski, & Waite, 2010; Maffiuletti, Herrero, Jubeau, Impellizzeri, & Bizzini, 2008). Although BMI did not correlate with pain thresholds averaged across intervals (r (49) = .05, *p* = .73), the analysis was repeated with BMI as a covariate, as this has been shown to influence sensory thresholds (e.g. Hodge et al., 1995; Cheng et al., 1999) and with age as covariate as age may also influence electrocutaneous thresholds (e.g. Takekuma et al., 2000; Lin, Hsieh, Chao, Chang, & Hsieh, 2005; Deshpande, Metter, Ling, Conwit, & Ferrucci, 2008; Sands et al., 1998).

ANOVAs were corrected for the assumption of independence of data points using Huynh-Feldt correction (ε). Significant results were followed by Newman-Keuls post hoc comparisons (all possible pairwise comparisons were computed) to further examine the patterning of tactile sensory thresholds across the cardiac cycle. Partial eta-squared (η_p^2), a measure of effect size, is reported. A significance level of .05 was adopted. Data were analysed using SPSS 20.0 and Statistica Version 10.

3.4 Results

3.4.1 Tactile sensory detection threshold across the cardiac cycle

A 7 Interval (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) repeated measures ANOVA revealed no significant variation in tactile sensory threshold across the cardiac cycle, ε = .816, $F(4.90, 234.97) = .470$, $p = .795$, $\eta_p^2 = .010$ (see Figure 3). The mean (*SD*) tactile sensory threshold across all cardiac cycle intervals was 0.37 (0.14) mA.

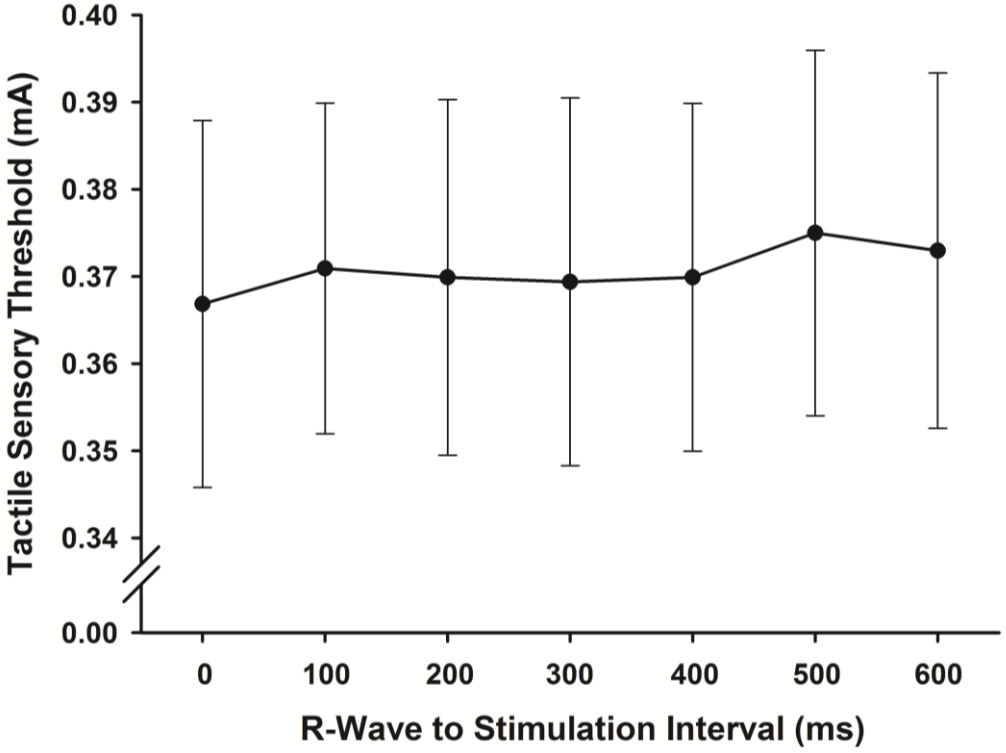

Figure 3. *Mean (SE) electrocutaneous tactile sensory thresholds as a function of phase of the cardiac cycle.*

3.4.2 Tonic blood pressure and cardiac cycle modulation of tactile sensory thresholds

The effect of tonic BP on tactile sensory thresholds across the cardiac cycle was investigated by splitting participants into low-normal and high-normal BP groups. A 2 Group (SBP_{low}, SBP_{high}) × 2 Sex (male, female) × 7 Interval (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) ANOVA revealed no Group effect, $F(1, 45) = 0.06$, $p = .82$, $\eta_p^2 = .001$. Mean (*SD*) tactile sensory thresholds were 0.36 (0.20) mA and 0.37 (0.18) mA in the SBP_{low} and SBP_{high} groups, respectively. However there was a Group × Interval interaction, $\varepsilon = .866$, $F(5.20, 233.86) = 4.54$, $p = <.001$, $\eta_p^2 = .092$, although Newman-Keuls post hoc comparisons revealed no significant effects (Figure 4a). A Group × Sex × Interval interaction, $\varepsilon = .866$, $F(5.20, 233.86) = 3.88$, $p = .002$, $\eta_p^2 = .079$ also emerged. Newman-Keuls post hoc comparisons revealed that the cardiac cycle effects were confined only to the male participants with the SBP_{low} group demonstrating significantly lower tactile sensory thresholds at *R*+300 ms than

R+0, *R*+100, *R*+500 and *R*+600 ms and significantly higher tactile sensory thresholds at *R*+600 than *R*+400. In contrast the male SBP_{high} group showed higher tactile sensory thresholds at *R*+300 than *R*+600 (see Figure 5). There were no Sex × Interval (ε = .866, $F(5.20, 233.86) = .73$, $p = .610$, $\eta_p^2 = .016$) or Sex × Group ($F(1, 45) = .037$, $p = .849$, $\eta_p^2 = .001$) interactions.

Similar analysis using DBP also revealed no Group effect, $F(1, 45) = 0.20$, $p = .66$, $\eta_p^2 = .004$, mean (*SD*) tactile sensory thresholds were 0.36 (0.18) mA and 0.38 (0.20) mA in the DBP_{low} and DBP_{high} groups, respectively. However, there was a Group × Interval interaction, $\varepsilon = .840$, $F(5.04, 226.75) = 6.59$, $p = <.001$, $\eta_p^2 = .128$. Newman-Keuls post hoc comparisons revealed that tactile sensory thresholds in the DBP_{low} group were higher at *R*+600 ms than at *R*+300. (see Figure 4b). A Group × Sex × Interval interaction, $\varepsilon = .840$, $F(5.04, 226.75) = 3.69$, $p = .003$, $\eta_p^2 = .076$ also emerged. Newman-Keuls post hoc comparisons revealed that the cardiac cycle effects were confined only to the male participants with the DBP_{low} group demonstrating lower tactile sensory thresholds at *R*+300 ms than *R*+100, *R*+500 and *R*+600 ms. The male DBP_{high} group presented higher tactile sensory thresholds at *R*+300 ms than *R*+0, *R*+500 and *R*+600 ms (see Figure 6). There were no Sex × Interval ($\varepsilon = .840$, $F(5.04, 226.75) = .52$, $p = .759$, $\eta_p^2 = .012$.) or Sex × Group ($F(1, 45) = .38$, $p = .539$, $\eta_p^2 = .008$) interactions. These analyses were repeated using ANCOVA, with potential confounding variables BMI and age entered as covariates. The results of were the same as those yielded in the original analysis.

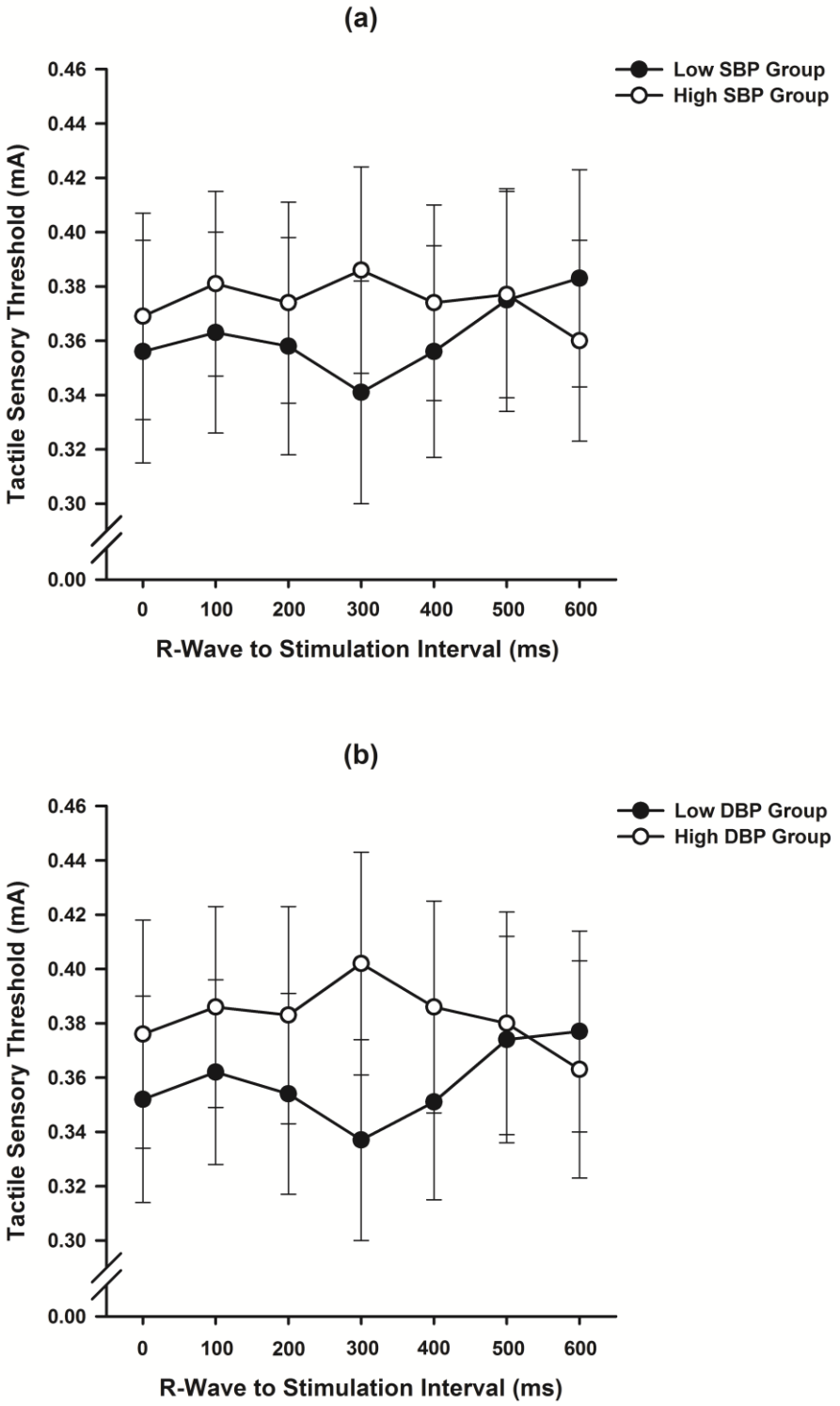

Figure 4. *Mean (SE) electrocutaneous tactile sensory thresholds at seven intervals across the cardiac cycle as a function of (a) systolic blood pressure (SBP) and (b) diastolic blood pressure (DBP).*

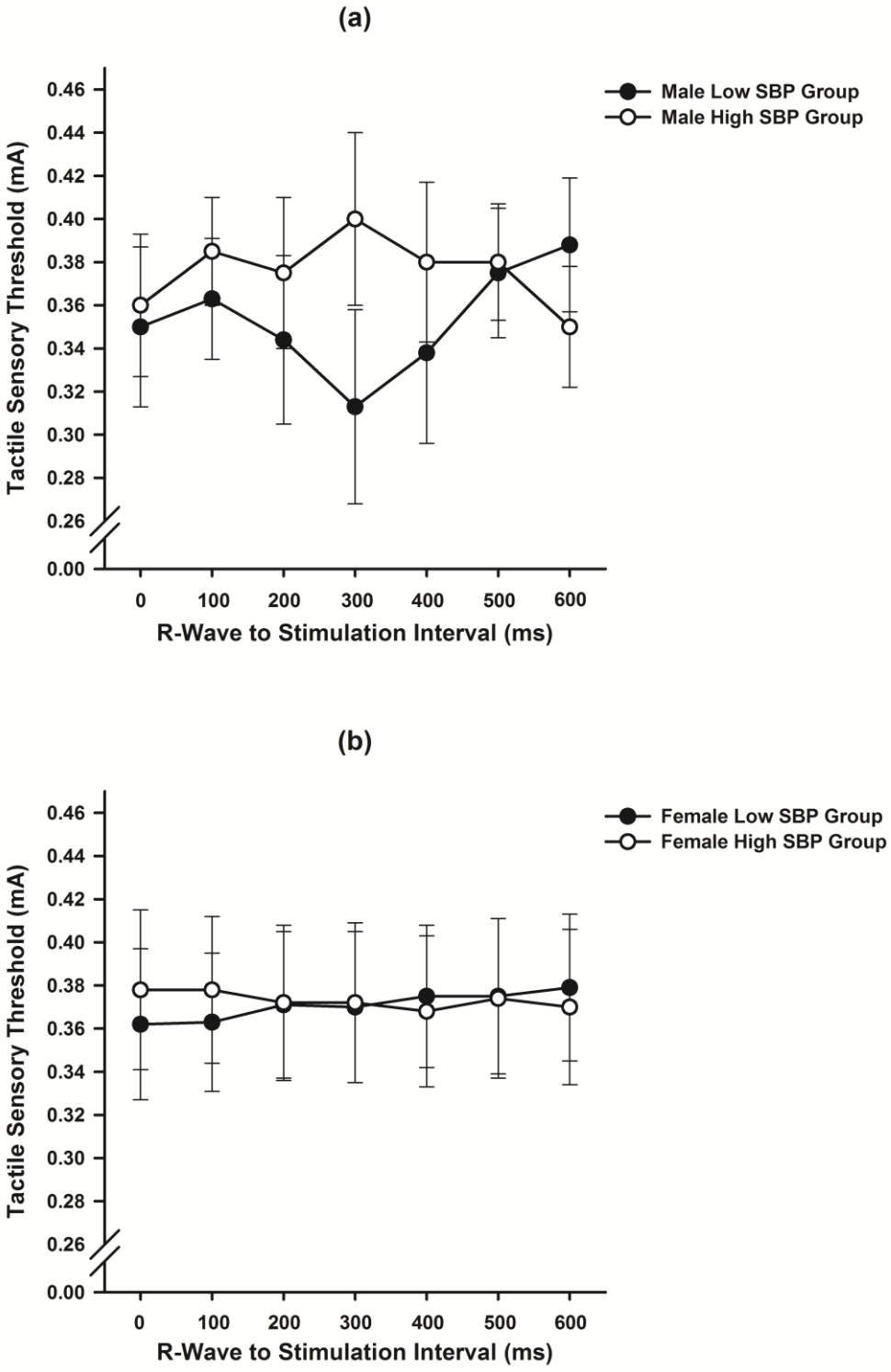

Figure 5. *Mean (SE) electrocutaneous tactile sensory thresholds at seven intervals across the cardiac cycle as a function of systolic blood pressure (SBP) in (a) male participants and (b) female participants.*

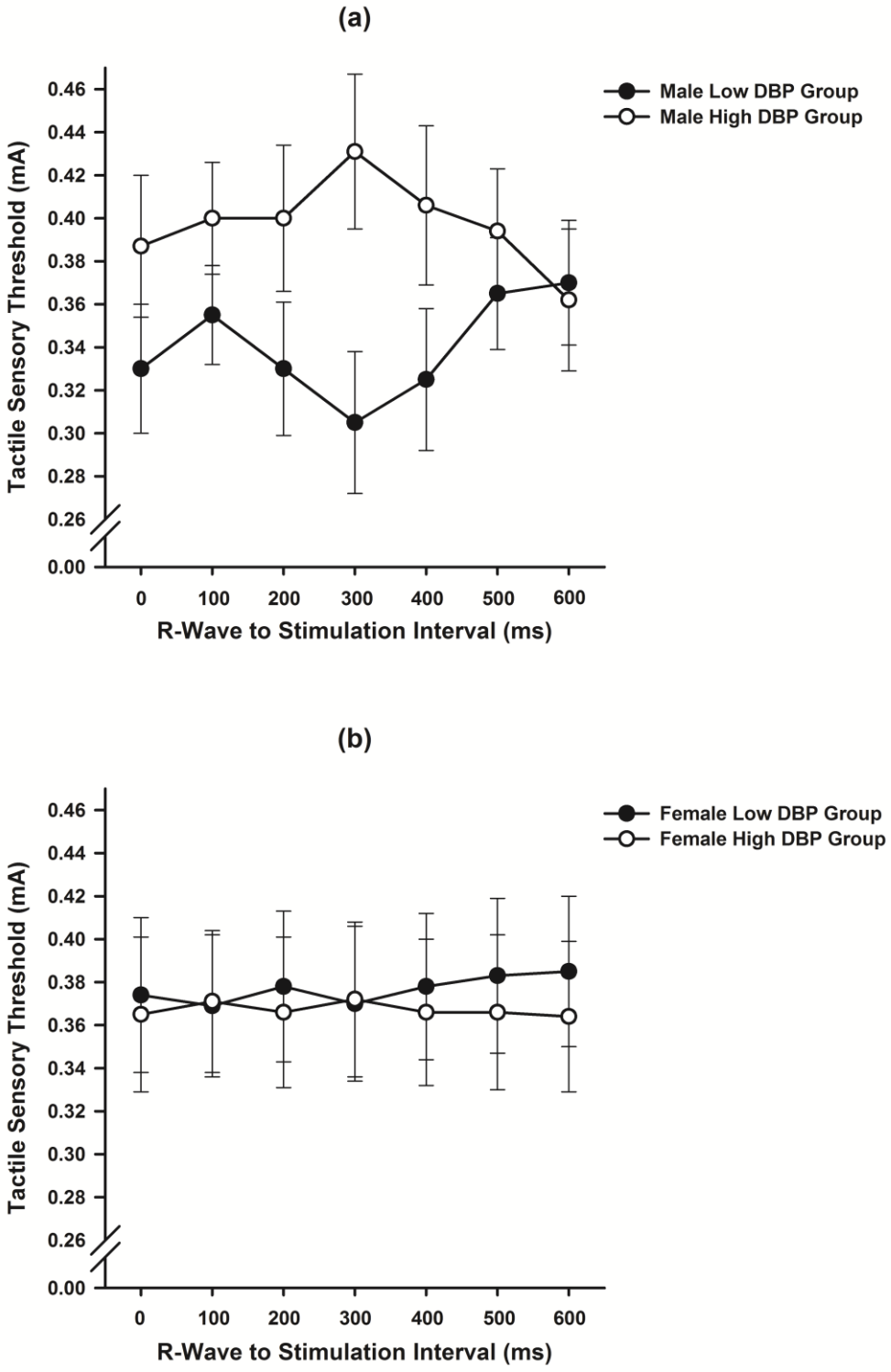

Figure 6. *Mean (SE) electrocutaneous tactile sensory thresholds at seven intervals across the cardiac cycle as a function of diastolic blood pressure (DBP) in (a) male participants and (b) female participants.*

3.5 Discussion

The current study found that there was no overall difference in tactile sensory thresholds across the cardiac cycle in normotensives, suggesting that within the normotensive BP range, tactile sensation is not influenced by the natural fluctuations in BP across the cardiac cycle. The lack of overall cardiac cycle modulation in the current study is in line with a previous study employing artificial baroreceptor stimulation via phase related external suction, which similarly reported no difference in intracutaneous electrical sensory detection thresholds between stimuli delivered during either mechanical stimulation, or inhibition of the carotid baroreceptors (Droste et al., 1994).

Conversely, the current findings are counter to the only previous study to specifically look at cutaneous thresholds across the cardiac cycle (Edwards et al., 2009) which reported reported that thresholds were reduced during systole compared to diastole, indicating heightened tactile sensitivity when baroreceptor activity was highest. The methods employed in the current study were very similar to Edwards et al., (2009); however, there are small differences that may help explain the differing results. Firstly, Edwards et al., (2009) examined tactile sensory thresholds at three intervals across the cardiac cycle (*R*+0, *R*+300 and *R*+600 ms) whereas the current study examined tactile sensory thresholds at seven intervals across the cardiac cycle (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms). To address this difference the current investigators analysed the data from the same intervals as Edwards et al., (2009) i.e. *R*+0, *R*+300 and *R*+600 ms using repeated measures ANOVA. These analyses also revealed no significant variation in sensory thresholds across the cardiac cycle (ε = 1.000, $F(2, 96) = .50$, $p = .607$, $\eta_p^2 = .010$). Therefore, the different range of cardiac cycle intervals used does not appear to be an explanation for the differences. A second consideration regarding the greater number of cardiac cycle intervals investigated in the current study is that the total number of stimuli and the total duration of the study would be longer than Edwards et al. (2009). A longer study duration may have resulted in participants disengaging with the task and/or possibly lead to fatigue of sensory fibres or central habituation to the stimulus which has previously been shown to occur for non-pain stimuli (Milne, Kay, & Irwin, 1991). However, as the inter-trial interval was 3 s, plus a 1 s delay as the

programme searched for the R-wave, plus the participants' response time, the inter-stimulus interval in the current study was relatively long (short inter-stimulus intervals have been shown to increase habituation – Milne et al., 1991) and as a variable stimulus intensity was employed in the current study, habituation should have kept to a low level (Milne et al., 1991). Therefore, study duration is not likely to be a major factor determining the differing results between the studies.

Thirdly, although both studies stimulated the hand electrocutaneously, the stimulating electrodes used were different as was the exact electrode location. The current study used a bar electrode (Nicolet) with 9 mm diameter contacts and a 22 mm inter-contact spacing secured to the dorsal surface of the intermediate and proximal phalanges of the right index finger, whereas Edwards et al., (2009) used a stimulating electrode comprising two 10 mm stainless steel disks (Nicolet) secured to the dorsolateral surface of distal phalanges. Although small, the slight differences in location may influence the sensitivity of the area as it has been shown that the density of cutaneous innervation in man varies considerably from one area to another (Mountcastle, 1974). Indeed, the over-all density of sensory units in the hand increases in the proximo-distal direction, showing a slight increase from the palm to the main part of the finger (where the current study electrode was sited) and an abrupt increase from the main part of the finger to the distal phalanges (where the electrode in the Edwards et al., (2009) was sited) (Johansson & Vallbo, 1979). Therefore, it is possible that the lack of modulation in the current study is due to the electrode being positioned on a less sensitive area of the hand. A further acknowledgement should be made to the mean sensory threshold in the current study being lower (0.37 mA) than that in the Edwards et al., (2009) study (0.59 mA). Although the same stimulation parameters were used in both studies (1 ms square wave pulses at 250 Hz for 60 ms), the higher stimulation intensities reported in the Edwards et al., (2009) study may have stimulated the A-δ nociceptive fibres as well as the A-β tactile fibres. However as the stimulation intensity was kept below 4 mA, it is unlikely that nociceptive A-δ fibres would be stimulated (Ring et al., 2008). Additionally, the reported cardiac cycle modulation of tactile sensory thresholds by Edwards et al., (2009) was the opposite (i.e. lower tactile sensory thresholds during systole) to that reported for the NFR (Edwards et al., 2001, 2002, 2003, McIntyre et

al., 2006, 2008) and pain thresholds (i.e. elevated pain thresholds during systole) (Study 1 - Wilkinson et al., 2013) further questioning the stimulation of A-δ nociceptive fibres in the Edwards et al., (2009) study as a possible explanation for the differences between the findings.

The methodological differences between the two studies are small, and therefore unlikely to fully explain the contrasting findings. It seems plausible that the difference between the current findings and those of Edwards et al., (2009) may be due to participant characteristic differences. First, differences in the age of the participants in the two studies may have influenced the results. The average age of the participants in the current study was lower, 28 (SD=11.7) years compared to Edwards et al., (2009) 38 (SD=10.25) years. It has been repeatedly shown that there is a reduction in most sensory modalities with age including vision (e.g. Weale, 1986), hearing (e.g. Helzner et al., 2005), smell (e.g. Schiffman, 1997) and tactile sensitivity (Stevens & Cruz, 1996; Gescheider, Bolanowski, Hall, Hoffman, & Verrillo, 1994; Takekuma et al, 2000). The 10 year difference in average age may not seem much, but it has been reported that tactile acuity threshold decreases, on average, by about 1% each year between the ages of 20 and 80 (Stevens & Cruz, 1996). Therefore the lower sensory threshold reported in the younger subjects in the current study may be expected. Additionally, baroreceptor sensitivity has also been shown to reduce with increasing age (Gribbin et al., 1971; Korner, West, Shaw, & Uther, 1974; Randall et al., 1976; Randall, Esler, Culp, Julius, & Zweifler, 1978). Therefore, one may expect to find less cardiac cycle modulation in the older individuals with potentially diminished sensation and reduced baroreceptor sensitivity, thus it does not seem likely that the different ages of participants may have contributed to the differing patterns of cardiac cycle modulation reported between the two studies. However, it is suggested that further research is required to investigate the possible influence of age on the cardiac cycle modulation of sensory detection thresholds.

Second, it must be acknowledged that the participants in the current study were predominately female, whereas in the Edwards et al., (2009) study there was a relatively even split of males (N=31) and females (N=28). Gender has been shown to significantly influence electrocutaneous thresholds, with women typically presenting

lower thresholds than men (Takekuma et al., 2000) and as such the differences in the distribution of males and females may have influenced the results of the two studies. In line with this, the only reported modulation in the current study was reported in the male sub-sets during the BP-median split analysis. However due to the small sample of males in these sub-sets (N=9) these data should be interpreted with extreme caution (see below for further discussion).

Third, the participants in the current study were normotensive, whereas the participants in the Edwards et al. (2009) study comprised 30 hypertensives and 29 normotensives. Given that hypertension is characterised by disruption to the baroreflex (Eckberg & Sleight, 1992) and that an inverse relationship has been reported between BP and baroreceptor sensitivity (Bristow et al., 1969; Gribbin et al., 1971), it is not unreasonable to assume that results may be different in a normotensive sample.

To further investigate the possible influence of tonic BP on the cardiac cycle modulation of tactile sensory thresholds, the current study split the sample into low-normal and high-normal BP groups. The BP group analysis revealed differences in tactile sensory threshold modulation across the cardiac cycle. The patterning of modulation illustrated in Figure 4 suggests that the cardiac cycle effects on tactile sensory thresholds may be differing at high-normal versus low-normal BP's. Specifically, only individuals in the DBPlow group demonstrated a significant variation in tactile sensory thresholds across the cardiac cycle, presenting lower tactile sensory thresholds during systole (R+300 ms) compared to diastole (R+600 ms), whereas the DBP_{high} group tended to have higher tactile sensory thresholds during systole compared to diastole, although post hoc analysis revealed these differences were not significant. These differing findings between the high and low DBP groups may contribute to the overall null finding in the current study. Indeed, we found a significant SBP × Interval interaction suggesting a similar patterning to DBP but post hocs were not significant. This explanation is partially supported by findings that electrocutaneous (Edwards, Ring et al., 2008) and electrical tooth pulp (Ghione et al., 1985) sensory thresholds are increased in unmedicated essential hypertension patients compared to normotensives and a significant correlation between mean

arterial BP and sensory threshold in response to electrical tooth pulp has also been reported (Ghione, Rosa, Mezzasalma, & Panattoni, 1988). However, the findings in the current study regarding the effects of tonic BP contrast with those of Edwards et al., (2009) who reported that individuals with higher diastolic BP had larger reductions in tactile sensory thresholds during systole compared to diastole, which may suggest that baroreceptor influence becomes greater as tonic BP increases.

It should be noted that further analysis revealed that the modulation reported in the current study appears to be driven by the males in the sample as evidenced by Figures 5 and 6 showing that cardiac cycle effects were confined to the male participants. The male DBPlow group demonstrated lower tactile sensory thresholds during systole (R+300 ms) than diastole (R+100, R+500 and R+600 ms), whereas the male DBPhigh group presented opposing modulation with higher tactile sensory thresholds at during systole (R+300 ms) than diastole (R+0, R+500 and R+600 ms). No significant differences were found between the DBPhigh and DBPlow female groups (see Figure 6). The same differing patterns of cardiac cycle modulation of tactile sensory thresholds were found in the male SBPlow and SBPhigh but not females (see Figure 5). In partial support of this finding, when using artificial baroreceptor stimulation Elbert et al. (1988) found that young men with high-normal BP were slower to detect discomfort following electrocutaneous stimulation compared to those with normal BP, with the group difference greatest during artificial baroreceptor stimulation. However, due to the low sample sizes in the male subsets (N=9), these results should be interpreted with extreme caution and further research is required to further investigate the true extent of tonic BP and gender interaction effects on the cardiac cycle modulation of tactile sensory thresholds.

Despite the contrasting findings, both the current results and those of Edwards et al. (2009) support a moderating influence of tonic BP on the cardiac cycle modulation of tactile perception. The mechanism for cardiac cycle-related modulation of tactile sensory thresholds in each BP group is unclear, but may relate to a baroreceptor mechanism (see Edwards et al., 2009). The baroreceptors are stimulated during systole when the pulse pressure wave stretches the walls of the aortic arch and carotid sinus (Angell James, 1971; Mancia & Mark, 1983), resulting

in increased baroreceptor activation during systole and subsequent cortical inhibition (e.g. Rau, Elbert, & Birbaumer, 1995). The diverging patterns of cutaneous sensitivity across the cardiac cycle between the male low and high BP groups may be due to BP group differences in baroreceptor afferent activity reaching brain areas affected by baroreceptor activity. In line with this hypothesis, an inverse relationship between BP and both baroreceptor sensitivity (Sleight, Robinson, Brooks, & Rees, 1977) and baroreflex sensitivity (Bristow et al., 1969; Gribbin et al., 1971) has been reported.

Taken together with the previous study by Edwards et al., (2009), the current findings provide preliminary evidence that tonic BP has a moderating influence on the cardiac cycle modulation of tactile sensibility. Further studies are required to further investigate the mechanisms underlying this relationship.

REFERENCES

Angell James, J. E. (1971). Effects of altering mean pressure, pulse pressure and pulse frequency on impulse activity in baroreceptor fibres from aortic arch and right subclavian artery in rabbit. *Journal of Physiology-London, 214*(1), 65-88.

Angell James, J. E., & Lumley, J. S. P. (1974). Effects of carotid endarterectomy on mechanical-properties of carotid-sinus and carotid-sinus nerve activity in atherosclerotic patients. *British Journal of Surgery, 61*(10), 805-810.

Bonvallet, M., Dell, P., & Hiebel, G. (1954). Tonus sympathique et activite electrique corticale. *Electroencephalography and Clinical Neurophysiology, 6*(1), 119-144.

Bonvallet, M., & Allen, M. B., Jr. (1963). Prolonged spontaneous and evoked reticular activation following discrete bulbar lesions. *Electroencephalography and Clinical Neurophysiology, 15*, 969-988.

Bonvallet, M., & Bloch, V. (1961). Bulbar control of cortical arousal. *Science (New York, N.Y.), 133*(3459), 1133-1134. doi: 10.1126/science.133.3459.1133

Bristow, J. D., Honour, A. J., Pickerin, G. W., Sleight, P., & Smyth, H. S. (1969). Diminished baroreflex sensitivity in high blood pressure. *Circulation, 39*(1), 48-&.

Brody, S., & Rau, H. (1994). Behavioral and psychophysiological predictors of self-monitored 19 month blood pressure change in normotensives. *Journal of Psychosomatic Research, 38*(8), 885-891.

Cheng, W. Y., Jiang, Y.D., Chuang, L. M., Huang, C. N., Heng, L. T., Wu, H. P., Tai, T.Y., & Lin, B.J. (1999). Quantitative sensory testing and risk factors of diabetic sensory neuropathy. *Journal of Neurology*, 246, 394-398.

Cohen, R., Lieb, H., & Rist, F. (1980). Loudness judgments, evoked potentials, and reaction time to acoustic stimuli early and late in the cardiac cycle in chronic schizophrenics. *Psychiatry Research, 3*(1), 23-29.

Coleridge, H. M., Coleridge, J. C. G., & Schultz, H. D. (1987). Characteristics of C-fiber baroreceptors in the carotid-sinus of dogs. *Journal of Physiology-London, 394*, 291-313.

Delfini, L. F., & Campos, J. J. (1972). Signal detection and cardiac arousal cycle. *Psychophysiology, 9*(5), 484-491.

Deshpande, N., Metter, E.J., Ling, S., Conwit, R., & Ferrucci, L. (2008). Physiological correlates of age-related decline in vibrotactile sensitivity. *Neurobiology of Aging*, *29*(5), 765–773.

Droste, C., Kardos, A., Brody, S., Greenlee, M. W., Roskamm, H., & Rau, H. (1994). Baroreceptor stimulation - pain perception and sensory thresholds. *Biological Psychology, 37*(2), 101-113.

Eckberg, D. L., Cavanaugh, M. S., Mark, A. L., & Abboud, F. M. (1975). A simplified neck suction device for activation of carotid baroreceptors. *The Journal of Laboratory and Clinical Medicine, 85*(1), 167-173.

Eckberg, D. L., & Sleight, P. (1992). *Human baroreflexes in health and disease*. Oxford: Clarendon Press.

Edwards, L., Inui, K., Ring, C., Wang, X. H., & Kakigi, R. (2008). Pain-related evoked potentials are modulated across the cardiac cycle. *Pain, 137*, 488-494. doi:10.1016/j.pain.2007.10.010

Edwards, L., McIntyre, D., Carroll, D., Ring, C., France, C. R., & Martin, U. (2003). Effects of artificial and natural baroreceptor stimulation on nociceptive responding and pain. *Psychophysiology, 40*, 762-769.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology, 39*, 678-681. doi:10.1017/S0048577202011770

Edwards, L., Ring, C., France, C. R., McIntyre, D., & Martin, U. (2008). Effects of opioid blockade on nociceptive flexion reflex thresholds and nociceptive responding in hypertensive and normotensive individuals. International Journal of Psychophysiology, 69(2), 96-100. doi:10.1016/j.ijpsycho.2008.03.005 ER

Edwards, L., Ring, C., McIntyre, D., & Carroll, D. (2001). Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology, 38*, 712-718.

Edwards, L., Ring, C., McIntyre, D., Winer, J. B., & Martin, U. (2009). Sensory detection thresholds are modulated across the cardiac cycle: Evidence that cutaneous sensibility is greatest for systolic stimulation. *Psychophysiology, 46*, 252-256. doi:10.1111/j.1469-8986.2008.00769.x

Elbert, T., Rockstroh, B., Lutzenberger, W., Kessler, M., Pietrowsky, R., & Birbaumer, N. (1988). Baroreceptor stimulation alters pain sensation depending on tonic blood-pressure. *Psychophysiology, 25*(1), 25-29.

Elliott, R., & Graf, V. (1972). Visual sensitivity as a function of phase of cardiac cycle. *Psychophysiology, 9*(3), 357-361.

France, C. R. (1999). Decreased pain perception and risk for hypertension: Considering a common physiological mechanism. *Psychophysiology, 36*(6), 683-692.

France, C. R., & Ditto, B. (1996). Risk for high blood pressure and decreased pain perception. *Current Directions in Psychological Science, 5*(4), 120-125.

Gescheider, G. A., Bolanowski, S. J., Hall, K. L., Hoffman, K. E., & Verrillo, R. T. (1994). The effects of aging on information-processing channels in the sense of touch: I. absolute sensitivity. *Somatosensory & Motor Research, 11*(4), 345-357.

Ghione, S. (1996). Hypertension-associated hypoalgesia - evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension, 28*, 494-504.

Ghione, S., Rosa, C., Mezzasalma, L., & Panattoni, E. (1988). Arterial hypertension is associated with hypoalgesia in humans. *Hypertension, 12*(5), 491-497.

Ghione, S., Rosa, C., Panattoni, E., Nuti, M., Mezzasalma, L., & Giuliano, G. (1985). Comparison of sensory and pain threshold in tooth pulp stimulation in normotensive man and essential hypertension. *Journal of Hypertension. Supplement, 3*(3), S113-5.

Goldstein, D.S. (1983). Arterial baroreflex sensitivity, plasma catecholamines, and pressor responsiveness in essential hypertension. *Circulation, 68,* 234-240.

Gribbin, B., Pickering, T.G., Sleight, P., & Peto, R. (1971). Effect of age and high blood pressure on baroreflex sensitivity in man. *Circulation Research, 29*, 424-431.

Helzner, E. P., Cauley, J. A., Pratt, S. R., Wisniewski, S. R., Zmuda, J. M., Talbott, E. O., . . . Newman, A. B. (2005). Race and sex differences in age-related hearing loss: The health, aging and body composition study. *Journal of the American Geriatrics Society, 53*(12), 2119-2127. doi:10.1111/j.1532-5415.2005.00525.x

Hodge, A. M., Dowse, G. K., Zimmet, P. Z., Gareeboo, H., Westerman, R. A., Tuomilehto, J., & Alberti, K. G. (1995). Factors associated with impaired vibration perception in Mauritians with normal and abnormal glucose tolerance. Mauritius NCD Study Group. Journal of Diabetes and its Complications, 9, 149-157.

Johansson, R. S., & Vallbo, A. B. (1979). Tactile sensibility in the human hand: Relative and absolute densities of four types of mechanoreceptive units in glabrous skin. *The Journal of Physiology, 286*, 283-300.

Koch, E.B. (1932). Die Irradiation der pressorezeptorischen kreislaufreflexe. *Klinische Wochenschrift, 2*, 225-227.

Korner, P. I., West, M. J., Shaw, J., & Uther, J. B. (1974). "Steady-state" properties of the baroreceptor-heart rate reflex in essential hypertension in man. *Clinical and Experimental Pharmacology & Physiology, 1*(1), 65-76.

Lacey, B. C., & Lacey, J. I. (1967). Somatic response patterning and stress: Some revisions of activity theory. In M. H. Apply, & R. Trumbull (Eds.), *Psychophysiological stress: Issues in research*. New York: Appleton Century Crofts.

Leong, G. W. S., Lauschke, J., Rutowski, S. B., & Waite, P. M. (2010). Age, Gender, and Side Differences of Cutaneous Electrical Perceptual Threshold Testing in an Able-Bodied Population. *Journal of Spinal Cord Medicine, 33*(3):249–255.

Levitt, H. (1971). Transformed up-down methods in psychoacoustics. *Journal of the Acoustical Society of America, 49*, 467-477.

Lin, Y. H., Hsieh, S. C., Chao, C. C., Chang, Y. C., & Hsieh, S. T., (2005). Influence of aging on thermal and vibratory thresholds of quantitative sensory testing. *Journal of the Peripheral Nervous System, 10*(3), 269–281.

Maffiuletti, N. A., Herrero, A. J., Jubeau, M., Impellizzeri, F. M., & Bizzini, M. (2008). Differences in electrical stimulation thresholds between men and women. *Annals of Neurology, 63*(4), 507-512. doi: 10.1002/ana.21346; 10.1002/ana.21346

Mancia, G., & Mark, A. L. (1983). Arterial baroreflexes in humans. In J. T. Shepherd, & F. M. Abboud (Eds.), *Handbook of physiology: The cardiovascular system* (pp. 755-793). Bethesda, Maryland: American Physiological Society.

Martins, A. Q, Ring, C., McIntyre, D., Edwards, L., & Martin, U. (2009). Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. *Pain, 147*, 84-90. doi:10.1016/j.pain.2009.08.016

Mazzucchi, A., Mutti, A., Poletti, A., Ravanetti, C., Novarini, A., & Parma, M. (1986). Neuropsychological deficits in arterial hypertension. *Acta Neurologica Scandinavica, 73*(6), 619-627.

McIntyre, D., Edwards, L., Ring, C., Parvin, B., & Carroll, D. (2006). Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology, 43*, 314-319. doi:10.1111/j.1469-8986.2006.00407.x

McIntyre, D., Kavussanu, M., & Ring, C. (2008). Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. *Pain, 137*(3), 550-555. doi:10.1016/j.pain.2007.10.018

Milne, R. J., Kay, N. E., & Irwin, R. J. (1991). Habituation to repeated painful and non-painful cutaneous stimuli: A quantitative psychophysical study. Experimental Brain Research, 87(2), 438-444.

Mountcastle, V. B. (1974). *Medical physiology.* Mosby: Saint Louis.

Persson, P. B., & Kirchheim, H. R. (1991). *Baroreceptor reflexes: integrative functions and clinical aspects*. Berlin: Springer-Verlag.

Radloff, L. S. (1977). The center for epidemiologic studies depression scale a self report depression scale for research in the general population. *Applied Psychological Measurement, 1*(3), 385-401.

Randall, O., Esler, M., Culp, B., Julius, S., & Zweifler, A. (1978). Determinants of baroreflex sensitivity in man. *The Journal of Laboratory and Clinical Medicine, 91*(3), 514-519.

Randall, O. S., Esler, M. D., Bulloch, E. G., Maisel, A. S., Ellis, C. N., Zweifler, A. J., & Julius, S. (1976). Relationship of age and blood pressure to baroreflex sensitivity and arterial compliance in man. *Clinical Science and Molecular Medicine. Supplement, 3*, 357s-360s.

Rau, H., Elbert, T., & Birbaumer, N. (1995). Baroreceptor activity and nociception. In D. Vaitl, & R. Schandry (Eds.), *From the heart to the brain: The psychophysiology of circulation brain interaction.* (pp. 151-168). Frankfurt: Peter Lang.

Raven, P. B., Fadel, P. J., & Ogoh, S. (2006). Arterial baroreflex resetting during exercise: A current perspective. *Experimental Physiology, 91*(1), 37-49. doi:10.1113/expphysiol.2005.032250

Ring, C., France, C. R., al'Absi, M., Edwards, L., McIntyre, D., Carroll, D., & Martin, U. (2008). Effects of naltrexone on electrocutaneous pain in patients with hypertension compared to normotensive individuals. Biological Psychology, 77(2), 191-196. doi:10.1016/j.biopsycho.2007.10.006

Rosa, C., Vignocchi, G., Panattoni, E., Rossi, B., & Ghione, S. (1994). Relationship between increased blood pressure and hypoalgesia: Additional evidence for the existence of an abnormality of pain perception in arterial hypertension in humans. *Journal of Human Hypertension, 8*(2), 119-126.

Sands, M. L., Schwatz, A. V., Brown, B. W., Nevitt, M.C., Seeley, D. G., & Kelsey, J. L. (1998). Relationship of neurological function and age in older women: the study of osteoporotic fractures. *Neuroepidemiology*, 17(6), 318–329.

Sandman, C. A., Walker, B. B., & Berka, C. (1982). Influence of afferent cardiovascular feedback on behaviour and the cortical evoked potential. In V. Cacioppo & R. E. Petty (Eds.), *Perspectives in Cardiovascular Psychology* (pp. 189-221). London: Guilford Press.

Sandman, C. A., Mccanne, T. R., Kaiser, D. N., & Diamond, B. (1977). Heart-rate and cardiac phase influences on visual-perception. *Journal of Comparative and Physiological Psychology, 91*(1), 189-202.

Saxon, S. A. (1970). Detection of near threshold signals during 4 phases of cardiac cycle. *Alabama Journal of Medical Sciences, 7*(4), 427-430.

Schiffman, S. S. (1997). Taste and smell losses in normal aging and disease. *JAMA : The Journal of the American Medical Association, 278*(16), 1357-1362.

Shapiro, A. P., Miller, R. E., King, H. E., Ginchereau, E. H., & Fitzgibbon, K. (1982). Behavioral consequences of mild hypertension. *Hypertension, 4*(3), 355-360.

Simon, G., Kiowski, W., & Julius, S. (1977). Effect of beta adrenoreceptor antagonists on baroreceptor reflex sensitivity in hypertension. *Clinical Pharmacology & Therapeutics, 22*, 293-298.

Sleight, P., Robinson, J. L., Brooks, D. E., & Rees, P. M. (1977). Characteristics of single carotid sinus baroreceptor fibers and whole nerve activity in the normotensive and the renal hypertensive dog. *Circulation Research, 41*(6), 750-758.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *Manual for the state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists Press, 1970.

Stevens, J. C., & Cruz, L. A. (1996). Spatial acuity of touch: Ubiquitous decline with aging revealed by repeated threshold testing. *Somatosensory & Motor Research, 13*(1), 1-10.

Takekuma, K., Ando, F., Niino, N., & Shimokata, H. (2000). Age and gender differences in skin sensory threshold assessed by current perception in community-dwelling japanese. *Journal of Epidemiology, 10*(1 Suppl), S33-8.

Velden, M., & Juris, M. (1975). Perceptual performance as a function of intra-cycle cardiac activity. *Psychophysiology, 12*(6), 685-692.

Walker, B. B., & Sandman, C. A. (1979). Human visual evoked responses are related to heart rate. *Journal of Comparative and Physiological Psychology, 93*(4), 18-25.

Weale, R. A. (1986). Aging and vision. *Vision Research, 26*(9), 1507-1512.

Wilkinson, M., McIntyre, D., & Edwards, L. (2013). Electrocutaneous pain thresholds are higher during systole than diastole. *Biological Psychology, 94*(1), 71-73. doi:10.1016/j.biopsycho.2013.05.002; 10.1016/j.biopsycho.2013.05.002

Zamir, N., & Shuber, E. (1980). Altered pain perception in hypertensive humans. *Brain Research, 201*(2), 471-474.

FOUR

Effects of blood pressure across the cardiac cycle on electrocutaneous pain-related evoked potentials

4.1 Abstract

Natural variations in blood pressure (BP) across the cardiac cycle have been shown to modulate nociception and pain. A recent study reported dampened N2 and N2-P2 laser-evoked potential amplitudes at Cz during systole compared to diastole in men. The current study examined the effects of natural variations in BP across the cardiac cycle on electrocutaneous pain-related evoked potentials (PREPs) using multi-channel recordings in 11 men and 15 women. Following determination of electrocutaneous pain thresholds using an up-down staircase method, stimuli equal to twice the individual pain threshold were delivered to the right hand in 7 blocks of 21 trials to elicit PREPs. Stimuli were delivered pseudorandomly at 7 cardiac cycle intervals (*R*-wave plus 50, 150, 250, 350, 450, 550, 650 ms). Separate repeated-measures ANOVAs revealed no significant variations in N2 or P2 peak amplitudes or N2-P2 peak-to-peak amplitude across the cardiac cycle at scalp recording sites Cz, C3, or C4 (all *p*'s > .05). Median BP splits were used to examine tonic BP effects on the cardiac cycle-related modulation of PREPs. Separate 2 Group (low-normal BP, high-normal BP) by 2 Sex (male, female) by 7 Interval repeated-measures ANOVAs for systolic and diastolic BP revealed no BP Group or interaction effects for N2 or P2 peak amplitudes or N2-P2 difference at Cz, C3 or C4 (all *p*'s > .05). These data suggest that the cardiac cycle-related modulation of PREPs may not be as robust as other measures of pain such as the nociceptive flexion reflex, and that the modality of stimulation may influence the cardiac cycle-related modulation of pain processing.

Descriptors: Arterial baroreceptors; Blood pressure; Cardiac cycle; Electrocutaneous; Pain-related evoked potentials

4.2 Introduction

It is well established that individuals with hypertension have a reduced sensitivity to both clinical and experimental pain (Ghione, 1996). A baroreceptor mechanism may provide an explanation for this hypertension hypoalgesia (France & Ditto, 1996; France, 1999; Ghione, 1996). Baroreceptors are stretch receptors located in the aortic arch and carotid sinus and are responsible for regulating blood pressure (BP) (Persson & Kirchheim, 1991). At rest, baroreceptors are stimulated during the systolic phase of the cardiac cycle by the arrival of the pulse pressure wave (Eckberg & Sleight, 1992; Mancia & Mark, 1983) and have reduced output during diastole (Angell James & Lumley, 1974) resulting in a pulsatile discharge (Angell James, 1971; Coleridge, Coleridge, & Schultz, 1987). As well as maintaining cardiovascular homeostasis, baroreceptors may also modulate the activity in areas of the brain related to pain (Ghione, 1996). There appears to be a significant overlap between the areas of the brain involved in cardiovascular and pain regulation, for example stimulation of the nucleus tractus solitarius induces antinociception (Aicher & Randich, 1990) as does the periaqueductal grey matter (Bandler, Carrive, & Zhang, 1991), which is also an important modulator of the arterial baroreflex (Inui, Murase, & Nosaka, 1994; Nosaka, Murata, Inui, & Murase, 1993).

A growing body of evidence suggests that natural fluctuations in BP across the cardiac cycle, which cause variations in baroreceptor activity, influence pain and nociception (e.g. Edwards et al., 2008). Cardiac cycle time studies utilise the natural variations in BP across the cardiac cycle, and thus, variations in the combined aortic and carotid baroreceptor stimulation. The cardiac cycle paradigm involves timing the delivering stimuli to coincide with systole, when BP and baroreceptor activation is highest, and diastole, when BP and baroreceptor is lowest, and comparing the respective responses. Several studies (Edwards, Ring, McIntyre, & Carroll, 2001; Edwards, McIntyre, Carroll, Ring, & Martin, 2002; Edwards et al., 2003; McIntyre, Edwards, Ring, Parvin, & Carroll, 2006; McIntyre, Kavussanu, & Ring, 2008a) have examined the influence of the cardiac cycle on the nociceptive flexion reflex (NFR). The NFR is defined as a polysynaptic spinal reflex sub-serving withdrawal from noxious stimuli (Sandrini et al., 2005), the threshold for which serves as a physiological correlate of pain (Hugon, 1973; Willer, 1977). These studies have

reported the NFR to be attenuated during systole compared to diastole (Edwards et al., 2001; 2002; 2003; McIntyre et al., 2006; 2008a) suggesting nociceptive responding may be dampened when arterial baroreceptor activity is maximal.

Pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage” (Merskey & Bogduk, 1994, p. 210) and as such, subjective in nature. Therefore, pain perception is inherently different to its neurophysiological correlates (Chen, Arendt-Nielsen, & Plaghki, 1998; Iannetti, Hughes, Lee, & Mouraux, 2008; Sandrini et al., 2005) described in the cardiac cycle time studies above (Edwards et al., 2001; 2002; 2003; McIntyre et al., 2006; 2008a). In response to this, a recent study by our group reported that pain perception thresholds were also attenuated during systole compared to diastole (Chapter 2 - Wilkinson, McIntyre, & Edwards, 2013).

To further the understanding of the mechanisms underlying the baroreceptor modulation of pain and nociception, investigators have also examined the pain-related evoked potential (PREP) response to painful stimuli. Pain-related evoked potentials elicited by noxious stimuli are thought to represent the central processing of nociception and as such, many researchers have used PREPs as objective measures of pain. The most commonly studied components of the PREP waveform are the second negative (N2) and positive (P2) peaks (Kanda et al., 1996; Garcia-Larrea, Peyron, Laurent, & Mauguiere, 1997; Fila & Bogucki, 2009), with N2 occurring approximately 130–240 ms post stimulus and P2 approximately 230–390 ms post stimulus (Bromm, 1985; Zaslansky et al., 1996). The amplitude of the N2 and P2 PREP components have been shown to correlate with the intensity of pain stimulus (Becker, Haley, Urena, & Yingling, 2000; Bromm, 1984; Stowell, 1977; Zaslansky et al., 1996), as well as with subjective ratings of pain (Kanda et al., 2002). In response to painful stimuli of the hand, both the N2 and P2 components have been found to be maximal at the midline central area, specifically scalp electrode site Cz (Bromm & Treede, 1987; Carmon, Mor, & Goldberg, 1976; Carmon, Dotan, & Sarne, 1978; Carmon, Friedman, Coger, & Kenton, 1980; Kakigi, Shibasaki, & Ikeda, 1989; Kanda et al., 1996; Kanda et al., 1999; Miyazaki et al., 1994; Treede, Kief, Holzer, & Bromm, 1988). Supporting evidence suggesting the

origin of the N2 and P2 components is mainly the anterior cingulate cortex (ACC), whilst the secondary somatosensory cortex (SII) and insula cortex, bilaterally, also contribute to the N2 component (Bromm & Chen, 1995; Tarkka & Treede, 1993; Valeriani, Rambaud, & Mauguiere, 1996).

Studies employing artificial baroreceptor stimulation have reported that the N2 and P2 amplitudes (Mini, Rau, Montoya, Palomba, & Birbaumer, 1995) and the N2-P2 peak-to-peak amplitude (Angrilli, Mini, Mucha, & Rau, 1997) elicited by noxious intracutaneous electrical stimulation of the finger were reduced during neck suction, whereas another study reported that the N2–P2 peak-to-peak amplitude elicited by noxious intracutaneous electrical stimulation of the finger was increased during neck suction (Brody et al., 1997). However, it is unclear what effect, if any, the artificial baroreceptor stimulation may have on the study participants and subsequent results. For example, the pressures exerted during the neck suction and compression may have made the procedure more aversive and distracting to participants, which may have influenced results. Additionally, Edwards and colleagues (2003) suggest that the neck cuff method for artificial baroreceptor stimulation may induce widespread physiological effects such as increased muscle tension indicated by increased muscle activity during trials when the neck cuff was applied compared to control trials. Such increase in muscle activity may contribute noise to the PREP recording as well as directly influencing baroreceptor effects of nociception.

To our knowledge only two studies have utilised the natural variations in BP across the cardiac cycle to investigate the cortical processing of noxious stimuli (Edwards, Inui, Ring, Wang, & Kakigi, 2008; Gray, Minati, Paoletti, & Critchley, 2010). The study by Edward et al. (2008) delivered noxious thulium-evoked laser stimulations were delivered to the dorsum of the right hand of 10 male participants and PREPs recorded at the vertex (Cz scalp electrode). The results reported that N2 amplitudes and N2-P2 peak-to-peak amplitudes were attenuated mid cardiac cycle, corresponding to maximal baroreceptor activation, compared to early and late cardiac cycle when baroreceptor activation is lowest.

Gray and colleagues (2010) investigated the effect of cue-induced expectancy and natural variations in BP across the cardiac cycle on electrocutaneously evoked

PREPs in a group of 11 female participants. Stimuli were delivered to the right ventral wrist during either the systolic or diastolic phase of the cardiac cycle, either with or without a visual cue prior to the pain stimulus. The results indicated that P2 amplitudes were significantly greater for cued stimuli than for uncued stimuli. However, when cued stimuli were presented during systole, the larger P2 amplitude was abolished without any corresponding changes in BP or heart rate. In contrast to Edwards et al. (2009) no amplitude differences were reported for the N2 component between cued or uncued stimuli and there was no significant cardiac cycle effect. The findings of these two studies concurred with several previous artificial baroreceptor stimulation studies investigating PREP responses to noxious stimulation (Angrilli et al., 1997; Mini et al., 1995), and to previous cardiac cycle studies investigating NFR responses to noxious stimulation (Edwards et al., 2001; 2002; 2003; McIntyre et al., 2006; 2008a). Taken together, these studies suggest that stimulation of the arterial baroreceptors modulates cortical processing of noxious stimuli.

It should be noted that the aforementioned cardiac cycle studies investigating baroreceptor effects on PREP responses to noxious stimuli employed different methods of stimulation, namely thulium-evoked laser (Edwards et al., 2008) and electrical (Gray et al., 2010). Gray et al. (2010) only delivered stimuli at 2 intervals within the cardiac cycle (Baroreceptor active & baroreceptor silent), whereas Edwards et al. (2008) delivered stimuli at 8 intervals (R+50, R+150....R+750 ms). Therefore, it is important to consider investigating the effects of natural variations in BP across the cardiac cycle on electrocutaneous stimulations at a greater number of cardiac cycle intervals to determine if the patterning of PREPs across the cardiac cycle is consistent across pain modalities. In addition, Edwards et al. (2008) included only male participants in their sample and Gray et al. (2010) only included females. As males and females present differing pain sensitivities (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009) it is important to investigate the PREP response across the cardiac cycle in a mixed sex sample to increase the generalisation of the results. Furthermore, Edwards et al. (2008) only determined the PREP response at scalp electrode Cz, whereas Gray et al. investigated possible modulation at sites lateral to the midline i.e. electrode sites C3 and C4.

Research regarding the precise location of pain processing in the brain is somewhat inconclusive with many studies indicating that following painful stimulation some of the pain processing areas are activated bilaterally, some contralaterally and some with a left or right hemisphere dominance regardless of side of stimulation. For example, Symonds and colleagues (Symonds, Gordon, Bixby, & Mande, 2006) reported using fMRI that following painful electrical stimulation, of the right and left hands, the somatosensory cortex and posterior insula were activated contralateral to the pain stimuli, whereas the mid/posterior insula, anterior insula, and posterior cingulate were activated bilaterally. Additionally, the middle frontal gyrus, anterior cingulate, inferior frontal gyrus, medial/superior frontal gyri, and inferior parietal lobule showed either an exclusive or strong lateralisation to the right hemisphere (Symonds et al., 2006). Additionally, further studies utilising fMRI have found bilateral responses within the SI, SII and insula but with a significantly greater contralateral response in SI and the thalamus in response to painful laser stimulation applied to the right and left hands (Bingel et al., 2003) and right and left lower legs (Youell et al., 2004). Left insula activity was also elevated following lower leg stimulation in the later study (Youell et al., 2004). Similar mixed findings were reported using PET, with increased cerebral blood flow following painful contact thermal stimulation of both the left and right arms in contralateral regions of the primary somatosensory cortex (SI), SII, insular cortex and bilateral regions of the cerebellum, putamen, thalamus, ACC, and frontal operculum regardless of side of stimulation (Coghill, Gilron, & Iadarola, 2001).Consequently, there is no clear consensus on the precise location of pain processing, although a meta-analysis of pain imaging studies suggests that in humans and primates the SI, SII ACC and Insula are consistently activated contralateral to the side of pain stimulation (see Peyron et al., 2000b for review).

In addition to the imaging studies discussed above, in specific relation to PREPs, studies have consistently identified the ACC as generating the N2 and P2 components, and SII and insular cortex, bilaterally additionally contributing to the N2 component (Bromm & Chen, 1995; Tarkka & Treede, 1993; Valeriani et al., 1996). Therefore, considering that the key pain regions (i.e. the SI, SII, ACC and Insula) as these extend anatomically laterally beyond the midline of the scalp (Nolte, 2002),

and previous studies have consistently identified contralateral, bilateral and lateralised activation of these pain processing areas (Apkarian et al., 2005; Bushnell & Apkarian, 2006; García-Larrea et al., 2003; Ingvar, 1999; Peyron et al., 2000b; Porro, 19 2003; Rainville, 2002; Tracey & Mantyh, 2007; Treede et al., 1999) electrode sites C3 and C4 would be expected to overlay these regions of possible pain processing.

The current study aimed to further the knowledge relating to the effects of natural fluctuations in BP across the cardiac cycle, on the cortical processing of noxious stimulation. Several methodological features were included to address the questions raised above, specifically, (a) to determine if stimulus modality influences the cardiac cycle modulation of PREPs, electrical stimulations were delivered via a concentric planar electrode (Kaube, Katsarava, Kaufer, Diener, & Ellrich, 2000) which has been shown to selectively stimulate A-δ nociceptive fibres (Katsarava et al., 2006; Kaube et al., 2000), (b) to increase the generalisation of the findings participants included both males and females, and (c) to determine if the cardiac cycle modulation of PREPs is evident in brain areas beyond Cz, PREP responses will be analysed at scalp electrode sites identified as overlaying potential sites of pain and cardiovascular system overlap i.e. Cz, C3 and C4. Based on previous findings that the N2-P2 peak-to-peak amplitude and the N2 amplitude, both shown to correlate with subjective pain reports and stimulus intensity (Bromm & Meier, 1984; Bromm & Lorenz, 1998; Granovsky, Granot, Nir, & Yarnitsky, 2008; Greffrath, Baumgartner, & Treede, 2007; Kanda et al., 2002), were attenuated during systole in response to noxious laser stimulation (Edwards et al., 2008) and that the NFR (Edwards et al., 2001; 2002; 2003; McIntyre et al., 2006; 2008a) and pain (Wilkinson et al., 2013) have been found to be reduced during systole, it was hypothesised that the N2–P2 peak-to-peak amplitude and N2 amplitudes would be reduced during systole compared to diastole at Cz. As previous studies have reported contributions to the N2 component of the PREP from SII and insular cortex bilaterally (Bromm & Chen, 1995; Tarkka & Treede, 1993; Valeriani et al., 1996) it was also hypothesised that the N2–P2 peak-to-peak amplitude and the N2 amplitudes would be reduced during systole compared to diastole at C3 and C4.

4.3 Methods

4.3.1 Participants

Twenty-Six (11 men, 15 women) normotensive adults with a mean (SD) age of 19.3 (2.3) years and body mass index (BMI) of 24 (5.4) kg/m^2 were recruited from the Loughborough University campus and local community to participate in the study. Mean (SD) resting systolic BP (SBP) was 120.3 (12.0) mmHg, diastolic BP (DBP) was 71.4 (7.2) mmHg, and resting heart rate (HR) was 71.4 (12.0) bpm. Individuals were excluded if they had any known health problems including chronic pain disorders, cerebrovascular, cardiovascular or neurological diseases, had a cardiac pacemaker, history of major psychiatric disorders, were pregnant or had missed their last menstrual cycle, were taking routine prescription medicine except for birth control, were currently using any narcotic substances, had an alcohol intake greater than 28 units per week for men and 21 units per week for women or had a resting HR above 92 bpm. Participants were asked to refrain from analgesic medication for 24 hrs and caffeine, nicotine and vigorous exercise for 2 hrs prior to testing. The Loughborough University Ethical Advisory Committee approved the study, and all participants provided written informed consent.

4.3.2 Apparatus and measurements

Resting BP (mmHg) and HR (bpm) were obtained using an automated oscillometeric sphygmomanometer (Omron 705-IT, Omron Healthcare Europe) and a brachial cuff attached around the upper non-dominant arm. An electrocardiogram (ECG) was recorded continuously at 2500 Hz using three disposable spot electrodes (Cleartrace, ConMed) placed in a modified chest configuration and connected to an AC amplifier (LP511, Grass). Pain stimuli (triple 1 ms monopolar square wave pulse with 5 ms inter-pulse interval at 200Hz) were delivered electrocutaneously by a constant current stimulator (DS7A, Digitimer) via a concentric planar electrode (Kaube et al., 2000). The concentric planar electrode was secured with tape (Transpore, 3M) to the dorsal surface of the right hand between the metacarpals of the index and middle fingers. Electrode sites were prepared by exfoliating (Nuprep, D.O. Weaver & Co) then degreasing the skin using isopropyl alcohol swabs (Sterets, Medlock Medical Ltd.). Electroencephalographic (EEG) data was recorded via a flexible nylon headcap (Biosemi) containing 32 electrode holders positioned

according to the internationally recognised 10-20 coordinate system (Jasper, 1958). A blunted needle (16G ¾ blunt square grind, Becton Dickinson and Company) and syringe (5ml syringe luer-lok tip, BD) were used to part the participants hair and fill each electrode holder (Biosemi Active electrode holders) with conductive gel (Electro-Gel, ECI). Thirty-two active version pin electrodes - sintered Ag-AgCl electrode tip (Biosemi) plus two feedback loop electrodes; a) Common Mode Sense (CMS) active electrode and b) Driven Right Leg (DRL) passive electrode were inserted into the corresponding electrode holders and checked to ensure electrode offset was below 25 mV. In addition to the head cap electrodes, six external electrodes flat type active electrodes - 4mm diameter sintered Ag-AgCl electrode pallet (Biosemi) were used to measure horizontal (HEOG) eye movement (2 electrodes) and vertical (VEOG) eye movement (2 electrodes) and to later act as reference for the scalp electrodes (2 electrodes). The cavity of each flat-type active electrode was filled with conductive gel and secured in place with double sided adhesive disks (Biosense Medical Ltd.) and with tape (Medipore, 3M). Both the Biosemi pin and flat electrodes are designed to provide very low noise, low offset voltages and very stable DC performance due to signal amplification occurring at the electrode which results in high electrode impedances not influencing the signal quality (Metting van Rijn, Kuiper, Dankers, & Grimbergen, 1996).

4.3.3 Procedure

Participants were tested in a single 2.5-hr session. At the start of the session participants sat quietly whilst completing the following questionnaires: (a) Demographics questionnaire containing questions about age, sex, health habits, education, (b) Spielberger State and Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970) , a 40-item inventory which assesses levels of state and trait anxiety and (c) the Center for Epidemiologic Studies Depression (CES-D) Scale (Radloff, 1977), a 20-item scale which is designed to measure depressive symptomatology in the general population (10 min total). Next, participants rested quietly while baseline BP and HR were measured at 60, 180, and 300 s (6 min). If a participant's HR exceeded 92 bpm, they were excluded from the study, as HR's above 92bpm would mean that stimuli presented at *R*-wave + 650ms would fall within the next cardiac cycle; however, none were excluded. Following instrumentation (30 min) participants

firstly undertook a pain threshold determination procedure (15 min). Participants then rested for 5mins after which a PREP assessment was completed (70 min).

4.3.4 Instrumentation

Electrode sites for the ECG were prepared by exfoliating then degreasing the skin to reduce impedance. Three electrodes were placed in a modified chest configuration; the two active electrodes were placed on the right clavicle and a rib below the heart on the left side of the torso and the ground electrode was placed on the left clavicle.

The same skin preparation procedure was applied to the ear lobes, an area between the outer canthus of the eyes and the temples, areas directly below each eye (infra-orbital point) and the forehead. Subsequently, the two external HEOG electrodes were attached to the skin approximately 1 cm out from the outer canthus of each eye, the two external VEOG electrodes were attached on the infra-orbital point below each eye and the two external reference electrodes were attached to the ear lobes. Next, the circumference of the participants head was measured to determine the appropriate size EEG head cap. The head cap was then placed on the participants head and secured in place with the chin strap. The location of the central (Cz) electrode was identified as half way between two anatomical landmarks; (a) the nasion, which is the distinctly depressed area between the eyes, just above the bridge of the nose, and (b) the inion, which is the lowest point of the skull from the back of the head and is normally indicated by a prominent bump. Lateral positioning of the Cz electrode was determined by investigator positioning and participant feedback from the insertion of the blunt needle into the Cz electrode holder. Once the head cap was in position the blunt needle and syringe were used to part the hair and fill each electrode holder with conductive gel to ensure contact with the scalp. The 32 electrodes, plus the CMS and DRL electrodes were then inserted into the corresponding electrode holder and their electrode offset checked.

Finally, the skin on the dorsal surface of the right hand between the metacarpals of the index and middle fingers was cleaned as previously described and the concentric planar electrode secured in position with tape.

4.3.5 Pain threshold assessment

Participants sat upright and supported their right forearm on a table while their hand rested on the response box. Mounted on the response box (16 cm × 16 cm × 3 cm) were a piezo-oscillator (top middle), a red light emitting diode (top left), a green light emitting diode (top right), and buttons marked “Yes” and “No” (centre left and right, respectively). A computer was programmed in Spike2 (CED) to record responses and trigger stimuli using a Micro II 1401 (Cambridge Electronic Design).

Pain detection thresholds were determined using an up-down staircase procedure (Levitt, 1971). A green warning light (1000 ms duration) illuminated to signify the start of each trial and a red light (variable duration; the light remained illuminated until the participant made a response, up to a maximum of 7500 ms) indicated the end of each trial. Following illumination of the green light, a 1s delay occurred after which the search for the *R*-wave of the ECG commenced and subsequently the participants hand was stimulated by a series of 3 square-wave pulses of 1 ms duration and 5 ms interval (200 Hz) at *R*+50 ms. Participants were informed that the stimulus could occur at any time between the illumination of the green and red lights. Once the red light was illuminated participants pressed the “*Yes*” button if they perceived the stimulation as painful or the “*No*” button if they did not perceive it as painful. The next trial commenced following the participants response. On the first trial the stimulus intensity was 0 mA, and subsequently increased in 0.8 mA steps until the participant first reported a painful sensation (first reversal). The stimulus intensity then decreased in 0.4 mA steps until the stimulus was no longer reported as painful (second reversal). Each staircase then continued in 0.1 mA steps until the staircase had completed two further ascending and descending series (i.e. four more reversals). The pain threshold (mA) was defined as the average of the peaks during the second and third series (i.e. the third and fifth reversal points) of each staircase. The maximum allowable stimulus intensity was 30 mA; however, this stimulus intensity was never reached.

4.3.6 Pain-related evoked potential procedure

Stimulation intensity was calculated as two times each individuals pain threshold and electrocutaneous stimulations were delivered using the same parameters as for the

threshold assessment procedure. Participants sat upright in a quiet room with their hands resting on their knees. They were instructed to focus on a fixation point (a black circle 2cm in diameter) positioned on the wall directly in front of them throughout each experimental block, to relax their muscles and to remain as still as possible. Each participant completed 7 experimental blocks of 21 trials, separated by a 5 minute rest period. During each block the participants hand was stimulated at each of seven *R*-wave intervals (*R*+50, *R*+150, *R*+250, *R*+350, *R*+450, *R*+550, *R*+650 ms) three times, thus over the 7 experimental blocks each interval was presented 21 times. The *R*-wave interval used in each trial was selected pseudorandomly and the inter-stimulus interval 12, 16, or 20s was pseudorandomly selected. At the start of each block an additional pain stimulus was delivered, but no data recorded, to prevent startle contamination affecting subsequent EEG activity. Participants were informed that the stimulus could occur at any time during the experimental period and that the experimental block would last approximately five minutes.

The Spike 2 programme marked the EEG data when each pain stimulus was delivered and also marked the EEG data with a beat-before trigger at the same *R*-wave interval in the preceding cardiac cycle as the subsequent pain stimulus. One problem with cardiac cycle studies is the potential for contamination of the EEG data by ECG artefacts (Gray, Minati, Paoletti, & Critchley, 2010). Marking the data during a cardiac cycle when no pain stimuli where delivered meant we were able to generate a beat-before average of EEG data representing the ECG-related artefacts and subtract this from the corresponding PREP average and thus remove any potential ECG contamination.

To ensure the stimulus remained painful, participants completed the Short-Form McGill Pain Questionnaire (Melzack, 1987) after each experimental block. The questionnaire included participants rating the average intensity of the pain stimuli during the proceeding block on a rating scale of 0-100 with anchors of ‘0’ (no sensation), ‘1’ (first sensation), ‘25’ (uncomfortable), ‘50’ (just noticeable pain), ‘75’ (very painful), and ‘100’ (maximum tolerable pain). If participants rated the intensity of the stimuli less than 50 (just noticeable pain) the stimulation in the following

experimental block was increased to double that used in the proceeding block. However, no participants rated the intensity below 50.

4.3.7 Electroencephalographic data recording and analysis

The EEG activity was recorded reference free, continuously using 32 electrodes via a battery-powered amplifier (Biosemi ActiveTwo AD-box, Mk 2) at a sample rate of 2048 Hz. Raw EEG and external electrode data were processed offline using BESA Research 5.2.2. The data were re-referenced to a linked ears reference off-line and filtered using a 30 Hz, 12 db/oct, ZeroType low-pass filter and a 0.1 Hz, 12 db/oct, Forward Type high-pass filter. Beat-before and pain stimulus data were segmented into 900 ms epochs (− 100 ms to 800 ms). The baseline was defined as 100ms pre-stimulus to stimulus onset. EEG data were corrected for ocular movement artifacts using manual definition of each participants blink topography and applying an adaptive artifact correction (Ille, Berg, & Scherg, 2002). Following correction of ocular movement artifacts, data were automatically scanned for epochs containing a voltage change of greater than 100 μV and these were rejected. Remaining trials were averaged according to *R*-wave interval (*R*+50, *R*+150, *R*+250, *R*+350, *R*+450, *R*+550, *R*+650 ms) resulting in a single PREP and one corresponding beat-before average for each *R*-wave interval. If more than 50% of the total pain or beat before trials were rejected, the participant was removed from further analysis. No participants were removed. The mean number of accepted trials per R-wave interval was 20 for both pain stimulus and beat-before (range = 13 to 21). Figure 7 shows, (a) the variation in one representative participants PREP potentials in consecutive trials across one experimental block, and (b) the average PREP waveform generated for an individual cardiac cycle interval after averaging 21 individual trials.

Prior to analysis of the PREP components, each *R*-wave beat-before average (e.g. Figure 8a) was subtracted from the corresponding PREP average (e.g. Figure 8a) to generate a difference PREP (e.g. Figure 8c). Such subtraction aimed to remove any ECG artefacts from the PREP for each individual cardiac cycle interval. However as shown in Figure 8, the beat-before waveform (8b) actually appears to contain very few ECG artifacts and there is therefore little difference between the original PREP waveform (8a) and the PREP minus beat-before waveform (8c).

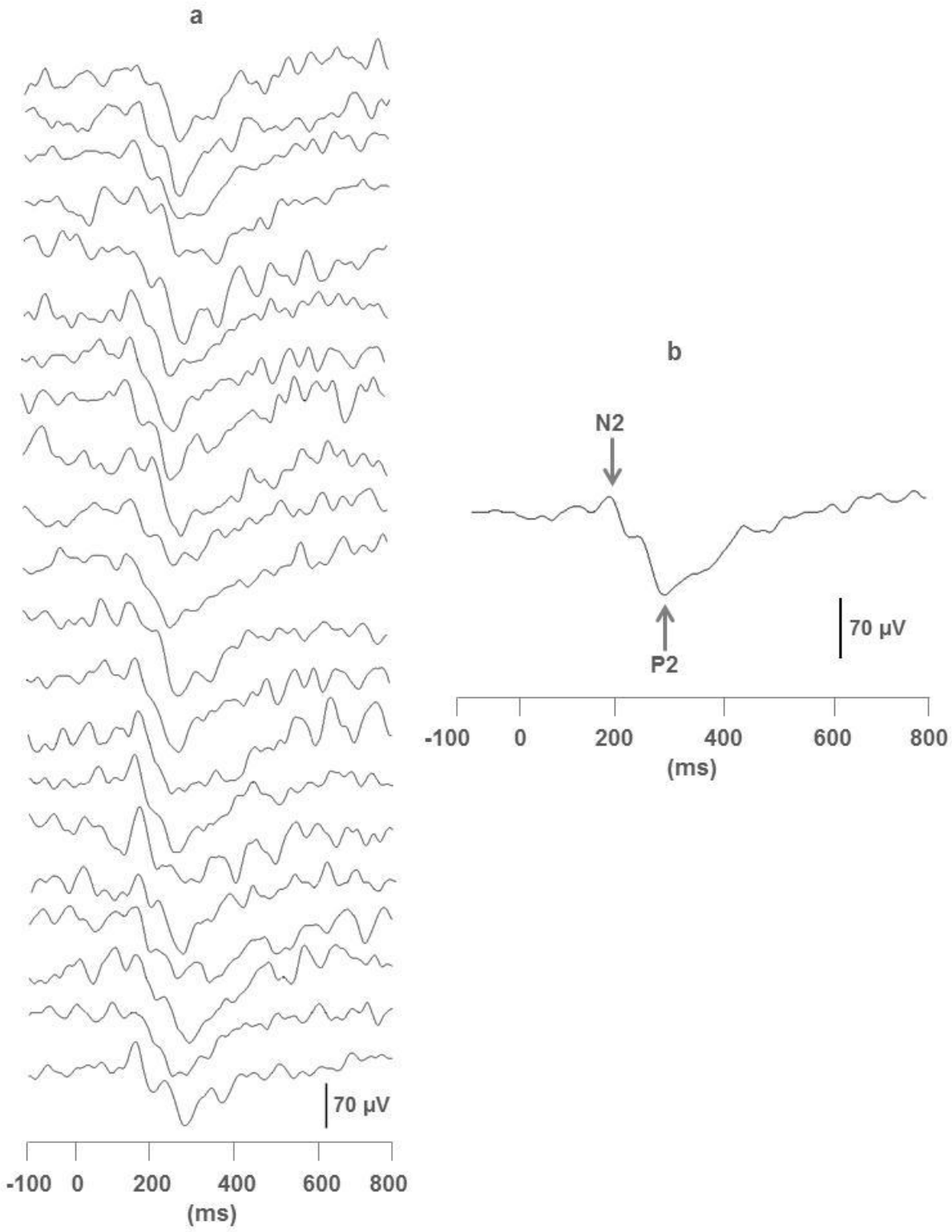

Figure 7. *Pain-related evoked potential waveforms for (a) 21 consecutive trials in a single experimental block (Block 1) and, (b) the average PREP for a single cardiac cycle interval (R+350ms) when 21 trials are combined in a representative participant.*

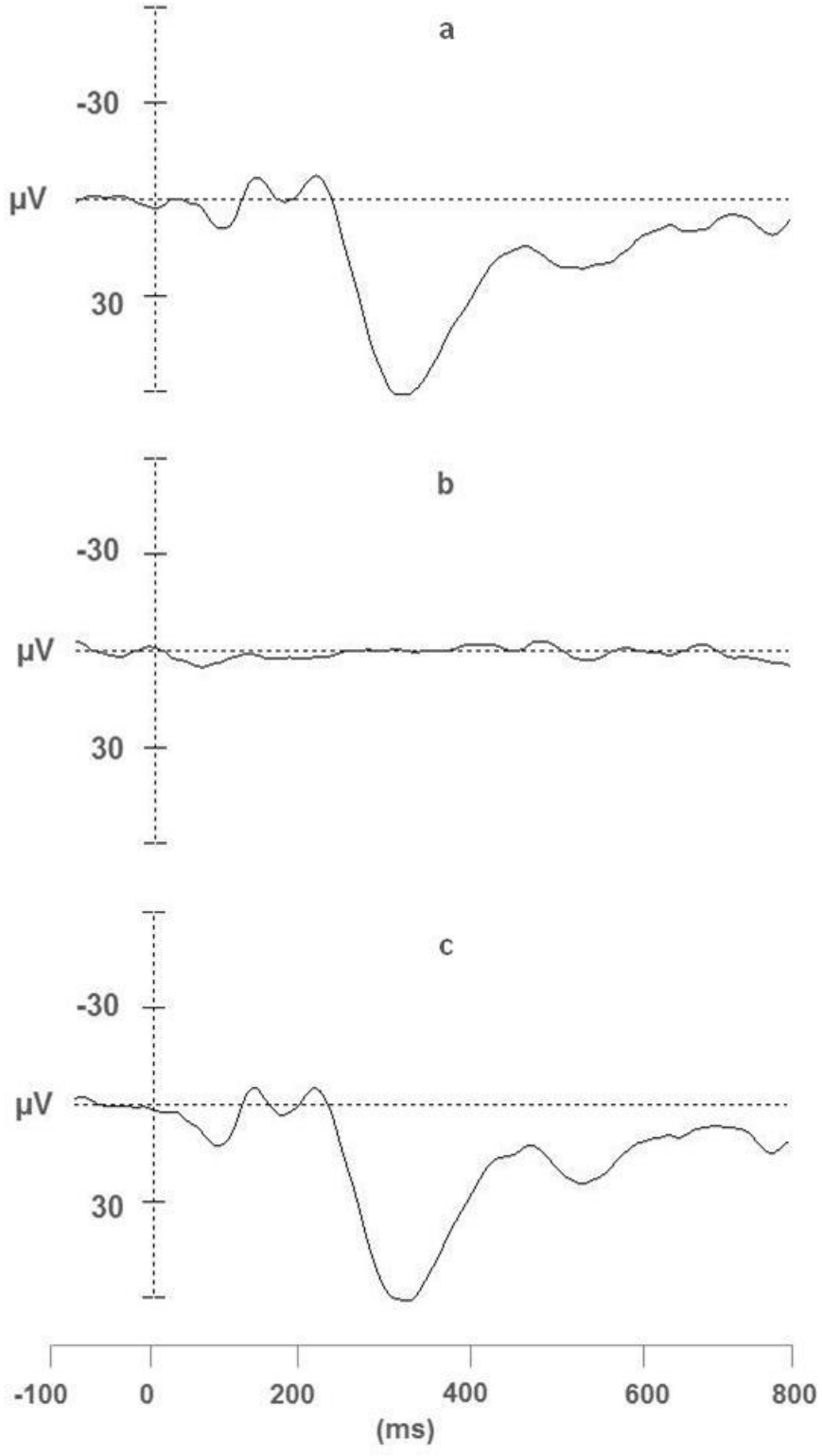

Figure 8. *Pain-related evoked potential waveforms for a) an average PREP waveform, b) average beat-before waveform and c) an average PREP minus beat-before waveform for a representative participant*

4.3.8 Pain-related evoked potential analysis

There are three main approaches to analysing PREPs, (a) peak amplitudes, (b) mean amplitudes, and (c) area analysis. Mean amplitudes are a linear measure and may be more reflective of components extending over time, especially if the time window is longer, compared to the single peak amplitude value which may be distorted by high frequency noise as mean amplitudes are less susceptible to high frequency noise (Luck, 2005). Area analysis reduces the influence of latency variability reducing amplitudes, as the area under the curve calculated from the waveform generated from several trials is always equal to the average of the area under the curve in each individual trial (Luck, 2005). However, as the pain response is generally a well defined, high frequency component the peak amplitude is likely to be the most appropriate method of analysis (Luck, 2005) and therefore in order to focus and streamline the results only the peak amplitude data will be reported in this chapter.

4.3.9 Peak Amplitude Detection

Peak detection windows for identification of the N2 and P2 component of the difference PREPs were identified as the latency of the visually identified N2 and P2 peak amplitudes from the grand average difference waveform for all participants and all intervals $\pm$40ms. Therefore the analysis windows for the N2 and P2 components of the averaged difference waveforms were 97-177ms and 263-343ms respectively. This fits with the typical electrically induced PREPS's being characterised by a negative peak (N2) at approximately 130–240 ms post stimulus followed by a positive peak (P2) approximately 230–390 ms post stimulus (Bromm, 1985; Zaslansky et al., 1996). Peak amplitudes were identified automatically within each analysis window for each R-wave interval. Peak amplitudes were defined as the baseline to highest peak in the detection window. Peak-to-peak measurements were also calculated as the difference between the peak in the 97-177ms window (N2) and the peak in the 263-343ms window (P2).

On the basis of previous neuroimaging studies identifying cerebral regions activated by painful stimulation (see Introduction), analyses were conducted on the following electrodes; Cz, C3 and C4. These electrodes are thought to reflect the

activity of brain areas associated with pain perception i.e. the ACC, SII, insular cortex bilaterally (Bentley, Derbyshire, Youell, & Jones, 2003; Bromm & Chen, 1995; Garcia-Larrea, et al., 2003; Ohara, Crone, Weiss, & Lenz, 2006; Tarkka & Treede, 1993; Treede, Lorenz, & Baumgartner, 2003; Valeriani et al., 1996) and the contralateral SI (Bushnell & Apkarian, 2006; Craig, 2002; Kakigi et al., 2005; Peyron et al., 2000a; Rainville, 2002).

4.3.10 Data reduction and analyses

The BP and HR readings were averaged to provide measures of resting SBP, DBP and HR. A series of repeated measures analysis of variance (ANOVAs) with *R*-wave to stimulation interval (i.e., *R*+0 ms, *R*+150 ms, *R*+250 ms, *R*+350 ms, *R*+450 ms, *R*+550 ms, *R*+650 ms) as a within subjects factor were performed separately on N2 and P2 peak amplitudes and N2-P2 amplitudes for Cz, C3 and C4 scalp electrodes.

Tonic BP has been shown to effect baroreceptor modulation of pain (e.g., Droste et al., 1994). Therefore, to examine the effect of tonic BP on PREPs across the cardiac cycle, participants were classified as having relatively low and high BP based on a median split of SBP and DBP. Median SBP was 120.17 mmHg; the SBP_{low} and SBP_{high} group comprised 13 participants each. For DBP the median was 70.00 mmHg; the DBP_{low} and DBP_{high} group comprised 13 participants each. Chi-squared analysis revealed differences for sex between the SBP_{low} and SBP_{high} groups, $\chi^2(1) = 7.72$, $p = .005$. The SBP_{low} group comprised 2 Males and 11 Females, whereas the SBP_{high} group contained 9 Males and 4 Females. There were no significant differences for sex between the DBP groups, $\chi^2(1) = 0.16$, $p = .691$. The DBP_{low} group contained 5 Males and 8 Females, and the DBP_{high} group consisted of 6 Males and 7 Females. A series of ANOVA's were performed on the continuous variables; SBP, DBP, BMI, height, weight and mean pain threshold across all intervals. Analyses revealed that the SBP_{high} group had significantly higher SBP, DBP, weight, height, BMI and mean pain thresholds than the SBP_{low} group. There were no significant differences between the groups in terms of age (Table 2). Similar ANOVA's using DBP group revealed significant differences between the groups only in terms of SBP and DBP, there were no other significant group differences (Table 3).

Table 2. *Mean (SE) Characteristics of the SBP_{low} and SBP_{high} Groups as well as Degrees of Freedom, F Values & Statistical Significance Level of the Group Effects and Associated Effect Size*

Variable	SBP_{low}	SBP_{high}	*df*	*F*	*p*	η_p^2
Systolic blood pressure (mmHg)	109.59 (1.05)	131.10 (1.64)	1, 24	121.34	<.001*	.835
Diastolic blood pressure (mmHg)	67.36 (1.41)	75.46 (1.87)	1, 24	11.96	.002*	.333
Age (years)	19.62 (0.84)	18.92 (1.12)	1, 24	0.59	.449	.024
Weight (kg)	59.54 (10.59)	83.15 (23.64)	1, 24	10.80	.003*	.310
Height (m)	1.67 (0.09)	1.78 (0.11)	1, 24	7.22	.013*	.231
BMI (kg/m^2)	21.18 (0.63)	26.20 (1.78)	1, 24	7.05	.014*	.227
Mean Pain Threshold (mA)	1.00 (0.12)	1.52 (0.22)	1, 24	4.32	.049*	.153

*significant at 0.05

Table 3. *Mean (SE) Characteristics of the DBP_{low} and DBP_{high} Groups as well as Degrees of Freedom, F Values & Statistical Significance Level of the Group Effects and Associated Effect Size*

Variable	DBP_{low}	DBP_{high}	*df*	*F*	*p*	η_p^2
Systolic blood pressure (mmHg)	114.62 (3.30)	126.08 (2.59)	1, 24	7.45	.012*	.237
Diastolic blood pressure (mmHg)	65.33(0.68)	77.49 (1.27)	1, 24	71.37	<.001*	.748
Age (years)	19.39 (0.83)	19.15 (0.37)	1, 24	0.07	.802	.003
Weight (kg)	64.58 (5.22)	78.12 (6.33)	1, 24	2.73	.112	.102
Height (m)	1.70 (0.29)	1.75 (0.33)	1, 24	1.21	.282	.048
BMI	22.49 (0.89)	24.89 (1.90)	1, 24	1.32	.263	.052
Mean Pain Threshold (mA)	1.25 (0.17)	1.28 (0.21)	1, 24	0.01	.910	.001

*significant at 0.05

A series of 2 BP Group (low, high) × 2 Sex (male, female) × 7 Interval (*R*+50, *R*+150, *R*+250, *R*+350, *R*+450, *R*+550, *R*+650 ms) repeated measures ANOVA, with Group and Sex as between-subjects factors and Interval as the within-subjects factor, were performed on N2 and P2 peak amplitudes and N2-P2 difference for Cz, C3 and C4 scalp electrodes. Sex was used as a between-subjects factor due to the SBP group differences identified, because men typically have higher BPs (e.g. Reckelhoff, 2001) and because there is good evidence that pain sensitivity is greater in women (Fillingim et al., 2009). As BMI has been shown to influence pain thresholds (e.g. Hodge & Zimmet, 1994) and in the current study BMI correlated with pain thresholds averaged across intervals (r (26) = .479, p = .013), BMI was entered as a covariate.

ANOVAs were corrected for the assumption of independence of data points using Huynh-Feldt correction (ε). In addition to significance levels, partial eta-squared (η_p^2), a measure of effect size, is also reported, indicating the proportion of total variation attributable to the factor, partialling out (excluding) other factors from the total non-error and range from 0 to 1 (Cohen, 1973). As partial eta-squared may over estimate effect sizes in repeated measures studies, effect sizes of 0.01, 0.09 and 0.25 are accepted as representing small, medium and large effects respectively (Hanna & Dempster, 2012). A significance level of .05 was adopted. Data were analysed using SPSS 20.0 and Statistica Version 10.

4.4 Results

4.4.1 Cardiac cycle effects on pain-related evoked potentials

Separate 7 Interval (*R*+50, *R*+150, *R*+250, *R*+350, *R*+450, *R*+550, *R*+650 ms) repeated measures ANOVA's were performed on N2, P2 amplitudes and N2-P2 peak-to-peak amplitude for Cz, C3 and C4 scalp electrodes, means (SD) are shown in table 4 and presented graphically in figure 9. As shown in table 5, these analyses revealed no significant variations in any of the variables of interest across the cardiac cycle (all *p*'s >.05).

Table 4. *Mean (SD) N2, P2 peak amplitudes and N2-P2 peak-to-peak amplitudes at seven intervals across the cardiac cycle for electrocutaneous PREPs at Cz, C3 and C4 electrodes.*

Variable	Mean (SD) Pain Thresholds (mA)						
	R+50 ms	*R*+150 ms	*R*+250 ms	*R*+350 ms	*R*+450 ms	*R*+550 ms	*R*+650 ms
Cz Electrode							
N2 Amplitude (μV)	-13.04 (9.21)	-11.83 (5.93)	-14.05 (6.92)	-13.92 (7.81)	-12.43 (6.50)	-14.23 (7.60)	-12.34 (6.34)
P2 Amplitude (μV)	11.24 (8.26)	12.24 (8.90)	11.28 (9.06)	11.25 (9.74)	11.87 (8.14)	11.35 (9.19)	11.13 (8.54)
N2-P2 peak-to-peak Amplitude (μV)	26.47 (13.44)	26.44 (10.47)	27.65 (11.00)	27.54 (12.42)	26.40 (9.72)	27.83 (12.14)	25.94 (10.91)
C3 Electrode							
N2 Amplitude (μV)	-12.41 (8.03)	-11.74 (5.88)	-12.78 (5.97)	-13.00 (7.56)	-12.03 (5.76)	-12.95 (6.27)	-11.59 (5.90)
P2 Amplitude (μV)	7.63 (7.45)	7.86 (7.37)	7.59 (7.00)	7.89 (7.95)	7.54 (6.40)	7.72 (7.74)	7.00 (7.20)
N2-P2 peak-to-peak Amplitude (μV)	21.40 (11.75)	21.46 (9.75)	21.98 (9.07)	22.72 (10.62)	20.92 (9.84)	22.29 (10.14)	20.35 (9.53)
C4 Electrode							
N2 Amplitude (μV)	-11.19 (7.05)	-10.98 (5.17)	-11.87 (6.35)	-12.08 (6.22)	-10.78 (5.07)	-11.90 (6.21)	-11.30 (5.58)
P2 Amplitude (μV)	7.01 (5.91)	6.85 (6.21)	5.90 (5.87)	6.34 (6.98)	7.82 (6.76)	6.27 (5.86)	6.84 (7.23)
N2-P2 peak-to-peak Amplitude (μV)	18.79 (10.50)	19.48 (8.66)	19.19 (9.19)	20.20 (8.98)	20.12 (7.62)	19.86 (9.49)	20.03 (9.90)

Table 5. *Seven Interval repeated measure ANOVA statistics for N2, P2 peak amplitudes and N2-P2 peak-to-peak amplitudes across the cardiac cycle for electrocutaneous PREPs at Cz, C3 and C4 electrodes.*

Variable	ε	*df*	*F*	*p*	η_p^2
Cz Electrode					
N2 Amplitude (μV)	.902	5.41, 135.28	1.61	.156	.061
P2 Amplitude (μV)	.774	4.64, 116.05	0.51	.753	.020
N2-P2 peak-to-peak Amplitude (μV)	.944	5.66, 141.54	0.71	.637	.027
C3 Electrode					
N2 Amplitude (μV)	.878	5.27, 131.66	0.71	.627	.027
P2 Amplitude (μV)	.933	5.60, 139.94	0.24	.955	.010
N2-P2 peak-to-peak Amplitude (μV)	.953	5.72, 142.93	0.99	.433	.0.38
C4 Electrode					
N2 Amplitude (μV)	.978	5.87, 146.75	0.58	.744	.023
P2 Amplitude (μV)	.862	5.17, 129.31	1.11	.357	.043
N2-P2 peak-to-peak Amplitude (μV)	.963	5.78, 144.43	0.41	.866	.016

*significant at 0.05

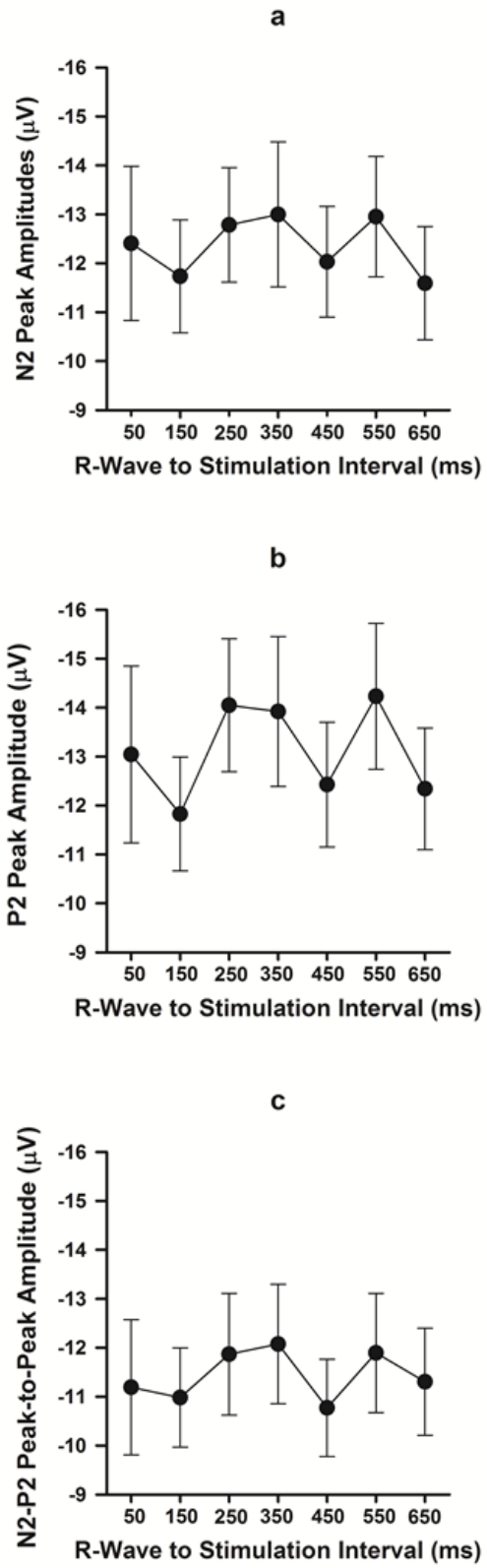

FIGURE 9. *Mean (SE) a) N2 peak amplitude, b) P2 peak amplitude, and c) N2-P2 peak-to-peak amplitude electrocutaneous PREP components as a function of phase of the cardiac cycle.*

4.4.2 Tonic BP and pain-related evoked potentials across the cardiac cycle

The effect of tonic BP on PREP amplitudes across the cardiac cycle was investigated by splitting the participants into low-normal and high-normal SBP and DBP groups. A series of separate 2 BP Group (low, high) × 2 Sex (male, female) × 7 Interval (*R*+50, *R*+150, *R*+250, *R*+350, *R*+450, *R*+550, *R*+650 ms) ANOVA's were performed on N2, P2 amplitudes and the N2-P2 peak-to-peak amplitude for Cz, C3 and C4 electrodes.

4.4.3 N2 *peak amplitudes across the cardiac cycle*

A series of 2 Group × 2 Sex × 7 Interval ANOVA's revealed no main effects for Group (all *p*'s >.05) or Sex (all *p*'s >.05) at any scalp electrode of interest (Cz, C3, C4) for N2 peak amplitudes. Analysis also revealed no Group × Interval, Sex × Interval or Group × Sex × Interval interactions (all *p*'s >.05) at any electrode of interest (Cz, C3, C4). Similar analysis using a DBP median split, also revealed no main or interaction effects at Cz, C3 or C4 (all *p*'s >.05).

4.4.4 P2 *peak amplitudes across the cardiac cycle*

As with N2 peak amplitudes, a series 2 Group × 2 Sex × 7 Interval ANOVA's revealed no main or interaction effects at Cz, C3 or C4 electrodes (all *p*'s >.05) for both SBP and DBP analyses.

4.4.5 N2-P2 peak-to-peak amplitude across the cardiac cycle

Further 2 Group × 2 Sex × 7 Interval ANOVA's on N2-P2 peak-to-peak amplitude revealed a significant main effect for Sex at C3, $F(1, 21) = 5.22$, $p = .033$, $\eta_p^2 = .199$. Males had higher N2-P2 peak-to-peak amplitudes than females at C3, mean (SD) 26.49μV (11.28) and 17.99 μV (11.97) respectively. No other main or interaction effects were significant at Cz, C3 or C4 (all *p*'s >.05).

When the ANOVA analyses was repeated using a DBP median split, no main or interaction effects were significant at any of the electrodes of interest (Cz, C3, C4) (all *p*'s >.05).

4.5 Discussion

The current study examined the effect of natural fluctuations in BP across the cardiac cycle on electrocutaneous PREPs in a group of healthy participants at scalp electrodes Cz, C3 and C4 which are hypothesised to overlay cortical areas involved in pain processing i.e. Somatosensory areas located in the parietal operculum (Bushnell & Apkarian, 2006; Craig, 2002; Peyron et al., 2000b; Rainville, 2002), the insula and the ACC (Bentley et al., 2003; Frot, Rambaud, Guenot, & Mauguiere, 1999; Garcia-Larrea et al., 2003; Lenz et al., 1998; Ohara et al., 2006; Treede et al., 2003). The main findings were that the N2 and P2 peak amplitudes and the N2-P2 peak-to-peak amplitude, which have been shown to correlate with the intensity of pain stimulus (Becker et al., 2000; Bromm, 1984; Stowell, 1977; Zaslansky et al., 1996) as well as with subjective ratings of pain (Kanda et al., 2002), were unaffected by natural variations in BP across the cardiac cycle.

The current findings partially agree with those of Gray and colleagues (2010) who reported no cardiac cycle effects for cued or uncued N2 peak amplitudes or for uncued P2 peak amplitudes. However, cued P2 peak amplitudes were modulated across the cardiac cycle, with reduced amplitudes during systole. No cue was presented prior to delivery of the pain stimulus in the current study. Therefore, presenting a cue prior to stimulation may be important in determining cardiac cycle modulation. In a second previous study which reported a systolic dampening of laser evoked PREPs (Edwards et al., 2008), participants were presented with a fixation point from 10 to 15 s before each stimulus, therefore the participants were aware that the stimuli was going to be presented and thus, is in line with Gray et al. (2010) suggests that cueing stimuli may be an important factor in cardiac cycle-related modulation. The reasons why cueing may influence cardiac cycle related modulation may relate to cardiac deceleration being induced by the cue (Graham & Clifton, 1966; Lacey & Lacey, 1970). Such cardiac deceleration has been shown to reduce the cardiac cycle effects on visual evoked potentials (Walker & Sandman, 1982). Alternatively, cueing may lead to a shift in attention towards the pain (Gray et al., 2010) which has been shown to increase its perceived intensity of pain (e.g. Angrilli, Mini, Mucha, & Rau, 1997) and increases PREP amplitudes (e.g. Lorenz & Garcia-Larrea, 2003).

As mentioned above, the lack of cardiac cycle-related modulation of PREPs in the current study contrasts with a reported reduction in electrocutaneous evoked P2 peak amplitudes during systole compared to diastole (Gray et al., 2010) and the systolic dampening of N2 peak amplitudes and N2-P2 peak-to-peak amplitudes in response to noxious laser stimulation at Cz electrode (Edwards et al., 2008). The findings are also contrasting to reports of a dampening of the NFR (Edwards et al., 2001; 2003; McIntyre et al., 2006; 2008a) and pain perception (Wilkinson et al., 2013) during systole compared to diastole. However, methodological differences may help explain the diverging results reported between the current study and the previous PREP cardiac cycle studies (Edwards et al., 2008; Gray et al., 2010). Firstly, the stimulus modality used to deliver the noxious stimulation was different; Edwards et al. (2008) used thulium-evoked laser stimulations whereas Gray et al. (2010) and the current study delivered electrocutaneous stimulations. However, it should be noted that the electrodes used to deliver the electrical stimulation were different. We used a concentric planar electrode (Kaube et al., 2000), were as Gray et al. (2010) used two standard EEG electrodes separate by approximately 1 cm, making its stimulation delivery very similar to a standard bar electrode.

The different types of stimulation may differentially active nociceptive and non-nociceptive fibres (e.g. Lefaucheur et al., 2012; Perchet et al., 2012). Laser stimulation has a rapid onset and excites a limited number of primary afferent fibres, primarily thin myelinated A-δ- and unmyelinated C-fibres (Meyer, Ringkamp, Campbell, & Raja, 2006). The myelinated A-δ afferents conduction velocity is approximately 15 m/s (Meyer, Walker, & Mountcastle, 1976), with high firing rates up to 30 Hz and reflect the fast “first pain” sensation being pinprick like (Arendt-Nielsen & Chen, 2003). Whereas the unmyelinated C fibres have slower conduction velocities approximately 0.86–1.25 m/s (Gybels, Handwerker, & Van Hees, 1979), have slower firing rates (15-20Hz) and reflect the “second pain” described as slow burning (Arendt-Nielsen & Chen, 2003). Traditional electrical stimulation, similar to that used by Gray et al. (2010) is known to cause powerful A-β fibre stimulation related to pressure-vibration which are felt like an aversive stab or vibration without actually being felt as pain (Gracely, 2006). Activation of A-β sensory fibres may have influenced the generation of PREPs in the current study, potentially reducing the

nociceptive nature of the stimulation and thus masking evidence of a cardiac cycle-related modulation of the electrocutaneous PREPs. In relation to Melzack & Wall's (1965) Gate Control Theory of Pain, stimulation of the larger A-β sensory fibres may result in stimulation of inhibitory interneurons within the dorsal horn. As stimulation of these inhibitory neurons inhibits the projection cells from sending nociceptive signals to the brain, the result is a closing of the gate and thus a reducing the pain signals reaching the brain. However, the concentric planar electrode used to deliver the pain stimuli in the current study is designed to selectively stimulate A-δ nociceptive fibres (Katsarava et al., 2006; Kaube et al., 2000) and indeed all participants described the sensation as painful and akin to a pin prick suggesting the activation of A-δ nociceptive fibres. Additionally, the PREP waveforms generated in the current study (Figure 7b) show the morphology and timings of the N2 and P2 components typically associated with electrocutaneous PREPs (Luck, 2005) and therefore it is likely that the electrical stimulation delivered in the current study was activating primarily A-δ pain fibres and thus generating PREPs reflecting brain responses to noxious rather than non-pain stimulation.

Laser stimulation is a thermal pain and two recent studies (de Tommaso et al., 2011; Lefaucheur et al., 2012) have reported differences in the latency of the N2 and P2 components between PREP responses evoked by laser stimuli and concentric planar electrode, with laser stimulation demonstrating later peak amplitudes than electrical. The latency differences could be attributed to differences in axon activation between the two stimulation modalities. Electrical stimulation directly activates peripheral afferents (Perchet et al., 2012), where as laser stimulation incurs a peripheral delay usually about 40 ms, although considerably less (approx.10-20 ms) when brief duration laser stimulations are used (Iannetti et al., 2004), due to signal transduction between thermoreceptor heating and action potential generation in A-δ nociceptive fibres (Bromm & Treede, 1991; Plaghki & Mouraux, 2003). However, recent studies (de Tommaso et al., 2011; Perchet et al., 2012) concluded that the difference in latency between laser and concentric planar electrode PREP's was greater (55-80 ms) than that generally associated with the receptor activation delay in brief laser stimulations (approx 10-20 ms). Therefore the authors conclude that the difference between the PREPs is likely indicative of a coactivation of A-β

fibres by the concentric planar electrode and thus suggest it may not be a specific nociceptive stimulation. Such differences in the PREP between laser and electrocutaneous stimulation may influence the cardiac cycle modulation of painful stimuli and help explain the lack of cardiac cycle modulation in the current study. If the concentric planar electrode coactivates a significant proportion of large myelinated sensory A-β fibres, which when stimulated dominate the cortical response, the results would concur with the lack of cardiac cycle modulation reported in Study 2 (Chapter 3) of this thesis in relation to sensory thresholds. However, it should also be noted that Gray et al. (2010) report a cardiac cycle modulation of P2 peak amplitudes using typical electrical stimulation electrodes. However, the PREP components which were modulated (P2) was different to the N2 and N2-P2 peak-to-peak components reported to be modulated by Edwards et al. (2008) perhaps suggesting that electrical and laser evoked PREPs are modulated differently across the cardiac cycle. To investigate this further, studies are required directly comparing the PREP response to laser, concentric planar electrode and tactile stimulation across the cardiac cycle in the same individuals.

Secondly, the current study aimed to increase the generalisation of the findings of Edwards et al. (2008) and Gray et al. (2010) who only included male and female participants respectively. The current study addressed this by including both male and female participants in the sample group. Consistent with previous literature suggesting women are more sensitive to pain than men, the current study revealed lower mean (SD) pain thresholds in females, 0.96 mA (0.40) compared to males, 1.67 mA (0.77). The present findings suggest sex does not influence PREP amplitudes across the cardiac cycle. This is in line with previous studies that have not found sex differences in the cardiac cycle modulation of pain ratings (Martins, Ring, McIntyre, Edwards, & Martin, 2009) , nociceptive responding (Edwards et al., 2001; Martins et al., 2009), pain thresholds (Study 1 – Wilkinson, McIntyre & Edwards, 2013) or reaction times (Birren, Phillips, & Cardon, 1963; Edwards, Ring, McIntyre, Carroll, & Martin, 2007; McIntyre, Ring, Hamer, & Carroll, 2007; McIntyre, Ring, Edwards, & Carroll, 2008b). However, this is not always the case as Study 2 (Chapter 3) in this thesis indicated that cardiac cycle modulation of tactile sensory detection thresholds was only evident in a subset of male participants with low-

normal BP. The only significant difference in the current study in relation to PREP amplitudes was that men had higher N2-P2 peak-to-peak amplitudes at C3 electrode than females when SBP median splits were applied. However, due to the relatively low number of male participants (n=11), these findings should be interpreted with caution. Further studies are needed with larger sample sizes to investigate the possible influence of sex on the cardiac cycle modulation of pain and PREPs.

Third, the duration and total number of noxious stimulations delivered was also different between the studies and these differences may result in varying levels of peripheral nerve fibre fatigue (Greffrath et al., 2007) and/or central habituation to the painful stimuli (Bingel, Schoell, Herken, Buchel, & May, 2007; Bingel, Herken, Teutsch, & May, 2008; Milne, Kay, & Irwin, 1991). The current study employed 7 blocks of 21 trials, Edwards et al. (2008) delivered 5 blocks of 12 trials and Gray et al. (2010) asked participants to complete 4 blocks of 50 trials. If the mean inter-stimulus interval used in the current study (16 s) is applied, each experimental block would total approximately 320 s. Similarly, with a mean inter-stimulus interval of 17.5 s, the Edwards et al. study would total 192s. Gray et al (2010) report that their experimental blocks lasted about 380 s. Therefore the total duration of the PREP task in the current study, including rest periods, was approximately 70 minutes (5 min rest periods between blocks), compared to approximately 55 minutes (10 min rest periods between blocks) for Edwards et al. (2008) and 30 minutes (140 s rest period between blocks) for Gray et al. (2010).Thus, the current study had a longer total study time.

A common occurrence following repetitive painful stimulation is habituation, i.e. a decrease in pain and pain-related responses to continuous or repetitive pain stimuli (LeBlanc & Potvin, 1966; Strempel, 1976; Strempel, 1978). In response to painful electrical stimulation applied to the same skin area every 30s, habituation was found over the course of 10 minutes (Milne et al., 1991). The response to painful stimuli has been shown to recover completely within 2 minutes of cessation of the stimulation (Milne et al., 1991) and thus the rest period (5 min) between each experimental block in the current study should have been sufficient to reduce habituation. However, the extended duration of the current study may have been

sufficient to dampen the pain responses to a greater extent than the previous studies (Edwards et al., 2008; Gray et al., 2010), although it should be noted that Gray et al. (2010) had longer individual experimental blocks (380 s vs. 320 s).

The source of pain habituation has not been fully determined. Repetitive stimulation at the same skin site has been shown to potentially induce fatigue of peripheral nociceptive neurons (Greffrath et al., 2007) as well as central habituation to the painful stimuli (Handwerker & Kobal, 1993; Milne et al., 1991; Valeriani et al., 2005). With regards to peripheral neuron fatigue due to the nature of laser stimulation and the potential for actual skin damage Edwards et al. (2008) moved the site of stimulation each time and thus may have reduced the effect of peripheral nociceptive neuron fatigue, whereas the current study stimulated at the same site throughout the experiment thus potentially exacerbating the effect of any neuron fatigue. Gray et al. (2010) did not move the stimulation site; however, the total experimental time was less. Recent functional neuroimaging studies have proposed that habituation to painful stimulation is related to central factors and specifically to increased activity in the rostral ACC (Bingel et al., 2007; Bingel et al., 2008). Indeed in response to electrical stimulation, habituation was shown not to depend on changes to peripheral nociceptive fibre recruitment or fatigue because the median nerve afferent volley remained constant throughout the period of stimulation (Milne et al., 1991). Therefore, it seems more plausible that the habituation to painful stimulation is a central mechanism which may be stimulus specific. In relation to central habituation, the longer experimental protocol may also have led to participant disengagement with the task indeed research has repeatedly shown that participant attention towards or away from the pain stimuli (Arntz, Dreessen, & Merckelbach, 1991; Bantick et al., 2002; Coen et al., 2008; Iannetti et al., 2008) modulates the neural correlates of pain. Therefore, if the participants in the current study became disengaged with the task over time, drawing attention away from the pain stimulus, this may have reduced the PREP amplitudes (Arntz et al., 1991; Rutter, Dahlquist, & Weiss, 2009; Tan, 1982) and thus affected the cardiac cycle effects in the present study.

It could be suggested that the shorter inter-stimulus interval and rest periods employed by Gray et al. (2010) would be expected to cause a greater habituation to the pain (Milne, Kay, & Irwin, 1991). However, the design of the Gray study included visual cues for 50% of the stimuli which may have helped maintain participant engagement with the task to a greater extent than the current study. Indeed, it was only for cued stimuli that Gray et al. (2010) found a cardiac cycle modulation of P2 amplitudes, and not for uncued stimuli, perhaps suggesting that participant focus is an important factor determining cardiac cycle effects.

Although it is not clear what type of EEG hardware Edwards et al. (2008) used, high impedance EEG systems such as the Biosemi system used in the current study require a greater number of trials to achieve significance than comparable low impedance systems because although high electrode impedances do not significantly reduce the amplitude of the EEG signal (Johnson et al., 2001), they may, due to an increased size or incidence of skin potentials increase the noise level, thus lowering the signal-to-noise ratio (Kappenman & Luck, 2010). Although the Biosemi system is specifically designed to tolerate high electrode impedances (Metting van Rijn, Kuiper, Dankers, & Grimbergen, 1996), Kappenman and Luck, (2010) suggest that to achieve an 80% chance of statistical significance for the P300 component using a high impedance system such as the Biosemi system with a 0.1 Hz high-pass filter as used in the current study would require approximately 25 individual trials per condition to be averaged whereas low impedance systems require approximately 10 trials. However, it should be noted that the number of required trials suggested by Kappenman and Luck (2010) was based on a study of just 12 particpants, whereas the current study had 26 participants. With respect to this, the current study demonstrated a higher number of accepted trials per cardiac cycle interval than Edwards et al. (2008), mean (SD) accepted trials for all cardiac cycle intervals was 19.9 (1.7) and 5.7 (2.4) respectively; data regarding accepted trials isn't available for Gray et al. (2010). Kappenman and Luck (2010) suggest that the chance of achieving statistical significance can be improved by increasing the number of trials and/or the number of participants. Therefore, the current study aimed to improve the signal to noise ratio by recruiting more participants (N=26) than both Edwards et al. (2008) and Gray et al. (2008) who had just 10 and 11

participants respectively. However, it should be acknowledged that there may be an insufficient number of trials and or participants to achieve a significant result. Therefore, it would be advisable for future studies to increase the number of trials averaged per cardiac cycle interval or collapse data across intervals to improve the chance of achieving significance. Indeed post hoc power analysis using the G*power computer program (Faul, Erdfelder, Buchner, & Lang, 2009) indicated that adopting the reported effect size for the main effect for interval in the current study (f(U) = .255), with an alpha of .05 and power of .80, the study would require a total sample of 40 to detect, by repeated measures ANOVA, a difference in N2 peak amplitudes across the cardiac cycle.

A significant aim of the current study was to determine if the proposed cardiac cycle-related modulation of PREPs extended beyond the single electrode (Cz) analysed by Edwards et al. (2008) indicating other brain areas potentially involved in the baroreceptor modulation of pain. Neuroimaging studies have documented a widespread “pain matrix” extending across both cortex hemispheres (Casey, 1999; Davis, 2000; Peyron et al., 2000b). To investigate the possible involvement of areas within this “pain matrix” beyond Cz in the cardiac cycle modulation of pain the current study assessed PREPs at C3 and C4 electrode sites. These sites were expected to overlay additional areas identified as interaction sites between the pain and cardiovascular systems i.e. ACC, SII and insular cortex bilaterally (Bromm & Chen, 1995; Tarkka & Treede, 1993; Valeriani et al., 1996), the SI areas located in the parietal operculum (Bushnell & Apkarian, 2006; Craig, 2002; Peyron et al., 2000a; Rainville, 2002). Counter to our hypothesis, there was no cardiac cycle modulation at either C3 or C4. However, as the current study also revealed no significant modulation at Cz, this may be due to the methodological differences discussed above rather than disproving any wider spread interaction of pain and cardiovascular systems.

As tonic BP is known to influence pain (e.g. France, 1999; Ghione, 1996), the present study also investigated the influence of tonic BP on the cardiac cycle-related modulation of PREPs. We found that participants in the high-normal SBP group had higher pain thresholds than the low-normal SBP group. However, we conclude that

this is likely to be due to the predominately male participants in the high-normal SBP group, who typically have higher pain thresholds than females (Fillingim et al., 2009). No BP group or interaction differences in PREPs were found. This is counter to several studies that have reported an inverse relationship between BP and pain within the normotensive range (for review see France, 1999). However, this relationship is not always evident (e.g., Bruehl, Chung, Diedrich, Diedrich, & Robertson, 2008; Bruehl et al., 2010; Edwards et al., 2002; France, 1999; Mechlin, Heymen, Edwards, & Girdler, 2011; Stewart & France, 1996). Future studies would do well to further investigate the possible influence of tonic BP on the cardiac cycle modulation of PREPs specifically looking at wider range of BP's from the hypotensive through to hypertensive.

In summary, the lack of cardiac cycle modulation of the N2 and P2 peak amplitude and N2-P2 peak-to-peak amplitude in the current study suggests that electrocutaneous PREPs are not influenced by the natural fluctuations in BP across the cardiac cycle. The data suggests that the cardiac cycle-related modulation of PREPs may not be as robust as other measures of pain such as the NFR, and that the modality of pain stimulation and attention, may influence the cardiac cycle-related modulation of pain processing. Indeed, it appears that the NFR which is a spinal withdrawal reflex is consistently modulated across the cardiac cycle, whereas other responses including pain thresholds and PREP's, which are generated cortically are less robust. This may suggest that the site of cardiac cycle modulation of pain is lower down the central nervous system than the central cortical regions investigated in the current study. Further studies are required to further investigate the possible differences between the cardiac cycle-related modulation of different pain modalities and possible sites of the modulation, and it would also be recommended to recruit larger numbers of participants.

REFERENCES

Aicher, S. A., & Randich, A. (1990). Antinociception and cardiovascular responses produced by electrical stimulation in the nucleus tractus solitarius, nucleus reticularis ventralis, and the caudal medulla. *Pain, 42*(1), 103-119.

Angell James, J. E. (1971). Effects of altering mean pressure, pulse pressure and pulse frequency on impulse activity in baroreceptor fibres from aortic arch and right subclavian artery in rabbit. *Journal of Physiology-London, 214*(1), 65-88.

Angell James, J. E., & Lumley, J. S. P. (1974). Effects of carotid endarterectomy on mechanical-properties of carotid-sinus and carotid-sinus nerve activity in atherosclerotic patients. *British Journal of Surgery, 61*(10), 805-810.

Angrilli, A., Mini, A., Mucha, R. F., & Rau, H. (1997). The influence of low blood pressure and baroreceptor activity on pain responses. *Physiology & Behavior, 62*(2), 391-397.

Apkarian, A.V., Bushnell, M.C., Treede, R.D. & Zubieta, J.K., (2005). Human brain mechanisms of pain perception and regulation in health and disease. European Journal of Pain, 8(9), 463-484.

Arendt-Nielsen, L., & Chen, A. C. (2003). Lasers and other thermal stimulators for activation of skin nociceptors in humans. *Neurophysiologie Clinique, 33*(6), 259-268.

Arntz, A., Dreessen, L., & Merckelbach, H. (1991). Attention, not anxiety, influences pain. *Behaviour Research and Therapy, 29*(1), 41-50.

Bandler, R., Carrive, P., & Zhang, S. P. (1991). Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: Viscerotopic, somatotopic and functional organization. *Progress in Brain Research, 87*, 269-305.

Bantick, S. J., Wise, R. G., Ploghaus, A., Clare, S., Smith, S. M., & Tracey, I. (2002). Imaging how attention modulates pain in humans using functional MRI. *Brain : A Journal of Neurology, 125*(Pt 2), 310-319.

Becker, D. E., Haley, D. W., Urena, V. M., & Yingling, C. D. (2000). Pain measurement with evoked potentials: Combination of subjective ratings, randomized intensities, and long interstimulus intervals produces a P300-like confound. *Pain, 84*(1), 37-47.

Bentley, D. E., Derbyshire, S. W. G., Youell, P. D., & Jones, A. K. P. (2003). Caudal cingulate cortex involvement in pain processing: An inter-individual laser evoked potential source localisation study using realistic head models. *Pain, 102*(3), 265-271. doi:10.1016/S0304-3959(02)00405-0 ER

Bingel, U., Herken, W., Teutsch, S., & May, A. (2008). Habituation to painful stimulation involves the antinociceptive system--a 1-year follow-up of 10 participants. *Pain, 140*(2), 393-394. doi:10.1016/j.pain.2008.09.030; 10.1016/j.pain.2008.09.030

Bingel, U., Schoell, E., Herken, W., Buchel, C., & May, A. (2007). Habituation to painful stimulation involves the antinociceptive system. *Pain, 131*(1-2), 21-30. doi:10.1016/j.pain.2006.12.005

Bingel, U., Quante, M., Knab, R., Bromm, B., Weiller, C., & Buchel, C. (2003). Single trial fMRI reveals significant contralateral bias in responses to laser pain within thalamus and somatosensory cortices. Neuroimage, 18(3), 740-748.

Birren, J. E., Phillips, S. L., & Cardon, P. V. (1963). Reaction time as a function of cardiac cycle in young adults. *Science, 140*(356), 195-196.

Brody, S., Angrilli, A., Weiss, U., Birbaumer, N., Mini, A., Veit, R., & Rau, H. (1997). Somatotosensory evoked potentials during baroreceptor stimulation in chronic low back pain patients and normal controls. *International Journal of Psychophysiology, 25*(3), 201-210.

Bromm, B. (1984). Pain-related components in the cerebral potential: Experimental and multivariate statistical approaches. In B. Bromm (Ed.), *Pain measurement in man: Neurophysiological correlates of pain* (pp. 257-290). Amsterdam: Elsevier.

Bromm, B. (1985). Modern techniques to measure pain in healthy man. *Methods and Findings in Experimental and Clinical Pharmacology, 7*(3), 161-169.

Bromm, B., & Chen, A. C. (1995). Brain electrical source analysis of laser evoked potentials in response to painful trigeminal nerve stimulation. *Electroencephalography and Clinical Neurophysiology, 95*(1), 14-26.

Bromm, B., & Lorenz, J. (1998). Neurophysiological evaluation of pain. *Electroencephalography and Clinical Neurophysiology, 107*(4), 227-253.

Bromm, B., & Meier, W. (1984). The intracutaneous stimulus: A new pain model for algesimetric studies. *Methods and Findings in Experimental and Clinical Pharmacology, 6*(7), 405-410.

Bromm, B., & Treede, R. D. (1987). Human cerebral potentials evoked by CO2 laser stimuli causing pain. *Experimental Brain Research, 67*(1), 153-162.

Bromm, B., & Treede, R. D. (1991). Laser-evoked cerebral potentials in the assessment of cutaneous pain sensitivity in normal subjects and patients. *Revue Neurologique, 147*(10), 625-643.

Bruehl, S., Burns, J. W., Chung, O. Y., Magid, E., Chont, M., Gilliam, W., . . . Cairl, H. (2010). Hypoalgesia associated with elevated resting blood pressure: Evidence for endogenous opioid involvement. *Journal of Behavioral Medicine, 33*(2), 168-176. doi:10.1007/s10865-009-9241-4

Bruehl, S., Chung, O. Y., Diedrich, L., Diedrich, A., & Robertson, D. (2008). The relationship between resting blood pressure and acute pain sensitivity: Effects of chronic pain and alpha-2 adrenergic blockade. *Journal of Behavioral Medicine, 31*(1), 71-80. doi:10.1007/s10865-007-9133-4

Bushnell M, C., & Apkarian, A,V,. (2006). Representation of pain in the brain. In S. McMahon B, & M. Koltzenburg (Eds.), (5th ed., pp. 107-124). Philadelphia: Elsevier.

Carmon, A., Dotan, Y., & Sarne, Y. (1978). Correlation of subjective pain experience with cerebral evoked responses to noxious thermal stimulations. *Experimental Brain Research, 33*(3-4), 445-453.

Carmon, A., Friedman, Y., Coger, R., & Kenton, B. (1980). Single trial analysis of evoked potentials to noxious thermal stimulation in man. *Pain, 8*(1), 21-32.

Carmon, A., Mor, J., & Goldberg, J. (1976). Evoked cerebral responses to noxious thermal stimuli in humans. *Experimental Brain Research, 25*(1), 103-107.

Casey, K. L. (1999). Forebrain mechanisms of nociception and pain: Analysis through imaging. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 7668-7674.

Chen, A. C. N., Arendt-Nielsen, L., & Plaghki, L. (1998). Laser-evoked potentials in human pain: I. use and possible misuse. *Pain Forum, 7*(4), 174-184. doi:10.1016/S1082-3174(98)70001-7

Coen, S. J., Aziz, Q., Yaguez, L., Brammer, M., Williams, S. C., & Gregory, L. J. (2008). Effects of attention on visceral stimulus intensity encoding in the male human brain. *Gastroenterology, 135*(6), 2065-74, 2074.e1. doi:10.1053/j.gastro.2008.08.005

Coghill, R. C., Gilron, I., & Iadarola, M. J. (2001). Hemispheric lateralization of somatosensory processing. Journal of Neurophysiology, 85(6), 2602-2612.

Coghill, R. C., Talbot, J. D., Evans, A. C., Meyer, E., Gjedde, A., Bushnell, M. C., & Duncan, G. H. (1994). Distributed processing of pain and vibration by the human brain. *The Journal of Neuroscience, 14*(7), 4095-4108.

Coleridge, H. M., Coleridge, J. C. G., & Schultz, H. D. (1987). Characteristics of C-fiber baroreceptors in the carotid-sinus of dogs. *Journal of Physiology-London, 394*, 291-313.

Craig, A. D. (2002). Pain mechanisms: Labeled lines versus convergence in central processing. *Annual Review of Neuroscience, 26*, 1-30.

Davis, K. D. (2000). The neural circuitry of pain as explored with functional MRI. *Neurological Research, 22*(3), 313-317.

Davis, K. D., Pope, G. E., Crawley, A. P., & Mikulis, D. J. (2002). Neural correlates of prickle sensation: A percept-related fMRI study. *Nature Neuroscience, 5*(11), 1121-1122. doi:10.1038/nn955

de Tommaso, M., Santostasi, R., Devitofrancesco, V., Franco, G., Vecchio, E., Delussi, M., . . . Katzarava, Z. (2011). A comparative study of cortical responses evoked by transcutaneous electrical vs CO(2) laser stimulation. *Clinical Neurophysiology, 122*(12), 2482-2487. doi:10.1016/j.clinph.2011.05.006

Droste, C., Kardos, A., Brody, S., Greenlee, M. W., Roskamm, H., & Rau, H. (1994). Baroreceptor stimulation - pain perception and sensory thresholds. *Biological Psychology, 37*(2), 101-113.

Eckberg, D. L., & Sleight, P. (1992). *Human baroreflexes in health and disease*. Oxford: Clarendon Press.

Edwards, L., Inui, K., Ring, C., Wang, X. H., & Kakigi, R. (2008). Pain-related evoked potentials are modulated across the cardiac cycle. *Pain, 137*(3), 488-494. doi:10.1016/j.pain.2007.10.010 ER

Edwards, L., McIntyre, D., Carroll, D., Ring, C., France, C. R., & Martin, U. (2003). Effects of artificial and natural baroreceptor stimulation on nociceptive responding and pain. *Psychophysiology, 40*(5), 762-769.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology, 39*(5), 678-681. doi:10.1017/S0048577202011770 ER

Edwards, L., Ring, C., McIntyre, D., & Carroll, D. (2001). Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology, 38*(4), 712-718.

Edwards, L., Ring, C., McIntyre, D., Carroll, D., & Martin, U. (2007). Psychomotor speed in hypertension: Effects of reaction time components, stimulus modality, and phase of the cardiac cycle. *Psychophysiology, 44*(3), 459-468. doi:10.1111/j.1469-8986.2007.00521.x ER

Fila, M., & Bogucki, A. (2009). Laser evoked potentials as a method of evaluating the function of small fibres - application technique and clinical implications. *Neurologia i Neurochirurgia Polska, 43*(4), 368-381.

Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B., & Riley, J. L. (2009). Sex, gender, and pain: A review of recent clinical and experimental findings. *Journal of Pain, 10*(5), 447-485. doi:10.1016/j.jpain.2008.12.001 ER

France, C. R. (1999). Decreased pain perception and risk for hypertension: Considering a common physiological mechanism. *Psychophysiology, 36*(6), 683-692.

France, C. R., & Ditto, B. (1996). Risk for high blood pressure and decreased pain perception. *Current Directions in Psychological Science, 5*(4), 120-125.

Frot, M., Rambaud, L., Guenot, M., & Mauguiere, F. (1999). Intracortical recordings of early pain-related CO2-laser evoked potentials in the human second somatosensory (SII) area. *Clinical Neurophysiology, 110*(1), 133-145.

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behavior Research Methods, 41(4), 1149-1160. doi:10.3758/BRM.41.4.1149

Garcia-Larrea, L., Frot, M., & Valeriani, M. (2003). Brain generators of laser-evoked potentials: From dipoles to functional significance. *Neurophysiologie Clinique, 33*(6), 279-292.

Ghione, S. (1996). Hypertension-associated hypoalgesia - evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension, 28*(3), 494-504.

Gracely, R. H. (2006). Studies of pain in human subjects. In S. McMahon B, & M. Koltzenburg (Eds.), *Wall and Melzack's textbook of pain* (5th ed., pp. 267-290). Philadelphia: Elsevier.

Granovsky, Y., Granot, M., Nir, R. R., & Yarnitsky, D. (2008). Objective correlate of subjective pain perception by contact heat-evoked potentials. *The Journal of Pain, 9*(1), 53-63. doi:10.1016/j.jpain.2007.08.010

Gray, M. A., Minati, L., Paoletti, G., & Critchley, H. D. (2010). Baroreceptor activation attenuates attentional effects on pain-evoked potentials. *Pain, 151*(3), 853-861. doi:10.1016/j.pain.2010.09.028

Gray, M. A., Rylander, K., Harrison, N. A., Wallin, B. G., & Critchley, H. D. (2009). Following one's heart: Cardiac rhythms gate central initiation of sympathetic reflexes. *The Journal of, 29*(6), 1817-1825. doi:10.1523/JNEUROSCI.3363-08.2009

Greffrath, W., Baumgartner, U., & Treede, R. D. (2007). Peripheral and central components of habituation of heat pain perception and evoked potentials in humans. *Pain, 132*(3), 301-311. doi:10.1016/j.pain.2007.04.026 ER

Gybels, J., Handwerker, H. O., & Van Hees, J. (1979). A comparison between the discharges of human nociceptive nerve fibres and the subject's ratings of his sensations. *The Journal of Physiology, 292*, 193-206.

Handwerker, H. O., & Kobal, G. (1993). Psychophysiology of experimentally induced pain. *Physiological Reviews, 73*(3), 639-671.

Hanna, D., & Dempster, M. (2012). *Psychology statistics for dummies*. Chichester: John Wiley & Sons.

Hodge, A. M., & Zimmet, P. Z. (1994). The epidemiology of obesity. *Bailliere's Clinical Endocrinology and Metabolism, 8*(3), 577-599.

Hugon, M. (1973). Exteroreceptive reflexes to stimulation of the sural nerve in normal man. In J. E. Desmedt (Ed.), *New developments in electromyography and clinical neurophysiology* (pp. 713--729). Basel: Karger.

Iannetti, G. D., Hughes, N. P., Lee, M. C., & Mouraux, A. (2008). Determinants of laser-evoked EEG responses: Pain perception or stimulus saliency? *Journal of Neurophysiology, 100*(2), 815-828. doi:10.1152/jn.00097.2008

Ille, N., Berg, P., & Scherg, M. (2002). Artifact correction of the ongoing EEG using spatial filters based on artifact and brain signal topographies. *Journal of Clinical Neurophysiology, 19*(2), 113-124.

Ingvar, M. (1999). Pain and functional imaging. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 354*(1387), 1347-1358. doi:10.1098/rstb.1999.0483

Jasper, H. H. (1958). The ten-twenty electrode system of the International Federation. *Electroencephalography and Clinical Neurophysiology*, 10, 370-375.

Johnson, M. H., de Haan, M., Oliver, A., Smith, W., Hatzakis, H., Tucker, L. A., & Csibra, G. (2001). Recording and analyzing high-density event-related potentials with infants. using the geodesic sensor net. *Developmental Neuropsychology, 19*(3), 295-323. doi:10.1207/S15326942DN1903_4

Kakigi, R., Inui, K., & Tamura, Y. (2005). Electrophysiological studies on human pain perception. *Clinical Neurophysiology, 116*(4), 743-763. doi:10.1016/j.clinph.2004.11.016

Kakigi, R., Shibasaki, H., & Ikeda, A. (1989). Pain-related somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology, 74*(2), 139-146.

Kanda, M., Fujiwara, N., Xu, X., Shindo, K., Nagamine, T., Ikeda, A., & Shibasaki, H. (1996). Pain-related and cognitive components of somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology, 100*(2), 105-114.

Kanda, M., Matsuhashi, M., Sawamoto, N., Oga, T., Mima, T., Nagamine, T., & Shibasaki, H. (2002). Cortical potentials related to assessment of pain intensity with visual analogue scale (VAS). *Clinical Neurophysiology, 113*(7), 1013-1024.

Kanda, M., Shindo, K., Xu, X., Fujiwara, N., Ikeda, A., Nagamine, T., & Shibasaki, H. (1999). Cortical mechanisms underlying point localization of pain spot as studied by event-related potentials following CO2 laser stimulation in man. *Experimental Brain Research, 127*(2), 131-140.

Kappenman, E. S., & Luck, S. J. (2010). The effects of electrode impedance on data quality and statistical significance in ERP recordings. *Psychophysiology, 47*(5), 888-904. doi:10.1111/j.1469-8986.2010.01009.x

Katsarava, Z., Ayzenberg, I., Sack, F., Limmroth, V., Diener, H. C., & Kaube, H. (2006). A novel method of eliciting pain-related potentials by transcutaneous electrical stimulation. *Headache, 46*(10), 1511-1517. doi:10.1111/j.1526-4610.2006.00446.x ER

Kaube, H., Katsarava, Z., Kaufer, T., Diener, H. C., & Ellrich, J. (2000). A new method to increase nociception specificity of the human blink reflex. *Clinical Neurophysiology, 111*(3), 413-416.

LeBlanc, J., & Potvin, P. (1966). Studies on habituation to cold pain. *Canadian Journal of Physiology and Pharmacology, 44*(2), 287-293.

Lefaucheur, J. P., Ahdab, R., Ayache, S. S., Lefaucheur-Menard, I., Rouie, D., Tebbal, D., . . . Ciampi de Andrade, D. (2012). Pain-related evoked potentials: A comparative study between electrical stimulation using a concentric planar electrode and laser stimulation using a CO2 laser. *Neurophysiologie Clinique, 42*(4), 199-206. doi:10.1016/j.neucli.2011.12.003

Lenz, F. A., Rios, M., Chau, D., Krauss, G. L., Zirh, T. A., & Lesser, R. P. (1998). Painful stimuli evoke potentials recorded from the parasylvian cortex in humans. *Journal of Neurophysiology, 80*(4), 2077-2088.

Levitt, H. (1971). Transformed up-down methods in psychoacoustics. *Journal of the Acoustical Society of America, 49*(2), 467-&.

Luck, S. J. (2005). *An introduction to the event-related potential technique*. Cambridge, MA: Massachusetts Institute of Technology.

Mancia, G., & Mark, A. L. (1983). Arterial baroreflexes in humans. In J. T. Shepherd, & F. M. Abboud (Eds.), *Handbook of physiology: The cardiovascular system* (pp. 755--793). Bethesda, Maryland: American Physiological Society.

Martins, A., Q, Ring, C., McIntyre, D., Edwards, L., & Martin, U. (2009). Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. *Pain, 147*(1-3), 84-90. doi:10.1016/j.pain.2009.08.016

McIntyre, D., Edwards, L., Ring, C., Parvin, B., & Carroll, D. (2006). Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology, 43*(3), 314-319. doi:10.1111/j.1469-8986.2006.00407.x ER

McIntyre, D., Kavussanu, M., & Ring, C. (2008). Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. *Pain, 137*(3), 550-555. doi:10.1016/j.pain.2007.10.018 ER

McIntyre, D., Ring, C., Edwards, L., & Carroll, D. (2008). Simple reaction time as a function of the phase of the cardiac cycle in young adults at risk for hypertension. *Psychophysiology, 45*(2), 333-336. doi:10.1111/j.1469-8986.2007.00619.x ER

McIntyre, D., Ring, C., Hamer, M., & Carroll, D. (2007). Effects of arterial and cardiopulmonary baroreceptor activation on simple and choice reaction times. *Psychophysiology, 44*(6), 874-879. doi:10.1111/j.1469-8986.2007.00547.x

Mechlin, B., Heymen, S., Edwards, C. L., & Girdler, S. S. (2011). Ethnic differences in cardiovascular-somatosensory interactions and in the central processing of noxious stimuli. *Psychophysiology, 48*(6), 762-773. doi:10.1111/j.1469-8986.2010.01140.x; 10.1111/j.1469-8986.2010.01140.x

Melzack, R. (1987). The short-form mcgill pain questionnaire. *Pain, 30*(2), 191-197.

Melzack, R., & Wall, P. D. (1965). Pain mechanisms: A new theory. *Science (New York, N.Y.), 150*(3699), 971-979.

Merskey, H., & Bogduk, N. (1994). Part III: Pain terms, A current list with definitions and notes on usage. In H. Merskey, & N. Bogduk (Eds.), *Classification of chronic pain, IASP task force on taxonomy* (2nd ed., pp. 209-214). Seattle: WA: IASP.

Metting van Rijn, A. C., Kuiper, A. P., Dankers, T. E., & Grimbergen, C. A. (1996). Low-cost active electrode improves the resolution in biopotential recordings. *Biosemi Research,* May 2013.

Meyer, R. A., Ringkamp, M., Campbell, J. N., & Raja, S. N. (2006). Peripheral mechanisms of cutaneous nociception. In S. B. McMahon, & M. Koltzenburg (Eds.), *Wall and Melzack's textbook of pain* (5th ed., pp. 3-34). Philadelphia: Elsevier.

Meyer, R. A., Walker, R. E., & Mountcastle, V. B., Jr. (1976). A laser stimulator for the study of cutaneous thermal and pain sensations. *IEEE Transactions on Bio-Medical Engineering, 23*(1), 54-60.

Milne, R. J., Kay, N. E., & Irwin, R. J. (1991). Habituation to repeated painful and non-painful cutaneous stimuli: A quantitative psychophysical study. *Experimental Brain Research, 87*(2), 438-444.

Mini, A., Rau, H., Montoya, P., Palomba, D., & Birbaumer, N. (1995). Baroreceptor cortical effects, emotions and pain. *International Journal of Psychophysiology, 19*(1), 67-77.

Miyazaki, M., Shibasaki, H., Kanda, M., Xu, X., Shindo, K., Honda, M., . . . Kimura, J. (1994). Generator mechanism of pain-related evoked potentials following CO2 laser stimulation of the hand: Scalp topography and effect of predictive warning signal. *Journal of Clinical Neurophysiology, 11*(2), 242-254.

Nolte, J. (2002). *The Human Brain: An Introduction to Its Functional.* 5th edition. St. Louis : Mosby

Nosaka, S., Murata, K., Inui, K., & Murase, S. (1993). Arterial baroreflex inhibition by midbrain periaqueductal grey in anaesthetized rats. *Pflugers Archiv : European Journal of Physiology, 424*(3-4), 266-275.

Ogino, Y., Nemoto, H., Inui, K., Saito, S., Kakigi, R., & Goto, F. (2007). Inner experience of pain: Imagination of pain while viewing images showing painful events forms subjective pain representation in human brain. *Cerebral Cortex (New York, N.Y.: 1991), 17*(5), 1139-1146. doi:10.1093/cercor/bhl023

Ohara, S., Crone, N. E., Weiss, N., & Lenz, F. A. (2006). Analysis of synchrony demonstrates 'pain networks' defined by rapidly switching, task-specific, functional connectivity between pain-related cortical structures. *Pain, 123*(3), 244-253. doi:10.1016/j.pain.2006.02.012

Perchet, C., Frot, M., Charmarty, A., Flores, C., Mazza, S., Magnin, M., & Garcia-Larrea, L. (2012). Do we activate specifically somatosensory thin fibres with the concentric planar electrode? A scalp and intracranial EEG study. *Pain, 153*(6), 1244-1252. doi:10.1016/j.pain.2012.03.004; 10.1016/j.pain.2012.03.004

Persson, P. B., & Kirchheim, H. R. (1991). *Baroreceptor reflexes: integrative functions and clinical aspects*. Berlin: Springer-Verlag.

Peyron, R., Garcia-Larrea, L., Gregoire, M. C., Convers, P., Richard, A., Lavenne, F., . . . Laurent, B. (2000a). Parietal and cingulate processes in central pain. A combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) study of an unusual case. *Pain, 84*(1), 77-87.

Peyron, R., Laurent, B. & Garcia-Larrea, L. (2000b). Functional imaging of brain responses to pain. A review and meta-analysis. Journal of Clinical Neurophysiology, 30, 263-288.

Porro, C.A. (2003). Functional imaging and pain: behavior, perception, and modulation. *Neuroscientist,* 9, 354-369.

Qiu, Y., Noguchi, Y., Honda, M., Nakata, H., Tamura, Y., Tanaka, S., . . . Kakigi, R. (2006). Brain processing of the signals ascending through unmyelinated C fibers in humans: An event-related functional magnetic resonance imaging study. *Cerebral Cortex, 16*(9), 1289-1295. doi:10.1093/cercor/bhj071

Radloff, L. S. (1977). The center for epidemiologic studies depression scale a self report depression scale for research in the general population. *Applied Psychological Measurement, 1*(3), 385-401.

Rainville, P. (2002). Brain mechanisms of pain affect and pain modulation. *Current Opinion in Neurobiology, 12*(2), 195-204.

Reckelhoff, J. F. (2001). Gender differences in the regulation of blood pressure. *Hypertension, 37*(5), 1199-1208.

Rutter, C. E., Dahlquist, L. M., & Weiss, K. E. (2009). Sustained efficacy of virtual reality distraction. *The Journal of Pain, 10*(4), 391-397. doi:10.1016/j.jpain.2008.09.016

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., & Willer, J. C. (2005). The lower limb flexion reflex in humans. *Progress in Neurobiology, 77*(6), 353-395. doi:10.1016/j.pneurobio.2005.11.003 ER

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *Manual for the state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists Press, 1970.

Stewart, K. M., & France, C. R. (1996). Resting systolic blood pressure, parental history of hypertension, and sensitivity to noxious stimuli. *Pain, 68*(2-3), 369-374.

Stowell, H. (1977). Cerebral slow waves related to the perception of pain in man. *Brain Research Bulletin, 2*(1), 23-30.

Strempel, H. (1976). Adaptive modifications of cold pain (author's transl). *European Journal of Applied Physiology and Occupational Physiology, 36*(1), 19-25.

Strempel, H. (1978). Adaptive modifications of cold pain. III. communication: Short-term experiments with 1-min-intervals (author's transl). *European Journal of Applied Physiology and Occupational Physiology, 39*(1), 63-72.

Symonds, L. L., Gordon, N. S., Bixby, J. C., & Mande, M. M. (2006). Right-lateralized pain processing in the human cortex: An FMRI study. Journal of Neurophysiology, 95(6), 3823-3830. doi:10.1152/jn.01162.2005

Tan, S. Y. (1982). Cognitive and cognitive-behavioral methods for pain control: A selective review. *Pain, 12*(3), 201-228.

Tarkka, I. M., & Treede, R. D. (1993). Equivalent electrical source analysis of pain-related somatosensory evoked potentials elicited by a CO2 laser. *Journal of Clinical Neurophysiology, 10*(4), 513-519.

Tracey, I., & Mantyh, P.W. (2007). The cerebral signature for pain perception and its modulation. *Neuron,* 55, 377-391.

Treede, R.D., Kenshalo, D.R., Gracely, R.H. & Jones, A.K. (1999). The cortical 8 representation of pain. *Pain,* 79, 105-111.

Treede, R. D., Kief, S., Holzer, T., & Bromm, B. (1988). Late somatosensory evoked cerebral potentials in response to cutaneous heat stimuli. *Electroencephalography and Clinical Neurophysiology, 70*(5), 429-441.

Treede, R. D., Lorenz, J., & Baumgartner, U. (2003). Clinical usefulness of laser-evoked potentials. *Neurophysiologie Clinique, 33*(6), 303-314.

Valeriani, M., Rambaud, L., & Mauguiere, F. (1996). Scalp topography and dipolar source modelling of potentials evoked by CO2 laser stimulation of the hand. *Electroencephalography and Clinical Neurophysiology, 100*(4), 343-353.

Valeriani, M., Sestito, A., Le Pera, D., De Armas, L., Infusino, F., Maiese, T., . . . Lanza, G. A. (2005). Abnormal cortical pain processing in patients with cardiac syndrome X. *European Heart Journal, 26*(10), 975-982. doi:10.1093/eurheartj/ehi229

Wilkinson, M., McIntyre, D., & Edwards, L. (2013). Electrocutaneous pain thresholds are higher during systole than diastole. *Biological Psychology, 94*(1), 71-73. doi:10.1016/j.biopsycho.2013.05.002

Willer, J. C. (1977). Comparative-study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

Youell, P. D., Wise, R. G., Bentley, D. E., Dickinson, M. R., King, T. A., Tracey, I., & Jones, A. K. (2004). Lateralisation of nociceptive processing in the human brain: A functional magnetic resonance imaging study. Neuroimage, 23(3), 1068-1077. doi:10.1016/j.neuroimage.2004.07.004

Zaslansky, R., Sprecher, E., Katz, Y., Rozenberg, B., Hemli, J. A., & Yarnitsky, D. (1996). Pain-evoked potentials: What do they really measure? *Evoked Potentials-Electroencephalography and Clinical Neurophysiology, 100*(5), 384-391.

FIVE

Lateralisation effects on the cardiac cycle modulation of electrocutaneous pain-related evoked potentials

5.1 Abstract

Natural variations in blood pressure (BP) across the cardiac cycle have been shown to modulate nociception and pain. However, the findings for cortical measures i.e. pain-related evoked potentials (PREPs) may be less robust than the nociceptive flexion reflex (a spinal reflex response). One possible reason for this may be a lateralisation of pain and baroreceptor processing in the brain. Studies appear to suggest a right hemisphere dominance for both pain and baroreceptor input. The current study examined the effects of natural variations in BP across the cardiac cycle on electrocutaneous PREPs delivered to the right and left hand using multi-channel recordings in 7 men and 10 women. Following determination of electrocutaneous pain thresholds using an up-down staircase method, stimuli equal to twice individual pain threshold were delivered to each hand on separate days in 7 blocks of 21 trials to elicit PREPs. Stimuli were delivered pseudorandomly at 7 cardiac cycle intervals (R-wave plus 50, 150, 250, 350, 450, 550, 650 ms). Separate 2 Hand (Left, Right) × 3 Scalp Electrode Site (C3, Cz, C4) × 7 Interval repeated-measures ANOVAs for N2 and P2 peak amplitudes and N2-P2 peak-to-peak amplitudes, revealed a significant main effect for scalp electrode site for N2 and P2 peak amplitudes and N2-P2 peak-to-peak amplitudes, with amplitudes being highest at Cz scalp electrode. Additionally, a significant 2 Hand × 3 Scalp Electrode Site × 7 Interval interaction for N2 peak amplitudes was also found, although subsequent ANOVA analyses to interrogate this finding were not significant. The findings tentatively suggest that both the side of stimulation and the site of scalp recording may be important in explaining the significant 2 Hand × 3 Scalp Electrode Site × 7 Interval interaction for N2 peak amplitudes, and thus the cardiac cycle-related patterning of pain processing.

Descriptors: Arterial baroreceptors; Lateralisation; Cardiac cycle; Electrocutaneous; Pain-related evoked potentials

5.2 Introduction

It is well established that blood pressure (BP) influences pain. Individuals with essential hypertension present a reduced sensitivity to pain (Ghione, 1996). The precise mechanisms underlying this phenomenon are yet to be clearly identified. However, there is growing evidence that a baroreceptor mechanism may contribute to the reduced pain sensitivity associated with elevated BP (France & Ditto, 1996; France, 1999; Ghione, 1996). Arterial baroreceptors are stretch receptors located in the aortic arch and carotid sinus and are important in the regulation of BP (Katona, Poitras, Barnett, & Terry, 1970; Persson & Kirchheim, 1991). At rest, baroreceptors are stimulated during the systolic phase of the cardiac cycle by the arrival of the pulse pressure wave (Eckberg & Sleight, 1992; Mancia & Mark, 1983) and have reduced output during diastole (Angell James & Lumley, 1974), resulting in a pulsatile discharge (Angell James, 1971; Coleridge, Coleridge, & Schultz, 1987).

As well as maintaining cardiovascular homeostasis, baroreceptors may also modulate the activity in areas of the brain related to pain (Ghione, 1996). There appears to be a significant overlap between the areas of the brain involved in cardiovascular and pain regulation. The BP regulating role of baroreceptors primarily occurs in the brainstem (Gilbey & Spyer, 1993; McAllen & Malpas, 1997; Janig, 2006; Gilbey, 2007), specifically baroreceptor afferents project to the nucleus tractus solitarius (NTS) in the medulla of the brainstem (Eckberg & Sleight, 1992; Benarroch, 2008; Bell, 2009; Klabunde, 2011) and both sympathetic and parasympathetic neuron activity in the medulla is modulated by the NTS (Klabunde, 2011). Stimulation of the NTS has also been shown to induce antinociception (Aicher & Randich, 1990). Baroreceptor afferents also project beyond the brainstem into multiple brain regions within both forebrain hemispheres including the thalamus (Oppenheimer et al., 1998; Zhang & Oppenheimer, 2000) and insular cortex (Zhang, Dougherty, & Oppenheimer, 1998, 1999), specifically the anterior cingulate cortex (ACC), bilateral insular cortex, amygdala and orbitofrontal cortices (Gray, Rylander, Harrison, Wallin, & Critchley, 2009; Henderson et al., 2004; Kimmerly, O'Leary, Menon, Gati, & Shoemaker, 2005; Sykora, Diedler, Rupp, Turcani, & Steiner, 2009;, Zhang et al., 1998).

Although the mechanisms through which baroreceptor activity effects sensation is less well established than their control of BP, recent studies utilising neuroimaging techniques suggest that the anterior insular cortex and ACC are brain areas central to integrating the baroreceptor afferents and influencing feeling states (Craig, 2002; Critchley, 2005; Gianaros, Jennings, Sheu, Derbyshire, & Matthews, 2007). Previous research using functional magnetic resonance imaging (fMRI) has also identified a baroreceptor influence on neural activity in response to painful electrocutaneous shocks in the periaqueductal grey (PAG) matter, amygdala and insular cortex (Gray et al., 2009). Direct stimulation of the PAG matter has also been found to induce antinociception (Bandler, Carrive, & Zhang, 1991). Taken together these data suggest that several brainstem and cortical areas may be involved in the integration of somatosensory and baroreceptor information and thus deserve further investigation in relation to understanding the mechanisms underlying hypertension hypoalgesia.

The cardiac cycle experimental paradigm provides an opportunity to study the effects of baroreceptor activity on various stimuli. Cardiac cycle studies take advantage of the natural variations in BP within an individual heart beat. Stimuli are delivered to coincide with systole, when BP and baroreceptor activation is highest, and diastole, when BP and baroreceptor activation is lowest, and the response compared. Recent studies have examined the effect of the cardiac cycle on the nociceptive flexion reflex (NFR), a polysynaptic spinal reflex sub-serving withdrawal from noxious stimuli (Sandrini et al., 2005), the threshold for which serves as a physiological correlate of pain (Hugon, 1973; Willer, 1977). These studies found the NFR to be attenuated during systole compared to diastole (Edwards, Ring, McIntyre, & Carroll, 2001; Edwards, McIntyre, Carroll, Ring, & Martin, 2002; Edwards et al., 2003; McIntyre, Edwards, Ring, Parvin, & Carroll, 2006; McIntyre, Kavussanu, & Ring, 2008). Taken together the findings discussed above suggest that nociceptive responding may be dampened when arterial baroreceptor activity is maximal.

The International Association of the Study of Pain (IASP) define pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage” (Merskey & Bogduk, 1994). As such pain has a strong emotional-

affective component (e.g. Ossipov, Dussor & Porreca, 2010; Ohara, Vit & Jasmin, 2005), it is subjective in nature (IASP, 2011) and thus includes a significant element of cortical modulation rather than being only a reflex response (see Ohara et al., 2005 for review). As such pain is inherently different to its neurophysiological correlates i.e. the NFR and PREPs (Chen, Arendt-Nielsen, & Plaghki, 1998; Iannetti, Hughes, Lee, & Mouraux, 2008; Sandrini et al., 2005) described in the cardiac cycle time studies above (Edwards et al., 2001; 2002; 2003; 2008; McIntyre et al., 2006; 2008) and thus may be influenced differently by baroreceptor activation. In relation to this, the first study in this thesis (Chapter 2) found that pain thresholds were modulated across the cardiac cycle, specifically pain was attenuated during systole (i.e. baroreceptor activation). This contrasts NFR studies which concurrently assessed pain perception and reported that although the NFR, which is considered a correlate of pain (e.g. Willer, 1977), was modulated across the cardiac cycle pain ratings were not (Edwards et al., 2001, 2002, 2003). However these studies were not specifically designed to investigate pain perception (Edwards et al., 2001, 2002, 2003).

To further the understanding regarding the cortical processing of nociception across the cardiac cycle, investigators have examined the pain-related evoked potential (PREP) response to painful stimuli. Pain-related evoked potentials elicited by noxious stimuli are thought to represent the central processing of nociception and as such, many researchers have used PREPs as objective measures of pain (Miltner, Larbig, & Braun, 1987; Granovsky, Granot, Nir, & Yarnitsky, 2008). The most commonly studied components of the PREP waveform are the second negative (N2) and positive (P2) peaks (Kanda et al., 1996; Garcia-Larrea, Peyron, Laurent, & Mauguiere, 1997; Fila & Bogucki, 2009), with N2 occurring approximately 130–240 ms post stimulus and P2 approximately 230–390 ms post stimulus (Bromm, 1985; Zaslansky et al., 1996). The amplitude of the N2 and P2 PREP components have been shown to correlate with the intensity of pain stimulus (Becker, Haley, Urena, & Yingling, 2000; Bromm, 1984; Stowell, 1977; Zaslansky et al., 1996), as well as with subjective ratings of pain (Kanda et al., 2002). In response to painful stimuli of the hand, both the N2 and P2 components have been found to be maximal at the midline central area, specifically scalp electrode site Cz (Bromm & Treede, 1987; Carmon,

Mor, & Goldberg, 1976; Carmon, Dotan, & Sarne, 1978; Carmon, Friedman, Coger, & Kenton, 1980; Kakigi, Shibasaki, & Ikeda, 1989; Kanda et al., 1996; Kanda et al., 1999; Miyazaki et al., 1994; Treede, Kief, Holzer, & Bromm, 1988). Supporting evidence suggesting the origin of the N2 and P2 components is mainly the ACC, whilst the secondary somatosensory cortex (SII) and insular cortex, bilaterally, also contribute to the N2 component (Bromm & Chen, 1995; Tarkka & Treede, 1993; Valeriani, Rambaud, & Mauguiere, 1996).

Two recent studies reported that N2 and N2-P2 peak-to-peak amplitudes (Edwards et al., 2008) or P2 peak amplitudes (Gray et al., 2010) were reduced during systole compared to diastole. This finding agrees with earlier studies indicating cardiac-related cortical inhibition (Koriath & Lindholm, 1986; Koriath, Lindholm, & Landers, 1987). Further studies also reported reduced visual (Walker & Sandman, 1982) and auditory (Sandman, 1984) evoked potential amplitudes during systole compared to diastole. However, it appears that modulation of cortical responses to painful stimulation across the cardiac cycle may be less robust than the NFR indeed within in this thesis, PREPs generated in response to painful electrocutaneous stimulation were found not to be modulated across the cardiac cycle (Chapter 4) which contrasts the findings of Edwards et al. (2008) using painful laser stimulation and Gray et al. (2010) using electrocutaneous pain. The reasons for these different results may relate to methodological differences between the studies. For a full discussion regarding the methodological differences see the previous chapter (Chapter 4). However, to summarise, first, noxious stimulation was delivered via different methods. We (Chapter 4) used a concentric planar electrode (Kaube, Katsarava, Kaufer, Diener, & Ellrich, 2000) to deliver electrocutaneous stimulation, whereas Edwards et al. (2008) used thulium-evoked laser stimulations and Gray et al. (2010) used a different type of electrocutaneous electrode. It is possible that the differing pain delivery modes may differentially stimulate nociceptive and non-nociceptive fibres (e.g. Lefaucheur et al., 2012; Perchet et al., 2012) which may have influenced the generation of the PREPs and thus the cardiac cycle modulation may also be affected. Second, the duration of the studies was also different, our study (Chapter 4) having a longer total duration than both Edwards et al. (2008) and Gray et al. (2010). This may have lead to habituation to the painful stimulation due to

fatigue at a peripheral nociceptive neuron level (Greffrath, Baumgartner, & Treede, 2007) and /or at a central/cortical level (Handwerker & Kobal, 1993; Milne, Kay, & Irwin, 1991; Valeriani et al., 2005) which may have influenced the PREPs. The extended duration may also lead to disengagement with the task, drawing attention away from the task and this may influence PREP generation (Arntz, Dreessen, & Merckelbach, 1991; Rutter, Dahlquist, & Weiss, 2009; Tan, 1982). Related to this point, the possible role of attention influencing the cardiac cycle related modulation of PREPs was also considered. Gray et al. (2010) only reported a cardiac cycle effect for P2 when pain stimuli were cued, no modulation was present for uncued stimuli. Edwards et al. also presented a focal point prior to delivery of the pain stimuli, whereas study 3 (Chapter 4) presented the pain stimuli totally uncued and thus cueing may influence the cardiac cycle modulation of PREPs. Finally, the studies used different EEG systems. We (Chapter 4) used a Biosemi system which is a high impedance EEG system which requires a greater number of trials to achieve significance than comparable low impedance systems. It should be noted that although we (Chapter 4) achieved a high number of accepted trials (20 per condition) and had greater participant numbers (N = 26) this may not have been enough to achieve an 80% chance of statistical significance which Kappenman and Luck (2010) suggest would require 25 trials per condition. However, it should be noted that the number of suggested trials was based on a sample of just 12 (Kappenman & Luck, 2010) and additionally, the N2 and P2 components may require a different number of trials to achieve significance compared to the P300. Additionally, although, the greater number of participants in Study 4 aimed to offset the lower number of trials, it is still possible that the lack of cardiac cycle-related modulation of PREPs may be due to low power.

Although the methodological differences between the studies may go some way to explaining the differential findings, a further area which may contribute to the contrasting findings is a possible lateralisation effect. Both Edwards et al. (2008), Gray et al. (2010) and ourselves (Chapter 4) delivered stimulation to the right hand and although Edwards et al. and Gray et al. found modulations, there is evidence to suggest that there may be a right hemispheric dominance for cardiac cycle-related modulation. For example it has been found that visual evoked potential amplitudes

recorded in the right hemisphere during systolic and diastolic pressure differed significantly, whereas those recorded in the left hemisphere did not (Walker & Sandman, 1982). However, as Edwards et al. (2008) only measured PREPs at Cz electrode, it isn't possible to determine if there were any recording side laterality effects. Additionally, Gray et al. (2010) measured PREPs at electrode sites C3, Cz and C4 but pooled the data for analyse and also only stimulated one hand, meaning it is not possible to fully analysis lateralisation effects. Although we measured PREPs in both the left and right hemisphere, it may be that the cardiac cycle-related effects on PREPs may be more pronounced for left hand stimulation.

The insular cortex is important in integrating baroreceptor information in the brain (Zhang et al., 1998; Saleh & Connell, 1998) and there is growing evidence to suggest that processing of baroreceptor afferents is lateralised with a right sided dominance (Critchley, Corfield, Chandler, Mathias, & Dolan, 2000; Henderson et al., 2004; Weisz et al., 2001). Functional magnetic resonance imaging in cats following baroreceptor activation has shown the baroreflex-mediated BP regulation in the right insular cortex (Henderson et al., 2004). Similarly, in rats (Zhang, Tang, Yuan, & Jia, 1997) and monkeys (Zhang et al., 1998) more baroreceptor neurons in the right insular cortex responded to changes in BP than in the left insular cortex. Evidence for lateralisation of baroreceptor processing in humans has been aided significantly by the development of neuroimaging techniques. Critchley and colleagues (2000) employed PET to observe increased activity in the right ACC and right insular cortex in response to changes in systemic BP generated via exercise and mental arithmetic tasks. Also using PET, Weisz and colleagues (2001) reported that following external neck suction to stimulate the carotid sinus baroreceptors regional cerebral blood flow increased in the right anterior–inferior prefrontal cortex. However this is not always the case. Other studies have suggested a left hemisphere dominance in relation to baroreflex sensitivity (Hilz et al., 2001; Sykora et al., 2009), although the majority of findings indicate a predominantly right sided baroreceptor processing.

It has been the general consensus for many years that somatosensory stimulation of the body surface is largely processed by brain regions contralateral to the side of stimulation (Bingel et al., 2003; Youell et al., 2004; Coghill, Gilron, &

Iadarola, 2001). However, there is evidence for pain-related lateralisation having a right hemisphere dominance. Studies have indicated pain thresholds are lower and pain ratings higher when noxious stimuli are presented to the left side of the body than when applied to the right, independent of handedness (e.g., Lugo, Isturiz, Lara, Garcia, & Eblen-Zaijur, 2002; Pauli, Wiedemann, & Nickola, 1999; Sarlani, Farooq, & Greenspan, 2003; Spernal, Krieg, & Lautenbacher, 2003; Merskey &Watson, 1979; Schiff & Gagliese, 1994). However, other studies have failed to find any lateralisation effects for pain (Coghill et al, 2001; Hall, Hayward, & Chapman, 1981; Seltzer, Yarczower, Woo, & Seltzer, 1992).

Research has been somewhat inconclusive when it comes to identifying specific locations of pain processing. However Peyron and colleagues (Peyron, Laurent, & Garcia-Larrea, 2000) suggest that in humans and primates the SI, SII ACC and Insula are the most consistently activated, typically contralateral to the side of pain stimulation. More recently, research has sought to identify if the processing of painful stimuli may be lateralised. Most relevant to this study is work by Symonds and colleagues (Symonds, Nakia, Bixby, & Mande, 2006), who utilised fMRI to investigate lateralisation effects following electrocutaneous stimulation of the right and left index fingers. As many previous studies have found, Symonds et al. (2006) report that different pain processing areas were activated uniquely by the same pain stimuli i.e. some areas were activated bilaterally, some contralaterally and some showed a hemispheric bias regardless of side of stimulation. Specifically, the somatosensory cortex and posterior insula were activated contralateral to the pain stimuli, whereas the mid/posterior insula, anterior insula, and posterior cingulate were activated bilaterally. Additionally, the middle frontal gyrus, anterior cingulate, inferior frontal gyrus, medial/superior frontal gyri, and inferior parietal lobule showed either an exclusive or strong lateralisation to the right hemisphere (Symonds et al., 2006). Symonds et al. (2006) also reported that activity in the right somatosensory cortex, during left hand stimulation was greater than activity seen in the left hemisphere during right hand stimulation. Similarly, right sided anterior cingulate activity during left hand stimulation was significantly greater than right hand stimulation. In support of the findings by Symonds et al. (2006), an fMRI study investigating the lateralisation of thermal pain delivered to both the right and left

hands (Brooks, Nurmikko, Bimson, Singh, & Roberts, 2002) reported a right lateralisation in the anterior insula when pain was attended to, and in the ACC regardless of attentional focus, whereas the posterior insula was found to be activated contralateral to the stimulus and no significant activation in response to painful stimulation was detected in the primary somatosensory cortex (SI) or the thalamus. Taken together these studies suggest that pain may be processed predominately in the right hemisphere.

Despite indicating a preferential processing of pain in the right hemisphere for certain pain processing areas, as evidenced by the Symonds et al (2006) and Brooks et al. (2002) studies, not all pain processing areas demonstrate a right sided bias, and indeed several studies have reported no lateralisation effects. For example Coghill and colleagues (2001) reported an increase in cerebral blood flow assessed via PET in contralateral regions of SI, SII, insular cortex and bilateral regions of the cerebellum, putamen, thalamus, ACC, and frontal operculum regardless of side of stimulation. Similar findings from fMRI studies have reported bilateral responses within the SI, SII and insula but with a significantly greater contralateral response in SI and the thalamus in response to painful laser stimulation applied to the right and left hands (Bingel et al., 2003) and right and left lower legs (Youell et al., 2004). Furthermore, Youell and colleagues (2004) also reported an increase in left insula activity following lower leg stimulation.

Supporting evidence for a potential lateralisation of pain processing comes from the well documented cerebral hemispheric lateralisation of emotional processing which has a proposed right hemispheric dominance (Ji & Neugebauer, 2009). Affective stimuli have been found to be more accurately processed when presented to the left ear, which projects mainly onto right-hemispheric brain structures (Carmon & Nachshon, 1973; Haggard & Parkinson, 1971; Joseph, 1988). In addition, affective startle modulation appears to be specific to left ear stimulation, whereas presentation to the right ear produced none or inconsistent affective startle modulation (Bradley, Cuthbert, & Lang, 1991, 1996; Kettle, Andrewes, & Allen, 2006).

Cardiac cycle studies have taken into account the proposed lateralised processing of baroreceptor input and investigated if this may contribute to a lateralisation of cardiac cycle modulation. Early work by Walker and Sandman (1982) indicated that baroreceptor activation (systole) appears to impact the processing of visual input in the right hemisphere to a greater extent than the left. They reported that visual evoked potentials recorded in the right hemisphere were found to be smaller during the systolic phase of the cardiac cycle than the diastolic phase, whereas the visual evoked potentials recorded from the left hemisphere were unaffected by the phase of the cardiac cycle (Walker & Sandman, 1982). More recently the cardiac cycle modulation of startle eye blink was suggested to show a right hemispheric dominance, as the startle eye blink response was found to be reduced during systole only following presentation to the left ear (Schulz et al., 2009). However, a right side bias is not always evident. Weisz and Adam (1996) propose greater influence on the cardiac cycle modulation of reaction times for processing in the left hemisphere. They found that reaction time was marginally longer during systole than diastole when stimuli were presented to the right, or responses were made with the right hand, whereas there was no difference for central and left stimuli or for left hand responses.

Based on the discussions above, it appears reasonable to conclude that research supports a right sided processing of baroreceptor information, and that evidence for greater activation of the right hemisphere pain areas (e.g. right ACC) when the left hand is stimulated. Therefore, it is possible that the lack of cardiac cycle-related modulation in our previous study (Chapter 4) may be due to the stimulation of the participants right hand, which may present a less evident cardiac cycle-related modulation than pain delivered to the left hand. Taken together this suggests the increased activity in response to pain stimulation of the left hand would offer greater opportunity for convergence during systole, when baroreceptor afferent input to the right hemisphere is proposed to be maximal (e.g. Zhang & Oppenheimer, 1997). Therefore it may be hypothesised that cardiac cycle related modulation would be most evident following left hand stimulation. Whereas right hand stimulation would be processed both contralateral to the stimulation (i.e., in the left hemisphere) and although right hand stimulation would likely activate right

hemisphere pain areas (e.g. ACC), this activation appears not to be as pronounced in the right hemisphere and thus convergence with the baroreceptor input would be reduced and thus cardiac cycle-related modulation not as pronounced.

It is also interesting to note that the majority of previous cardiac cycle research indicating a reduction in the NFR (Edwards et al., 2001; 2002; 2003; McIntyre et al., 2006) delivered stimuli to the left side. However, there are also a studies demonstrating a cardiac cycle-relation attenuation of the NFR (McIntyre et al., 2008), PREPs (Edwards et al., 2008; Gray et al., 2010), neural activity in the brain (Gray et al., 2009) and pain thresholds (Wilkinson, McIntyre & Edwards, 2013) when stimuli were delivered to the right hand.

Therefore, the aim of the current study was to directly compare PREP responses to painful electrocutaneous stimulation delivered to both the left and right hands at 7 intervals across the cardiac cycle in the same subjects. The current study specifically aimed to determine if the cardiac cycle-related modulation of PREPs is influenced by the side of stimulation i.e. left and right hand, and/or location of cerebral processing of noxious afferent information i.e. scalp electrode sites C3, Cz and C4. If the proposed right hemispheric dominance of baroreceptor afferent information processing and pain processing outlined above is considered, it is hypothesised that the cardiac cycle-related modulation of pain would be greater following stimulation of the left hand than the right hand and may be more evident in the scalp electrodes covering the central and right sites i.e. scalp electrode C4 compared to C3.

5.3 Methods

5.3.1 Participants

Seventeen (7 men, 10 women) normotensive adults with a mean (SD) age of 18.4 (0.6) years and body mass index (BMI) of 22 (2.7) kg/m^2 were recruited from the Loughborough University campus and local community to participate in the study. Four of the participants had participated in the original PREP study reported in this thesis (Chapter 4) and were recalled to undertake the assessment with their left hand, the remainder of the participants were new recruits. Across the two testing

sessions mean (SD) resting systolic BP (SBP) was 118.7 (8.9) mmHg, diastolic BP (DBP) was 70.5 (7.2) mmHg, and resting heart rate (HR) was 70.9 (12.8) bpm. Individuals were excluded if they had any known health problems including chronic pain disorders, cerebrovascular, cardiovascular or neurological diseases, had a cardiac pacemaker, history of major psychiatric disorders, were pregnant or had missed their last menstrual cycle, were taking routine prescription medicine except for birth control, were currently using any narcotic substances, had an alcohol intake greater than 28 units per week for men and 21 units per week for women or had a resting HR above 92 bpm. Participants were asked to refrain from analgesic medication for 24 hrs and caffeine, nicotine and vigorous exercise for 2 hrs prior to testing. The Loughborough University Ethical Advisory Committee approved the study, and all participants provided written informed consent. Participants returning from the original PREP study (Chapter 4) were paid an inconvenience payment of £5 (4 participants) and new recruits who were first year psychology students received credits towards their course (13 participants).

5.3.2 Apparatus and measurements

Resting BP (mmHg) and HR (bpm) were obtained using an automated oscillometeric sphygmomanometer (Omron 705-IT, Omron Healthcare Europe) and a brachial cuff attached around the upper non-dominant arm.

An electrocardiogram (ECG) was recorded continuously at 2500 Hz using three disposable spot electrodes (Cleartrace, ConMed) placed in a modified chest configuration; the two active electrodes were placed on the right clavicle and a rib below the heart on the left side of the torso and the ground electrode was placed on the left clavicle, and connected to an AC amplifier (LP511, Grass).

Pain stimuli (triple 1 ms monopolar square wave pulse with 5 ms inter-pulse interval at 200Hz) were delivered electrocutaneously by a constant current stimulator (DS7A, Digitimer) via a concentric planar electrode (Kaube et al., 2000). The concentric planar electrode was secured with tape (Transpore, 3M) to the dorsal surface of the hand between the metacarpals of the index and middle fingers.

Electrode sites were prepared by exfoliating (Nuprep, D.O. Weaver & Co) then degreasing the skin using isopropyl alcohol swabs (Sterets, Medlock Medical Ltd.).

During the determination of pain thresholds participants indicated if each stimulus was painful or not via a push button response box (16 cm ×16 cm × 3 cm). Mounted on the response box were a piezo-oscillator (top middle), a red light emitting diode (top left), a green light emitting diode (top right), and buttons marked “Yes” and “No” (centre left and right, respectively). A computer was programmed in Spike2 (CED) to record responses and trigger stimuli using a Micro II 1401 (Cambridge Electronic Design).

Electroencephalographic data was recorded via a flexible nylon headcap (Biosemi) containing 32 electrode holders positioned according to the internationally recognised 10-20 coordinate system (Jasper, 1958). A blunted needle (16G ¾ blunt square grind, Becton Dickinson and Company) and syringe (5ml syringe luer-lok tip, BD) were used to part the participants hair and fill each electrode holder with conductive gel (Electro-Gel, ECI). Thirty-two active version pin electrodes sintered - Ag-AgCl electrode tip (Biosemi) plus two feedback loop electrodes; a) Common Mode Sense (CMS) active electrode and b) Driven Right Leg (DRL) passive electrode were inserted into the corresponding electrode holders and checked to ensure electrode offset was below 25 mV. In addition to the head cap electrodes, six external flat type active electrodes - 4mm diameter sintered Ag-AgCl electrode pallet (Biosemi) were used to measure horizontal (HEOG) eye movement (2 electrodes) and vertical (VEOG) eye movement (2 electrodes) and to later act as reference for the scalp electrodes (2 electrodes). The cavity of each flat-type active electrode was filled with conductive gel and secured in place with double sided adhesive disks (Biosense Medical Ltd.) and with tape (Medipore, 3M). Both the Biosemi pin and flat electrodes signal was amplified at the electrode, reducing the impact of high electrode impedance influencing the signal quality (Metting van Rijn, Kuiper, Dankers, & Grimbergen, 1996).

5.3.3 Procedure

Participants were tested in two 2-hr sessions separated by at least 2 days (typical range 2-4days). Testing was conducted in a quiet room with no windows and the participants were separated from the experimenter with access via a closed door. At the start of the first session participants completed an informed consent form (also completed at the start of the second session for returning participants) and sat quietly whilst completing the following questionnaires: (a) a brief questionnaire containing questions about age, sex, health habits, education, (b) Spielberger State and Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970), a 40-item inventory used to assess levels of state and trait anxiety and (c) the Center for Epidemiologic Studies Depression (CES-D) Scale (Radloff, 1977), a 20-item scale designed to measure depressive symptomatology in the general population (10 min total). During both sessions participants rested quietly while baseline BP and HR were measured at 60, 180, and 300 s (6 min). If a participant's HR exceeded 92 bpm, they were excluded from the study, as HR's above 92bpm would mean that stimuli presented at *R*-wave + 650ms would fall within the next cardiac cycle; however, none were excluded. Following instrumentation (30 min) participants firstly undertook a pain threshold determination procedure (15 min). Participants then rested for 5mins after which a PREP assessment was completed (70 min). The hand to which the pain stimulus was delivered first was counterbalanced throughout the study to eliminate order effects.

5.3.4 Instrumentation

Following attachment of the ECG electrodes, the same skin preparation procedure described above was applied to the ear lobes, an area between the outer canthus of the eyes and the temples, areas directly below each eye (infra-orbital point) and the forehead. Subsequently, the two external HEOG electrodes were attached to the skin approximately1 cm out from the outer canthus of each eye, the two external VEOG electrodes were attached on the infra-orbital point below each eye and the two external reference electrodes were attached to the ear lobes. Next, the circumference of the participants head was measured to determine the appropriate size EEG head cap. The head cap was then placed on the participants head and secured in place with the chin strap. The location of the central (Cz) electrode was

identified as half way between two anatomical landmarks; (a) the nasion, which is the distinctly depressed area between the eyes, just above the bridge of the nose, and (b) the inion, which is the lowest point of the skull from the back of the head and is normally indicated by a prominent bump. Lateral positioning of the Cz electrode was determined by investigator positioning and participant feedback from the insertion of the blunt needle into the Cz electrode holder. Once the head cap was in position the blunt needle and syringe were used to part the hair and fill each electrode holder with conductive gel to ensure contact with the scalp. The 32 electrodes, plus the CMS and DRL electrodes were then inserted into the corresponding electrode holder and their electrode offset checked.

Finally, the skin on the dorsal surface of the hand to which the pain stimulus was to be delivered was cleaned in an area between the metacarpals of the index and middle fingers as previously described and the concentric planar electrode secured in position with tape.

5.3.5 Pain threshold assessment

Participants sat upright and supported the forearm of the hand for stimulation testing on a table while their opposite hand rested on the response box. Pain detection thresholds were determined using an up-down staircase procedure (Levitt, 1971). A green warning light (1000 ms duration) illuminated to signify the start of each trial and a red light (variable duration; the light remained illuminated until the participant made a response, up to a maximum of 7500 ms) indicated the end of each trial. Following illumination of the green light, a 1s delay occurred after which the programme searched for the *R*-wave of the ECG and subsequently the participants hand was stimulated at *R*+0ms. Participants were informed that the stimulus could occur at any time between the illumination of the green and red lights. Once the red light was illuminated participants pressed the "*Yes*" button if they perceived the stimulation as painful or the "*No*" button if they did not perceive it as painful. The next trial commenced following the participants response. On the first trial the stimulus intensity was 0 mA, and subsequently increased in 0.8 mA steps until the participant first reported a painful sensation (first reversal). The stimulus intensity then decreased in 0.4 mA steps until the stimulus was no longer reported as painful

(second reversal). Each staircase then continued in 0.1 mA steps until the staircase had completed two further ascending and descending series (i.e. four more reversals). The pain threshold (mA) was defined as the average of the peaks during the second and third series (i.e. the third and fifth reversal points) of each staircase. The maximum allowable stimulus intensity was 30 mA; however, this stimulus intensity was never reached. Mean (SD) pain threshold for the right hand was 1.15 (0.65) mA and for the left hand 1.12 (0.54) mA. An Independent Samples t-test found no significant difference between the mean pain thresholds for the right and left hands (t (32) = .157, p = .876).

5.3.6 Pain-related evoked potential procedure

Stimulation intensity for PREP elicitation was calculated as two times each individual's pain threshold and electrocutaneous stimulations were delivered to the dorsal surface of the experimental hand with the same stimulus parameters as for the threshold assessment procedure. Participants sat quietly upright with their hands resting on their knees. They were instructed to focus on a fixation point (a black circle 2cm in diameter) positioned on the wall directly in front of them throughout each experimental block, to relax their muscles, remain as still as possible and minimise the number of blinks they took. Each participant completed 7 experimental blocks of 21 trials, with each block separated by a 5 minute rest period. During each block the participants hand was pseudorandomly stimulated 3 times at each of seven *R*-wave intervals (*R*+50, *R*+150, *R*+250, *R*+350, *R*+450, *R*+550, *R*+650 ms), thus over the 7 experimental blocks each interval was presented 21 times. The inter-stimulus interval of 12, 16, or 20s was pseudorandomly selected. At the start of each block an additional pain stimulus was delivered, but no data recorded, to reduce startle contamination affecting subsequent EEG activity. Participants were informed that the stimuli could occur at any time during the experimental block, which would last approximately five minutes. The Spike 2 programme marked the EEG data with event codes, sent by the 1401. These event codes were evident; (a) when pain stimuli were elicited, and (b) in the cardiac cycle before a pain stimulation (i.e. a beat-before event) at the same *R*-wave interval at which the pain stimuli was to be subsequently delivered. The beat-before event code enabled us to subsequently generate an ERP waveform containing EEG data without a pain stimuli presented

and containing any ECG-related artifact and subtract this from an ERP waveform containing EEG data in which a pain stimulus was delivered. The subtraction of the beat-before ERP waveform from the stimulation ERP waveform was undertaken because one problem with cardiac cycle studies is the potential for contamination of the EEG data by ECG artefacts (Allen et al., 1998).

It has been reported that a common occurrence following repetitive painful stimulation is habituation, i.e. a decrease in pain and pain-related responses to continuous or repetitive pain stimuli (LeBlanc & Potvin, 1966; Strempel, 1976; Strempel, 1978). This may be due to peripheral nerve fibre fatigue (Greffrath et al., 2007) and/or central habituation to the painful stimuli (Bingel, Schoell, Herken, Buchel, & May, 2007; Bingel, Herken, Teutsch, & May, 2008; Milne et al., 1991). Therefore to ensure the stimulus remained painful, participants completed the Short-Form McGill Pain Questionnaire (Melzack, 1987) after each experimental block. The questionnaire included participants rating the average intensity of the pain stimuli during the preceding block using a rating scale of 0-100 with anchors of '0' (no sensation), '1' (first sensation), '25' (uncomfortable), '50' (just noticeable pain), '75' (very painful), and '100' (maximum tolerable pain). If participants rated the intensity of the stimuli less than 50 (just noticeable pain) the stimulation in the following experimental block was increased to double that used in the proceeding block. However, no participants rated the intensity below 50.

5.3.7 Electroencephalographic data recording and analysis

The EEG activity, VEOG, HEOG and ear lobe references was recorded reference free, continuously via a battery-powered amplifier (Biosemi ActiveTwo AD-box, Mk 2) and Actiview acquisition software (Biosemi) at a sample rate of 2048 Hz. Raw EEG, VEOG, HEOG and reference electrode data were processed offline using BESA Research 5.2.2. The EEG data were re-referenced to linked ears reference off-line and filtered using a 30 Hz, 12 db/oct, ZeroType low-pass filter and a 0.1 Hz, 12 db/oct, Forward Type high-pass filter. Beat-before and pain stimulus data were segmented into 900 ms epochs (− 100 ms to 800 ms). The baseline epoch was defined as 100ms pre-stimulus to stimulus onset. EEG data were corrected for ocular movement artifacts using manual definition of each participants blink

topography and applying an adaptive artifact correction (Ille, Berg, & Scherg, 2002). Following correction of ocular movement artifacts, data were automatically scanned for epochs containing a voltage change of greater than 100 μV and these were rejected. Remaining beat-before and pain stimuli trials were separately averaged according to *R*-wave interval (*R*+50, *R*+150, *R*+250, *R*+350, *R*+450, *R*+550, *R*+650 ms) resulting in a single PREP waveform and one corresponding beat-before average waveform for each *R*-wave interval. If more than 50% of the total pain or beat before trials were rejected, the participant was removed from further analysis. No participants were removed. The mean number of accepted trials per R-wave interval was 19 for both pain stimulus and beat-before (range = 14 to 21).

Prior to peak detection analysis of the PREP components, for each *R*-wave interval the beat-before average waveform was subtracted from the corresponding PREP waveform to generate a difference waveform with ECG artifacts removed whilst preserving the PREP components for each *R*-wave interval (Figures outlining the effects of the subtraction of the beat-before average waveform from the corresponding PREP waveform can be seen in Chapter 4 of the thesis – Figures 7 & 8).

As discussed in the Chapter 4 there are three main approaches for the measurement of PREP components, (a) peak amplitudes, (b) mean amplitudes, and (c) area analysis. However, as the pain response is generally a well defined, high frequency component the peak amplitude is likely to be the most appropriate method of PREP component measurement (Luck, 2005). Therefore in order to focus and streamline the results only the peak amplitude data will be reported in this chapter.

5.3.8 Peak amplitude detection

Peak detection windows for identification of the N2 and P2 component of the difference PREPs were identified as the latency of the visually identified N2 and P2 peak amplitudes from the grand average difference waveform averaged across all participants and all intervals $\pm$40ms. Separate analysis windows for the right and left hand data were determined based on the grand average waveforms for each side. Therefore, the analysis windows for the N2 and P2 components of the averaged

difference waveforms were 97-177 ms and 105-185 ms for N2 in the right and left hand respectively, and 263-343 ms and 258-338ms for P2 in the right and left hands respectively. These timings fit with the typical electrically induced PREPs being characterised by a negative-going peak (N2) at approximately 130–240 ms post stimulus followed by a positive-going peak (P2) approximately 230–390 ms post stimulus (Bromm, 1985; Zaslansky et al., 1996). The largest negative-going peak was automatically identified in the N2 window and the largest positive-going peak automatically in the P2 window for each *R*-wave interval for each hand. Peak amplitudes were defined as the baseline to highest peak in the detection window. Peak-to-peak measurements were also calculated as the difference between the N2 peak amplitude and the P2 peak amplitude.

On the basis of previous neuroimaging studies identifying cerebral regions activated by painful stimulation (see Introduction) and the focus of the current study on lateralisation effects, analyses were conducted on the following scalp electrodes; Cz, C3 and C4. These electrodes are thought to reflect the activity of brain areas associated with pain perception i.e. the ACC, SII, insular cortex bilaterally (Bentley, Derbyshire, Youell, & Jones, 2003; Bromm & Chen, 1995; Garcia-Larrea, Frot, & Valeriani, 2003; Ohara, Crone, Weiss, & Lenz, 2006; Tarkka & Treede, 1993; Treede, Lorenz, & Baumgartner, 2003; Valeriani et al., 1996) and the contralateral SI (Kakigi, Inui, & Tamura, 2005). Specifically, Cz overlays the central cerebral cortex, C3 electrode overlays the left cerebral hemisphere and C4 overlays the right cerebral hemisphere.

5.3.9 Data reduction and analyses

The BP and HR readings were averaged to provide measures of resting SBP, DBP and HR over both sessions. A series of repeated measures analysis of variance (ANOVAs) with 2 Hand (right, left) × 3 Scalp Electrode Site (C3, Cz, C4) × 7 R-wave to stimulation Interval (i.e., *R*+0 ms, *R*+150 ms, *R*+250 ms, *R*+350 ms, *R*+450 ms, *R*+550 ms, *R*+650 ms) were performed separately on N2 and P2 peak amplitudes and the N2-P2 difference amplitudes. Such analysis enabled the following to be investigated; 1) the 2 × 7 interaction determined if there is a difference in the cardiac cycle modulation of PREP components of interest between hands regardless of

scalp electrode location i.e. stimulus-related laterality; 2) the 3 × 7 interaction investigates if there is a difference in the cardiac cycle modulation of PREP components between the three scalp electrode locations regardless of which hand was stimulated i.e. hemisphere-related laterality; 3) the 2 × 3 interaction determines if PREP component amplitudes across scalp electrode sites differ between hands regardless of cardiac cycle phase and; 4) the 2 × 3 × 7 interaction determines if there is a difference in the cardiac cycle-related modulation of PREP components between hands with scalp electrode site.

Subsequently, significant 2 × 3 × 7 findings were followed by separate 2 Hand (right, left) × 7 Interval (*R*+0 ms, *R*+150 ms, *R*+250 ms, *R*+350 ms, *R*+450 ms, *R*+550 ms, *R*+650 ms) at each scalp electrode site to test for an interaction effect in isolation. This would determine if any difference in cardiac cycle modulation of the PREP components between hands depends on scalp electrode. Following these analyses, separate 3 Scalp Electrode Site (C3, Cz, C4) × 7 Interval (*R*+0 ms, *R*+150 ms, *R*+250 ms, *R*+350 ms, *R*+450 ms, *R*+550 ms, *R*+650 ms) repeated measures ANOVA's were conducted for each hand (i.e. right and left) to determine if any difference in the cardiac cycle modulation of PREP components between the three scalp electrode locations depended on which hand was stimulated. In addition 6 separate 7 Interval (*R*+0 ms, *R*+150 ms, *R*+250 ms, *R*+350 ms, *R*+450 ms, *R*+550 ms, *R*+650 ms) repeated measures ANOVAs on N2 and P2 peak amplitudes and N2-P2 difference amplitudes were conducted to investigate if, when tested in isolation, the Interval effect may help to explain any interactions in the preceding 2 × 3 × 7 ANOVAs. The following individual 7 Interval repeated measures ANOVAs were conducted; a) left hand stimulation recorded in right hemisphere (C4 scalp electrode), b) left hand stimulation recorded in left hemisphere (C3 scalp electrode), c) left hand stimulation recorded at midline (Cz scalp electrode), d) right hand stimulation recorded in right hemisphere (C4 scalp electrode), e) right hand stimulation recorded in left hemisphere (C3 scalp electrode) and f) right hand stimulation recorded at midline (Cz scalp electrode).

ANOVAs were corrected for the assumption of independence of data points using Huynh-Feldt correction (ε). Significant results were followed by Newman-Keuls

post hoc comparisons (all possible pairwise comparisons were computed). In addition to significance levels, partial eta-squared (η_p^2), a measure of effect size, is also reported, indicating the proportion of total variation attributable to the factor, partialling out (excluding) other factors from the total non-error and range from 0 to 1 (Cohen, 1973). As partial eta-squared may over estimate effect sizes in repeated measures studies, effect sizes of 0.01, 0.09 and 0.25 are accepted as representing small, medium and large effects respectively (Hanna & Dempster, 2012). A significance level of .05 was adopted. Data were analysed using SPSS 20.0 and Statistica Version 10.

5.4 Results

5.4.1 N2 peak amplitudes

As shown in Table 6, a 2 Hand × 3 Scalp Electrode × 7 Interval repeated measures ANOVA revealed a main effect for scalp electrode site. Post hoc pairwise comparisons showed N2 peak amplitudes to be significantly higher at scalp electrode Cz than scalp electrode C3 (Figure 10a). Mean (SD) N2 peak amplitudes for scalp electrode site C3, Cz and C4 were -7.45 (4.82) µV, -8.56 (5.52) µV and -8.31 (5.97) µV respectively. In addition, the 2 Hand × 3 Scalp Electrode Site × 7 Interval interaction was also significant. No other main or interaction effects were significant.

5.4.2 P2 peak amplitudes

The results of the 2 Hand × 3 Scalp Electrode Site × 7 Interval repeated measures ANOVA can be found in Table 6. Analysis revealed a main effect for scalp electrode site. As shown in Figure 10b post hoc pairwise comparisons showed P2 peak amplitudes were higher at scalp electrode site Cz than C3 and C4 scalp electrodes. Mean (SD) P2 peak amplitudes for scalp electrode site C3, Cz and C4 were 18.89 (4.53) µV, 24.31 (5.52) µV and 19.06 (4.71) µV respectively. No other main or interaction effects were significant.

5.4.3 N2-P2 peak-to-peak amplitude

As shown in Table 6, a 2 Hand × 3 Scalp Electrode Site × 7 Interval repeated measures ANOVA revealed a main effect for scalp electrode site. Post hoc pairwise

comparisons showed N2-P2 difference amplitudes were higher at scalp electrode site Cz than C3 and C4 scalp electrodes (Figure 10c). Mean (SD) P2 amplitudes for scalp electrode site C3, Cz and C4 were 26.34 (7.76) µV, 32.87 (9.33) µV and 27.37 (8.99) µV respectively. No other main or interaction effects were significant.

5.4.4 Separate 2 Hand (Right, Left) × 7 Cardiac Cycle Interval (R+50, R+150, R+250, R+350, R+450, R+550, R+650 ms) repeated measures ANOVAs at 3 scalp electrode sites (C3, Cz, C4)

To further investigate the significant 2 Hand × 3 Scalp Electrode Site × 7 Interval interaction identified above for N2 peak amplitudes, separate 2 Hand × 7 Interval repeated measures ANOVAs were conducted at each scalp electrode site for N2 peak amplitudes. These analyses would help to further understand if the significant Hand × Scalp Electrode × Interval interaction for N2 amplitudes could be driven by a 2 × 7 interaction that varies across the scalp electrode sites. As shown in Table 7 and Figure 11 below, none of the main effects (interval or hand) or interactions (hand × interval) at any scalp electrode site (C3, Cz, C4) were significant.

Table 6. *Statistical values for separate 2 Hand (right, left) × 3 Scalp Electrode Site (C3, Cz, C4) × 7 Interval (R+50, R+150, R+250, R+350, R+450, R+550, R+650 ms) repeated measures ANOVAs for N2, P2 and N2-P2 peak-to-peak amplitudes.*

Variable	ε	*df*	*F*	*p*	η_p^2
N2 Peak amplitude					
Interval	1.00	6, 96	.913	.489	.054
Hand	1.00	1, 16	2.86	.110	.151
Scalp Electrode Site	.995	2, 31.8	3.75	.035*	.190
Interval × Hand	.860	5.2, 82.5	1.00	.422	.059
Interval × Scalp Electrode Site	.777	9.3, 149.2	.768	.651	.046
Hand × Scalp Electrode Site	1.00	2, 32	.213	.810	.013
Interval × Hand x Scalp Electrode Site	.738	5.6, 141.7	2.38	.016*	.129
P2 Peak amplitude					
Interval	.807	4.9, 77.5	.202	.958	.012
Hand	1.00	1, 16	2.5	.133	.135
Scalp Electrode Site	.832	1.7, 26.6	94.28	<.001*	.885
Interval × Hand	1.00	6, 96	1.54	.174	.088
Interval × Scalp Electrode Site	.799	9.6, 153.4	.763	.659	.045
Hand × Scalp Electrode Site	.864	1.7, 27.7	1.42	.258	.081
Interval × Hand x Scalp Electrode Site	.748	9, 143.7	.859	.563	.051
N2-P2 Peak-to-Peak amplitude					
Interval	.819	4.9, 78.6	.803	.549	.048
Hand	1.00	1, 16	3.36	.085	.174
Scalp Electrode Site	1.00	2, 32	54.53	<.001*	.773
Interval × Hand	1.00	6, 96	1.66	.139	.094
Interval × Scalp Electrode Site	.754	9, 144.8	1.42	.186	.081
Hand × Scalp Electrode Site	.946	1.9, 30.3	.285	.742	.017
Interval × Hand × Scalp Electrode Site	.973	11.7, 186.8	1.35	.196	.078

*significant at 0.05

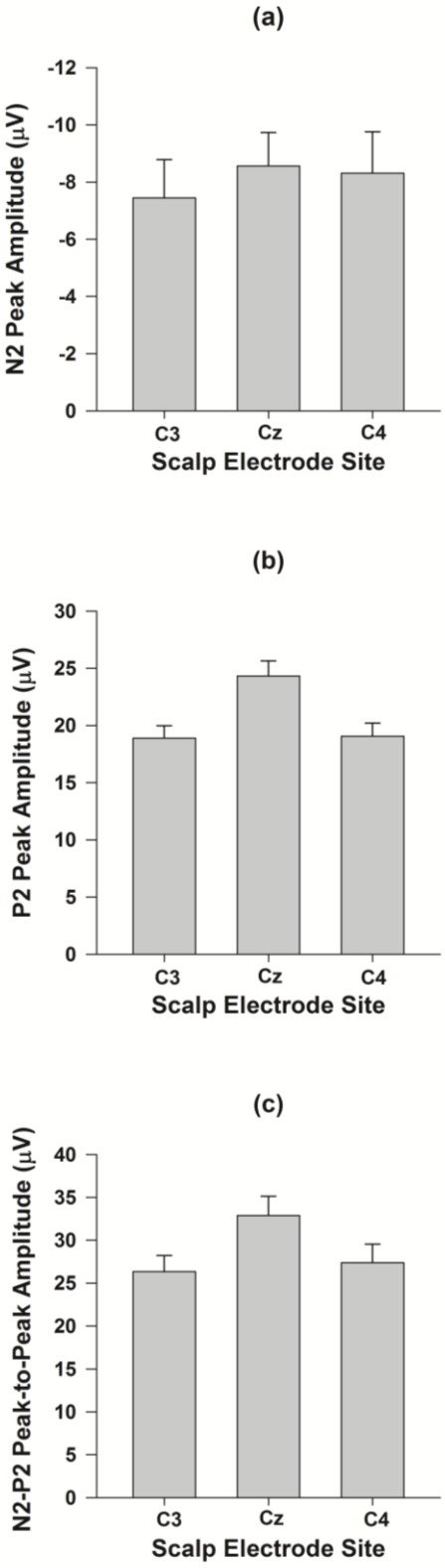

Figure 10. *Mean (SE) (a) N2 peak amplitudes, (b) P2 peak amplitudes and (c) N2-P2 peak-to-peak amplitudes at 3 scalp electrode sites (C3, Cz, C4) in response to painful electrocutaneous stimulation.*

Table 7. *Statistical values for separate 2 hand (right, left) × 7 Interval (R+50, R+150, R+250, R+350, R+450, R+550, R+650 ms) repeated measures ANOVAs for N2 peak amplitudes at each scalp electrode site (C3, Cz, C4).*

Variable	ε	*df*	*F*	*p*	η_p^2
C3 Scalp Electrode					
Interval	1.00	6, 96	.805	.568	.048
Hand	1.00	1, 16	2.89	.109	.153
Interval × Hand	.760	4.6, 72.9	1.61	.173	.092
Cz Scalp Electrode					
Interval	1.00	6,96	1.28	.273	.074
Hand	1.00	1, 16	1.12	.306	.065
Interval × Hand	.825	5, 79.2	1.63	.162	.093
C4 Scalp Electrode					
Interval	.902	5.4, 86.6	.521	.773	.032
Hand	1.00	1, 16	3.26	.090	.169
Interval × Hand	1.00	6, 96	.372	.895	.023

*significant at 0.05

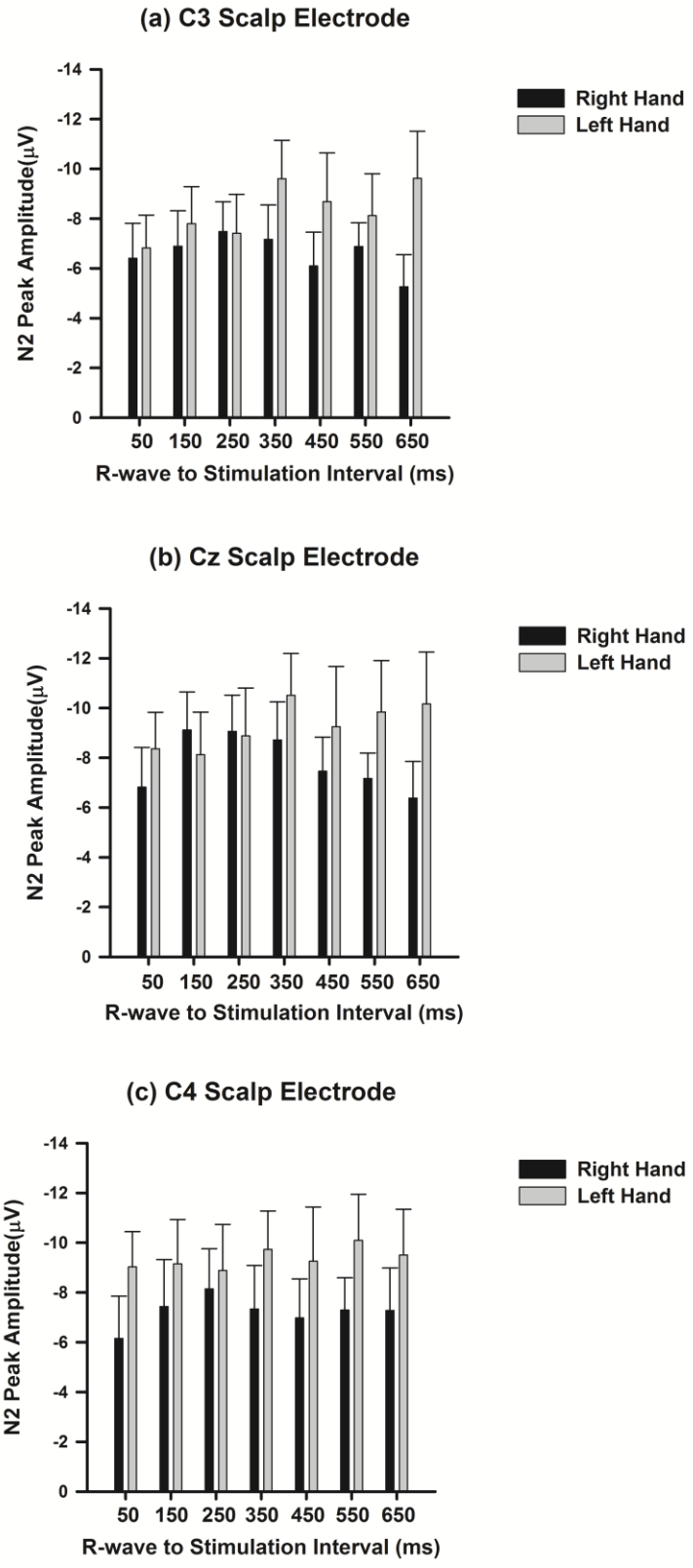

Figure 11. *Mean (SE) N2 peak amplitudes as a function of the cardiac cycle following painful stimulation of the right and left hands recorded at scalp electrode sites (a) C3, (b) Cz and (c) C4.*

5.4.5 Separate 3 Scalp Electrode Site (C3, Cz, C4) × 7 Cardiac Cycle Interval (R+50, R+150, R+250, R+350, R+450, R+550, R+650 ms) repeated measures ANOVAs in the right and left hands

To further explore if the significant 2 × 3 × 7 interaction identified above may be explained by a 3 x 7 interaction that varies in left and right hands, two separate 3 Scalp Electrode Site × 7 Interval repeated measures ANOVAs were performed on N2 peak amplitudes in the left and right hands. As shown in Table 8, a 3 Scalp Electrode Site × 7 Interval repeated measures ANOVA for N2 peak amplitudes for pain stimuli delivered to the right hand revealed no significant main (Interval or scalp electrode site) or interaction (Interval × Scalp Electrode Site) effects (Figure 12a). Similarly, for pain stimuli delivered to the left hand no main (cardiac cycle interval or scalp electrode site) significant effects. However, there was a marginal interaction effect (Interval × Scalp Electrode Site). Newman-Keuls post hoc analysis revealed that N2 peak amplitudes at scalp electrode site C3 were significantly smaller at cardiac cycle interval R+50ms than all cardiac cycle intervals at scalp electrode site C4 and at cardiac cycle intervals R+250, R+350, R+450, R+550 and R+650ms at scalp electrode site Cz. Similarly, N2 peak amplitudes at scalp electrode C3 cardiac cycle interval R+150ms were significantly smaller than at C4 electrode site at cardiac cycle intervals R+350 and R+550ms, and at Cz cardiac cycle intervals R+350, R+550 and R+650ms. At scalp electrode site C3 N2 peak amplitudes at cardiac cycle interval R+250ms were significantly smaller than R+150, R+350, R+450, R+550 and R+650 cardiac cycle intervals at scalp electrode site C4 and smaller than Cz scalp electrode site cardiac cycle intervals R+350, R+450, R+550 and R+650ms. Finally at cardiac cycle interval R+550ms at scalp electrode site C3 N2 amplitudes were smaller than cardiac cycle interval R+550ms at C4 scalp electrode site and R+350 and R+650ms at scalp electrode site Cz (Figure 12b). Planned orthogonal comparisons indicated that the Scalp Electrode Site × Interval interaction was characterised by a combined linear and quintic trend for scalp electrode site and interval, respectively, $F(1,16) = 7.45$, $p = .01$ and also by a combined quadratic and quartic trend for scalp electrode site and interval, respectively, $F(1,16) = 5.40$, $p = .03$.

Table 8. *Statistical values for two separate 3 Scalp Electrode Site (C3, Cz, C4)× 7 Interval (R+50, R+150, R+250, R+350, R+450, R+550, R+650 ms) repeated measures ANOVAs for N2 peak amplitudes in the right and left hands.*

Variable	ε	*df*	*F*	*p*	η_p^2
Right Hand					
Interval	.821	4.9, 78.8	.872	.502	.052
Scalp Electrode Site	.985	2, 31.5	1.88	.170	.105
Interval x Scalp electrode site	.784	9.4, 150.1	1.54	.135	.088
Left Hand					
Interval	1.00	6, 96	1.08	.383	.063
Scalp Electrode Site	1.00	2, 32	1.47	.246	.084
Interval x Scalp electrode site	.743	8.9, 142.7	1.73	.088	.098

*significant at 0.05

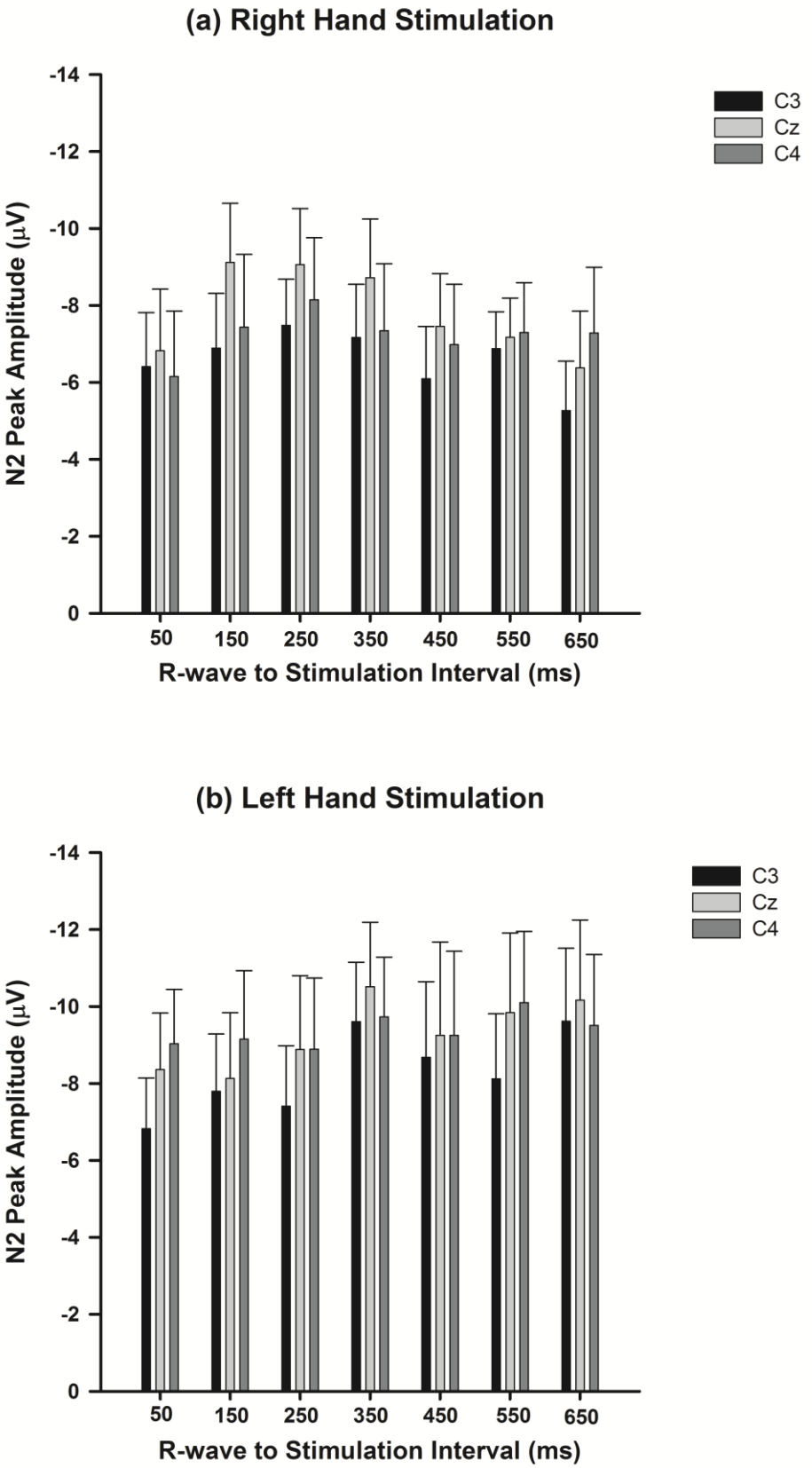

Figure 12. *Mean (SE) N2 peak amplitudes as a function of the cardiac cycle recorded at 3 scalp electrode site (C3, Cz, C4) in the a) right and b) left hands.*

5.4.6 Individual cardiac cycle interval effects

A series of 6 separate 7 Interval repeated measures ANOVAs were conducted for each different combination of hand and scalp electrode site for N2 peak amplitudes. The rationale for testing each combination of hand and scalp electrode site in isolation was to explore if there was any disparity in cardiac cycle-related modulation when different combinations of hand and scalp positions were tested (i.e. was there a significant cardiac cycle effect in some conditions but not others conditions) that may help us understand the 2 × 3 × 7 interaction.

As shown in Table 9, the following combinations of hand and scalp electrode location were subjected to repeated measures ANOVA analysis; a) left hand stimulation recorded at C4 scalp electrode, b) left hand stimulation recorded at C3 scalp electrode, c) left hand stimulation recorded at Cz scalp electrode, d) right hand stimulation recorded at C4 scalp electrode, e) right hand stimulation recorded at C3 scalp electrode, and f) right hand stimulation recorded at Cz scalp electrode. There were no Interval effects at any of the hand / scalp electrode recording site combinations.

Table 9. *Statistical values for six separate 7 Interval (R+50, R+150, R+250, R+350, R+450, R+550, R+650 ms) repeated measures ANOVAs for N2 peak amplitudes at each hand / scalp electrode site combination.*

Variable	ε	*df*	*F*	*p*	η_p^2
Left hand / C4 scalp electrode	.995	6, 95.6	.348	.909	.021
Left hand / C3 scalp electrode	.979	5.9, 94	1.81	.106	.102
Left hand / Cz scalp electrode	1.00	6, 96	1.24	.292	.072
Right hand / C4 scalp electrode	.947	5.7, 90.9	.498	.799	.030
Right hand / C3 scalp electrode	.819	6, 78.6	.739	.594	.044
Right hand / Cz scalp electrode	.810	4.9, 77.8	1.69	.148	.096

*significant at 0.05

5.5 Discussion

The first significant finding from the current study was a significant variation in N2 and P2 peak amplitudes, and N2-P2 peak-to-peak amplitudes between the three scalp electrodes assessed (C3, Cz, C4) following painful electrocutaneous stimulation. For all three PREP components, amplitudes at scalp electrode site Cz were significantly larger than C3 and for P2 peak amplitudes and N2-P2 peak-to-peak amplitudes Cz amplitudes were also larger than those measured at C4. These findings are in agreement with a large proportion of previous pain studies which have reported that both the N2 and P2 PREP components were found to be maximal at the midline central area, specifically scalp electrode site Cz (Bromm & Treede, 1987; Carmon et al., 1976; Carmon et al., 1978; et al., 1980; Kakigi et al., 1989; Kanda et al., 1996; Kanda et al., 1999; Miyazaki et al., 1994; Treede et al., 1988). It has been suggested that the origin of the N2 and P2 components is primarily the ACC, whilst the SII and insular cortex, bilaterally, also contribute to the N2 component (Bromm & Chen, 1995; Tarkka & Treede, 1993; Valeriani et al., 1996). These areas correspond to the scalp measurement at Cz and thus are in line with the findings from the current study. However, findings from fMRI studies have reported a right hemisphere dominance for pain processing (Brooks et al., 2002; Symonds et al., 2006), which is different to our results. Specifically right lateralisation was evident in the right anterior insula when pain was attended to, and in the ACC regardless of attentional focus (Brooks et al., 2002). Symonds et al. (2006) found the middle frontal gyrus, anterior cingulate, inferior frontal gyrus, medial/superior frontal gyri, and inferior parietal lobule showed either an exclusive or strong lateralisation to the right hemisphere. At least some of these are areas would have been expected to underlay the right electrode site (C4) in the current study (Bentley, Derbyshire, Youell, & Jones, 2003; Bromm & Chen, 1995; Garcia-Larrea, Frot, & Valeriani, 2003; Ohara, Crone, Weiss, & Lenz, 2006; Tarkka & Treede, 1993; Treede, Lorenz, & Baumgartner, 2003; Valeriani et al., 1996) and thus, greater PREP amplitudes had been hypothesised from electrode site C4 than C3. However, other studies utilising fMRI (Bingel et al., 2003; Youell et al., 2004) and PET (Coghill et al., 2001) imaging techniques have failed to find a right lateralisation effect for pain. Coghill and colleagues (2001) reported bilateral processing in the regions of the cerebellum, putamen, thalamus, ACC, and frontal operculum and contralateral processing in the regions of the SI, SII

and insular cortex regardless of side of stimulation. Similarly, bilateral processing was evident in SI, SII and insular cortex but with a significantly greater contralateral response in SI and the thalamus (Bingel et al., 2003; Youell et al., 2004).

As we had found no cardiac cycle-related modulation of electrocutaneous PREPs in our previous study (Chapter 4), we suggested that this may be due to a potential lateralisation of cardiac cycle-related modulation. In our previous study only the right hand was stimulated, thus if we take into consideration the crossing of neural pathways following stimulation, whereby most of the axons carrying afferent information cross transversely through the anterior white comissure of the spinal cord within several segments of their origin (Almeida, Roizenblatt & Tufik, 2003), pain afferent information from the right side of the body would terminate in the left hemisphere of the brain. However, as discussed in the introduction, there is also good evidence proposing a right hemispheric dominance (Brooks et al., 2002; Symonds et al., 2006) or contralateral (Bingel et al., 2003; Youell et al., 2004; Coghill et al., 2001) dominance of pain processing. As well as strong support indicating that processing of baroreceptor afferent information processing is predominately in the right cerebral hemisphere (e.g. Critchley et al., 2000; Henderson et al., 2004; Weisz et al., 2001). Therefore, the main hypothesis for the current study was that the cardiac cycle-related modulation of pain would be more apparent following stimulation of the left hand than the right hand and be more evident in the scalp electrodes covering the right side of the cerebral cortex i.e. scalp electrode C4. This is due to the suggested greater convergence of the baroreceptor and pain processing input in the right side of the brain following left hand stimulation. As the analysis presented in Table 10 shows, the current study found that there was no significant variation in PREPs across the cardiac cycle at scalp electrode site C4 following left hand stimulation. Indeed, contra to our hypothesis, this study found no significant differences between the cardiac cycle modulation of painful stimuli delivered to the right or left hand, nor any difference between the cardiac cycle-related modulation of noxious stimuli at scalp electrode sites covering the left (C3), right (C4) or central (Cz) brain areas.

The second significant finding in the current study was a significant three-way interaction between hand, scalp electrode site and cardiac cycle interval for N2 peak amplitudes suggesting that although neither hand nor scalp electrode site in isolation appear to be important in the cardiac cycle patterning of pain, together stimulation side and scalp electrode site may influence the cardiac cycle patterning of N2 peak amplitudes. To further investigate the significant Hand × Scalp Electrode Site × Interval interaction, a series of separate ANOVA s were conducted on each individual interaction i.e. Hand × Interval ANOVAs at each scalp electrode site, Scalp Electrode Site × Interval ANOVAs for each hand, and Hand × Scalp Electrode Site ANOVAs at each cardiac cycle interval. These analyses revealed no significant interaction effects and thus we must interpret the significant Hand × Scalp Electrode Site × Interval interaction with extreme caution.

However, the separate Hand × Interval ANOVAs at each scalp electrode site did reveal a marginal main effect for hand at scalp electrode site C4 (Table 7). Visual inspection of the graphs associated with the Hand x Interval ANOVAs (Figure 11) appears to suggest that there may be weak disparity in the Hand × Interval interaction across electrode sites. At C3 (Figure 11a) and Cz (Figure 11b) electrode sites there appears to be a greater divergence of N2 peak amplitudes between the left and right hands later in the cardiac cycle i.e. *R*+350ms to *R*+650ms than at electrode site C4 which appears to show consistently smaller amplitudes across the cardiac cycle in the right hand (Figure 11c). In addition, Scalp Electrode Site × Interval analysis indicated a marginal interaction effect in the left hand (Table 8) and subsequent post hocs (described in the “Separate 3 Scalp Electrode Site (C3, Cz, C4) × 7 cardiac cycle Interval (R+50, R+150, R+250, R+350, R+450, R+550, R+650 ms) repeated measures ANOVAs in the right and left hands” results section above) confirmed that N2 peak amplitudes were significantly smaller at scalp electrode site C3 than scalp electrodes C4 and Cz particularly at the early cardiac cycle intervals i.e. *R*+50, *R*+150 and *R*+250ms in the left hand (Figure 12). Taken together these marginally significant effects very tentatively suggest that the significant Hand × Scalp Electrode Site × Interval interaction could be the result of a combination of both side of stimulation and scalp electrode site influencing the patterning of N2 peak amplitudes across the cardiac cycle. However, due to lack of statistical significance it

is not appropriate to draw any definitive conclusions from these suggestions and further research with greater numbers of participants is required to further investigate the origins of this possible interaction.

Very few studies have investigated the potential lateralisation of cardiac cycle-related modulation but those that have typical report a lateralisation effect. However, the side of the lateralisation is yet to be determined. Specifically, Walker and Sandman (1982) reported that baroreceptor activation (systole) appeared to impact the processing of visual stimuli in the right hemisphere but not in the left. This conclusion was evidenced by visual evoked potentials recorded from the right hemisphere being larger during the diastolic phase of the cardiac cycle than the systolic phase, whereas visual evoked potentials recorded from the left hemisphere were uninfluenced by the phase of the cardiac cycle. These findings suggest that the side of recording visual evoked potentials, rather than the side of presentation is important in determining the lateralisation of the cardiac cycle-related modulation. This right hemispheric bias for cardiac cycle modulation was also found to be evident for startle responding (Schulz et al., 2009). Startle response was found to be reduced during systole compared to diastole only following stimulation presented to the left ear and this was independent of recording side (Schulz et al., 2009). The authors conclude that this finding is likely to reflect the crossing of sensory information from the left side of presentation to the right cerebral hemisphere (as discussed above) combining with the visceral afferent and baroreceptor afferent signals which are predominately processed in the right hemisphere (Schulz et al., 2009). These findings suggest that the side of stimulus presentation is important in determining the lateralisation of the cardiac cycle-related modulation of startle responding rather than stimulus presentation side. In contrast simple visual reaction time for stimuli presented to the right side and for right hand responses was marginally longer during systole than diastole, compared to no differences between systole and diastole for central and left stimuli or for left hand responses (Weisz & Adam, 1996). This finding suggests that reaction time was influenced to a greater extent by cardiac cycle-related modulation in the left cerebral hemisphere than the right hemisphere (Weisz & Adam, 1996) and that both side of stimulation and recording side are important in determining the lateralisation of the cardiac cycle-

related modulation of simple reaction times. Therefore, it may be suggested that the cardiac cycle modulation of different stimulation modalities may be lateralised differently or that both are important factors. Thus, the significant Hand × Scalp Electrode Site × Interval interaction for electrocutaneous PREPs in the current study may indicate that both the side of stimulation and the scalp recording site are important in the cardiac cycle-related modulation of pain.

The current study found no main effect for cardiac cycle interval in either hand or any electrode site for N2 and P2 peak amplitudes or the N2-P2 peak-to-peak amplitude, which have been shown to correlate with the intensity of pain stimulus (Becker et al., 2000; Bromm, 1984; Stowell, 1977; Zaslansky et al., 1996) as well as with subjective ratings of pain (Kanda et al., 2002). The lack of cardiac cycle-related modulation of PREPs in the current study is in line with our previous study investigating the cardiac cycle-related modulation of electrocutaneous PREPs (Chapter 4). However, it should be noted that the current study included participants who's data was also included in the first study (N=4).

The current findings also partially agree with those of Gray and colleagues (Gray, Minati, Paoletti, & Critchley, 2010) who reported no modulation of N2 peak amplitudes but that P2 amplitudes were modulated across the cardiac cycle, with reduced amplitudes during systole; however, this effect was only present for cued stimuli. No cardiac cycle effect was found for un-cued stimuli, which suggests that presenting a cue prior to stimulation may be important in determining the degree of cardiac cycle modulation. Specifically, the presentation of a cue stimuli may induce cardiac deceleration (Graham & Clifton, 1966; Lacey & Lacey, 1970) which it is suggested may reduce the impact of the cardiac cycle effects on visual evoked potentials (Walker & Sandman, 1982). Alternatively, as Gray et al. (2010) suggest the expectancy associated with the cueing of pain stimuli may shift attention towards the imminent pain stimuli. Attention towards pain has been shown to increase its perceived intensity (e.g. Angrilli, Mini, Mucha, & Rau, 1997) and increase the associated PREP amplitudes (e.g. Lorenz & Garcia-Larrea, 2003). Indeed Gray et al. (2010) reported increased PREP amplitudes when stimuli were cued, which was abolished during baroreceptor activation thus providing the cardiac cycle modulation.

They suggest baroreceptor firing disrupts the attentional modulation and thus accounts for the attenuated PREPs during baroreceptor activation. The current study, and the PREP study presented in Chapter 4 of this thesis presented un-cued stimuli, the stimuli occurred at variable intervals throughout each experimental block without warning and both studies failed to find a cardiac cycle modulation of PREPs. In a previous study reporting a systolic dampening of laser evoked N2 PREPs (Edwards et al., 2008), participants were presented with a fixation point from 10 to 15 s before each stimulus. This may have shifted participants attention towards the imminent pain compared to our studies and thus, is in line with Gray et al.'s (2010) suggestion, that cueing stimuli may be an important factor in cardiac cycle-related modulation.

However, the current findings contrast with the systolic dampening of N2 peak amplitudes and N2-P2 peak-to-peak amplitudes in response to noxious laser stimulation at Cz reported by Edwards et al. (2008). The reasons for the differing findings between the current study and Edwards et al. (2008) are discussed in detail in Chapter 4, but most likely relate to methodological differences. Specifically, the modality of pain stimuli (i.e. laser used by Edwards et al. (2008) vs. electrocutaneous used in the current study), sex of the participants (Edwards et al. (2008) only studied men, the current study included men and women), the duration of the study and the total number of pain stimuli was greater in the current study than Edwards et al. (2008) and the EEG systems used to record the PREPs were also different (the current study employed a high impedance system (Biosemi)). In addition, as discussed above, the different findings between the current study and Edwards et al. (2008) may be partially explained by the presentation of un-cued stimuli in the current study compared to the cued stimuli presented by Edwards et al.

The lack of cardiac cycle modulation in the current study also contrasts several previous cardiac cycle studies reporting a dampening of the NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006, 2008) and pain perception (Study 1 - Wilkinson et al., 2013) during systole compared to diastole. However, some of these studies were designed to investigate cardiac cycle-related modulation of NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006, 2008) and pain (Wilkinson

et al., 2013) thresholds and thus stimulation intensity may be lower than the painful stimuli (2 times pain threshold) delivered in the current study. The higher stimulation intensities may have induced physiological arousal which has been shown to influence pain perception and moderate the midcycle dampening of the NFR, probably through reduced transmission of baroreceptor afferents (McIntyre et al., 2006). Indeed, a recent study (Martins et al., 2009) employing presentation of unpredictable pain stimuli at different pain intensities up to pain tolerance failed to find a cardiac cycle modulation of the NFR and contra to the typical systolic dampening of the NFR in previous studies (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006, 2008) the pain intensity was heightened during systole compared to diastole (Martins et al., 2009). These findings suggest that higher stimulation intensities when presented un-cued may differentially influence the previously demonstrated cardiac cycle-related modulation. However, as participants in the current study rated the intensity of the painful stimuli as 56 (average of all experimental blocks), this does not appear to be notably higher than threshold (50) and thus, a very high pain stimulation appears an unlikely explanation for the lack of cardiac cycle modulation in the current study. However, further studies investigating the possible effects of cued and un-cued stimulation, and different pain stimulation intensities moderating the cardiac cycle-related modulation requires further investigation.

The current study should be viewed in the light of several limitations. Firstly, the sample size was only 17. Although a previous cardiac cycle study investigating laterality effects had a similar sample size (Walker & Sandman, 1982, N=18) found effects, as did recent studies regarding the cardiac cycle modulation of laser (Edwards et al., 2008) and electrocutaneous (Gray et al., 2010) PREPs which only had 10 and 11 participants respectively, it maybe that the lack of significant findings in the current study was due to a lack of power. Indeed post hoc power analysis using the G*power computer program (Faul, Erdfelder, Buchner, & Lang, 2009) indicated that adopting the reported effect size for the main effect for interval in the current study (f(U) = .239), with an alpha of .05 and power of .80, the study would require a total sample of 42 to detect, by repeated measures ANOVA, a difference in N2 peak amplitudes across the cardiac cycle.

Additionally, the lack of cardiac cycle modulation in the current study may also be partially explained, as discussed in Chapter 4, by the EEG system used in the current study (Biosemi) being a high impedance system. Such systems require a greater number of trials to achieve significance than comparable low impedance systems (Kappenman & Luck, 2010). The Biosemi system is designed to tolerate high electrode impedances (Metting van Rijn et al., 1996), but it is still suggested that to achieve an 80% chance of statistical significance for the P300 component, approximately 25 trials per condition are required (Kappenman & Luck, 2010) although this was in a sample of just 12 participants. Although it must be acknowledged that this was calculated for the P300 rather than N2 or P2 which may require a different number of trials to achieve significance. However, the average number of trials accepted per cardiac cycle interval in the current study (Mean across all cardiac cycle intervals = 19) is below this suggested level. Although the increased number of participants compared to Edwards et al. (2008) and Gray et al. (2010) aimed to improve the chances of reaching statistical significance, it appears that future studies would do well to either increase the number of trials averaged per cardiac cycle interval or collapse data across intervals to improve the signal-to-noise ratio and subsequently the chance of achieving significance.

Notwithstanding the limitations outlined above, the findings of the current study suggest that although the cardiac cycle-related modulation of PREPs may not be as robust as other pain indices, such as the NFR, further study is warranted to investigate a possible role of lateralisation indicated in the current study.

REFERENCES

Aicher, S. A., & Randich, A. (1990). Antinociception and cardiovascular responses produced by electrical stimulation in the nucleus tractus solitarius, nucleus reticularis ventralis, and the caudal medulla. *Pain, 42*(1), 103-119.

Almeida, T. F., Roizenblatt, S., & Tufik, S. (2004). Afferent pain pathways: A neuroanatomical review. *Brain Research, 1000*(1–2), 40-56. doi:10.1016/j.brainres.2003.10.073

Angell James, J. E. (1971). Effects of altering mean pressure, pulse pressure and pulse frequency on impulse activity in baroreceptor fibres from aortic arch and right subclavian artery in rabbit. *Journal of Physiology-London, 214*(1), 65-88.

Angell James, J. E., & Lumley, J. S. P. (1974). Effects of carotid endarterectomy on mechanical-properties of carotid-sinus and carotid-sinus nerve activity in atherosclerotic patients. *British Journal of Surgery, 61*(10), 805-810.

Angrilli, A., Mini, A., Mucha, R. F., & Rau, H. (1997). The influence of low blood pressure and baroreceptor activity on pain responses. *Physiology & Behavior, 62*(2), 391-397.

Arbib, M. A., Érdi, P., & Szentágothai, J. (1998). *Neural Organization: Structure, Function, and Dynamics.* Cambridge, MA: A Bradford Books

Arntz, A., Dreessen, L., & Merckelbach, H. (1991). Attention, not anxiety, influences pain. *Behaviour Research and Therapy, 29*(1), 41-50.

Bandler, R., Carrive, P., & Zhang, S. P. (1991). Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: Viscerotopic, somatotopic and functional organization. *Progress in Brain Research, 87*, 269-305.

Barclay, J . N. (2012). *The influence of lateralised processing of the baroreceptor signal on cardiac cycle-related modulation of pain thresholds.* Unpublished B.Sc. dissertation. Loughborough University, United Kingdom.

Becker, D. E., Haley, D. W., Urena, V. M., & Yingling, C. D. (2000). Pain measurement with evoked potentials: Combination of subjective ratings, randomized intensities, and long interstimulus intervals produces a P300-like confound. *Pain, 84*(1), 37-47.

Bell, D. R. (2009). Control Mechanisms in Circulatory Function. In R. A. Rhoades & D. R. Bell D.R. (Eds.), *Medical Physiology: Principles for clinical medicine 3rd Edition.* Philadelphia: Lippincott Williams & Wilkins.

Benarroch, E. E. (2008). The arterial baroreflex: Functional organization and involvement in neurologic disease. *Neurology, 71*(21), 1733-1738. doi:10.1212/01.wnl.0000335246.93495.92

Bentley, D. E., Derbyshire, S. W. G., Youell, P. D., & Jones, A. K. P. (2003). Caudal cingulate cortex involvement in pain processing: An inter-individual laser evoked potential source localisation study using realistic head models. *Pain, 102*(3), 265-271. doi:10.1016/S0304-3959(02)00405-0 ER

Bingel, U., Herken, W., Teutsch, S., & May, A. (2008). Habituation to painful stimulation involves the antinociceptive system-a 1-year follow-up of 10 participants. *Pain, 140*(2), 393-394. doi:10.1016/j.pain.2008.09.030

Bingel, U., Quante, M., Knab, R., Bromm, B., Weiller, C., & Buchel, C. (2003). Single trial fMRI reveals significant contralateral bias in responses to laser pain within thalamus and somatosensory cortices. *Neuroimage, 18*(3), 740-748.

Bingel, U., Schoell, E., Herken, W., Buchel, C., & May, A. (2007). Habituation to painful stimulation involves the antinociceptive system. *Pain, 131*(1-2), 21-30. doi:10.1016/j.pain.2006.12.005

Bonmassar, G., Purdon, P. L., Jaaskelainen, I. P., Chiappa, K., Solo, V., Brown, E. N., & Belliveau, J. W. (2002). Motion and ballistocardiogram artifact removal for interleaved recording of EEG and EPs during MRI. *Neuroimage, 16*(4), 1127-1141.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1991). Startle and emotion: Lateral acoustic probes and the bilateral blink. *Psychophysiology, 28*(3), 285-295.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1996). Lateralized startle probes in the study of emotion. *Psychophysiology, 33*(2), 156-161.

Bromm, B. (1984). Pain-related components in the cerebral potential: Experimental and multivariate statistical approaches. In B. Bromm (Ed.), *Pain measurement in man: Neurophysiological correlates of pain* (pp. 257-290). Amsterdam: Elsevier.

Bromm, B. (1985). Modern techniques to measure pain in healthy man. *Methods and Findings in Experimental and Clinical Pharmacology, 7*(3), 161-169.

Bromm, B., & Chen, A. C. (1995). Brain electrical source analysis of laser evoked potentials in response to painful trigeminal nerve stimulation. *Electroencephalography and Clinical Neurophysiology, 95*(1), 14-26.

Bromm, B., & Treede, R. D. (1987). Human cerebral potentials evoked by CO2 laser stimuli causing pain. *Experimental Brain Research, 67*(1), 153-162.

Brooks, J. C., Nurmikko, T. J., Bimson, W. E., Singh, K. D., & Roberts, N. (2002). fMRI of thermal pain: Effects of stimulus laterality and attention. *Neuroimage, 15*(2), 293-301. doi:10.1006/nimg.2001.0974

Carmon, A., Dotan, Y., & Sarne, Y. (1978). Correlation of subjective pain experience with cerebral evoked responses to noxious thermal stimulations. *Experimental Brain Research, 33*(3-4), 445-453.

Carmon, A., Friedman, Y., Coger, R., & Kenton, B. (1980). Single trial analysis of evoked potentials to noxious thermal stimulation in man. *Pain, 8*(1), 21-32.

Carmon, A., Mor, J., & Goldberg, J. (1976). Evoked cerebral responses to noxious thermal stimuli in humans. *Experimental Brain Research, 25*(1), 103-107.

Carmon, A., & Nachshon, I. (1973). Ear asymmetry in perception of emotional non-verbal stimuli. *Acta Psychologica, 37*(6), 351-357.

Chen, A. C. N., Arendt-Nielsen, L., & Plaghki, L. (1998). Laser-evoked potentials in human pain: I. use and possible misuse. *Pain Forum, 7*(4), 174-184. doi:10.1016/S1082-3174(98)70001-7

Coghill, R. C., Gilron, I., & Iadarola, M. J. (2001). Hemispheric lateralization of somatosensory processing. *Journal of Neurophysiology, 85*(6), 2602-2612.

Cohen, J. (1973). Eta-squared and partial eta-squared in fixed factor ANOVA designs. *Educational and Psychological Measurement*, 33, 107-112.

Coleridge, H. M., Coleridge, J. C. G., & Schultz, H. D. (1987). Characteristics of C-fiber baroreceptors in the carotid-sinus of dogs. *Journal of Physiology-London, 394*, 291-313.

Craig, A. D. (2002). Pain mechanisms: Labeled lines versus convergence in central processing. *Annual Review of Neuroscience, 26*, 1-30.

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *The Journal of Comparative Neurology, 493*(1), 154-166. doi:10.1002/cne.20749

Critchley, H. D., Corfield, D. R., Chandler, M. P., Mathias, C. J., & Dolan, R. J. (2000). Cerebral correlates of autonomic cardiovascular arousal: A functional neuroimaging investigation in humans. *The Journal of Physiology, 523 (1)*, 259-270.

Eckberg, D. L., & Sleight, P. (1992). *Human baroreflexes in health and disease*. Oxford: Clarendon Press.

Edwards, L., Inui, K., Ring, C., Wang, X. H., & Kakigi, R. (2008). Pain-related evoked potentials are modulated across the cardiac cycle. *Pain, 137*(3), 488-494. doi:10.1016/j.pain.2007.10.010 ER

Edwards, L., McIntyre, D., Carroll, D., Ring, C., France, C. R., & Martin, U. (2003). Effects of artificial and natural baroreceptor stimulation on nociceptive responding and pain. *Psychophysiology, 40*(5), 762-769.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology, 39*(5), 678-681. doi:10.1017/S0048577202011770 ER

Edwards, L., Ring, C., McIntyre, D., & Carroll, D. (2001). Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology, 38*(4), 712-718.

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods, 41*(4), 1149-1160. doi:10.3758/BRM.41.4.1149

Fila, M., & Bogucki, A. (2009). Laser evoked potentials as a method of evaluating the function of small fibres - application technique and clinical implications. *Neurologia i Neurochirurgia Polska, 43*(4), 368-381.

France, C. R. (1999). Decreased pain perception and risk for hypertension: Considering a common physiological mechanism. *Psychophysiology, 36*(6), 683-692.

France, C. R., & Ditto, B. (1996). Risk for high blood pressure and decreased pain perception. *Current Directions in Psychological Science, 5*(4), 120-125.

Garcia-Larrea, L., Frot, M., & Valeriani, M. (2003). Brain generators of laser-evoked potentials: From dipoles to functional significance. *Clinical Neurophysiology, 33*(6), 279-292.

Garcia-Larrea, L., Peyron, R., Laurent, B., & Mauguiere, F. (1997). Association and dissociation between laser-evoked potentials and pain perception. *Neuroreport, 8*(17), 3785-3789.

Ghione, S. (1996). Hypertension-associated hypoalgesia - evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension, 28*(3), 494-504.

Gianaros, P. J., Jennings, J. R., Sheu, L. K., Derbyshire, S. W., & Matthews, K. A. (2007). Heightened functional neural activation to psychological stress covaries with exaggerated blood pressure reactivity. *Hypertension, 49*(1), 134-140. doi:10.1161/01.HYP.0000250984.14992.64

Gilbey, M. P. (2007). Sympathetic rhythms and nervous integration. *Clinical and Experimental Pharmacology & Physiology, 34*(4), 356-361.

Gilbey, M. P., & Spyer, K. M. (1993). Essential organization of the sympathetic nervous system. *Bailliere's Clinical Endocrinology and Metabolism, 7*(2), 259-278.

Graham, F. K., & Clifton, R. K. (1966). Heart-rate change as a component of the orienting response. *Psychological Bulletin, 65*(5), 305-320.

Granovsky, Y., Granot, M., Nir, R. R., & Yarnitsky, D. (2008). Objective correlate of subjective pain perception by contact heat-evoked potentials. *The Journal of Pain, 9*(1), 53-63. doi:10.1016/j.jpain.2007.08.010

Gray, M. A., Minati, L., Paoletti, G., & Critchley, H. D. (2010). Baroreceptor activation attenuates attentional effects on pain-evoked potentials. *Pain, 151*(3), 853-861. doi:10.1016/j.pain.2010.09.028

Gray, M. A., Rylander, K., Harrison, N. A., Wallin, B. G., & Critchley, H. D. (2009). Following one's heart: Cardiac rhythms gate central initiation of sympathetic reflexes. *The Journal of Neuroscience, 29*(6), 1817-1825. doi:10.1523/JNEUROSCI.3363-08.2009

Greffrath, W., Baumgartner, U., & Treede, R. D. (2007). Peripheral and central components of habituation of heat pain perception and evoked potentials in humans. *Pain, 132*(3), 301-311. doi:10.1016/j.pain.2007.04.026 ER

Haggard, M. P., & Parkinson, A. M. (1971). Stimulus and task factors as determinants of ear advantages. *The Quarterly Journal of Experimental Psychology, 23*(2), 168-177. doi:10.1080/14640747108400237

Hall, W., Hayward, L., & Chapman, C. R. (1981). On "the lateralization of pain". *Pain, 10*(3), 337-356.

Handwerker, H. O., & Kobal, G. (1993). Psychophysiology of experimentally induced pain. *Physiological Reviews, 73*(3), 639-671.

Hanna, D., & Dempster, M. (2012). *Psychology statistics for dummies*. Chichester: John Wiley & Sons.

Henderson, L. A., Richard, C. A., Macey, P. M., Runquist, M. L., Yu, P. L., Galons, J. P., & Harper, R. M. (2004). Functional magnetic resonance signal changes in neural structures to baroreceptor reflex activation. *Journal of Applied Physiology, 96*(2), 693-703. doi:10.1152/japplphysiol.00852.2003

Hilz, M. J., Dutsch, M., Perrine, K., Nelson, P. K., Rauhut, U., & Devinsky, O. (2001). Hemispheric influence on autonomic modulation and baroreflex sensitivity. *Annals of Neurology, 49*(5), 575-584.

Hugon, M. (1973). Exteroreceptive reflexes to stimulation of the sural nerve in normal man. In J. E. Desmedt (Ed.), *New developments in electromyography and clinical neurophysiology* (pp. 713--729). Basel: Karger.

Iannetti, G. D., Hughes, N. P., Lee, M. C., & Mouraux, A. (2008). Determinants of laser-evoked EEG responses: Pain perception or stimulus saliency? *Journal of Neurophysiology, 100*(2), 815-828. doi:10.1152/jn.00097.2008

Ille, N., Berg, P., & Scherg, M. (2002). Artifact correction of the ongoing EEG using spatial filters based on artifact and brain signal topographies. *Journal of Clinical Neurophysiology, 19*(2), 113-124.

Jänig W. (2006). *The integrative action of the autonomic nervous system: neurobiology of homeostasis.* Cambridge UP: Cambridge, UK.

Jasper, H. H. (1958). The ten-twenty electrode system of the International Federation. Electroencephalography and Clinical Neurophysiology, 10, 370-375.

Ji, G., & Neugebauer, V. (2009). Hemispheric lateralization of pain processing by amygdala neurons. *Journal of Neurophysiology, 102*(4), 2253-2264. doi:10.1152/jn.00166.2009

Joseph, R. (1988). The right cerebral hemisphere: Emotion, music, visual-spatial skills, body-image, dreams, and awareness. *Journal of Clinical Psychology, 44*(5), 630-673.

Kakigi, R., Inui, K., & Tamura, Y. (2005). Electrophysiological studies on human pain perception. *Clinical Neurophysiology, 116*(4), 743-763. doi:10.1016/j.clinph.2004.11.016

Kakigi, R., Shibasaki, H., & Ikeda, A. (1989). Pain-related somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology, 74*(2), 139-146.

Kanda, M., Fujiwara, N., Xu, X., Shindo, K., Nagamine, T., Ikeda, A., & Shibasaki, H. (1996). Pain-related and cognitive components of somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology, 100*(2), 105-114.

Kanda, M., Matsuhashi, M., Sawamoto, N., Oga, T., Mima, T., Nagamine, T., & Shibasaki, H. (2002). Cortical potentials related to assessment of pain intensity with visual analogue scale (VAS). *Clinical Neurophysiology, 113*(7), 1013-1024.

Kanda, M., Shindo, K., Xu, X., Fujiwara, N., Ikeda, A., Nagamine, T., & Shibasaki, H. (1999). Cortical mechanisms underlying point localization of pain spot as studied by event-related potentials following CO2 laser stimulation in man. *Experimental Brain Research, 127*(2), 131-140.

Kappenman, E. S., & Luck, S. J. (2010). The effects of electrode impedance on data quality and statistical significance in ERP recordings. *Psychophysiology, 47*(5), 888-904. doi:10.1111/j.1469-8986.2010.01009.x

Katona, P. G., Poitras, J. W., Barnett, G. O., & Terry, B. S. (1970). Cardiac vagal efferent activity and heart period in the carotid sinus reflex. *The American Journal of Physiology, 218*(4), 1030-1037.

Kaube, H., Katsarava, Z., Kaufer, T., Diener, H. C., & Ellrich, J. (2000). A new method to increase nociception specificity of the human blink reflex. *Clinical Neurophysiology, 111*(3), 413-416.

Kettle, J. W., Andrewes, D. G., & Allen, N. B. (2006). Lateralization of the startle reflex circuit in humans: An examination with monaural probes following unilateral temporal lobe resection. *Behavioral Neuroscience, 120*(1), 24-39. doi:10.1037/0735-7044.120.1.24

Kimmerly, D. S., O'Leary, D. D., Menon, R. S., Gati, J. S., & Shoemaker, J. K. (2005). Cortical regions associated with autonomic cardiovascular regulation during lower body negative pressure in humans. *The Journal of Physiology, 569*(1), 331-345. doi:10.1113/jphysiol.2005.091637

Klabunde, R. E. (2011). *Cardiovascular Physiology Concepts. Second Edition.* Philadelphia, PA: Lippincott Williams & Wilkins.

Koriath, J. J., & Lindholm, E. (1986). Cardiac-related cortical inhibition during a fixed foreperiod reaction time task. *International Journal of Psychophysiology, 4*(3), 183-195.

Koriath, J. J., Lindholm, E., & Landers, D. M. (1987). Cardiac-related cortical activity during variations in mean heart rate. *International Journal of Psychophysiology, 5*(4), 289-299.

Lacey, J. I., & Lacey, B. C. (1970). Some autonomic-central nervous system interrelationships. In P. Black (Ed.), *Physiological correlates of emotion* (pp. 205-227). New York: Academic Press.

LeBlanc, J., & Potvin, P. (1966). Studies on habituation to cold pain. *Canadian Journal of Physiology and Pharmacology, 44*(2), 287-293.

Lefaucheur, J. P., Ahdab, R., Ayache, S. S., Lefaucheur-Menard, I., Rouie, D., Tebbal, D., . . . Ciampi de Andrade, D. (2012). Pain-related evoked potentials: A comparative study between electrical stimulation using a concentric planar electrode and laser stimulation using a CO2 laser. *Clinical Neurophysiology, 42*(4), 199-206. doi:10.1016/j.neucli.2011.12.003

Levitt, H. (1971). Transformed up-down methods in psychoacoustics. *Journal of the Acoustical Society of America, 49*(2), 467-&.

Lorenz, J., & Garcia-Larrea, L. (2003). Contribution of attentional and cognitive factors to laser evoked brain potentials. *Clinical Neurophysiology, 33*(6), 293-301.

Luck, S. J. (2005). *An introduction to the event-related potential technique*. Cambridge, MA: Massachusetts Institute of Technology.

Lugo, M., Isturiz, G., Lara, C., Garcia, N., & Eblen-Zaijur, A. (2002). Sensory lateralization in pain subjective perception for noxious heat stimulus. *Somatosensory & Motor Research, 19*(3), 207-212. doi:10.1080/0899022021000009125

Mancia, G., & Mark, A. L. (1983). Arterial baroreflexes in humans. In J. T. Shepherd, & F. M. Abboud (Eds.), *Handbook of physiology: The cardiovascular system* (pp. 755--793). Bethesda, Maryland: American Physiological Society.

Martins, A., Q, Ring, C., McIntyre, D., Edwards, L., & Martin, U. (2009). Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. *Pain, 147*(1-3), 84-90. doi:10.1016/j.pain.2009.08.016

McAllen, R. M., & Malpas, S. C. (1997). Sympathetic burst activity: Characteristics and significance. *Clinical and Experimental Pharmacology & Physiology, 24*(11), 791-799.

McIntyre, D., Edwards, L., Ring, C., Parvin, B., & Carroll, D. (2006). Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology, 43*(3), 314-319. doi:10.1111/j.1469-8986.2006.00407.x ER

McIntyre, D., Kavussanu, M., & Ring, C. (2008). Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. *Pain, 137*(3), 550-555. doi:10.1016/j.pain.2007.10.018 ER

Melzack, R. (1987). The short-form mcgill pain questionnaire. *Pain, 30*(2), 191-197.

Merskey, H., & Bogduk, N. (1994). Part III: Pain terms, A current list with definitions and notes on usage. In H. Merskey, & N. Bogduk (Eds.), *Classification of chronic pain, IASP task force on taxonomy* (2nd ed., pp. 209-214). Seattle: WA: IASP.

Merskey, H., & Watson, G. D. (1979). The lateralisation of pain. *Pain, 7*(3), 271-280.

Metting van Rijn, A. C., Kuiper, A. P., Dankers, T. E., & Grimbergen, C. A. (1996). Low-cost active electrode improves the resolution in biopotential recordings. *Biosemi Research,* May 2013.

Milne, R. J., Kay, N. E., & Irwin, R. J. (1991). Habituation to repeated painful and non-painful cutaneous stimuli: A quantitative psychophysical study. *Experimental Brain Research, 87*(2), 438-444.

Miltner, W., Larbig, W., & Braun, C. (1987). Habituation of subjective rating and event related potentials to painful intracutaneous electrical stimulation of the skin. Journal of Psychophysiology, 1, 221-228.

Miyazaki, M., Shibasaki, H., Kanda, M., Xu, X., Shindo, K., Honda, M., . . . Kimura, J. (1994). Generator mechanism of pain-related evoked potentials following CO2 laser stimulation of the hand: Scalp topography and effect of predictive warning signal. *Journal of Clinical Neurophysiology, 11*(2), 242-254.

Ohara, P. T., Vit, J. P., & Jasmin, L. (2005). Cortical modulation of pain. *Cellular and Molecular Life Sciences : CMLS, 62*(1), 44-52. doi:10.1007/s00018-004-4283-9

Ohara, S., Crone, N. E., Weiss, N., & Lenz, F. A. (2006). Analysis of synchrony demonstrates 'pain networks' defined by rapidly switching, task-specific, functional connectivity between pain-related cortical structures. *Pain, 123*(3), 244-253. doi:10.1016/j.pain.2006.02.012

Oppenheimer, S. M., Kulshreshtha, N., Lenz, F. A., Zhang, Z., Rowland, L. H., & Dougherty, P. M. (1998). Distribution of cardiovascular related cells within the human thalamus. *Clinical Autonomic Research, 8*(3), 173-179.

Ossipov, M. H., Dussor, G. O., & Porreca, F. (2010). Central modulation of pain. *The Journal of Clinical Investigation, 120*(11), 3779-3787. doi:10.1172/JCI43766; 10.1172/JCI43766

Pauli, P., Wiedemann, G., & Nickola, M. (1999). Pain sensitivity, cerebral laterality, and negative affect. *Pain, 80*(1–2), 359-364. doi:10.1016/S0304-3959(98)00231-0

Perchet, C., Frot, M., Charmarty, A., Flores, C., Mazza, S., Magnin, M., & Garcia-Larrea, L. (2012). Do we activate specifically somatosensory thin fibres with the concentric planar electrode? A scalp and intracranial EEG study. *Pain, 153*(6), 1244-1252. doi:10.1016/j.pain.2012.03.004

Persson, P. B., & Kirchheim, H. R. (1991). *Baroreceptor reflexes: integrative functions and clinical aspects*. Berlin: Springer-Verlag.

Radloff, L. S. (1977). The center for epidemiologic studies depression scale a self report depression scale for research in the general population. *Applied Psychological Measurement, 1*(3), 385-401.

Rutter, C. E., Dahlquist, L. M., & Weiss, K. E. (2009). Sustained efficacy of virtual reality distraction. *The Journal of Pain, 10*(4), 391-397. doi:10.1016/j.jpain.2008.09.016

Saleh, T. M., & Connell, B. J. (1998). Role of the insular cortex in the modulation of baroreflex sensitivity. *The American Journal of Physiology, 274*(5 Pt 2), R1417-24.

Sandman, C. A. (1984). Augmentation of the auditory event related potentials of the brain during diastole. *International Journal of Psychophysiology, 2*(2), 111-119.

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., & Willer, J. C. (2005). The lower limb flexion reflex in humans. *Progress in Neurobiology, 77*(6), 353-395. doi:10.1016/j.pneurobio.2005.11.003 ER

Sarlani, E., Farooq, N., & Greenspan, J. D. (2003). Gender and laterality differences in thermosensation throughout the perceptible range. *Pain, 106*(1-2), 9-18.

Schiff, B. B., & Gagliese, L. (1994). The consequences of experimentally induced and chronic unilateral pain: Reflections of hemispheric lateralization of emotion. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior, 30*(2), 255-267.

Schulz, A., Reichert, C. F., Richter, S., Lass-Hennemann, J., Blumenthal, T. D., & Schaechinger, H. (2009). Cardiac modulation of startle: Effects on eye blink and higher cognitive processing. *Brain and Cognition, 71*(3), 265-271. doi:10.1016/j.bandc.2009.08.002

Seltzer, S. F., Yarczower, M., Woo, R., & Seltzer, J. L. (1992). Laterality and modality-specific effects of chronic pain. *Perception & Psychophysics, 51*(5), 500-503.

Spernal, J., Krieg, J. C., & Lautenbacher, S. (2003). Pain thresholds as a putative functional test for cerebral laterality in major depressive disorder and panic disorder. *Neuropsychobiology, 48*(3), 146-151.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *Manual for the state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists Press, 1970.

Stowell, H. (1977). Cerebral slow waves related to the perception of pain in man. *Brain Research Bulletin, 2*(1), 23-30.

Strempel, H. (1976). Adaptive modifications of cold pain (author's transl). *European Journal of Applied Physiology and Occupational Physiology, 36*(1), 19-25.

Strempel, H. (1978). Adaptive modifications of cold pain. III. communication: Short-term experiments with 1-min-intervals (author's transl). *European Journal of Applied Physiology and Occupational Physiology, 39*(1), 63-72.

Sykora, M., Diedler, J., Rupp, A., Turcani, P., & Steiner, T. (2009). Impaired baroreceptor reflex sensitivity in acute stroke is associated with insular involvement, but not with carotid atherosclerosis. *Stroke; a Journal of Cerebral Circulation, 40*(3), 737-742. doi:10.1161/STROKEAHA.108.519967

Symonds, L. L., Gordon, N. S., Bixby, J. C., & Mande, M. M. (2006). Right-lateralized pain processing in the human cortex: An FMRI study. *Journal of Neurophysiology, 95*(6), 3823-3830. doi:10.1152/jn.01162.2005

Tan, S. Y. (1982). Cognitive and cognitive-behavioral methods for pain control: A selective review. *Pain, 12*(3), 201-228.

Tarkka, I. M., & Treede, R. D. (1993). Equivalent electrical source analysis of pain-related somatosensory evoked potentials elicited by a CO2 laser. *Journal of Clinical Neurophysiology, 10*(4), 513-519.

Treede, R. D., Kief, S., Holzer, T., & Bromm, B. (1988). Late somatosensory evoked cerebral potentials in response to cutaneous heat stimuli. *Electroencephalography and Clinical Neurophysiology, 70*(5), 429-441.

Treede, R. D., Lorenz, J., & Baumgartner, U. (2003). Clinical usefulness of laser-evoked potentials. *Clinical Neurophysiology, 33*(6), 303-314.

Valeriani, M., Rambaud, L., & Mauguiere, F. (1996). Scalp topography and dipolar source modelling of potentials evoked by CO2 laser stimulation of the hand. *Electroencephalography and Clinical Neurophysiology, 100*(4), 343-353.

Valeriani, M., Sestito, A., Le Pera, D., De Armas, L., Infusino, F., Maiese, T., . . . Lanza, G. A. (2005). Abnormal cortical pain processing in patients with cardiac syndrome X. *European Heart Journal, 26*(10), 975-982. doi:10.1093/eurheartj/ehi229

Walker, B. B., & Sandman, C. A. (1982). Visual evoked-potentials change as heart-rate and carotid pressure change. *Psychophysiology, 19*(5), 520-527.

Weisz, J., & Adam, G. (1996). The influence of cardiac phase on reaction time depending on heart period length and on stimulus and response laterality. *Psychobiology, 24*(2), 169-175.

Weisz, J., Emri, M., Fent, J., Lengyel, Z., Marian, T., Horvath, G., . . . Adam, G. (2001). Right prefrontal activation produced by arterial baroreceptor stimulation: A PET study. *Neuroreport, 12*(15), 3233-3238.

Wilkinson, M., McIntyre, D., & Edwards, L. (2013). Electrocutaneous pain thresholds are higher during systole than diastole. *Biological Psychology, 94*(1), 71-73. doi:10.1016/j.biopsycho.2013.05.002

Willer, J. C. (1977). Comparative-study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

Youell, P. D., Wise, R. G., Bentley, D. E., Dickinson, M. R., King, T. A., Tracey, I., & Jones, A. K. (2004). Lateralisation of nociceptive processing in the human brain: A functional magnetic resonance imaging study. *Neuroimage, 23*(3), 1068-1077. doi:10.1016/j.neuroimage.2004.07.004

Zaslansky, R., Sprecher, E., Katz, Y., Rozenberg, B., Hemli, J. A., & Yarnitsky, D. (1996). Pain-evoked potentials: What do they really measure? *Evoked Potentials-Electroencephalography and Clinical Neurophysiology, 100*(5), 384-391.

Zhang, Y. Q., Tang, J. S., Yuan, B., & Jia, H. (1997). Inhibitory effects of electrically evoked activation of ventrolateral orbital cortex on the tail-flick reflex are mediated by periaqueductal gray in rats. *Pain, 72*(1-2), 127-135.

Zhang, Z. H., Dougherty, P. M., & Oppenheimer, S. M. (1998). Characterization of baroreceptor-related neurons in the monkey insular cortex. *Brain Research, 796*(1-2), 303-306.

Zhang, Z. H., Dougherty, P. M., & Oppenheimer, S. M. (1999). Monkey insular cortex neurons respond to baroreceptive and somatosensory convergent inputs. *Neuroscience, 94*(2), 351-360.

Zhang, Z. H., & Oppenheimer, S. M. (2000). Baroreceptive and somatosensory convergent thalamic neurons project to the posterior insular cortex in the rat. *Brain Research, 861*(2), 241-256.

SIX

General Discussion

The main purpose of this thesis was to expand the current knowledge regarding the role of natural fluctuations in blood pressure (BP) across the cardiac cycle on the modulation of pain and tactile stimuli. This concluding chapter aims to summarise the findings obtained from the experimental chapters within this thesis, and to discuss these in relation to the current understanding and future directions of the cardiac cycle-related modulation of sensation. Finally, the chapter concludes with acknowledgment of the main limitations of the studies present and suggestions for future research.

6.1 Summary of Findings

6.1.1 Study 1

The first study (Chapter 2) was designed to further investigate the cardiac cycle-related modulation of electrocutaneous pain thresholds and thus pain perception. Specifically, the study re-investigated the findings of a recent study by Martins and colleagues (Martins, Ring, McIntyre, Edwards, & Martin, 2009) who reported, unexpectedly, that pain ratings were elevated during systole compared to diastole. This finding was contra to the majority of previous research indicating a systolic dampening of the nociceptive flexion reflex (NFR) (Edwards, Ring, McIntyre, & Carroll, 2001; Edwards, McIntyre, Carroll, Ring, & Martin, 2002; Edwards et al., 2003 McIntyre, Edwards, Ring, Parvin, & Carroll, 2006; McIntyre, Kavussanu, & Ring, 2008a) and laser evoked pain-related evoked potentials (PREPs) (Edwards, Inui, Ring, Wang, & Kakigi, 2008a). Despite the different patterning of modulation, the Martins et al. (2009) finding's suggested that pain perception may be modulated across the cardiac cycle. However, the specific patterning of the cardiac cycle modulation of pain perception remained to be established by further research. Therefore, the first study employed several different methodological features to address possible reasons for the unexpected findings reported by Martins and colleagues (2009).

The study determined electrocutaneous pain thresholds in 49 healthy adults at seven intervals after the *R*-wave of the electrocardiogram (EEG) (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms), using an interleaved up-down staircase

procedure. Stimuli were pseudorandomly delivered to the right hand and participants indicated the presence or absence of pain via a button press.

Results indicated that pain thresholds were higher mid-cycle (*R*+200 and *R*+300ms), indicating that pain perception was attenuated during systole compared to diastole. Furthermore, analysis using BP median splits revealed no difference between pain thresholds in the high and low BP groups, however, a Group × Interval interaction was found. Further analysis revealed that only participants with low Systolic BP (SBP) displayed the reported cardiac cycle modulation of pain.

6.1.2 Study 2

Having reported a cardiac cycle modulation of pain perception in Study 1 (Chapter 2), the second study (Chapter 3) aimed to further explore if the cardiac cycle-related modulation identified for pain thresholds (Study 1) and for other modalities including the NFR (Edwards et al., 2001, 2002, 2003), PREPs (Edwards et al., 2008a) and pain (Martins et al., 2009) was evident for tactile thresholds. The study aimed to expand previous work by Edwards and colleagues (Edwards, Ring, McIntyre, Winer, Martin, 2009) which was the first to report a cardiac cycle-related modulation of cutaneous sensory thresholds. They found that tactile thresholds were lower during systole compared to diastole, which indicates that in contrast to pain, tactile sensitivity was increased during baroreceptor activation. However, the effect of the inclusion of unmedicated hypertensives in the sample assessed by Edwards et al. (2009) is unknown and therefore Study 2 (Chapter 3) aimed to determine if electrocutaneous tactile sensation was modulated across the cardiac cycle by natural variation in baroreceptor activity in a normotensive group. The second study also sought to provide greater resolution regarding the modulation of tactile thresholds across the cardiac cycle by presenting stimuli at seven cardiac cycle intervals as opposed to the three used by Edwards et al. (2009).

Electrocutaneous stimuli were delivered via a bar electrode to the participants right index finger and they indicated if they felt anything or nothing via a button press. Tactile thresholds were determined concurrently at seven cardiac cycle intervals after the R-wave of the ECG (*R*+0 ms, *R*+100 ms, *R*+200 ms, *R*+300 ms, *R*+400 ms, *R*+500 ms, *R*+600 ms) by interleaving seven up-down staircases (Levitt, 1971).

Stimuli were delivered pseudorandomly to ensure variability in stimulus intensity and cardiac cycle interval.

The main finding from this study was no overall cardiac cycle-related modulation of electrocutaneous tactile thresholds across the cardiac cycle in normotensives. Similar to the first study in this thesis (Chapter 2), when investigating the possible influence of tonic BP on the cardiac cycle modulation of tactile thresholds, we found no group differences in tactile thresholds, however, we did report significant Group × Interval interactions for both the SBP and Diastoic BP (DBP) groups. Further analysis confirmed a diverging pattern of tactile threshold modulation across the cardiac cycle between the DBP groups. Only the DBP_{low} group showed significant variation in tactile thresholds across the cardiac cycle, with lower tactile thresholds during systole (*R*+300 ms) compared to diastole (*R*+600 ms), whereas the DPB_{high} group tended to have higher tactile thresholds during systole compared to diastole, although this wasn't significant. Such opposing patterns may have contributed to the null overall findings in the study. Additional analysis revealed that the cardiac cycle effects were limited to males, but as the participant split in the study was heavily biased towards females (Female = 40, Male = 10), interpretation of the tonic BP effects should be treated with caution.

6.1.3 Study 3

Having found a significant variation in pain thresholds across the cardiac cycle in the first study (Chapter 2), the third study (Chapter 4) aimed to further understand the factors underlying the cardiac cycle modulation of pain. Specifically, the study aimed to expand the knowledge of the cortical processing of painful stimuli across the cardiac cycle. As previous research has reported a systolic dampening of the N2 and N2-P2 peak-to-peak components of laser-evoked PREPs at the central scalp electrode site, Cz, in men (Edwards et al., 2008a), and a systolic dampening of P2 peak amplitudes in females following electrocutaneous stimulation (Gray, Minati, Paoletti, & Critchley, 2010) the third study sought to determine if the same modulation was evident for electrocutaneous PREPs as a greater number of cardiac cycle intervals than the 2 used by Gray et al. (2010). To further the findings of these two studies (Edwards et al., 2008a; Gray et al., 2010), and based on several functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG) and positron

emission tomography (PET) imaging studies indicating that areas beyond the midline are activated by painful stimulation, Study 3 used multi-channel electroencephalogram (EEG) recording to determine if modulation of PREPs was more evident at scalp sites located more laterally. Specifically, PREPs were also analysed at scalp electrode sites positioned lateral to Cz i.e. scalp electrode sites C3 and C4, which were proposed to overlay brain areas involved in the processing of both pain and baroreceptor afferent information i.e. anterior cingulate cortex, the secondary somatosensory and insular cortex bilaterally (Bromm & Chen, 1995; Tarkka & Treede, 1993; Valeriani, Rambaud, & Mauguiere, 1996), and the primary somatosensory areas located in the parietal operculum (Bushnell & Apkarian, 2006; Craig, 2002; Peyron et al., 2000; Rainville, 2002). Finally, the study recruited both male and female participants to increase the generalisation of the findings as Edwards et al. (2009) who had only studied males and Gray et al. (2010) who only studied females.

Following determination of individual pain thresholds via an up-down staircase procedure (Levitt, 1971), EEG activity was recorded via 32 electrodes. Participants completed 7 separate experimental blocks each containing 21 painful stimuli delivered to the right hand at an intensity equal to two times pain threshold. Within each block pain stimuli were delivered three times at each of seven intervals after the *R*-wave of the ECG (*R*+50, *R*+150, *R*+250, *R*+350, *R*+450, *R*+550, *R*+650 ms) resulting in 21 stimuli being presented at each interval over the 7 experimental blocks. EEG data was subsequently corrected for ocular artifacts, beat-before epochs were subtracted from stimulation epochs to remove ECG artefacts from contaminating the EEG data (Gray et al., 2010) and analysed off-line with BESA. Peak N2, and P2 amplitudes and N2-P2 peak-to-peak amplitudes for the average waveforms for each cardiac cycle interval were then determined.

The results of the study showed that there was no significant variation in the N2 or P2 peak amplitudes or N2-P2 peak-to-peak amplitudes across the cardiac cycle at scalp recording sites Cz, C3, or C4. As with studies 1 (Chapter 2) and 2 (Chapter 3) the possible influence of tonic BP on the cardiac cycle modulation of PREPs was investigated, however, in contrast to the previous two studies (Chapters

2 & 3) median BP split analysis revealed no main effect for BP Group or interaction effects between BP Group × Cardiac Cycle Interval for N2 or P2 peak amplitudes or N2-P2 peak-to-peak amplitudes at Cz, C3 or C4.

6.1.4 Study 4

Due to the lack of cardiac cycle-related modulation of PREPs reported in Study 3 (Chapter 4), the fourth study (Chapter 5) sought to determine if this may be due to a lateralisation of cardiac cycle-related modulation in the brain. Specifically, in Study 3 (Chapter 4) only the right hand was stimulated and although a cardiac cycle-related modulation of laser evoked (Edwards et al., 2008a) and electrocutaneous (Gray et al., 2010) PREPs has been reported following right hand stimulation, it is possible that modulation may be more evident following left hand stimulation. A full discussion regarding the evidence justifying this hypothesis is presented in the Introduction of Chapter 5 in this thesis. By delivering stimuli to both the left and right hands, and measuring cortical responses at Cz, and at sites lateral to Cz (i.e., C3 & C4) in the same subjects allowed us to directly compare responses and thus investigate the importance of both side of stimulation and site of recording in the cardiac cycle-related modulation of PREPs, and possibly contribute to explaining the lack of cardiac cycle-related modulation of PREPs in Study 3 (Chapter 4). In addition, previous studies indicating a systolic dampening of the NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006) delivered stimuli to the left side. Further support for a hypothesised laterality of PREP processing came from the few studies that have looked at the lateralisation of cardiac cycle-related modulation. The site of recording visual evoked potentials was found to be important in the cardiac cycle modulation of these visual evoked potentials (Walker & Sandman, 1982). With visual evoked potentials recorded from the right hemisphere being larger during the diastolic phase of the cardiac cycle than the systolic phase, whereas visual evoked potentials recorded from the left hemisphere were uninfluenced by the phase of the cardiac cycle. Similarly, startle response was found to be reduced during systole compared to diastole only following stimulation presented to the left ear and this was independent of recording side (Schultz et al., 2009), which suggest that the side of stimulus presentation is important in determining the lateralisation of the cardiac cycle-related modulation. Finally, both side of stimulation and recording side may be

important in determining the lateralisation of the cardiac cycle-related modulation of simple visual reaction time (Weisz & Adam, 1996). Reaction time for stimuli presented to the right side and for right hand responses was marginally longer during systole than diastole, compared to no differences between systole and diastole for central and left stimuli or for left hand responses (Weisz & Adam, 1996). Although Edwards et al. (2008a) and Gray et al. (2010) reported cardiac cycle effects for PREPs, as both studies only stimulated the right hand and Edwards et al. (2008a) only recorded PREPs at Cz, it is not possible to determine if PREPs delivered to the left hand may have shown a greater cardiac cycle modulation than that reported for the right hand. Furthermore, as suggested in the Discussion of Study 3 (Chapter 4), laser evoked PREPs may be modulated differently to electrocutaneous PREPs due to differentially stimulating nociceptive and non-nociceptive fibres (e.g. Lefaucheur et al., 2012; Perchet et al., 2012).

The EEG methodology and procedures used in Study 4 (Chapter 5) were the same as those used in Study 3 (Chapter 4), the only difference being that subjects were required to attend the laboratory twice and PREPs were recorded following stimulation of the left and right hands on separate days. As in Study 3 (Chapter 4) PREP responses were recorded and compared at scalp electrode sites Cz, C3 and C4.

The results of the study indicated that N2 and P2 peak amplitudes, and N2-P2 peak-to-peak amplitudes were significantly larger at scalp electrode site Cz than C3, and P2 peak amplitudes and N2-P2 peak-to-peak amplitudes at Cz were also larger than those measured at C4.

The second result reported was that there was no significant differences between the cardiac cycle modulation of PREP amplitudes following painful stimuli delivered to the right or left hand, nor any difference among the cardiac cycle-related modulation of PREP amplitudes at scalp electrode sites covering the left (C3), right (C4) or central (Cz) brain areas.

Finally, there was no main effect for cardiac cycle interval for N2 peak amplitudes, P2 peak amplitudes or N2-P2 peak-to-peak amplitudes. However, analysis did reveal a significant interaction between the hand, scalp electrode site and cardiac cycle interval for N2 peak amplitudes.

6.2 Discussion and Interpretation

The results of Study 1 (Chapter 2) showed that pain thresholds were higher mid-cycle (*R*+200 and *R*+300ms), indicative of pain attenuation during systole compared to diastole. Thus, the results of Study 1 (Chapter 2) suggest that pain perception, at threshold level, is modulated by natural fluctuations in BP across the cardiac cycle. Whereas, Study 2 (Chapter 3) revealed that tactile detection thresholds were not modulated across the cardiac cycle and similarly studies 3 and 4 (Chapters 4 & 5) found that electrocutaneous PREPs were not modulated by natural fluctuations in BP across the cardiac cycle. Interestingly, the participants in studies 1 and 2 (Chapters 2 & 3) were the same, suggesting that in a group of normotensive individuals the influence of the cardiac cycle is different between pain and tactile detection, possibly indicting that pain perception is more sensitive to natural fluctuations in BP than tactile perception.

The pattern of modulation reported in Study 1 (Chapter 2) agrees with previous studies reporting a systolic dampening of the NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006; 2008a) and PREPs (Edwards et al., 2008a). However, the pattern of modulation reported in Study 1 is contra to the only previous study to specifically study the cardiac cycle modulation of pain (Martins et al., 2009) who reported an increase in pain ratings during systole.

The findings of Study 2 (Chapter 3), indicating no cardiac cycle-related modulation of electrocutaneous tactile thresholds was counter to the only previous study investigating the cardiac cycle-related modulation of sensory thresholds (Edwards et al., 2009) who reported cutaneous sensory thresholds were lower, indicating heightened sensitivity during systole compared to diastole. The lack of cardiac cycle-related modulation of tactile thresholds in Study 2 (Chapter 3) was, however, in line with a previous studying reporting no difference in intracutaneous

electrical sensory detection thresholds when stimuli were delivered during either mechanical stimulation or inhibition of the carotid baroreceptors (Droste et al., 1994). However, comparison of studies utilising the natural fluctuations in BP across the cardiac cycle with those employing artificial stimulation of baroreceptors should be done with caution due to the aversive nature of artificial baroreceptor stimulation. During the artificial baroreceptor stimulation procedure participants may become more aroused, be more distracted from the task and the action of compression and suction may induce wider spread physiological effects such as increased muscle tension (Edwards et al., 2003), the effects of which on the cardiac cycle-related modulation are not known.

The lack of cardiac cycle-related modulation of PREPs following painful electrocutaneous stimulation reported in studies 3 and 4 (Chapters 4 & 5), is contra to reports of reduced N2 and N2-P2 peak-to-peak amplitudes following painful laser stimulation (Edwards et al., 2008a). However, the lack of cardiac cycle modulation in studies 3 and 4 (Chapters 4 & 5) is in partial agreement with the findings of Gray et al. (2010) who reported that although P2 peak amplitudes were modulated, this patterning was only evident for pain stimuli delivered following presentation of a warning cue. Indeed, Gray et al. (2010) reported that N2 peak amplitudes were not modulated across the cardiac cycle, and neither N2 nor P2 peak amplitudes were modulated across the cardiac cycle when presented without a warning cue. These findings suggest that presentation of a cue prior to stimulus may be an important factor determining cardiac cycle effects. The null finding from studies 3 and 4 (Chapter 4 & 5) are also contra to the typically reported dampening of the NFR during systole compared to diastole (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006; 2008a) and the reduced pain thresholds during systole compared to diastole reported in Study 1 of this thesis (Chapter 2 – Wilkinson, McIntyre, & Edwards, 2013).

A possible explanation for the contrasting findings between Study 1 (Chapter 2) and the study closest related to it (Martins et al., 2009) may relate to differences in stimulation intensity. Martins et al. (2009) delivered pain stimuli unpredictably, up to and including pain tolerance and this may have induced physiological arousal which

has been shown to influence cardiac cycle modulation (McIntyre et al., 2006). Whereas Study 1 (Chapter 2) delivered stimuli that oscillated around pain threshold and thus the 'threat' of very high intensity stimulation was removed, potentially reducing participant arousal. Indeed, additional analysis (data not reported here but available in Wilkinson et al., 2013) in a subsample of 40 participants from Study 1 (Chapter 2) revealed a decrease in heart rate from baseline to pain assessment, which suggests participants did not experience increased physiological arousal during the pain task. The lack of cardiac cycle-related modulation of PREPs reported in studies 3 and 4 (Chapters 4 & 5) in the current thesis may provide partial support to the suggestion that stimulation intensity may be an important factor in determining the cardiac cycle modulation of pain. The stimuli delivered during studies 3 (Chapter 4) and 4 (Chapter 5) were equal to two times individual pain thresholds, and thus one could suggest that the lack of cardiac cycle modulation in these studies (Chapters 4 & 5) may in part, be due to the higher stimulation intensity inducing arousal and thus reducing the cardiac cycle effects as indicated by McIntyre et al. (2006) in relation to the cardiac cycle-related modulation of the NFR. However, during Study 3 and 4 (Chapter 5 & 6) we ensured that the average intensity of each experimental block of pain stimuli was rated at an intensity above 50 on the 0-100 VRS scale (with anchors '0' (no sensation), '1' (sensory threshold), '25' (uncomfortable), '50' (just noticeable pain), '75' (very painful), and '100' (maximum tolerable pain). The average pain rating for studies 3 and 4 (Chapters 4 & 5) was 56.8 (Range 50-77), which one might suggest is not significantly greater than pain threshold thus, differing pain stimuli intensities may not be an explanatory reason for the differing results. Additionally, it must be acknowledged that a cardiac cycle-related modulation of PREPs has been reported following painful laser stimulation (Edwards et al., 2008a). The intensity of the stimuli delivered by Edwards et al. (2008a) was equal to a rating of 50 on the visual analogue scale which has anchors at '0' (no painful sensation) and '100' (imaginary intolerable pain sensation). A rating of 50 equates to 'moderate pain' (Jensen, Chen, & Brugger, 2003). Gray et al. (2010) asked participants to rate the intensity of stimuli using a VRS scale ranging from 1 (barely identifiable as pain) to 10 (imaginary worst possible pain) and the average (SD) rating given by participants was 4.1 ± 1.0 which would be similarly defined as moderate pain. Although the use of different rating scales makes direct comparison

difficult, the average pain rating of 56.7 in studies 3 and 4 (Chapters 4 & 5) also appears to equate to moderate pain, thus suggesting that the perceived intensity of the stimuli in studies 3 and 4 was similar to that of Edwards et al. (2008a) and Gray et al. (2010) further suggesting pain intensity may not be the primary factor influencing the different results between the studies.

The finding that PREPs were not modulated across the cardiac cycle in studies 3 and 4 (Chapters 4 & 5), whilst we did find a modulation of pain thresholds in Study 1 (Chapter 2), may partially relate to the fact studies 3 and 4 (Chapters 4 & 5) were examining the PREP response to painful stimuli and not pain ratings, which due to the subjective nature of pain ratings are inherently different (Chen, Arendt-Nielsen, & Plafhki, 1998; Iannetti, Hughes, Lee, & Mouraux, 2008; Sandrini et al., 2005) and thus the difference in cardiac cycle-related modulation may be understandable. Indeed, when pain ratings have previously been assessed concurrently to the NFR (Edwards et al., 2001, 2002, 2003, McIntyre et al., 2006; 2008a) and PREPs (Edwards et al., 2008a), which are thought to serve as a physiological correlate of pain (Hugon, 1973; Willer, 1977), pain ratings, in contrast to the NFR and PREPs, were not modulated across the cardiac cycle. Similarly, Martins et al. (2009) found that although pain ratings were modulated across the cardiac cycle, the NFR wasn't. This suggests that pain perception and the neurophysiological correlates of pain may be modulated differently across the cardiac cycle and thus contribute to explaining the differing results between studies 3 & 4 (Chapters 4 & 5) in the current thesis and those of Study 1 (Chapter 2) and Martins et al. (2009).

It is possible that methodological differences may partially explain the differing results from studies 3 and 4 (Chapters 4 & 5) reported in this thesis and Edwards et al. (2008a). Firstly, pain modality was different. All studies in this thesis delivered electrocutaneous stimuli, whereas Edwards et al. (2008a) delivered noxious thulium laser stimuli. Differences in the activation of nociceptive and non-nociceptive fibres following electrocutaneous and laser stimulation (e.g. Lefaucheur et al., 2012; Perchet et al., 2012) may help explain the differing results. A detailed discussion about the differences between electrocutaneous and laser stimulation is presented in chapter 4 of this thesis, but to summarise, laser stimulation has a rapid onset and

excites a limited number of primary afferent fibres, primarily thin myelinated A-δ and unmyelinated C-fibres (Meyer, Ringkamp, Campbell, & Raja, 2006). Myelinated A-δ fibres have a rapid conduction velocity (Meyer, Walker, & Mountcastle, 1976), high firing rates and reflect “first, pinprick” pain (Arendt-Nielsen & Chen, 2003), unmyelinated C fibres have slower conduction velocities (Gybels, Handwerker, & Van Hees, 1979), slower firing rates and reflect “second, slow burning” (Arendt-Nielsen & Chen, 2003). Electrical stimulation is known to stimulate A-β fibres which are felt like pressure or vibration without actually being painful (Gracely, 2006). Such A-β fibre activation may have influenced the modulation of PREPs across the cardiac cycle in studies 3 and 4 (Chapters 4 & 5) in the current thesis. Additionally, electrical stimulation directly activates A-δ afferents (Perchet et al., 2012), whereas laser stimulation incurs a peripheral delay due to laser initially stimulating thermoreceptors which subsequently generate an action potential in A-δ nociceptive fibres (Bromm & Treede, 1991; Plaghki & Mouraux, 2003). Although the concentric planar electrode, used in studies 1, 3 and 4 (Chapters 2, 4 & 5) is thought to more selectively stimulate A-δ nociceptive fibres than a bar electrode (Katsarava et al., 2006; Kaube, Katsarava, Kaufer, Diener, & Ellrich, 2000), it is possible differential activation of A-δ nociceptive- and A-β sensory-fibres may partially explain the different cardiac cycle modulation results between studies 3 and 4 (Chapters 4 & 5) in the current thesis and Edwards et al. (2008a). However, it should be acknowledged that Gray et al. (2010) delivered electrocutaneous stimuli via a more traditional electrode design and reported a cardiac cycle-related modulation of the P2 peak amplitudes. As the electrode used by Gray et al. (2010) would be expected to activate A-β sensory-fibres to a greater extent than the concentric electrode used in the current studies (Katsarava et al., 2006; Kaube et al., 2000), this suggests that differing patterns of fibre activation may not explain the differing results. However, as Gray et al. (2010) only reported a cardiac cycle modulation following cued pain stimulation, as stimuli in the current thesis were uncued, this may contribute to the differing findings. Also, the two previous studies (Edwards et al., 2008a; Gray et al., 2010) reported modulation of different PREP components (Edwards et al. (2008a) reported modulation of N2 peak amplitude & N2-P2 peak-to-peak amplitude, Gray et al. (2010) reported modulation for P2 peak amplitudes) it may be suggested that pain stimulation delivered by different electrodes is modulated differently, possibly due to different

fibre type activation. Further studies directly comparing the cardiac cycle related modulation of PREPs evoked by different electrode types is required to further investigate this suggestion. Additionally, it should however be noted that Martins et al. (2009) found a cardiac cycle-related modulation of pain ratings following pain stimuli delivered via a bar electrode, and Study 1 (Chapter 2) also reported a modulation when delivering stimuli via the concentric planar electrode. However, as discussed above the different stimulation intensities used in Study 1 (Chapter 2) (i.e. pain threshold) and by Martins et al. (2009) (i.e. up to pain tolerance) may partially explain the differing results. Furthermore, in partial support for the co-activation of A-δ nociceptive- and A-β sensory-fibres by the concentric planar electrode contributing to the null findings in studies 3 and 4 of this thesis, Study 2 (Chapter 3) reported that electrocutaneous tactile thresholds showed no cardiac cycle-related modulation, suggesting, perhaps, that stimulation of A-β tactile fibres is less sensitive to cardiac cycle-related effects than A-δ pain fibres. However, it should be noted that contra to this hypothesis previous work has indicated that cutaneous tactile thresholds evoked via a bar electrode were elevated during systole compared to diastole (Edwards et al., 2009). If we accept that at tactile detection levels the bar electrode stimulates A-β sensory-fibres, the findings of Edwards et al. (2009) suggest that stimulation of A-β sensory-fibres is influenced by variations in BP across the cardiac cycle. However, the inclusion of hypertensive participants in the Edwards et al. (2009) study may have influenced the results (see below for further discussion regarding this).

Alternatively, the lack of cardiac cycle-related modulation of electrocutaneous PREPs may be explained to some extent by further methodological differences, a full discussion is presented in chapter 4, but to summarise these may include; a) Studies 3 and 4 (Chapters 4 & 5) had a longer total study duration compared to Edwards et al. (2008a) and Gray et al. (2010) and additionally a greater number of total number of noxious stimulations compared to Edwards et al. (2008a). These may have lead to differing levels of peripheral nerve fibre fatigue (Greffrath, Baumgartner, & Treede, 2007) and/or central habituation to the painful stimuli (Bingel, Schoell, Herken, Buchel, & May, 2007; Bingel, Herken, Teutsch, & May, 2008; Milne, Kay, & Irwin, 1991). However due to Study 3 and 4 (Chapters 4 & 5) recording EEG via the Biosemi system which is a high impedance EEG system, a greater number of trials

were required to achieve significance than comparable low impedance systems, due to an increased size or incidence of skin potentials increasing the noise level, thus lowering the signal-to-noise ratio (Kappenman & Luck, 2010). Therefore, based on previous research using the P300 component, Study 3 and 4 (Chapters 4 & 5) needed to deliver approximately 25 stimuli per condition to achieve an 80% chance of statistical significance (Kappenman and Luck, 2010). However, the suggested number of trials related to P300 and it is possible that N2 and P2 components require a different number of trials to reach significance and we also included a greater number of participants (N=26) to increase the chance of reaching significance. None the less, as the mean accepted number of trials for all cardiac cycle intervals in Study 3 and 4 (Chapters 4 & 5) was 20 and 19 respectively, reducing the total number of trials would have reduced the chance of attaining significance and thus the potential for habituation must be acknowledged as a potential confounding factor for the differences between Study 3 and Edwards et al. (2008a).

The patterning of modulation in the Edwards et al. (2009) study, i.e. heightened tactile sensibility during systole, was perhaps surprising considering the well documented systolic inhibition of the NFR (Edwards et al., 2001, 2002, 2003; McIntyre et al., 2006; 2008a) and PREPs (Edwards et al., 2008a). Study 2 (Chapter 3) aimed to closely replicate the methods of the study (Edwards et al., 2009) to further the understanding of the cardiac cycle modulation of tactile sensation. Therefore, the differing patterns of cardiac cycle-related modulation between Study 2 (Chapter 3) and Edwards et al. (2009) are unlikely to relate to big methodological differences. However, it should be acknowledged that to increase the resolution of the cardiac cycle-related modulation of tactile thresholds Study 2 (Chapter 3) delivered stimuli at 7 intervals across the cardiac cycle compared to just 3 used by Edwards et al. (2009). The total duration nor the total number of stimuli delivered are reported by Edwards et al., (2009), however, one can assume that a greater number of trials would be required to determine tactile thresholds at 7 intervals as opposed to 3. The mean (SD) number of trials required to determine all 7 tactile thresholds was 51.06 (12.9) and the task typically lasted 10-15 minutes and thus the total duration of Study 2 (Chapter 3) is likely to be longer than Edwards et al. (2009). Thus, it could

be suggested that the longer duration of Study 2 (Chapter 3) may have lead to participant disengagement or habituation to the stimuli, which has been shown to reduce pain in pain studies (LeBlanc & Potvin, 1966; Strempel, 1976; Strempel, 1978) and discussed above in relation to the PREPs studies in this thesis (Chapters 4 & 5). Indeed Gray et al. (2010) found that P2 amplitudes were only modulated when stimuli were delivered following a cue. The authors suggest that drawing attention to the imminent stimulation may be an important factor determining cardiac cycle-related modulation and thus if disengagement occurred and attention was drawn away from the stimuli this may help explain the null findings in the current thesis.

However, the most probable explanation for the different results between Study 2 (Chapter 3) and Edwards et al. (2009) is differing participant characteristics. First, when comparing Study 2 (Chapter 3) and the Edwards et al. (2009) study, the participants in Study 2 were on average 10 years younger than those in the Edwards et al. (2009). Although this may seem a small difference, sensory acuity has been shown to reduce in most sensory modalities with age, including vision (e.g. Weale, 1986), hearing (e.g. Helzner et al., 2005), smell (e.g. Schiffman, 1997) and tactile sensitivity (Stevens & Cruz, 1996; Gescheider, Bolanowski, Hall, Hoffman, & Verrillo, 1994; Takekuma, Ando, Niino, & Shimokata, 2000), as has baroreceptor sensitivity (Gribbin, Pickerin, Sleight, & Peto 1971; Korner, West, Shaw, & Uther, 1974; Randall et al., 1976; Randall, Esler, Culp, Julius, & Zweifler, 1978). However, this reduced sensitivity with increasing age may suggest we would be expected to see less cardiac cycle modulation in older adults. Therefore, the differing ages of participants is an unlikely explanation for the differences. Second, the tonic BP status of the participants was also different between the two studies. Participants in Study 2 (Chapter 3) were normotensive, whereas Edwards et al. (2009) included newly diagnosed, unmedicated hypertensives. As hypertension is characterised by disruption to the baroreflex (Eckberg & Sleight, 1992) and an inverse relationship between BP and baroreceptor sensitivity has been reported (Bristow, Honour, Pickering, Sleight, & Smyth, 1969; Gribbin et al., 1971), it may be to be expected that results would differ between a normotensive and a hypertensive/normotensive mixed sample. Indeed research has shown heightened electrocutaneous (Edwards, Ring,

France, McIntyre, & Martin, 2008b) and electrical tooth pulp (Ghione et al., 1985) sensory thresholds in unmedicated essential hypertension patients compared to normotensives.

One of the main aims of the third Study (Chapter 4) was to determine if the cardiac cycle-related modulation of PREPs extended beyond the Cz analysed by Edwards et al. (2008a). However, the results indicated there was no cardiac cycle-related modulation at C3 or C4 electrode sites, this finding was also repeated in Study 4 (Chapter 5) following electrocutaneous noxious stimulation of the right and left hands. As we did not find a modulation of PREPs at Cz, a site which has previously shown maximal N2 and P2 amplitudes following hand stimulation (Carmon, Mor, & Goldberg, 1976, 1978; Bromm & Treede, 1987; Treede, Kief, Holzer, & Bromm, 1988; Kakigi, Shibasaki, & Ikeda, 1989; Miyazaki et al., 1994; Xu et al., 1995; Kanda et al., 1996, 1999), it is perhaps not surprising that we did not see modulation at C3 or C4 either. We propose that this is, at least in part, due to the methodological differences (see chapter 4 for a full discussion) discussed above rather than necessarily disproving any wider spread interaction of pain and baroreceptor systems. Indeed, we hypothesised that the overall lack of cardiac cycle-related modulation of electrocutaneous PREPs in Study 3 (Chapter 4) may be due to lateralisation of the baroreceptor and pain processing in the brain and thus the cardiac cycle-related modulation may be more evident following stimulation of the left hand rather than the right which was stimulated in Study 3 (Chapter 4). In line with the previous Study (Chapter 4), Study 4 (Chapter 5) also reported no overall cardiac cycle modulation of any electrocutaneous PREP component (N2 peak amplitude, P2 peak amplitude or N2-P2 difference) at any scalp electrode site following stimulation of the right or left hand. These results were contra to our hypothesis that cardiac cycle-related modulation of electrocutaneous PREP amplitudes would be more evident following stimulation of the left hand and in the scalp electrodes covering the central (Cz) and right side of the cerebral cortex (C4) than in scalp electrodes over the left hemisphere (C3). As some of the participants in Study 4 (Chapter 5) also took part in Study 3 (Chapter 4) (N=4, 24% of the total participants), and the remaining participants in Study 4 (Chapter 5) were recruited from the same university pool, this finding should perhaps not be unexpected.

However, analysis revealed an interaction between hand, scalp electrode site and cardiac cycle interval, suggesting that together stimulation side and site of recording are both important in determining the patterning of N2 peak amplitudes across the cardiac cycle. Study 4 (Chapter 5) is the first study to our knowledge to investigate a potential lateralisation of the cardiac cycle-related modulation of pain and the findings provide initial, although limited evidence for a potential cardiac cycle lateralisation. The few previous studies that have studied the phenomenon of cardiac cycle related lateralisation have reported differing conclusions regarding the relative importance of the side of stimulation versus the side of cerebral recording in determining the cardiac cycle-related modulation. For example the side of recording visual evoked potentials appears to be more important than side of stimulation (Walker & Sandman, 1982), whereas side of presentation appears to be the determining factor for startle response (Schultz et al., 2009) and similar to our suggestion in relation to PREPs, both side of presentation and recording side appear important in determining the cardiac cycle-related modulation of simple visual reaction time (Weisz & Adam, 1996). Therefore, it may be suggested that the cardiac cycle modulation of different stimulation modalities may be lateralised differently.

With regards to the influence of natural fluctuations in BP across the cardiac cycle, it may be concluded that the temporal patterning of systolic pain dampening reported in Study 1 (Chapter 2) is consistent with the hypothesis that pain is inhibited during systole due to arterial baroreceptor activation of pain inhibition pathways (Ghione, 1996). A full description of the specific timings relating to the divergence of the baroreceptor and pain processing pathways following painful stimulation during systole is presented in chapter 2 of this thesis. Thus, the results from Study 1 provide further support for a baroreceptor role in the hypertensive hypoalgesia phenomenon (see Introduction (Chapter 1) for a full description of the hypertensive hypoalgesia phenomenon). Despite not presenting a pattern of cardiac cycle-related modulation consistent with a baroreceptor mediated modulation of tactile thresholds and PREPs, the lack of cardiac cycle-related modulation reported in studies 2, 3 and 4 (Chapters 3, 4 and 5) suggests that tactile sensibility and PREPs may not be as sensitive to natural fluctuations in BP across the cardiac cycle as the NFR and pain perception (Study 1). However, it is possible that differing methodology and

participant characteristics may explain the contrasting findings and that PREPs specifically may be influenced by the combination of stimulation and recording sites.

As tonic BP has been shown to influence the baroreceptor modulation of pain (e.g., Droste et al., 1994), a second aim of the current thesis was to further understand the role of tonic BP in cardiac cycle-related modulation. Such an aim also enabled the suggested role of tonic BP contributing to the lack of cardiac cycle modulation of tactile thresholds in Study 2 (Chapter 3) to be further investigated. Participants in studies 1, 2 and 3 were split into high-normal and low-normal DBP and SBP groups using a median BP split for comparison. With regards to Study 2 (Chapter 3), only participants in the DBP_{low} group presented a cardiac cycle modulation of tactile thresholds. The DBP_{low} group displayed a pattern similar to the overall cardiac cycle related modulation reported by Edwards et al. (2009), i.e. lower tactile thresholds at *R*+300 ms compared to *R*+600 ms. In contrast, the DBP_{high} group tended to have higher tactile thresholds during systole compared to diastole, although these differences were not significant. These results suggest that cardiac cycle effects on tactile sensibility become less as tonic BP increases. Contrastingly, when investigating tonic BP effects Edwards et al. (2009) reported that individuals with higher diastolic BP had larger reductions in sensory threshold during systole compared to diastole. However, as mentioned before the inclusion of hypertensives in the subject group for this study is likely to have influenced the results. It should be noted that the male participants appeared to drive the cardiac cycle modulation in the DBP_{low} group, and as the sample of males was small (N=9, 18% of the total sample) this finding should be interpreted with caution. Regardless, the opposing findings between the high and low BP groups in Study 2 (Chapter 3) may have contributed to the overall null finding in the current study and supports the suggestion that the conflicting results reported between Study 2 (Chapter 3) and Edwards et al., (2009) may be due, in part, to differences in tonic BP status of the participants.

Similar BP group analysis was also conducted in studies 1 and 3 in relation to the cardiac cycle-related modulation of pain thresholds and PREPs, respectively. In relation to pain thresholds, similar to tactile thresholds, only participants with low-

normal SBP displayed the systolic dampening of pain thresholds. However, as the participants in Studies 1 and 2 (Chapters 3 & 4) were the same, this may not be surprising. Whereas, in relation to PREPs no BP group or group × Interval interaction was found.

The mechanism for the differing cardiac cycle-related modulation of pain and tactile thresholds in the low-normal and high-normal BP group is unclear, but may relate to a baroreceptor mechanism. Baroreceptors are stimulated during systole when the pulse pressure wave stretches the walls of the aortic arch and carotid sinus (Angell James, 1971; Mancia & Mark, 1983), resulting in increased baroreceptor activation during systole and subsequent cortical inhibition (e.g. Rau, Elbert, & Birbaumer, 1995). The differing patterns of cardiac cycle-related modulation between the low-normal and high-normal BP groups may be due to BP group differences in baroreceptor afferent activity reaching brain areas affected by baroreceptor activity. Evidence supporting this hypothesis comes from the reported inverse relationship between BP and both baroreceptor sensitivity (Sleight, Robinson, Brooks, & Rees, 1977) and baroreflex sensitivity (Bristow et al., 1969; Gribbin et al., 1971).

Taken together the findings from Studies 1, 2 and 3 (Chapters 2, 3 & 4) suggest that tonic BP may moderate the cardiac cycle-related modulation of subjective perception of sensation, but that objective measures of pain, i.e. PREPs, may not be subject to the same tonic BP influence.

6.3 Limitations and recommendations for future research

It should be acknowledged that the studies presented in this thesis have a few limitations. Considering the first two studies, a major critique is the ratio of males and females in the participant group, which was predominately female (Study 1 Males N = 10, Female N = 39; Study 2 Males = 10, Female = 40). Typically men have higher pain (e.g. Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009) and tactile (e.g. Takekuma et al, 2000) thresholds than women. Indeed we reported that women were more sensitive to pain and presented lower tactile thresholds than men. However, in line with previous studies that have not found sex differences in the cardiac cycle modulation of pain ratings (Martins et al., 2009), nociceptive

responding (Edwards et al., 2001; Martins et al., 2009) or reaction times (Birren, Phillips, & Cardon, 1963; Edwards, Ring, McIntyre, Carroll, & Martin, 2007; McIntyre, Ring, Hamer, & Carroll, 2007, McIntyre, Ring, Edwards, & Carroll, 2008b), we found no sex differences for the modulation of pain thresholds in Study 1. However, in relation to the cardiac cycle modulation of tactile thresholds, we reported a Group × Sex × Interval interaction. Males with low-normal diastolic BP were the only sub-set of participants to present a cardiac cycle modulation. But due to the limited number of participants in this group (N=5) this finding should be interpreted with extreme caution and future studies would do well to examine the cardiac cycle modulation of both pain and tactile thresholds in a more evenly balanced sample of males and females to increase the understanding of any potential sex effects and increase the generalisation of the findings. With regards to this, studies 3 and 4 (Chapters 4 & 5) had a better ratio of males to females (Study 3 Males N = 11, Female N = 15; Study 4 Male N = 7, Female N = 10) and in line with study 1, we reported in study 3 (Chapter 4) we found no sex differences in the cardiac cycle influence on PREPs.

Secondly, none of the studies in this thesis assessed parental history of hypertension, which has been found to influence pain perception (France, 1999) and baroreflex sensitivity (Parmer, Cervenka, & Stone, 1992). Therefore, we cannot rule out that individuals with a parental history of hypertension did not influence the results of the studies in this thesis. However, several prior studies have indicated that parental history may not influence the cardiac cycle modulation of reaction times (McIntyre et al., 2008b; Stewart, France, & Suhr, 2006). Future studies would be necessary to examine the influence of parental history of hypertension on pain modulation across the cardiac cycle.

Third, the effects of tonic BP were investigated using a median split design which transforms a continuous variable into a categorical variable and thus has limitations. A median split results in all participants above and below the median being considered the same and thus resolution of the effects is reduced. By grouping the data, there is also a loss of power (Aiken & West, 1991) and therefore effects are harder to find. Additionally, when using a median split, participants positioned close to the median may actually be more similar in terms of the factor of interest than

individuals at the extremes of each high and low group. Thus, it is worth acknowledging that the data could have been split into three groups with the middle group removed from analysis or regression analysis conducted. However, the limited sample sizes, especially in Studies 3 and 4 (Chapter 4 & 5) limited the use of these statistical techniques.

The PREP methodology employed in studies 3 and 4 (Chapters 4 & 5) has many strengths, especially when studying stimulus-responses activated in milliseconds as is the case with cardiac cycle studies, as they have exceptional temporal resolution. However, it should be acknowledged that PREPs also have limitations. The PREP response is a measure of the electrical activity of the brain recorded at scalp level and it represents the averaged cortical processing of the nociceptive stimulus (Iannetti et al., 2008). Therefore, the PREP does not provide information about exactly which areas of the brain are generating the response i.e. it has poor spatial resolution and thus makes source localisation of the areas generating the PREP difficult (Devinsky & D'Esposito, 2004). To improve the ability of studies to identify which areas of the brain are central to the cardiac cycle-related modulation of pain, future studies would be advised to utilise the growing methodology incorporating concurrent fMRI and PREP analysis.

Time constraints prevent a full analysis of the PREP data regarding habituation or disengagement with task as a potential factor contributing to the lack of cardiac cycle related modulation reported in studies 3 and 4 (Chapter 4 & 5). Indeed, changes in PREP amplitudes across the seven experimental blocks could have analysed to identify if there was any changes in the amplitudes of interest. A reduction in the amplitude of the PREP components across the seven blocks may suggest that habituation to the pain stimuli and/or distraction or disengagement with the task was occurring (Bingel et al., 2007; 2008; Milne et al., 1991). Indeed, as discussed above, Gray et al. (2010) propose that the cardiac cycle-related modulation of P2 peak amplitudes only following cued stimulation may be due to participants attention being drawn to the imminent pain. Future studies would do well to further investigate a possible role of attention and distraction on the cardiac cycle modulation of PREPs by directly comparing responses when attention is focused on

the pain stimuli, away from the pain stimuli or presented unexpectedly. Additionally, future studies would be required to explore if PREPs do indeed habituate over the duration of a cardiac cycle study and aim to identify what the cause of any changes in PREP amplitudes may be i.e. fibre fatigue, central fatigue, distraction etc. If such an habituation in PREPs over the duration of a study was confirmed, further research would also be required to investigate the cardiac cycle effects during conditions when habituation was not occurring.

As acknowledged in the discussion of Study 4 (Chapter 5), the sample size for this study, and this may equally apply to Study 3 (Chapter 4) was relatively small (Study 3 N= 26, Study 4 N = 17). Thus, the lack of significant findings may be at least part attributable to low power. Although Edwards et al. (2008a) found significant effects for laser evoked PREPs with just 10 subjects and Gray et al. (2010) also reported effects for electrocutaneous PREPs in 11 subjects, it may be possible that the high impedance Biosemi EEG system used in the studies in this thesis may mean that either a larger number of trials per interval or a greater number of participants is required to achieve statistical significance. Indeed, post hoc analysis using the G*power computer program (Faul, Erdfelder, Buchner, & Lang, 2009) indicated that to detect, by repeated measures ANOVA, a difference in N2 peak amplitudes across the cardiac cycle in Study 3 (Chapter 4) would require a total sample of 40 and in Study 4 (Chapter 5) a total sample of 42. Therefore, although we increased the number of trials per interval, Studies 3 and 4 (Chapters 4 & 5) presented 21 stimuli per cardiac cycle interval, with an acceptance of 10 (Study 3) and 19 (Study 4) compared to 12 stimuli presented, but just 5 accepted per interval by Edwards et al. (2008a), this was still below the 25 suggested to reach statistical significance for the P300 (Kappenman & Luck, 2010). The inclusion of 7 cardiac cycle intervals in studies 3 and 4 sought to increase the resolution of the cardiac cycle-related modulation of electrocutaneous PREPs compared to Gray et al. (2010) who only delivered stimuli at 2 cardiac cycle intervals. However, assessing 7 intervals across the cardiac cycle made it impractical to increase the total number of trials further and indeed as discussed above an even greater number of trials may have lead to habituation or disengagement with the task. Therefore, future studies employing high impedance EEG systems into study PREPs would be advised to

increase the number of participants and maybe deliver stimuli at fewer intervals across the cardiac cycle to reduce the duration of the study whilst increase the total number of trials per condition.

In relation to all the studies in this thesis, stimuli were delivered based on the assumption that baroreceptor stimulation would be minimal at R+0/R+50 ms and R+600/R+650 ms and maximal at around R+300/R+350 ms. This assumption is well grounded in the literature, but it should be acknowledged that we did not directly measure BP or baroreceptor activity during the experimental studies and therefore there may be some individuals for whom this assumption did not hold true.

It should be acknowledged that had time not been a limiting factor, it would have been interesting to analyse the data from the current thesis further. Specifically, with regards to studies 3 and 4 (Chapters 4 & 5), by re-analysing the PREP data and binning the individual cardiac cycle intervals into early, mid and late periods would have increased the number of trials per condition, improving the signal to noise ratio and thus the chance of reaching significance. Furthermore, although we did not find cardiac cycle effect at scalp electrode sites C3, Cz or C4 as we had hypothesised, it maybe that baroreceptor and pain interactions would be evident at other scalp sites and thus further work could have been conducted at further electrode sites thought to overlay potential sites of interaction. For example elements of the anterior and posterior ACC and insula may also be potential sites of interaction (Symonds, Gordon, Bixby, & Mande, 2006) and therefore examination of sites positioned to the front and back of Cz would also be worth investigating.

It should be noted that during the studies in the current thesis, data regarding state and trait anxiety were collected via the Spielberger State Trait Anxiety Index (STAI) (Spielberger, Gorsuch, & Lushene, 1970) and data regarding depressive symptomatology in the general population via the Center for Epidemiologic Studies Depression (CES-D) Scale (Radloff, 1977). However, aside from being analysed for extreme outliers, these data were not used to investigate the possible influence of these emotional factors on the results.

Many environmental and psychological factors have been shown to interact to influence an individual's perception of pain and one of the most extensively studied psychological factors influencing pain perception is anxiety (Tang & Gibson, 2005). Anxiety is an emotion involving appraisal of a threat that is uncertain or uncontrollable and is typically associated with feelings of fear about impending or anticipated harm (Spielberger, 1972). It is proposed that anxiety can be divided into state and trait anxiety. State anxiety is the level of anxiety an individual is experiencing at the present time, measuring subjective feelings of tension, worry, apprehensiveness and autonomic arousal, and is influenced by situational factors. Whereas trait anxiety is an individual's general disposition to be anxious and measures proneness to be anxious and personality variables, such as low self esteem and low self-confidence (Spielberger & Rickman, 1990).

In relation to pain, research has typically found that state anxiety which is directly relevant to the source of pain (e.g. highlighting the pain that is to come) increases reported pain, whereas anxiety which is irrelevant to the source of pain (e.g. highlighting a shock that may be about to occur) reduces pain reports (e.g. Absi & Rokke, 1991). Further support suggesting that state anxiety effects pain comes from several studies that have reported that higher levels of state anxiety results in higher pain intensity ratings for cold pressor pain (Jones, Spindler, Jorgensen, & Zachariae, 2002), decreased pressure pain tolerance (Carter et al., 2002) and pressure pain thresholds (Michelotti, Farella, Tedesco, Cimino, & Martina, 2000). There has been less research regarding the effects of trait anxiety on pain; however, trait and state anxiety are inter-linked with individuals recording higher trait anxiety typically reporting higher state anxiety for the same potentially threatening situation compared to low trait anxiety individuals (Spielberger, 1972) suggesting that trait anxiety may have a similar effect on pain as state anxiety. Indeed, research has shown that individuals with higher trait anxiety have lower pain tolerance during a cold pressor test, compared to individuals with low trait anxiety (James & Hardardottir, 2002).

Similarly, research has also reported that depression affects pain (Dickens, McGowan, & Dale, 2003). An initial experimental study reported that depressed

participants had higher thermal pain perception thresholds than non-depressed participants, suggesting that depression reduced pain sensitivity (Hemphill & Crookes, 1952). Further experimental studies produced inconsistent results with some studies reporting that pain perception was reduced in depressed participants, whereas others reported heightened pain perception in participants with depression compared to those without depression (see Lautenbacher & Krieg, 1994 for review). However, a recent meta-analysis regarding the impact of depression on experimental pain perception (Dickens et al., 2003) concluded that participants with depression had higher pain perception thresholds, following experimental pain compared to non-depressed participants. Notwithstanding the variable findings, it is reasonable to suggest that as with anxiety, depression may influence pain perception. Therefore, it is recommended that further research would do well to examine the possible effects of both state and trait anxiety and depression on the cardiac cycle related modulation of pain thresholds and PREPs. Indeed the data collected within the experimental chapters of this thesis could be analysed to examine what, if any, effect state and trait anxiety and depression within the normal range may have on the pain indices examined.

6.4 Future Research

In addition to the suggestions made above regarding addressing the limitations identified in the studies of this thesis, below are further ideas for future research based on the findings of the studies in this thesis. As all of the studies in this thesis should be considered preliminary, future research would do well to replicate the studies to increase the strength of the findings.

Study 1 is the first study to our knowledge to indicate that pain perception, at threshold levels, is modulated across the cardiac cycle in the manner expected if a baroreceptor mechanism is accepted as explaining the modulation i.e. a dampening of pain thresholds during systole compared to diastole. Whereas Martins et al. (2009) reported increased perception of pain during systole compared to diastole when using a range of pain intensities up to and including pain tolerance. Taken together these findings suggest that different intensities of electrocutaneous stimulation may be modulated differently by natural fluctuations in BP. Therefore, to

further investigate this suggestion, future studies would do well to assess a range of electrocutaneous stimulation intensities such as pain threshold, half way between pain threshold and pain tolerance and pain tolerance in the same subjects.

Based on the suggestions made above regarding the possibility that different pain stimulation modalities may be modulated differently, future studies would do well to directly compare the cardiac cycle-related modulation of electrocutaneous and laser stimulation at the same relative intensity in the same participants. This potential difference would be best investigated for both a perceptual measure of pain (i.e. pain threshold) and objective measures of pain (i.e. PREPs) to provide a fuller picture regarding the potential factors influencing the cardiac cycle modulation effects. Additionally, as is suggested above, different electrocutaneous electrodes may activate different proportions of A-δ nociceptive fibres and A-β sensory fibres and thus may be modulated differently across the cardiac cycle. Studies would be advised to directly compare the cardiac cycle related modulation of pain perception and PREPs for stimuli delivered via a bar electrode and a concentric planar electrode (Kaube et al., 2000).

Studies 1 and 2 suggest that tonic BP may be an important moderator of cardiac cycle effects and previous research suggests a linear relationship between increasing BP levels and decreasing pain (France, 1999). Therefore, although there have already been a few studies investigating differences in cardiac cycle effects in hypertensives versus normotensives (e.g. Edwards et al., 2007) and in hypotensives vs. normotensives using mechanical baroreceptor stimulation (Angrilli, Mini, Mucha, & Rau, 1997), future research would be recommended to look at the cardiac cycle effects on pain and tactile thresholds in a wider range of tonic BPs including hypotensives, a spectrum of normotensives, pre-hypertensives and clinical hypertensives.

Finally, having suggested that it would be reasonable to potentially expect a lateralisation of cardiac cycle effects, although we did not find a lateralisation effect for PREPs (Study 4), as we did not originally find a cardiac cycle modulation of PREPs (Study 3) this may, as discussed above relate to methodological factors.

However, as we did report a cardiac cycle effect for pain thresholds, future research would be recommended to determine if cardiac cycle-related modulation of pain thresholds may be a lateralised phenomenon.

REFERENCES

Absi, M. A. & Rokke, P. D. (1991). Can anxiety help us tolerate pain? *Pain* 46, 43-51.

Aiken, L. S., & West, S. G. (1991). *Multiple regression: Testing and interpreting interactions.* Newbury Park, CA: Sage.

Angell James, J. E. (1971). Effects of altering mean pressure, pulse pressure and pulse frequency on impulse activity in baroreceptor fibres from aortic arch and right subclavian artery in rabbit. *Journal of Physiology, 214*(1), 65-88.

Angrilli, A., Mini, A., Mucha, R. F., & Rau, H. (1997). The influence of low blood pressure and baroreceptor activity on pain responses. *Physiology & Behavior, 62*(2), 391-397.

Arendt-Nielsen, L., & Chen, A. C. (2003). Lasers and other thermal stimulators for activation of skin nociceptors in humans. *Clinical Neurophysiology, 33*(6), 259-268.

Bingel, U., Herken, W., Teutsch, S., & May, A. (2008). Habituation to painful stimulation involves the antinociceptive system-a 1-year follow-up of 10 participants. *Pain, 140*(2), 393-394. doi:10.1016/j.pain.2008.09.030

Bingel, U., Schoell, E., Herken, W., Buchel, C., & May, A. (2007). Habituation to painful stimulation involves the antinociceptive system. *Pain, 131*(1-2), 21-30. doi:10.1016/j.pain.2006.12.005

Birren, J. E., Phillips, S. L., & Cardon, P. V. (1963). Reaction time as a function of cardiac cycle in young adults. *Science, 140*(356), 195-196.

Bristow, J. D., Honour, A. J., Pickerin.Gw, Sleight, P., & Smyth, H. S. (1969). Diminished baroreflex sensitivity in high blood pressure. *Circulation, 39*(1), 48.

Bromm, B., & Chen, A. C. (1995). Brain electrical source analysis of laser evoked potentials in response to painful trigeminal nerve stimulation. *Electroencephalography and Clinical Neurophysiology, 95*(1), 14-26.

Bromm, B., & Treede, R. D. (1987). Human cerebral potentials evoked by CO2 laser stimuli causing pain. *Experimental Brain Research, 67*(1), 153-162.

Bromm, B., & Treede, R. D. (1991). Laser-evoked cerebral potentials in the assessment of cutaneous pain sensitivity in normal subjects and patients. *Revue Neurologique, 147*(10), 625-643.

Bushnell M, C., & Apkarian, A,V,. (2006). Representation of pain in the brain. In S. McMahon B, & M. Koltzenburg (Eds.), (5th ed., pp. 107-124). Philadelphia: Elsevier.

Carmon, A., Mor, J., & Goldberg, J. (1976). Evoked cerebral responses to noxious thermal stimuli in humans. *Experimental Brain Research, 25*(1), 103-107.

Carmon, A., Dotan, Y., & Sarne, Y. (1978). Correlation of subjective pain experience with cerebral evoked responses to noxious thermal stimulations. *Experimental Brain Research, 33*(3-4), 445-453.

Carter, L. E., McNeil, D. W., Vowles, K. E., Sorrell, J. T., Turk, C. L., Ries, B. J. & Hopko, D. R. (2002). Effects of emotion on pain reports, tolerance and physiology. *Pain Research and Management*, 7, 21-30.

Chen, A. C. N., Arendt-Nielsen, L., & Plaghki, L. (1998). Laser-evoked potentials in human pain: I. use and possible misuse. *Pain Forum, 7*(4), 174-184. doi:10.1016/S1082-3174(98)70001-7

Craig, A. D. (2002). Pain mechanisms: Labeled lines versus convergence in central processing. *Annual Review of Neuroscience, 26*, 1-30.

Devinsky, O, & D'Esposito, M. (2004). *Neurology of Cognitive and Behavioral Disorders*. Oxford University Press: New York.

Dickens, C., McGowan, L. & Dale, S. (2003). Impact of Depression on Experimental Pain Perception: A Systematic Review of the Literature with Meta-Analysis. *Psychosomatic Medicine*, 65, 369–375.

Droste, C., Kardos, A., Brody, S., Greenlee, M. W., Roskamm, H., & Rau, H. (1994). Baroreceptor stimulation - pain perception and sensory thresholds. *Biological Psychology, 37*(2), 101-113.

Eckberg, D. L., & Sleight, P. (1992). *Human baroreflexes in health and disease*. Oxford: Clarendon Press.

Edwards, L., Inui, K., Ring, C., Wang, X. H., & Kakigi, R. (2008a). Pain-related evoked potentials are modulated across the cardiac cycle. *Pain, 137*(3), 488-494. doi:10.1016/j.pain.2007.10.010 ER

Edwards, L., McIntyre, D., Carroll, D., Ring, C., France, C. R., & Martin, U. (2003). Effects of artificial and natural baroreceptor stimulation on nociceptive responding and pain. *Psychophysiology, 40*(5), 762-769.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology, 39*(5), 678-681. doi:10.1017/S0048577202011770 ER

Edwards, L., Ring, C., McIntyre, D., & Carroll, D. (2001). Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology, 38*(4), 712-718.

Edwards, L., Ring, C., McIntyre, D., Carroll, D., & Martin, U. (2007). Psychomotor speed in hypertension: Effects of reaction time components, stimulus modality,

and phase of the cardiac cycle. *Psychophysiology, 44*(3), 459-468. doi:10.1111/j.1469-8986.2007.00521.x ER

Edwards, L., Ring, C., France, C. R., McIntyre, D., & Martin, U. (2008b). Effects of opioid blockade on nociceptive flexion reflex thresholds and nociceptive responding in hypertensive and normotensive individuals. *International Journal of Psychophysiology, 69*(2), 96-100. doi:10.1016/j.ijpsycho.2008.03.005 ER

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods, 41*(4), 1149-1160. doi:10.3758/BRM.41.4.1149; 10.3758/BRM.41.4.1149

Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B., & Riley, J. L. (2009). Sex, gender, and pain: A review of recent clinical and experimental findings. *Journal of Pain, 10*(5), 447-485. doi:10.1016/j.jpain.2008.12.001 ER

France, C. R. (1999). Decreased pain perception and risk for hypertension: Considering a common physiological mechanism. *Psychophysiology, 36*(6), 683-692.

Gescheider, G. A., Bolanowski, S. J., Hall, K. L., Hoffman, K. E., & Verrillo, R. T. (1994). The effects of aging on information-processing channels in the sense of touch: I. absolute sensitivity. *Somatosensory & Motor Research, 11*(4), 345-357.

Ghione, S. (1996). Hypertension-associated hypalgesia - evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension, 28*(3), 494-504.

Ghione, S., Rosa, C., Panattoni, E., Nuti, M., Mezzasalma, L., & Giuliano, G. (1985). Comparison of sensory and pain threshold in tooth pulp stimulation in normotensive man and essential hypertension. *Journal of*

Hypertension.Supplement : Official Journal of the International Society of Hypertension, 3(3), S113-5.

Gracely, R. H. (2006). Studies of pain in human subjects. In S. McMahon B, & M. Koltzenburg (Eds.), *Wall and Melzack's textbook of pain* (5th ed., pp. 267-290). Philadelphia: Elsevier.

Gray, M. A., Minati, L., Paoletti, G., & Critchley, H. D. (2010). Baroreceptor activation attenuates attentional effects on pain-evoked potentials. *Pain, 151*(3), 853-861. doi:10.1016/j.pain.2010.09.028

Greffrath, W., Baumgartner, U., & Treede, R. D. (2007). Peripheral and central components of habituation of heat pain perception and evoked potentials in humans. *Pain, 132*(3), 301-311. doi:10.1016/j.pain.2007.04.026 ER

Gribbin, B., Pickerin.Tg, Sleight, P., & Peto, R. (1971). Effect of age and high blood pressure on baroreflex sensitivity in man. *Circulation Research, 29*(4), 424-&.

Gybels, J., Handwerker, H. O., & Van Hees, J. (1979). A comparison between the discharges of human nociceptive nerve fibres and the subject's ratings of his sensations. *The Journal of Physiology, 292*, 193-206.

Helzner, E. P., Cauley, J. A., Pratt, S. R., Wisniewski, S. R., Zmuda, J. M., Talbott, E. O., . . . Newman, A. B. (2005). Race and sex differences in age-related hearing loss: The health, aging and body composition study. *Journal of the American Geriatrics Society, 53*(12), 2119-2127. doi:10.1111/j.1532-5415.2005.00525.x

Hemphill, R. E., & Crookes, T. G. (1952). A preliminary report on fatigue and pain tolerances in depressives and psychoneurotic patients. *Journal of Mental Science,* 98, 433–40.

Hugon, M. (1973). Exteroreceptive reflexes to stimulation of the sural nerve in normal man. In J. E. Desmedt (Ed.), *New developments in electromyography and clinical neurophysiology* (pp. 713-729). Basel: Karger.

Iannetti, G. D., Hughes, N. P., Lee, M. C., & Mouraux, A. (2008). Determinants of laser-evoked EEG responses: Pain perception or stimulus saliency? *Journal of Neurophysiology, 100*(2), 815-828. doi:10.1152/jn.00097.2008

James, J. E. & Hardardottir, D. (2002). Influence of attention focus and trait anxiety on tolerance of acute pain. *British Journal of Health Psychology*, 7,149-162.

Jensen, M. P., Chen, C., & Brugger, A. M. (2003). Interpretation of visual analog scale ratings and change scores: A reanalysis of two clinical trials of postoperative pain. *The Journal of Pain, 4*(7), 407-414.

Jones, A., Spindler, H., Jorgensen, M. M. & Zachariae, R. (2002). The effect of situation-evoked anxiety and gender on pain report using the cold pressor test. *Scandinavian Journal of Psychology*, 43, 307-313.

Kakigi, R., Shibasaki, H., & Ikeda, A. (1989). Pain-related somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology, 74*(2), 139-146.

Kanda, M., Fujiwara, N., Xu, X., Shindo, K., Nagamine, T., Ikeda, A., & Shibasaki, H. (1996). Pain-related and cognitive components of somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalography and Clinical Neurophysiology, 100*(2), 105-114.

Kanda, M., Shindo, K., Xu, X., Fujiwara, N., Ikeda, A., Nagamine, T., & Shibasaki, H. (1999). Cortical mechanisms underlying point localization of pain spot as studied

by event-related potentials following CO2 laser stimulation in man. *Experimental Brain Research, 127*(2), 131-140.

Kappenman, E. S., & Luck, S. J. (2010). The effects of electrode impedance on data quality and statistical significance in ERP recordings. *Psychophysiology, 47*(5), 888-904. doi:10.1111/j.1469-8986.2010.01009.x

Katsarava, Z., Ayzenberg, I., Sack, F., Limmroth, V., Diener, H. C., & Kaube, H. (2006). A novel method of eliciting pain-related potentials by transcutaneous electrical stimulation. *Headache, 46*(10), 1511-1517. doi:10.1111/j.1526-4610.2006.00446.x ER

Kaube, H., Katsarava, Z., Kaufer, T., Diener, H. C., & Ellrich, J. (2000). A new method to increase nociception specificity of the human blink reflex. *Clinical Neurophysiology, 111*(3), 413-416.

Korner, P. I., West, M. J., Shaw, J., & Uther, J. B. (1974). "Steady-state" properties of the baroreceptor-heart rate reflex in essential hypertension in man. *Clinical and Experimental Pharmacology & Physiology, 1*(1), 65-76.

Lautenbacher, S. & Krieg, J. C. (1994). Pain perception in psychiatric disorders: a review of the literature. *Journal of Psychiatric Research*, 28, 109–22.

LeBlanc, J., & Potvin, P. (1966). Studies on habituation to cold pain. *Canadian Journal of Physiology and Pharmacology, 44*(2), 287-293.

Lefaucheur, J. P., Ahdab, R., Ayache, S. S., Lefaucheur-Menard, I., Rouie, D., Tebbal, D., . . . Ciampi de Andrade, D. (2012). Pain-related evoked potentials: A comparative study between electrical stimulation using a concentric planar electrode and laser stimulation using a CO2 laser. *Clinical Neurophysiology, 42*(4), 199-206. doi:10.1016/j.neucli.2011.12.003

Levitt, H. (1971). Transformed up-down methods in psychoacoustics. *Journal of the Acoustical Society of America, 49*(2), 467.

Mancia, G., & Mark, A. L. (1983). Arterial baroreflexes in humans. In J. T. Shepherd, & F. M. Abboud (Eds.), *Handbook of physiology: The cardiovascular system* (pp. 755-793). Bethesda, Maryland: American Physiological Society.

Martins, A., Q, Ring, C., McIntyre, D., Edwards, L., & Martin, U. (2009). Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. *Pain, 147*(1-3), 84-90. doi:10.1016/j.pain.2009.08.016

McIntyre, D., Edwards, L., Ring, C., Parvin, B., & Carroll, D. (2006). Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology, 43*(3), 314-319. doi:10.1111/j.1469-8986.2006.00407.x ER

McIntyre, D., Kavussanu, M., & Ring, C. (2008a). Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. *Pain, 137*(3), 550-555. doi:10.1016/j.pain.2007.10.018 ER

Mcintyre, D., Ring, C., Edwards, L., & Carroll, D. (2008b). Simple reaction time as a function of the phase of the cardiac cycle in young adults at risk for hypertension. *Psychophysiology, 45*(2), 333-336. doi:10.1111/j.1469-8986.2007.00619.x ER

McIntyre, D., Ring, C., Hamer, M., & Carroll, D. (2007). Effects of arterial and cardiopulmonary baroreceptor activation on simple and choice reaction times. *Psychophysiology, 44*(6), 874-879. doi:10.1111/j.1469-8986.2007.00547.x

Meyer, R. A., Ringkamp, M., Campbell, J. N., & Raja, S. N. (2006). Peripheral mechanisms of cutaneous nociception. In S. B. McMahon, & M. Koltzenburg (Eds.), *Wall and Melzack's textbook of pain* (5th ed., pp. 3-34). Philadelphia: Elsevier.

Meyer, R. A., Walker, R. E., & Mountcastle, V. B.,Jr. (1976). A laser stimulator for the study of cutaneous thermal and pain sensations. *IEEE Transactions on Bio-Medical Engineering, 23*(1), 54-60.

Michelotti, A., Farella, M., Tedesco, A., Cimino, R. & Martina, R. (2000). Changes in pressure-pain thresholds of the jaw muscles during a natural stressful condition in a group of symptom-free subjects. *Journal of Orofacial Pain*, 14, 279-285.

Milne, R. J., Kay, N. E., & Irwin, R. J. (1991). Habituation to repeated painful and non-painful cutaneous stimuli: A quantitative psychophysical study. *Experimental Brain Research, 87*(2), 438-444.

Miyazaki, M., Shibasaki, H., Kanda, M., Xu, X., Shindo, K., Honda, M., . . . Kimura, J. (1994). Generator mechanism of pain-related evoked potentials following CO2 laser stimulation of the hand: Scalp topography and effect of predictive warning signal. *Journal of Clinical Neurophysiology, 11*(2), 242-254.

Parmer, R. J., Cervenka, J. H., & Stone, R. A. (1992). Baroreflex sensitivity and heredity in essential hypertension. *Circulation, 85*(2), 497-503.

Perchet, C., Frot, M., Charmarty, A., Flores, C., Mazza, S., Magnin, M., & Garcia-Larrea, L. (2012). Do we activate specifically somatosensory thin fibres with the concentric planar electrode? A scalp and intracranial EEG study. *Pain, 153*(6), 1244-1252. doi:10.1016/j.pain.2012.03.004

Peyron, R., Garcia-Larrea, L., Gregoire, M. C., Convers, P., Richard, A., Lavenne, F., . . . Laurent, B. (2000). Parietal and cingulate processes in central pain. A combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) study of an unusual case. *Pain, 84*(1), 77-87.

Plaghki, L., & Mouraux, A. (2003). How do we selectively activate skin nociceptors with a high power infrared laser? physiology and biophysics of laser stimulation. *Clinical Neurophysiology, 33*(6), 269-277.

Radloff, L. S. (1977). The center for epidemiologic studies depression scale a self report depression scale for research in the general population. *Applied Psychological Measurement,* 1(3), 385-401.

Rainville, P. (2002). Brain mechanisms of pain affect and pain modulation. *Current Opinion in Neurobiology, 12*(2), 195-204.

Randall, O., Esler, M., Culp, B., Julius, S., & Zweifler, A. (1978). Determinants of baroreflex sensitivity in man. *The Journal of Laboratory and Clinical Medicine, 91*(3), 514-519.

Randall, O. S., Esler, M. D., Bulloch, E. G., Maisel, A. S., Ellis, C. N., Zweifler, A. J., & Julius, S. (1976). Relationship of age and blood pressure to baroreflex sensitivity and arterial compliance in man. *Clinical Science and Molecular Medicine.Supplement, 3*, 357s-360s.

Rau, H., Elbert, T., & Birbaumer, N. (1995). Baroreceptor activity and nociception. In D. Vaitl, & R. Schandry (Eds.), *From the heart to the brain: The psychophysiology of circulation brain interaction.* (pp. 151-168). Frankfurt: Peter Lang.

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., & Willer, J. C. (2005). The lower limb flexion reflex in humans. *Progress in Neurobiology, 77*(6), 353-395. doi:10.1016/j.pneurobio.2005.11.003 ER

Schiffman, S. S. (1997). Taste and smell losses in normal aging and disease. *JAMA : The Journal of the American Medical Association, 278*(16), 1357-1362.

Schulz, A., Reichert, C. F., Richter, S., Lass-Hennemann, J., Blumenthal, T. D., & Schaechinger, H. (2009). Cardiac modulation of startle: Effects on eye blink and higher cognitive processing. *Brain and Cognition, 71*(3), 265-271. doi:10.1016/j.bandc.2009.08.002

Sleight, P., Robinson, J. L., Brooks, D. E., & Rees, P. M. (1977). Characteristics of single carotid sinus baroreceptor fibers and whole nerve activity in the normotensive and the renal hypertensive dog. *Circulation Research, 41*(6), 750-758.

Spielberger, C. D. *Anxiety. Current Trends in Theory and Research: Vol1*. New York, NY: Academic Press, 1972.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *Manual for the state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists Press, 1970.

Spielberger, C. D. & Rickman, R. L. Assessment of state and trait anxiety in cardiovascular disorders (pp. 73–92). In: Byrne DG, Rosenman RH (editors). *Anxiety and the heart*. New York: Hemisphere Publishing Corporation, 1990.

Stevens, J. C., & Cruz, L. A. (1996). Spatial acuity of touch: Ubiquitous decline with aging revealed by repeated threshold testing. *Somatosensory & Motor Research, 13*(1), 1-10.

Stewart, J. C., France, C. R., & Suhr, J. A. (2006). The effect of cardiac cycle phase on reaction time among individuals at varying risk for hypertension. *Journal of Psychophysiology, 20*(1), 1-8. doi:10.1027/0269-8803.20.1.1 ER

Strempel, H. (1976). Adaptive modifications of cold pain. *European Journal of Applied Physiology and Occupational Physiology, 36*(1), 19-25.

Strempel, H. (1978). Adaptive modifications of cold pain. III. communication: Short-term experiments with 1-min-intervals. *European Journal of Applied Physiology and Occupational Physiology, 39*(1), 63-72.

Symonds, L. L., Gordon, N. S., Bixby, J. C., & Mande, M. M. (2006). Right-lateralized pain processing in the human cortex: An FMRI study. *Journal of Neurophysiology, 95*(6), 3823-3830. doi:10.1152/jn.01162.2005

Takekuma, K., Ando, F., Niino, N., & Shimokata, H. (2000). Age and gender differences in skin sensory threshold assessed by current perception in community-dwelling japanese. *Journal of Epidemiology, 10*(1 Suppl), S33-8.

Tarkka, I. M., & Treede, R. D. (1993). Equivalent electrical source analysis of pain-related somatosensory evoked potentials elicited by a CO2 laser. *Journal of Clinical Neurophysiology, 10*(4), 513-519.

Tang, J. & Gibson, S.J. (2005). A Psychophysical Evaluation of the Relationship Between Trait Anxiety, Pain Perception, and Induced State Anxiety. The Journal of Pain, 6,(9), 612-619.

Treede, R. D., Kief, S., Holzer, T., & Bromm, B. (1988). Late somatosensory evoked cerebral potentials in response to cutaneous heat stimuli. *Electroencephalography and Clinical Neurophysiology, 70*(5), 429-441.

Valeriani, M., Rambaud, L., & Mauguiere, F. (1996). Scalp topography and dipolar source modelling of potentials evoked by CO2 laser stimulation of the hand. *Electroencephalography and Clinical Neurophysiology, 100*(4), 343-353.

Walker, B. B., & Sandman, C. A. (1982). Visual evoked-potentials change as heart-rate and carotid pressure change. *Psychophysiology, 19*(5), 520-527.

Weale, R. A. (1986). Aging and vision. *Vision Research, 26*(9), 1507-1512.

Weisz, J., & Adam, G. (1996). The influence of cardiac phase on reaction time depending on heart period length and on stimulus and response laterality. *Psychobiology, 24*(2), 169-175.

Wilkinson, M., McIntyre, D., & Edwards, L. (2013). Electrocutaneous pain thresholds are higher during systole than diastole. *Biological Psychology, 94*(1), 71-73. doi:10.1016/j.biopsycho.2013.05.002

Willer, J. C. (1977). Comparative-study of perceived pain and nociceptive flexion reflex in man. *Pain, 3*(1), 69-80.

Xu, X., Kanda, M., Shindo, K., Fujiwara, N., Nagamine, T., Ikeda, A., . . . Shibasaki, H. (1995). Pain-related somatosensory evoked potentials following CO2 laser stimulation of foot in man. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section, 96*(1), 12-23. doi: 10.1016/0013-4694(94)00223-8

APPENDIX A

Published Abstracts & Papers

ELECTROCUTANEOUS PAIN THRESHOLDS ARE HIGHER DURING SYSTOLE THAN DIASTOLE

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The nociceptive flexion reflex and pain-related evoked potentials have been shown to be dampened during the systolic phase of the cardiac cycle compared to diastole. The impact of the cardiac cycle on pain perception is less clear. The current study examined the effects of natural variations in blood pressure (BP) across the cardiac cycle on pain thresholds in 49 healthy adults. Pain thresholds were determined concurrently at 7 cardiac cycle intervals (*R*-wave plus 0, 100, 200, 300, 400, 500 and 600 ms) using an interleaved up-down staircase procedure. Electrocutaneous stimuli were delivered to the back of the hand using a concentric planar electrode. After each stimulation participants indicated the presence or absence of pain using a response box. Repeated-measures ANOVA revealed variations in pain thresholds across the cardiac cycle ($p = .002$); pain thresholds were higher mid-cycle compared to early and late cycle. Further analyses, using BP median splits, revealed that only participants with low-normal systolic BP ($p = .002$), diastolic BP ($p = .0005$) and mean arterial pressure ($p = .004$) displayed this cardiac cycle-related pain modulation. The present study provides preliminary evidence that pain perception, at least at threshold levels, is attenuated during systole compared to diastole. Further, these data suggest that tonic BP may have a moderating effect on cardiac cycle-related pain modulation. The current findings provide further support for the hypothesis that natural variations in arterial baroreceptor activity across the cardiac cycle influence pain.

EFFECTS OF THE CARDIAC CYCLE ON ELECTROCUTANEOUS PAIN RELATED EVOKED POTENTIALS

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Natural variations in blood pressure (BP) across the cardiac cycle have been shown to modulate pain. A recent study reported dampened N2 and N2-P2 laser evoked potential amplitudes at Cz during systole compared to diastole in men. The current study examined the effects of natural variations in BP across the cardiac cycle on electrocutaneous pain-related evoked potentials (PREPs) using multi-channel recordings in 10 men and 10 women. Following determination of pain thresholds using an up-down staircase method, PREPs were elicited at seven cardiac cycle intervals (*R*-wave plus 50, 150, 250, 350, 450, 550, 650 ms). Electrocutaneous stimuli equal to twice individual pain threshold were pseudorandomly delivered to the right index finger in 7 blocks of 21 trials. Separate repeated-measures ANOVAs revealed no variation in N2, P2 or N2-P2 amplitudes across the cardiac cycle at Cz (all $p > .05$) or C3, a recording site considered to cover the contralateral primary and secondary somatosensory cortices (all $p > .05$). Median BP splits were used to examine tonic BP effects on the cardiac cycle-related modulation of PREPs. Separate 2 Group (low-normal BP, high-normal BP) by 7 Interval repeated-measures ANOVAs for systolic and diastolic BP revealed no Group or Interaction effects for N2, P2 or N2-P2 amplitudes at Cz or C3 (all $p > .05$). Similar 2 Sex by 7 Interval ANOVAs revealed no Group or Interaction effects for N2, P2 or N2-P2 amplitudes at Cz or C3 (all $p > .05$). These data suggest the modality of pain stimulation may influence the cardiac cycle-related modulation of pain processing.

Electrocutaneous pain thresholds are higher during systole than diastole

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Abstract

Arterial baroreceptors may modulate pain. Evidence suggests the neurophysiological correlates of pain are dampened during systole, when baroreceptors are stimulated, compared to diastole, when stimulation is minimal. However, the influence of the cardiac cycle on perception of pain remains unclear. This study examined pain thresholds in 49 healthy adults at seven intervals after the *R*-wave of the electrocardiogram, using an interleaved up-down staircase procedure. Electrocutaneous stimuli were delivered to the hand and participants indicated the presence or absence of pain. Pain thresholds were higher mid-cycle, indicative of pain attenuation during systole compared to diastole. Analyses using blood pressure median splits revealed only participants with low systolic blood pressure displayed cardiac cycle modulation of pain, suggesting that tonic blood pressure may moderate cardiac cycle-related pain modulation. These findings suggest fluctuations in arterial baroreceptor activity across the cardiac cycle may influence pain in normotensive individuals.

Descriptors: Baroreceptor; Blood pressure; Cardiac cycle; Pain threshold

1. Introduction

A baroreceptor mechanism may account for hypertensive hypoalgesia (France and Ditto, 1996). Arterial baroreceptors are stimulated during the systolic phase of the cardiac cycle and show a pulsatile discharge (Eckberg and Sleight, 1992; Coleridge et al., 1987). Studies investigating the influence of the cardiac cycle on neurophysiological correlates of pain have reported dampening of the nociceptive flexion reflex (NFR) (Edwards et al., 2001; 2002; 2003; McIntyre et al., 2006; 2008) and reduced amplitude pain-related evoked potentials (PREPs) (Edwards et al., 2008) for stimuli delivered during systole compared to diastole.

The subjective nature of pain is different to its neurophysiological correlates (Chen et al., 1998; Iannetti et al., 2008; Sandrini et al., 2005). Some cardiac cycle studies concurrently measured the NFR or PREPs and pain but found no cardiac cycle-related pain modulation (Edwards et al., 2001; 2002; 2003; 2008). Crucially, these studies (Edwards et al., 2001; 2002; 2003; 2008) were not specifically designed to investigate pain modulation with some presenting stimuli at intensities relative to NFR thresholds that were not necessarily painful (Edwards et al., 2001; 2002; 2003), or, having employed painful stimuli, used constant stimulus intensities that may have caused participant disengagement with the task (Edwards et al. 2008). Although one study (Martins et al., 2009) reported pain increasing during systole, methodological differences may explain this unexpected result (see Discussion). The current study examined pain thresholds across the cardiac cycle and comprised several methodological features, including (a) a stimulating electrode that more selectively stimulates Aδ fibres (Katsarava et al., 2006; Kaube et al., 2000), (b) pain intensities focussed at threshold levels to minimise physiological arousal, and (c) variable stimulus intensities to limit participant disengagement. It was hypothesised that pain thresholds would be higher during systole than diastole.

2. Methods

2.1 Participants

Forty nine healthy adults (10 men, 39 women) were tested with a mean (*SD*) age of 27.98 (11.6) years, resting systolic blood pressure (SBP) of 116.2 (11.3) mmHg, diastolic blood pressure (DBP) of 70.6 (11.2) mmHg, and heart rate (HR) of 71.2 (11.8) bpm. Individuals with HRs ≥100 bpm were excluded from the study. Participants refrained from analgesics for 24 hrs and caffeine, nicotine and vigorous exercise for 2 hrs before testing. Loughborough University ethics committee approved the study and participants provided written consent.

2.2 Apparatus and Measurements

Resting blood pressure (BP) and HR were obtained using an oscillometric sphygmomanometer (705-IT, Omron). An electrocardiogram (ECG) was recorded using a modified chest configuration (see Edwards et al., 2001). Electrocutaneous stimuli (triple 0.5 ms monopolar square-wave pulse at 200Hz) were delivered via a concentric planar electrode (Kaube et al., 2000), attached to the dorsal surface of the right hand between the metacarpals of the index and middle fingers. Participants sat upright with their hand on a response box with "Yes" and "No" buttons. A computer programmed with Spike2 (CED) recorded responses and presented stimuli using a Micro1401 II (CED).

2.3 Procedure

Following three baseline BP and HR measurements, participants first completed a sensory threshold assessment (not reported here) and, following attachment of the concentric stimulating electrode, completed the pain threshold assessment.

The pain threshold assessment determined pain thresholds at seven intervals after the *R*-wave of the ECG (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms). Thresholds were determined concurrently by interleaving seven up-down staircases (Levitt, 1971). During each trial the participant's hand was stimulated at one of the seven *R*-wave intervals following which participants

pressed the "*Yes*" button if they perceived the stimulation as painful or the "*No*" button otherwise. For each staircase, stimulation intensity increased from 0 mA in 1 mA steps until the participant first reported pain. Stimulus intensity then decreased in 0.4 mA steps until the stimulus was not painful then continued in 0.1 mA steps until the seven staircases completed two further ascending and descending series. Pain threshold (mA) was defined as the average of the peaks during the second and third series of each staircase.

2.4 Data Reduction and Analyses

The BP and HR readings were averaged to provide measures of tonic SBP, DBP and HR. Repeated measures analysis of variance (ANOVA) with *R*-wave to stimulation interval (*R*+0, *R*+100, *R*+200, *R*+300, *R*+400, *R*+500, *R*+600 ms) as a within-subjects factor were performed on pain thresholds. To examine the effect of tonic BP on cardiac cycle-related pain thresholds, participants were divided into low-normal and high-normal BP groups based on SBP median split. Separate 2 BP Group (low, high) × 2 Sex × 7 Interval repeated measures ANOVAs were performed on pain thresholds. Sex was included as a factor because men typically have higher BPs and women have greater pain sensitivity (Fillingim et al., 2009). ANOVAs were corrected with Huynh-Feldt correction (ε). Significant results were followed by Newman-Keuls post hoc comparisons and planned orthogonal comparisons. Partial eta-squared (η_p^2) is reported. A significance level of .05 was adopted.

3. Results

Repeated measures ANOVA revealed significant variation in pain thresholds across the cardiac cycle, $\varepsilon = .90$, $F(5.40, 259.21) = 3.74$, $p = .002$, $\eta_p^2 = .072$. Post hoc comparisons confirmed pain thresholds were higher at *R*+200 and *R*+300 ms than *R*+100 and *R*+500 ms and pain thresholds at *R*+300 ms were higher than *R*+600 ms (see Figure 1). Planned orthogonal comparisons revealed

significant quartic, $F(1, 48) = 12.95$, $p = .001$, $\eta_p^2 = .212$, and quadratic, $F(1, 48) = 5.19$, $p = .027$, $\eta_p^2 = .097$, trends.

Median SBP was 116.00 mmHg; accordingly the low-SBP group comprised 25 participants (Mean = 108.03, *SD* = 5.67 mmHg) and the high-SBP group comprised 24 participants (Mean = 124.72, *SD* = 9.15 mmHg). A 2 Group × 2 Sex × 7 Interval ANOVA revealed no Group effect. Mean (*SD*) pain thresholds were 2.11 (1.47) mA and 2.40 (1.77) mA in the low- and high-SBP groups, respectively. However, a Group × Interval interaction was found, $\varepsilon = .946$, $F(5.67, 255.34) = 2.23$, $p = .04$, $\eta_p^2 = .047$ (see Figure 2). Analysis also revealed a main effect for Sex, $F(1, 45) = 5.86$, $p = .02$, $\eta_p^2 = .115$. Mean (*SD*) pain thresholds were 3.32 (2.50) for men and 1.98 (1.20) mA for women.

To further investigate the differing pattern of cardiac cycle modulation between SBP groups, 7 Interval repeated measures ANOVAs were conducted separately for each SBP group. Pain thresholds varied across the cardiac cycle in the low-SBP group, $\varepsilon = .77$, $F(4.59, 110.26) = 4.40$, $p = .002$, $\eta_p^2 = .155$, but not in the high-SBP group. Post-hocs for the low-SBP group revealed higher pain thresholds at *R*+300 ms than *R*+100, 500 and 600 ms and higher pain thresholds at *R*+200 ms than *R*+100 and *R*+500 ms. Planned comparisons indicated this modulation of pain thresholds was characterised by quadratic, $F(1, 24) = 6.11$, $p = .02$, $\eta_p^2 = .203$ and quartic, $F(1, 24) = 21.51$, $p = .0001$, $\eta_p^2 = .473$, terms.

4. Discussion

Pain thresholds were higher, indicating reduced pain, during systole compared to diastole. These findings are consistent with previous studies reporting dampening of the NFR (Edwards et al., 2001; 2002; 2003; McIntyre et al., 2006; 2008) and PREPs (Edwards et al., 2008) during systole compared to diastole. However, our findings conflict with Martins et al. (2009), who reported increased pain midcycle. Methodological differences may explain this discrepancy. In our study, stimulus intensities oscillated around pain threshold whilst Martins et al. presented stimuli between pain threshold and

pain tolerance. High stimulus intensities may induce physiological arousal, which has been shown to change pain perception and moderate midcycle NFR dampening (McIntyre et al., 2006). Indeed, elevated HRs, indicative of increased arousal, were reported by Martins and colleagues. Further, we used a stimulus electrode designed to more selectively stimulate Aδ nociceptive fibres compared to the electrode used by Martins and colleagues, which may also stimulate Aβ tactile fibres (Kaube et al., 2000). Indeed, lower cutaneous sensory thresholds have been reported during systole (Edwards et al., 2009). Accordingly, the current study may reflect a cardiac cycle modulation more specific to pain. Regardless, the temporal patterning of systolic pain inhibition found in our study is consistent with the hypothesis that pain is inhibited due to baroreceptor activation of pain inhibition pathways (Ghione, 1996).

Interestingly, no BP group differences in pain threshold were found in our study, counter to studies reporting an inverse BP-pain relationship within the normotensive range (see France, 1999). However, this relationship is not always evident (e.g., Edwards et al., 2002; France, 1999). Notably, our study revealed differences in cardiac cycle-related pain modulation between SBP groups, suggesting cardiac cycle-related pain modulation may be reduced at higher-normal SBPs. This finding may be accounted for by the amount of baroreceptor afferent activity reaching the pain inhibition pathways. Indeed, an inverse relationship between BP and both baroreceptor sensitivity (Sleight et al., 1977) and baroreflex sensitivity (Bristow et al., 1969; Gribbin et al., 1971) has been reported. Regardless of the mechanism, the current data provide preliminary evidence that tonic BP may influence the cardiac cycle-related pain modulation within the normotensive range.

REFERENCES

Bristow, J. D., Honour, A. J., Pickering. G.W., Sleight, P., & Smyth, H. S. (1969). Diminished baroreflex sensitivity in high blood pressure. *Circulation, 39*, 48-54.

Chen, A.C.N., Arendt-Nielsen, L., Plafhki, L. (1998). Laser-evoked potentials in human pain: I. Use and possible misuse. *Pain Forum 7*(4), 174-184.

Coleridge, H. M., Coleridge, J. C. G., & Schultz, H. D. (1987). Characteristics of C-fiber baroreceptors in the carotid-sinus of dogs. *Journal of Physiology - London, 394*, 291-313.

Eckberg, D. L., & Sleight, P. (1992). *Human baroreflexes in health and disease*. Oxford: Clarendon Press.

Edwards, L., Inui, K., Ring, C., Wang, X. H., & Kakigi, R. (2008). Pain-related evoked potentials are modulated across the cardiac cycle. *Pain, 137*, 488-494. doi:10.1016/j.pain.2007.10.010

Edwards, L., McIntyre, D., Carroll, D., Ring, C., France, C. R., & Martin, U. (2003). Effects of artificial and natural baroreceptor stimulation on nociceptive responding and pain. *Psychophysiology, 40*, 762-769.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology, 39*, 678-681. doi:10.1017/S0048577202011770

Edwards, L., Ring, C., McIntyre, D., & Carroll, D. (2001). Modulation of the human nociceptive flexion reflex across the cardiac cycle. *Psychophysiology, 38*, 712-718.

Edwards, L., Ring, C., McIntyre, D., Winer, J. B., & Martin, U. (2009). Sensory detection thresholds are modulated across the cardiac cycle: Evidence that cutaneous sensibility is greatest for systolic stimulation. *Psychophysiology, 46*, 252-256. doi:10.1111/j.1469-8986.2008.00769.x

Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B. & Riley, J.L 3rd. (2009). Sex, gender, and pain: A review of recent clinical and experimental findings. J Pain, 10, 447-85. doi: 10.1016/j.jpain.2008.12.001

France, C. R. (1999). Decreased pain perception and risk for hypertension: Considering a common physiological mechanism. *Psychophysiology, 36*, 683-692.

France, C. R., & Ditto, B. (1996). Risk for high blood pressure and decreased pain perception. *Current Directions in Psychological Science, 5*, 120-125.

Ghione, S. (1996). Hypertension-associated hypalgesia - evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension, 28*, 494-504.

Gribbin, B., Pickering, T.G., Sleight, P., & Peto, R. (1971). Effect of age and high blood pressure on baroreflex sensitivity in man. *Circulation Research, 29*, 424-431.

Iannetti, G.D., Hughes, N.P., Lee, M.C., Mouraux, A. (2008). Determinants of Laser-Evoked EEG Responses: Pain Perception or Stimulus Saliency? *Journal of Neurophysiology, 100*(2), 815-828.

Katsarava, Z., Ayzenberg, I., Sack, F., Limmroth, V., Diener, H. C., & Kaube, H. (2006). A novel method of eliciting pain-related potentials by transcutaneous electrical stimulation. *Headache, 46,* 1511-1517. doi:10.1111/j.1526-4610.2006.00446.x

Kaube, H., Katsarava, Z., Kaufer, T., Diener, H. C., & Ellrich, J. (2000). A new method to increase nociception specificity of the human blink reflex. *Clinical Neurophysiology, 111*, 413-416.

Levitt, H. (1971). Transformed up-down methods in psychoacoustics. *Journal of the Acoustical Society of America, 49*, 467-477.

Martins, A. Q, Ring, C., McIntyre, D., Edwards, L., & Martin, U. (2009). Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. *Pain, 147*, 84-90. doi: 10.1016/j.pain.2009.08.016

McIntyre, D., Edwards, L., Ring, C., Parvin, B., & Carroll, D. (2006). Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology, 43*, 314-319. doi:10.1111/j.1469-8986.2006.00407.x

McIntyre, D., Kavussanu, M., & Ring, C. (2008). Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. *Pain, 137*, 550-555. doi:10.1016/j.pain.2007.10.018

Sandrini, G., Serrao, M., Rossi, P., Romaniello, A., Cruccu, G., & Willer, J. C. (2005). The lower limb flexion reflex in humans. *Progress in Neurobiology, 77*, 353-395. doi:10.1016/j.pneurobio.2005.11.003

Sleight, P., Robinson, J.L., Brooks, D.E., Rees, P.M. (1977). Characteristics of single carotid sinus baroreceptor fibers and whole nerve activity in the normotensive and the renal hypertensive dog. *Circulation Research, 41*, 750-758.

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Figure Captions

Figure 1. Mean (*SE*) electrocutaneous pain thresholds as a function of phase of the cardiac cycle.

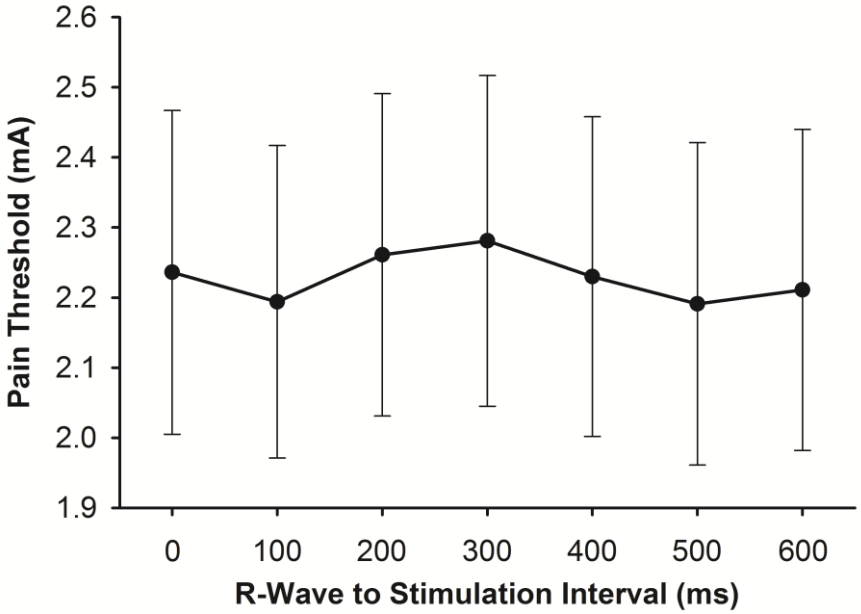
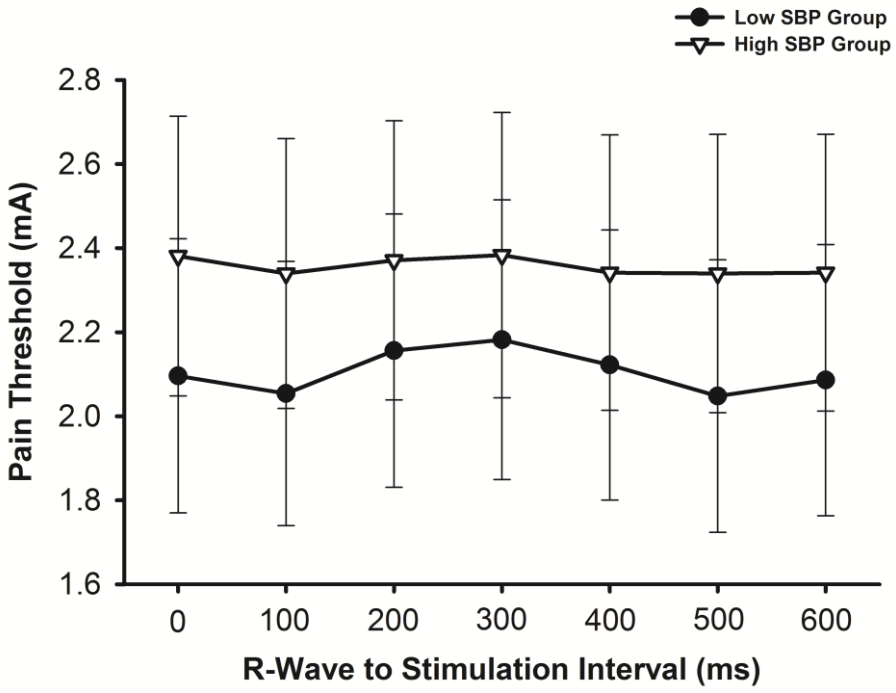


Figure 2. Mean (*SE*) electrocutaneous pain thresholds at seven intervals across the cardiac cycle as a function of systolic blood pressure (SBP).



APPENDIX B

Recruitment Material & Questionnaires

WANTED…

HEALTHY INDIVIDUALS AGED 18 - 65

FOR A STUDY INVESTIGATING HOW BLOOD PRESSURE INFLUENCES SENSATION

Can you spare just 1.5 hours & get information about your Blood Pressure, Heart Rate, Tactile & Pain thresholds?

WHAT WILL I HAVE TO DO?

1. Fill out some short questionnaires
2. Have your heart beat and blood pressure measured
3. Have your touch threshold assessed: we will assess when you first perceive the presence of a sensation
4. Have your pain threshold assessed: we will assess when you first perceive a pin-prick like sensation

You cannot participate if you have:

a chronic disease or major psychiatric disorder, an artificial cardiac pacemaker, you are pregnant or have missed your last menstrual cycle females), you currently take opiates or use any narcotic medication, you take prescription medication (excluding birth control), you drink >28 units/week if male, >21 units/week if female.

For more information contact **Mary Wilkinson** e-mail: m.j.wilkinson@lboro.ac.uk, School of Sport, Exercise and Health Sciences, Loughborough University

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Modulation of Tactile and Pain Thresholds across the Cardiac Cycle

INFORMED CONSENT FORM

(to be completed after Participant Information Sheet has been read)

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Advisory Committee.

I have read and understood the information sheet and this consent form.

I have had an opportunity to ask questions about my participation.

I understand that I am under no obligation to take part in the study.

I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

I understand that all the information I provide will be treated in strict confidence.

I agree to participate in this study.

Your name _____

Your signature _____

Signature of investigator _____

Date _____

Please address any complaints to the Secretary of the Loughborough University Ethical Advisory Committee (e-mail: m.r.coney@lboro.ac.uk).

Demographic / Health Behaviour / SES Questionnaire

Age _____ years

Sex Man ☐ Woman ☐

Ethnic Origin

African Caribbean	☐	Asian	☐
African	☐	Other Black	☐
White / UK	☐	White / Irish	☐
Other White	☐	Mixed Race	☐
		(Details: _____)	

Marital status

Single ☐
Married / Cohabiting ☐
Widowed / Divorced ☐

Smoking

Never smoked ☐
Used to smoke ☐
Currently smoke ☐
Cigarettes per day_____

How many units of alcohol do you drink in an average week? _____ units/week
(1 unit = 284 ml of beer, 125 ml of wine, or 25 ml of spirits)

How many units of alcohol would you usually drink in one session? _____

How many drinking sessions would you have in an average week? _____

For how many years were you in full-time education ? _____
(e.g., years in education starting from 1st year at primary school)

Centre for Epidemiologic Studies Depression (CES-D) Scale

Circle the number for each statement which best describes how often you felt or behaved this way **during the past week**.

	Rarely or none of the time (less than 1 day)	*Some or a little of the time (1 to 2 days)*	*Occasionally or a moderate amount of time (3 to 4 days)*	*Most or all of the time (5 to 7 days)*
During the past week…				
1. I was bothered by things that don't usually bother me	0	1	2	3
2. I did not feel like eating: my appetite was poor	0	1	2	3
3. I felt that I could not shake off the blues even with help from my family or friends	0	1	2	3
4. I felt that I was just as good as other people	0	1	2	3
5. I had trouble keeping my mind on what I was doing	0	1	2	3
6. I felt depressed	0	1	2	3
7. I felt that everything I did was an effort	0	1	2	3
8. I felt hopeful about the future	0	1	2	3
9. I thought my life had been a failure	0	1	2	3
10. I felt tearful	0	1	2	3
11. My sleep was restless	0	1	2	3
12. I was happy	0	1	2	3
13. I talked less than usual	0	1	2	3
14. I felt lonely	0	1	2	3
15. People are unfriendly	0	1	2	3
16. I enjoyed life	0	1	2	3
17. I had crying spells	0	1	2	3
18. I felt sad	0	1	2	3
19. I felt that people disliked me	0	1	2	3
20. I could not 'get going'	0	1	2	3

State Anxiety Inventory (title to be removed for participants)

DIRECTIONS: Please read the statements below and then circle the number that corresponds with how you feel right now, that is **at this moment**. There are no right or wrong answers. Use the following scale:

1 = Not at all
2 = Somewhat
3 = Moderately
4 = Very Much

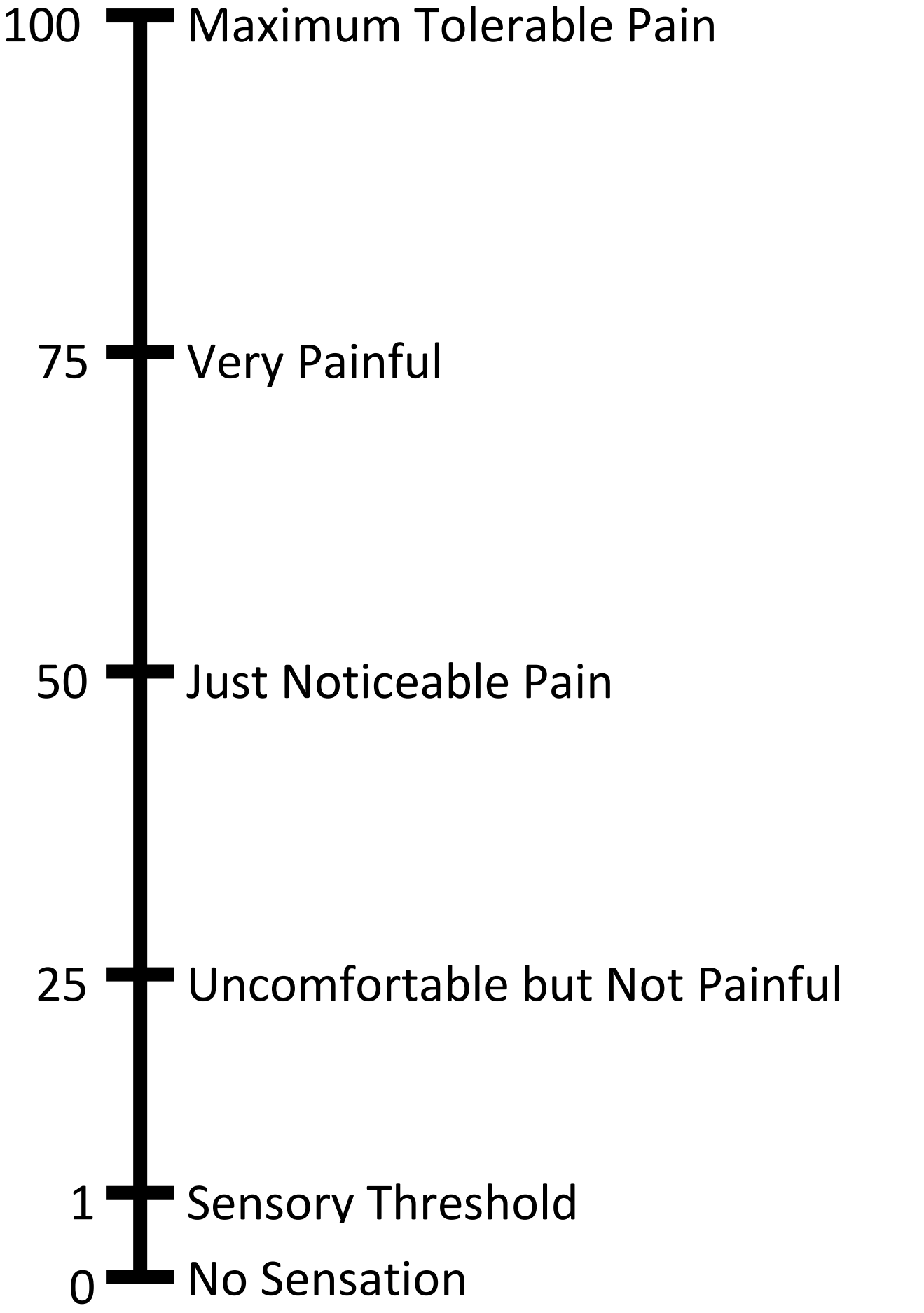
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I am regretful	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel rested	1	2	3	4
9. I feel anxious	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel “high strung”	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel over-excited and “rattled”	1	2	3	4
19. I feel joyful	1	2	3	4
20. I feel pleasant	1	2	3	4

Trait Anxiety Inventory (title to be removed for participants)

DIRECTIONS: Please read the statements below and then circle the number that corresponds with how you **generally** feel. There are no right or wrong answers. Use the following scale:

1 = Not at all 2 = Somewhat 3 = Moderately 4 = Very Much

1. I feel pleasant	1	2	3	4
2. I tire quickly	1	2	3	4
3. I feel like crying	1	2	3	4
4. I wish I could be as happy as others seem to be	1	2	3	4
5. I am losing out on things because I can't make up my mind soon enough	1	2	3	4
6. I feel rested	1	2	3	4
7. I am "calm, cool and collected"	1	2	3	4
8. I feel difficulties are piling up so that I cannot overcome them	1	2	3	4
9. I worry too much over something that doesn't really matter	1	2	3	4
10. I am happy	1	2	3	4
11. I am inclined to take things hard	1	2	3	4
12. I lack self-confidence	1	2	3	4
13. I feel secure	1	2	3	4
14. I try to avoid facing a crisis or difficulty	1	2	3	4
15. I feel blue	1	2	3	4
16. I am content	1	2	3	4
17. Some unimportant thoughts run through my mind & bother me	1	2	3	4
18. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
19. I am a steady person	1	2	3	4
20. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4


100
Maximum Tolerable Pain
75
Very Painful
50
Just Noticeable Pain
25
Uncomfortable but Not Painful
1
Sensory Threshold
0
No Sensation