THE IMPACT OF CARDIAC FEEDBACK ON HUMAN COGNITION

BY

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ABSTRACT

Natural activation of arterial baroreceptors has been shown to modulate basic sensorimotor responses. However, it is mostly unknown whether high-order cognitive processes are also interfered by afferent baroreceptor feedback. The present thesis investigated this question. In the first study, participants were exposed to five intensities (ranging from non-painful to very painful) of electrocutaneous stimuli, randomly delivered at five intervals (0, 150, 300, 450, 600 ms) after the R-wave of the EKG. For painful stimulation, ratings were highest at R+300 ms and lowest at R+0 and R+600 ms. For non-painful stimulation, ratings declined linearly as the cycle progressed. In addition, nociceptive responses did not vary across the cardiac cycle for both types of stimuli. The second study followed up these findings by only changing the schedule of stimulation within the procedure, i.e., stimuli were now presented in blocks of either an ascending or descending order of stimulus intensity. Nociceptive responding for painful stimuli was attenuated during systole whereas ratings did not differ across the cardiac cycle regardless of stimulus intensity. The previous data, representing an unpredictable (Study One) and a predictable (Study Two) schedule of stimulation, were compared in a third study independently of cardiac cycle timings. Unpredictable shocks elicited a stress-induced hypoalgesia whilst evoking the highest nociceptive responses, thereby demonstrating that pain can dissociate from nociception under stress. The fourth study combined two experiments that examined the effects of moderate intensity exercise on measures of attention control and working memory. Cognitive tasks were performed at rest and/or while cycling at different graded power outputs designed to produce different levels of cardiovascular arousal. Together, these experiments indicated that working memory and attention control are facilitated by moderate exercise, an effect likely moderated by task demands. Finally, the last study examined performance on the Sternberg working memory task as a function of the phase of the cardiac cycle. The zero intercept, indexing basic sensorimotor processing, was greater for probes presented temporally proximal to the R-wave of the EKG. Response latency per additional digit, i.e., the slope, was greatest for stimuli presented late in the cardiac cycle. In sum, these studies (a) provide further support for the afferent feedback hypothesis; and (b) extend the findings obtained with basic sensorimotor responses to high-order cognition.

"Go to your bosom; knock there, and ask your heart what it doth know"

William Shakespeare
In "Measure for Measure"

To my grandmother Julia and my daughter Matilde,
For their bond, the prettiest I've ever witnessed.

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LIST OF PAPERS

This thesis includes the following papers, each corresponding to an original study:

Quelhas Martins, A., 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.

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Quelhas Martins, A., McIntyre, D. & Ring, C. (2010). Effects of baroreceptor stimulation on performance of the Sternberg task: A cardiac cycle time study of working memory. In revision, *Psychophysiology*.

Quelhas Martins, A., Willoughby, A., Kavussanu, M., McIntyre, D. & Ring, C. (2010). Moderate Intensity Exercise Facilitates Attentional Control and Working Memory. Submitted to *Biological Psychology*.

Additionally, the following abstract refers to a presentation of material from this thesis:

Martins, A., Ring, C., McIntyre, D., Edwards, L. & Martin, U. (2009). Effects of experimental design on the nociceptive flexion reflex and electrocutaneous pain. *European Journal of Pain*, 13, S100.

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CHAPTER ONE

General Introduction

It is most surprising that centuries of common sense belief about the propensity "of the heart to govern upon mental processes" were not accompanied by a thoroughly scientific inspection of such claim. After losing "preponderance" to emotion theories (e.g., Cannon, 1927), the heart became progressively put aside from cognitive processes to be remitted solely to its pumping function. In fact, nowadays, much is known about the cardiovascular impact of stress, anxiety, and other emotional states, but little can be said about the extent to which cardiovascular activity and respective afferences affect the brain, and specifically, psychological processes.

Nonetheless, early animal experimentation reported profound lethargic effects on the behaviour of dogs resulting from prolonged mechanical stimulation of arterial baroreceptors (Koch, 1932). Further experiments in decerebrate cats indicated that this type of stimulation could decrease cortical arousal, as indexed by an increased electroencephalogram synchronisation (Bonvallet et al., 1954; Nakao et al., 1956). In humans, Weiss and Baker (1933) reported a loss of consciousness that could follow mechanical stimulation of the carotids, without concomitant cerebral ischemia or blood pressure changes. Further, Schlager and Meier (1947) also described a form of carotid massage employed by Balinese natives to induce sleep. Taken together, these preliminary studies suggested that increased stimulation of the arterial baroreceptors could attenuate cortical activity and the arousal state of the organism. This assumption later formed the

basis of the Laceys' hypothesis (Lacey & Lacey, 1974), who proposed an interference mechanism caused by afferent baroreceptor neural feedback being integrated into medullary and cortical structures.

This visceral afferent feedback mechanism has been the focus of several studies, which examined the influences of natural baroreceptor stimulation across the cardiac interbeat interval on distinct sensorimotor outcome measures, such as the nociceptive withdrawal reflex (e.g., Edwards et al., 2001; McIntyre et al., 2006), or simple (e.g., Edwards et al., 2007; McIntyre et al., 2008) and choice (e.g., McIntyre et al., 2007) reaction times. In all, these studies provided evidence that basic sensorimotor responses reveal a pattern of systolic inhibition consistent with the visceral afferent feedback hypothesis (Lacey & Lacey, 1974). However, most of this research was not designed to examine whether complex sensory-affective (e.g., pain) and cognitive (e.g., working memory) processes are susceptible to afferent baroreceptor neural interference. The present thesis addresses this question.

This General Introduction will describe (a) the main neurophysiological features of the arterial baroreceptors; (b) paradigms for studying phasic and tonic activation of the arterial baroreceptors, with an emphasis on studies employing the cardiac cycle time paradigm; (c) the outline of the present thesis.

The Arterial Baroreflex and the Neurophysiology of the Arterial Baroreceptors

Cardiovascular homeostasis results from a moment-by-moment regulation of both the arterial blood pressure and the blood flow to the viscera and muscles. The efficiency of this regulatory activity depends on the balance of feedforward (also known as "central

command") and feedback (also known as "reflex") mechanisms operating at rest and in response to metabolic, emotional, and environmental challenges (Benarroch, 2008).

In this context, the arterial baroreflex is a feedback mechanism operating to buffer acute variations of blood pressure, particularly during exercise, postural changes, and emotion (Benarroch, 2008). The arterial baroreflex controls blood pressure oscillations mainly by regulating the cardiac output and the total peripheral resistance, which both determine the arterial blood pressure (Dembowsky & Seller, 1995; Benarroch, 2008). When blood pressure rises, the arterial baroreceptors increase their firing rate and afferent inputs to the medulla, resulting in (a) vasodilation within the muscles, due to a decrease in the activity of sympathetic nerves innervating the heart, skeletal muscles and splanchnic vessels; and (b) a slowing of heart rate due to increased vagal output to the heart (Dembowsky & Seller, 1995; Benarroch, 2008).

The arterial baroreceptors as the afferent limb of the baroreflex

The arterial baroreceptors are mechanoreceptors located in the carotid sinuses and the aortic arch that respond to the tension of the arterial walls, being sensitive to both the absolute pressure and the rate of increase of blood pressure within the vessel (Angell James, 1971; Dembowsky & Seller, 1995). Afferents from both the carotid and the aortic baroreceptors send monosynaptic excitatory input to the nucleus tractus solitarius (Eckberg & Sleight, 1992; Benarroch, 2008). Barosensitive neurons within the nucleus tractus solitarius initiate a parasympathetic (cardioinhibitory) pathway by projecting to vagal parasympathetic neurones in the nucleus ambiguous. Increased activation of these neurons elicits bradycardia by decreasing the sinoatrial node pacemaker cells discharge rate (Jordan, 1995; Benarroch, 2008). In parallel, the nucleus tractus solitarius can also trigger a

sympathetic (sympathoinhibitory) pathway via a group of interneurons within the caudal ventrolateral medulla that inhibit the sympathoexcitatory neurones in the rostral ventrolateral medulla controlling the sympathetic preganglionic neurons in the intermediolateral column of the spinal cord (for further neuroanatomical and neurophysiological details on the arterial baroreflex see Benarroch, 2008). As such, increases in afferent baroreceptor input to the nucleus tractus solitarius results in decreased sympathetic outflow to the vessels (see Figure 1.1).

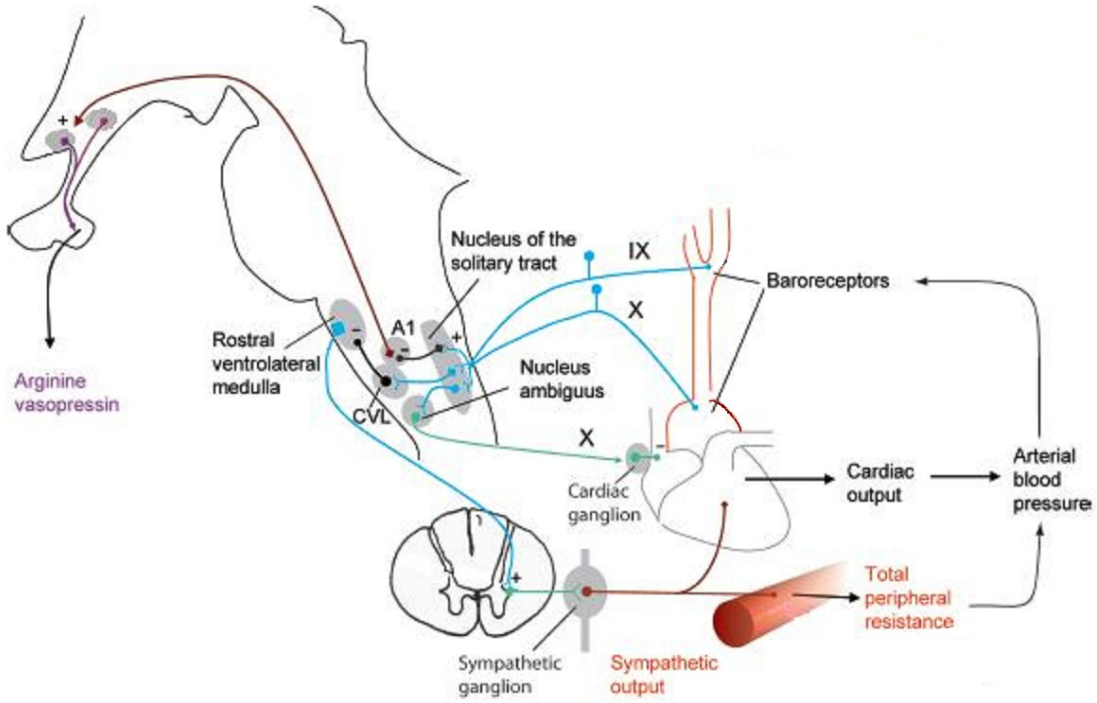

Figure 1.1. Pathways implicated in the arterial baroreflex. Afferents from the arterial baroreceptors (glossopharyngeal nerve, IX; vagus nerve, X) send input to the nucleus tractus solitarius (NTS). Sympathetic pathway: NTS neurons project to interneurons in the caudal ventrolateral medulla (CVL) that inhibit sympathoexcitatory neurons within the rostral ventrolateral medulla. Parasympathetic pathway: NTS neurons send a direct projection to vagal preganglionic neurons within the nucleus ambiguus (NA), which project to the cardiac ganglion eliciting bradycardia. The secretion of arginine vasopressin by hypothalamic nuclei can also be indirectly inhibited by the NTS, via an inhibitory projection to the noradrenergic cells of the A1 group. (Adapted from Benarroch, 2008).

Transmission of afferent baroreceptor information to the cortex

The nucleus tractus solitarius is also highly interconnected with the reticular formation, which conveys baroreceptor input to the thalamus (Rau & Elbert, 2001). In addition, the nucleus tractus solitarius sends direct projections to limbic structures, including the hypothalamus and the amygdala, and a major ascending projection to the lateral parabrachial nucleus (Dembowsky & Seller, 1995). Given that the lateral parabrachial nucleus also projects to the hypothalamus and the amygdala, this constitutes an indirect route for baroreceptor input to reach the limbic cortex. Furthermore, the lateral parabrachial nucleus also projects to the lateral ventroposterior thalamus, hence allowing baroreceptor input to reach the insular cortex via the thalamus (Dembowsky & Seller, 1995). Finally, there is also recent evidence that both the prefrontal and the somatosensory cortices integrate baroreceptor input (Kimmerly et al., 2005; Kimmerly et al., 2007; Wong et al., 2007).

Human paradigms for the study of phasic and tonic baroreceptor activation

During the last decades, human experimentation has resorted to a few indirect techniques to stimulate the arterial baroreceptors. Accordingly, pharmacological manipulation with phenylephrine and nitroprusside to respectively raise and lower the arterial blood pressure (known as the Oxford technique) has been employed (Raven et al, 2006). However, ethical constraints, particularly the inadequacy of the technique for use with clinical populations (e.g., patients with hypertension), limit its application. In turn, constant (e.g., Eckberg, Cavanaugh, Mark, & Abboud, 1975) and variable (e.g., Brody & Rau, 1994) neck suction/compression methods have also been designed to manipulate the

carotid sinus transmural pressure, mainly in the context of pain studies interested in the phenomenom of hypertensive hypoalgesia (see "Introduction" in Chapters Two and Three for a brief review of the paradigms employed in the study of tonically-elevated blood pressure effects in pain sensitivity). Overall, both the constant (c.f. Elbert et al., 1988; France, Ditto, & Adler, 1991) and the phasic (c.f. Edwards et al., 2003; Angrilli, Mini, Mucha, & Rau, 1997) methods produced inconsistent findings. A major limitation of these methods was the relatively unknown integrated baroreceptor output, given that they were not designed to stimulate the aortic arch baroreceptors.

The cardiac cycle time as an observational-like paradigm

The aforementioned limitations are avoided when a cardiac cycle time paradigm is employed because it takes advantage of naturally-occurring variations in both aortic and carotid baroreceptor stimulation. Specifically, within each cardiac cycle, the pulse pressure wave arrives at the aortic baroreceptors approximately 90 ms (value estimated for an average heart rate of 64 bpm, at rest; see Kroeker & Wood, 1955, for the respective timings of arrival of the pulse pressure wave at the aortic baroreceptors at different heart rates) after the R-wave and at the carotid baroreceptors approximately 140 ms (Edwards et al., 2007) after the R-wave. Both aortic and carotid groups prolong their activity for approximately 250 ms (Edwards et al., 2007). Therefore, peak afferent activity begins at 90 ms and endures for approximately 250 ms after the R-wave, until arterial blood pressure starts decreasing during the diastolic phase of the cardiac cycle. As such, the maximal pulse synchronous afferent firing from the arterial baroreceptors occurs during early systole (Langrehr, 1964). Consequently, in the cardiac cycle time paradigm, probe stimuli

are delivered when baroreceptors are activated (i.e., systole) and when they are quiescent (i.e., diastole), and the respective responses compared.

Early studies employing the cardiac cycle time paradigm demonstrated that simple reaction times to auditory (Birren et al., 1963) and visual (Callaway, III & Layne, 1964) stimuli were the slowest when presented early in the cardiac cycle. In fact, both auditory (Saxon, 1970) and visual (Requin & Brouchon, 1964) acuity were also the lowest for stimuli presented during the QRS complex of the electrocardiogram. These findings provided preliminary support for the visceral afferent feedback hypothesis (Lacey & Lacey, 1974). Yet, subsequent studies that failed to replicate such cardiac cycle time effects questioned the strength of the phenomenon (see "Introduction" in Chapter Six, for further detail). Nonetheless, more recent studies with adequate sample sizes and proper equipment have examined intra-cardiac cycle intervals with more detail and consistently found the simple reaction times to be the slowest early in the cardiac cycle (Edwards et al., 2007; McIntyre et al., 2007; McIntyre et al., 2008).

Another line of cardiac cycle time research has directly looked at neurophysiological markers of cortical activity to inspect whether baroreceptor-related cortical interference could be detected. Accordingly, evidence has now accumulated to support a pattern of systolic attenuation of auditory, visual and pain evoked potentials (Edwards et al., 2008; Sandman et al., 1982; Walker & Sandman, 1979; Walker & Sandman, 1982). Furthermore, decreases in frequency for electroencephalographic oscillations measured in the alpha band have also been reported (Walker & Walker, 1983).

Finally, cardiac cycle time studies have also examined whether peripheral reflexes vary across the cardiac cycle. Although null findings were reported for the muscle stretch-reflex (McIntyre et al, 2004), therefore excluding a possible merging of baroreceptor inputs with alpha-motoneuron neural activity, effects were found for the nociceptive flexion reflex, a polysynaptic protective withdrawal reflex. Edwards, Ring, Mclntyre and Carroll (2001) were the first to show an inhibition of the amplitude of this reflex between 200 and 400 ms after the R-wave of the electrocardiogram. This same pattern of systolic inhibition was subsequently replicated in several studies (al'Absi, France, Ring, France, Harju, Mclntyre, & Wittmers, 2005; Edwards, Mclntyre, Carroll, Ring & Martin, 2002; Edwards, Mclntyre, Carroll, Ring, France & Martin, 2003). Overall, a consistent cardiac cycle effect on the nociceptive flexion reflex has provided further support to the visceral afferent feedback hypothesis.

Steady-state dynamic exercise for the tonic activation of the arterial baroreceptors

At rest, the resulting effect of arterial baroreceptors stimulation by rises in blood pressure is a reflex bradycardia. However, during steady-state dynamic exercise, sustained levels of increased blood pressure are also accompanied by increased heart rate. Indeed, the cardiovascular response during exercise increases blood pressure to maintain adequate perfusion levels for muscle activity. For this to happen, a complex imbalance between the central command from the somatic motor cortex and the muscle mechanoreflex must promote a resetting of the arterial baroreflex to a higher operating point (see Rowell & O'Leary, 1990 and Raven et al, 2006, for detailed reviews on the mechanisms operating the arterial baroreflex resetting during acute bouts of exercise). On the one hand, the central command must inhibit the sensitivity of the cardiac component of the baroreflex to

produce cardiac acceleration. On the other hand, afferent input from the muscle mechanoreflex must contribute to the gain of the neural arc of the baroreflex (Yamamoto et al, 2008). Thus, steady-state dynamic exercise elicits vagal withdrawal to increase heart rate and cardiac output, and consequentially, blood pressure; if heart rate exceeds the range of vagal withdrawal, sympathetic nervous activity completes the rise in blood pressure, either by a sympathetically mediated rise in cardiac output or by sympathetically mediated vasoconstriction (Rowell & O'Leary, 1990). Moreover, not only the arterial baroreflex must be fully functional during exercise, but must also operate proportionally to the intensity of exercise (Fadel, 2008). Therefore, it is a logical conclusion that arterial baroreceptor function is also crucial during steady-state dynamic exercise, even more so considering that exaggerated blood pressure response to exercise is a valid risk marker for future hypertension (Singh et al, 1999).

At rest, dose-response relationships have been reported between increasing blood pressure levels and decreased cognitive performance in neuropsychological tests (Elias et al., 1993). In turn, studies that have looked at general cognitive function during steady-state dynamic exercise report inconsistent results (see "Introduction" on Chapter Five, Tomporowski, 2003, and Brisswalter et al., 2002, for a review of studies examining the effects of different types of acute exercise protocols on several cognitive functions). Some studies argue for a facilitating effect of moderate exercise-induced cardiovascular arousal on the attentional focus and the speed of responding to reaction time tasks (e.g., Davranche et al., 2006; Pesce et al., 2007), whereas others (e.g., Cote et al., 1992; Travlos & Marisi, 1995) find no effects. In addition, more controversy is apparent in the literature when higher cognitive processes are concerned, particularly, executive function (e.g., Pesce et al., 2002; Dietrich & Sparling, 2004; Coles & Tomporowski, 2008; see "Introduction" on

Chapter Five for a brief review of the conflicting perspectives about executive function performance during acute exercise protocols). Two facts are surprising among this literature. First, there are "islets" of cognitive performance poorly studied during steady-state dynamic exercise (e.g., working memory). Second, with occasional exceptions (e.g., Becque et al., 1993), the majority of studies does not report blood pressure levels assessed during the exercise protocols, albeit the heart rate is commonly reported. Clearly, this field warrants further research.

Thesis overview

Study One. Previous cardiac cycle time studies have shown a pattern of systolic modulation of neurophysiological correlates of pain (e.g., the nociceptive flexion reflex, Edwards et al, 2001; pain-related evoked potentials, Edwards et al, 2008). Such findings were interpreted as evidence for a baroreceptor mechanism of antinociception. However, intriguingly, the subjective evaluation of pain (pain ratings) did not yield any cardiac cycle modulation in these studies. Study One started by examining possible reasons for this discrepancy. After a close inspection to the methods and procedures employed, it soon became clear that these studies suffered from either one or two of the following limitations: (a) the stimulation was not reliably painful, as indexed by average ratings below pain threshold; (b) the stimulus intensity was always fixed, either corresponding to the nociceptive flexion reflex threshold (e.g., Edwards et al, 2001) or to a visual analogue scale rating of 50 (Edwards et al, 2008); it seemed odd to approach a subjective phenomenon with an invariant stimulus. Thus, in an effort to overcome these limitations, the present study followed a mixed block design to assess the effects of natural arterial baroreceptor activity on both the nociceptive flexion reflex and pain intensity and unpleasantness

reports. Specifically, electrocutaneous stimuli were randomly delivered to the sural nerve at one of five intensities (50% pain threshold, 75% pain threshold, pain threshold, midway between pain threshold and pain tolerance, pain tolerance) at five intervals (0, 150, 300, 450, 600 ms) after the R-wave of the electrocardiogram. From the visceral afferent feedback hypothesis, it was expected that both nociceptive flexion reflex responding and pain ratings would reveal a pattern of systolic modulation across the cardiac cycle, given that painful and non-painful stimuli were now triggered within a sufficiently variable schedule.

Study Two. The primary purpose of this study was to follow up the results obtained by the previous one. Particularly, it was reasoned that the experimental design (i.e., several intensities of stimulation, including painful ones, randomly presented) introduced in that study was somehow responsible for the unexpected findings. To test this assumption, the same properties of electrocutaneous stimulation (i.e., 50% pain threshold, 75% pain threshold, pain threshold, midway between pain threshold and pain tolerance, pain tolerance) and exactly the same intervals after the R-wave of the electrocardiogram (0, 150, 300, 450, 600 ms) were used. Yet, stimuli presentation followed a fixed block design, i.e., each participant was randomly assigned to receive five blocks of stimuli in either an ascending or descending order of intensity of stimulation. Combining the visceral afferent feedback hypothesis with the findings obtained in Study One, it was predicted that the nociceptive flexion reflex would be attenuated whereas pain ratings would be increased during systole.

Study Three. The accidental findings obtained in Study One followed by the results from Study Two, casted the attention for the stimulus (un)predictability as the core feature

that was changing in the paradigm. Previous research stemming from aversive learning paradigms (e.g., fear-potentiated startle; Bradley & Lang, 2007) has established that temporally predictable noxious stimuli (i.e., knowing *when* the noxious stimulus occurs) elicit fear, potentiation of defensive reflexes and hypoalgesia. However, studies examining the effects of event predictability (i.e., knowing *what* the stimulus sensory properties will be) have produced mixed findings (see Miller, 1981 for a review of studies manipulating the event predictability of stressful / aversive exposures and examining emotional and nociceptive outcomes). As such, the main goal of this study was methodological in nature, namely, to answer a few interesting questions: (a) What are the specific effects of an event predictable / unpredictable schedule of electrocutaneous stimulation on an objective (i.e., the nociceptive flexion reflex) and a subjective (i.e., pain ratings) measure of pain? (b) Can it be argued that stress-induced hypoalgesia occurs during any of the schedules? For this purpose, data from the two previous studies was collapsed across the cardiac cycle time intervals to permit a simplified, yet robust analysis; in addition, anticipatory heart rate data collected during each trial provided an indirect measure of the arousal experienced by the participants. Specifically, participants were pooled into two groups – event unpredictability / event predictability – according to the schedule of stimuli presentation experienced – random (Study One) / blocked ascending or descending (Study Two). From previous studies (Brown et al., 2008; Willer et al., 1981), it was hypothesized that the lowest pain ratings and nociceptive flexion reflex responses would be revealed by the event unpredictability group, at least for the extremely noxious stimuli.

Study Four. This study was originally a cardiac cycle time study designed to compare working memory performance in the Sternberg task (Sternberg, 1966) under conditions of superimposition of tonic gradual rises in blood pressure (obtained by low,

medium, and high dynamic exercise protocols) on phasic baroreceptor functioning with natural baroreceptor stimulation alone (control condition). However, two reasons made the cardiac cycle comparison impracticable. First, the cardiac interbeat intervals were progressively reduced by increasing exercise intensities. Second, physiological signals became gradually impoverished and noisy during exercise. As such, the analyses were restricted to the working memory performance of a large sample (N=120) across different intensities of steady-state dynamic exercise and a control condition. Specifically, each participant performed the Sternberg task under control and exercise. In the control condition, the task was completed while sitting on a cycle ergometer. In the exercise condition, participants were randomly assigned to one of three exercise intensity groups (low, medium, high) and completed the task once steady-state physiological load was achieved (see "Method" of "Experiment 2" on Chapter Five for further details). In addition, this chapter presents another dataset obtained with a smaller (N=24) sample whose performance to a similar working memory task (paced auditory serial addition test, PASAT; Gronwall, 1977) was assessed under control (sitting on a cycle ergometer) or moderate dynamic exercise conditions (see "Method" of "Experiment 1" on Chapter Five for further details). Together, these datasets combine to test predictions derived from two of the main theoretical perspectives on executive function performance during moderate aerobic exercise (see "Introduction" on Chapter Five for a brief description of these perspectives). Accordingly, from the "transient hypofrontality" hypothesis, it was predicted worse performance in both the PASAT and the Sternberg task during moderate exercise in comparison to control conditions; conversely, from an exercise-induced arousal perspective, it was expected that performance to both tasks would improve under moderate exercise.

Study Five. As indicated above, this dataset comprises the available cardiac cycle time data obtained under the control condition of Study Four. The purpose of this study was two-fold. First, occasional reports have established a link between vagal tone functioning and performance on tasks involving executive function (see Thayer et al, 2009 for a review of studies examining the effects of heart rate variability on the performance to several cognitive tasks). This line of research has mainly resorted to heart rate variability analyses and suggests that high heart rate variability is associated with better cognitive performance on this type of task (e.g., Hansen et al, 2003). As such, it would be reasonable to explore the performance on such type of cognitive task in the context of a cardiac cycle paradigm. Second, the present study would also add to the very limited data available on cardiac cycle time influences on high-order cognition. Moreover, given that the few studies conducted were all choice reaction time studies (see "Introduction" on Chapter Six for a brief description of these studies), the use of the Sternberg task would allow a cross-sectional comparison of results in terms of cardiac cycle time influences on measures of sensorimotor processing, because it is a reaction time-based task. Therefore, this study examined performance on the Sternberg task as a function of the phase of the cardiac cycle. Specifically, trials were scored retrospectively according to the timing of probe onset after the R-wave into one of six intervals (each labelled by its midpoint): R+50, R+150, R+250, R+350, R+450, and R+550 ms. Such procedure was required to standardize the retention period for every trial. The slope (ms per digit), a measure of the time required to process one additional digit in memory, and the zero intercept (ms), a measure of sensorimotor processing time, were computed for each interval (Sternberg, 1966). From the visceral afferent feedback hypothesis, it was expected that the cognitive

processing required for serial comparisons and probe assessment would be the slowest for probes presented during systole.

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CHAPTER TWO

Effects of Unpredictable Stimulation on Pain and Nociception across the Cardiac Cycle

Abstract

Previous research has demonstrated that the nociceptive flexion reflex (NFR) and pain-related evoked potentials are reduced in amplitude when elicited during the middle of the cardiac cycle. Despite these findings, suggesting a baroreceptor mechanism of antinociception during systole, pain intensity ratings reported in these studies were not modulated across the cardiac cycle. This discrepancy between the neurophysiological correlates of pain and its subjective experience was the focus of the current study that used a mixed block design to assess the effects of natural arterial baroreceptor activity on both the NFR and pain intensity and unpleasantness reports. Specifically, electrocutaneous stimuli were randomly delivered to the sural nerve at one of five intensities (50% pain threshold, 75% pain threshold, pain threshold, midway between pain threshold and pain tolerance, pain tolerance) at five intervals (0, 150, 300, 450, 600 ms) after the R-wave of the electrocardiogram. Under painful stimulation, intensity and unpleasantness varied in a quadratic manner across the cardiac cycle; pain was highest at R+300 ms and lowest at R+0 and R+600 ms. Under non-painful stimulation, ratings declined linearly as the cycle progressed. Finally, nociceptive responses did not differ among the R-wave to stimulation intervals for both painful and non-painful intensities. The observed phasic modulation of pain may be explained by a central nervous system alarm/defence reaction triggered by the unpredictability of the potentially damaging stimulation. The absence of systolic attenuation of nociceptive responding is compatible with previous evidence that baroreceptor modulation of the NFR is abolished under conditions of heightened arousal.

Introduction

Patients with essential hypertension exhibit reduced pain sensitivity (Ghione, 1996). Hypertensive hypoalgesia has clinical implications: these patients are less likely to recognize the symptoms of a heart attack (Kannel et al., 1985). Indeed, an inverse relation between blood pressure (BP) and reported chest pain has been demonstrated in individuals undergoing an exercise tolerance test to screen for myocardial ischemia (Ditto et al., 2007). Although the reasons for this phenomenon have yet to be established, a visceral afferent feedback (VAF) mechanism activating pain inhibition pathways has been proposed (France & Ditto, 1996; Koltyn & Umeda, 2006). According to this hypothesis, afferent inputs from phasic natural baroreceptor stimulation (Angell James, 1971; Mancia & Mark, 1983) are integrated into brain stem regions implicated in descending pain inhibition approximately 180-320 ms after the R-wave of the electrocardiogram (see Edwards et al., 2001).

Early studies examining baroreceptor stimulation effects on pain manipulated the carotid sinus transmural pressure by applying constant suction to the neck for several seconds (Eckberg et al., 1975). The findings were mixed with neck pressure manipulations reducing pain in borderline hypertensives (Elbert et al., 1988), while increasing pain (Elbert et al., 1988) or not affecting pain (France et al., 1991) in participants with normal BP. This inconsistency has been attributed to methodological weaknesses of the procedure (Rau & Elbert, 2001). However, later experiments employing more sophisticated phasic suction/compression methods have also yielded mixed findings: neck suction during systole reduced pain in some (Al'Absi et al., 2005; Brody & Rau, 1994; Dworkin et al., 1994; Edwards et al., 2003; Mini et al., 1995) but not all (Angrilli et al., 1997; Rau et al.,

1994; Rau et al., 1995) studies. Because these methods were not designed to stimulate aortic arch baroreceptors, the integrated baroreceptor output is unknown in these studies.

Cardiac cycle time studies capitalise on naturally-occurring variations in both aortic and carotid baroreceptor stimulation. In this paradigm, probe stimuli are delivered when baroreceptors are activated (i.e., systole) and when they are quiescent (i.e., diastole), and the respective responses compared. For example, studies have reported inhibited cortical activity (Koriath & Lindholm, 1986; Koriath et al., 1987), reduced visual and auditory evoked potentials (Sandman, 1984; Walker & Sandman, 1982) and reduced pain-related evoked potentials (Edwards et al., 2008a) during systole. We have found that the NFR, a polysynaptic spinal withdrawal reflex (Sandrini et al., 2005), is attenuated during systole (Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003; McIntyre et al., 2006; McIntyre et al., 2008). In sum, neurophysiological correlates of pain seem inhibited when noxious stimuli are delivered approximately 200-400 ms after the R-wave of the electrocardiogram.

However, the aforementioned studies found no evidence that pain ratings were modulated across the cardiac cycle, thus revealing a striking discrepancy between neurophysiological and psychological correlates of pain. Methodological factors may explain these null findings. First, the stimulation was not consistently painful; the average intensity ratings reported by participants were below pain threshold in most studies (Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003) with one exception (Edwards et al., 2008a). Second, only the perceived intensity has been assessed, being possible that other dimensions of pain, such as unpleasantness, are modulated across the cardiac cycle. Third, the stimulus intensity used by previous studies was always kept fixed, either corresponding to 100% of NFR threshold (Edwards et al., 2001; Edwards et al.,

2002; Edwards et al., 2003; McIntyre et al., 2006) or to a VAS rating of 50 (Edwards et al., 2008a). Under such predictable conditions, participants may soon learn the stimulus invariance and give constant ratings. The present study addresses these potential limitations. In particular (a) the sural nerve was stimulated at multiple intensities, ranging from non-painful to tolerance levels, (b) ratings of intensity and unpleasantness were collected, and (c) stimuli were pseudo-randomly presented. Based on the VAF hypothesis, it was expected that both NFR responding and pain ratings would be lower during systole compared to diastole.

Method

Participants

Thirty-three healthy normotensive adults (14 male, 19 female) with a mean age of 19.4 (*SD* = 1.0) years and a mean body mass index of 23.4 (*SD* = 2.3) kg/m^2 gave informed consent and participated in the study. They had a mean resting systolic blood pressure of 112 (*SD* = 11) mmHg, diastolic blood pressure of 65 (*SD* = 5) mmHg, and heart rate of 64 (*SD* = 10) bpm. Individuals were excluded if they had any known heart problems or chronic illnesses, or if they were on any medication except birth control. Participants were asked to refrain from caffeine, alcohol, and exercise for 2 hours before testing. The study protocol was approved by the local research ethics committee.

Physiological Measurements

Participants sat in a chair with an adjustable legrest. Their left leg was flexed to an angle of 35° and supported at the ankle. A Spike2 (Cambridge Electronic Design) computer program ran the experiment and collected physiological data via a Power1401

(Cambridge Electronic Design). All signals were digitised at 2500 Hz with 16-bit resolution. All electrode sites were exfoliated (Nuprep, D.O. Weaver & Co) and degreased with isopropyl alcohol swabs (Mediswab, Seton Healthcare) until contact impedance was <10 kΩ (Checktrode, UFI). Electromyographic (EMG) activity of the left biceps femoris muscle was recorded with an active differential surface electrode with two silver bar contacts, 10 mm in length and 1 mm in diameter, with an inter-contact spacing of 10 mm (DE 2.1, Delsys) placed 12 cm above the knee crease, with a separate reference electrode positioned 12 cm lateral to the active electrode. The contacts were mounted on a polycarbonate case (35 × 20 × 5 mm) that housed a ×10 pre-amplifier. The active electrodes were placed, with the contact bars perpendicular to the muscle fibres and secured using adhesive interfaces (Delsys). Conductive cream (Synapse, Nicolet Biomedical) was applied to the contacts of the active electrodes. The EMG signal was bandpass filtered (20–450 Hz) and amplified (×10000) using a Bagnoli-4 system (Delsys).

An electrocardiogram (ECG) was recorded with three spot electrodes (Cleartrace, ConMed) in a modified chest configuration. The active electrodes were placed on the right clavicle and lower left rib, and a reference electrode was placed on the left clavicle. The ECG signal was amplified and filtered (0.1–100 Hz plus 50Hz notch filter) by an AC amplifier (P511, Grass). Baseline BP and pulse rate were measured with an oscillometric sphygmomanometer (Dinamap Pro100, Critikon) and a brachial cuff (Dura-cuff, Critikon) attached to the left arm.

The sural nerve was electrocutaneously stimulated via a gold-plated stainless steel bar electrode (Nicolet) with 9 mm diameter contacts and a 22 mm inter-contact spacing. Conductive cream was applied to the contacts of the bar electrode and was secured with tape (Transpore) posterior to the ankle with the anode superior. Stimulations were

delivered by a constant current stimulator (DS7A, Digitimer) with 400 V compliance, equivalent to 40 mA into 10 kΩ.

Self-Report Measures

Pain Tolerance. A modified visual analogue scale (Janal et al., 1994) of 0 (no sensation), 1 (faint sensation), 2 (mild sensation), 3 (moderate sensation), 4 (strong sensation but not painful), 5 (faint pain), 6 (mild pain), 7 (moderate pain), 8 (strong pain), 9 (very strong pain), and 10 (maximum tolerable pain), was used to determine pain threshold and pain tolerance levels.

Pain Intensity and Pain Unpleasantness. Two modified visual analogue scales (Rainville et al., 1992) were used to assess both the intensity and the unpleasantness dimensions of pain. Participants rated the perceived intensity / unpleasantness on scales of 0 (NOT AT ALL painful / unpleasant), 25 (SLIGHTLY painful / unpleasant), 50 (MODERATELY painful / unpleasant), 75 (VERY painful / unpleasant), and 100 (EXTREMELY painful / unpleasant). Participants were instructed to use any number in between the categories that would give the most accurate rating.

Procedure

Participants completed a single 2-hr session. Demographic data were collected at the start of the session, and following instrumentation, participants rested for 5 minutes. During this baseline period, blood pressure and heart rate readings were initiated at the start of minutes 1, 3 and 5. These readings were averaged to yield mean systolic blood pressure, diastolic blood pressure, and heart rate.

Tolerance task. The pain tolerance scale (see above) was displayed 2 m in front of the participant. Participants received the following instructions: “We will measure your muscle activity by delivering several stimuli to your ankle. Please rate each stimulus using

the scale displayed in front of you. Give the number that most accurately represents the sensation you feel. You may use decimals if you wish. We will stop the task when you give a rating of 10". The sural nerve was stimulated electrocutaneously by five rectangular 1 ms pulses at 250 Hz, starting at 2 mA. Stimulus intensity was increased in 2 mA steps until a rating of 10 (maximum tolerable pain) was reported or a maximum intensity of 50 mA was reached. The inter-trial interval ranged from 20-30 s.

Cycle time task. The pain intensity and pain unpleasantness scales (see above) were displayed 2 m in front of the participant. The following instructions were used: "In general, the intensity and unpleasantness of pain seem to vary somehow together, but sometimes we feel a weak pain that is very unpleasant (e.g. a tight shoe), or at other times we may feel a strong pain that we don't consider unpleasant at all (e.g. winning a running race while wearing the same tight shoe). In this task, several stimuli will again be delivered to your left ankle and you will be asked to immediately rate both the intensity and unpleasantness of the sensations that you experience. First, you will evaluate the intensity of each sensation using this new pain intensity scale. You may use any number between the verbal descriptors that most accurately describes the sensation experienced. You will also evaluate the unpleasantness of each sensation using this pain unpleasantness scale. Again, you may use any number you wish to indicate your perception. It is very important you give the numbers that most accurately represent the sensation you feel." The properties of the stimulus applied were the same as in the previous task. Six seconds into each trial, the computer program initiated a search for an R-wave of the ECG and then triggered the electrocutaneous stimulation of the sural nerve at one of five intervals after the R-wave of the ECG (R+0, R+150, R+300, R+450, R+600 ms). Five intensities were used, namely, 50% of the pain threshold, 75% of the pain threshold, the pain threshold, the

mid-point between pain threshold and pain tolerance, and the pain tolerance. During each trial, baseline rectified EMG activity was measured online and if it exceeded 2 μV, stimuli were not presented. If this occurred, participants were asked to relax their leg, and the trial was repeated. A variable inter-stimulus interval of 20-30 s was used. Participants were stimulated at each of the five intensities, in ascending order of magnitude, to familiarise them with the task demands. Next, participants completed four blocks of 25 trials with a 5 minute rest after each block. In each block, a 5 intervals by 5 intensities Greco-Latin square was used to counterbalance the trial order. The same square was used in blocks 1 and 4, whereas the reversed square was used in blocks 2 and 3. Further, participants were randomised to one of five different squares.

Data Reduction and Analysis

In each trial of the cycle time task, EMG activity from the biceps femoris was rectified and the mean activity 65 to 5 ms pre-stimulation (baseline activity) and 90 to 150 ms post stimulation (RIII, nociceptive flexion reflex responding) was calculated. The mean EMG activity and pain ratings of the four trials for each cardiac cycle interval and each stimulus intensity were calculated. The data associated with the lowest two intensities (50% pain threshold, 75% pain threshold) were collapsed to create average non-pain condition responses. Similarly, data from the highest three intensities (pain threshold, difference between pain threshold and pain tolerance, and pain tolerance) were collapsed to create average pain condition responses.

Our rationale for reducing the data over trials and intensities was twofold. First, data were reduced over trials to increase reliability of measurement. Second, data were reduced over intensities into two qualitatively different categories of sensory experience, namely pain and non-pain.

Two participants were identified as statistical outliers (nociceptive flexion reflex responding > 3 *SD*s above mean) and, therefore, were excluded from all analyses. Thus, the effective sample size for the statistical analyses reported below was 33. A series of 2 Sex (male, female) by 5 Interval (R+0, R+150, R+300, R+450, R+600 ms) mixed-model (i.e., split plot) analysis of variance (ANOVAs), with sex as a between-subject factor and interval as a within-subject factor, were conducted on the key outcome variables. ANOVAs were corrected for the assumption of independence of data points using a Greenhouse-Geisser correction (ε). Although the original degrees of freedom are reported, the corrected degrees of freedom that were used to determine the probability levels can be obtained by multiplying the reported degrees of freedom by epsilon. Eta-squared (η^2), a measure of effect size, was reported. In ANOVA this equals the adjusted R^2 obtained in regression analyses; values of .02, .13 and .26 for η^2 indicate small, medium and large effect sizes respectively (Cohen, 1992). Polynomial trend analyses were performed to test for cardiac cycle time effects for the key outcome variables.

Results[1]

Pain threshold and pain tolerance

The mean (*SD*) pain threshold was 12.3 mA (5.7) for men and 9.7 mA (4.4) for women whereas the pain tolerance was 28.7 mA (8.8) for men and 25.5 mA (7.9) for women. Separate 2 Sex ANOVAs revealed no significant differences between men and women for either pain threshold, $F(1, 31) = 2.18$, $p = .15$, $\eta^2 = .07$, or pain tolerance, $F(1, 31) = 1.23$, $p = .28$, $\eta^2 = .04$.

[1] The mean (SD) intensity and unpleasantness ratings evoked by each level of stimulation were, respectively, 9.3 (6.3) and 7.7 (4.7) for level 1, 18.8 (8.7) and 16.2 (7.8) for level 2, 29.8 (12.7) and 27.2 (12.5) for level 3, 55.8 (15.1) and 53.5 (16.6) for level 4, and 76.6 (13.5) and 74.2 (17.5) for level 5. Similarly, the mean (SD) nociceptive flexion reflex responses (μV) were 12.6 (4.7), 16.5 (8.9), 22.1 (13.5), 31.7 (22.4), and 36.0 (21.5) μV, for stimulation levels 1, 2, 3, 4, and 5, respectively.

Pain condition

A series of 2 Sex (men, women) × 5 Interval (R+0, R+150, R+300, R+450, R+600 ms) repeated measures ANOVAs showed significant interval effects for both pain intensity ratings, $F(4, 124) = 2.93$, $p = .04$, $\varepsilon = .72$, $\eta^2 = .09$, and pain unpleasantness ratings, $F(4, 124) = 3.85$, $p = .01$, $\varepsilon = .80$, $\eta^2 = .11$, but not for nociceptive responding, $F(4, 124) = 0.21$, $p = .84$, $\varepsilon = .58$, $\eta^2 = .01$, or pre-stimulation baseline muscle activity, $F(4, 124) = 0.17$, $p = .95$, $\varepsilon = .96$, $\eta^2 = .01$. Figure 2.1 (panels A, B and C) shows the average pain ratings and nociceptive flexion reflex responses as a function of the R-wave to stimulation interval in the pain condition: pain was greatest at R+300 ms. Polynomial trend analyses confirmed significant quadratic effects for both pain intensity ratings, $F(1, 31) = 6.71$, $p = .01$, $\eta^2 = .18$, and pain unpleasantness ratings, $F(1, 31) = 12.90$, $p = .001$, $\eta^2 = .29$. Finally, no sex or sex × interval effects emerged for pain ratings, nociceptive flexion reflex responses, or pre-stimulation muscle activity.

Non-pain condition

The 2 Sex × 5 Interval repeated measures ANOVAs revealed a marginal interval effect for pain unpleasantness ratings, $F(4, 124) = 2.68$, $p = .05$, $\varepsilon = .77$, $\eta^2 = .08$, and a non-significant interval effect for pain intensity ratings, $F(4, 124) = 1.64$, $p = .19$, $\varepsilon = .69$, $\eta^2 = .05$. It is worth noting that the ratings were highest at R+300 ms. Polynomial trend analyses revealed that the unpleasantness ratings declined linearly across the cardiac cycle, $F(1, 31) = 6.98$, $p = .01$, $\eta^2 = .18$, and that the intensity ratings also yielded a similar, albeit nonsignificant, pattern, $F(1, 31) = 3.30$, $p = .08$, $\eta^2 = .10$. No interval effects were detected for either nociceptive flexion reflex responses, $F(4, 124) = 0.91$, $p = .45$, $\varepsilon = .83$, $\eta^2 = .03$, or pre-stimulation baseline muscle activity, $F(4, 124) = 0.60$, $p = .64$, $\varepsilon = .90$, $\eta^2 = .02$,.

Figure 2.1 (panels D, E and F) presents the key summary data for the non-pain condition. Again, no sex effects were detected.

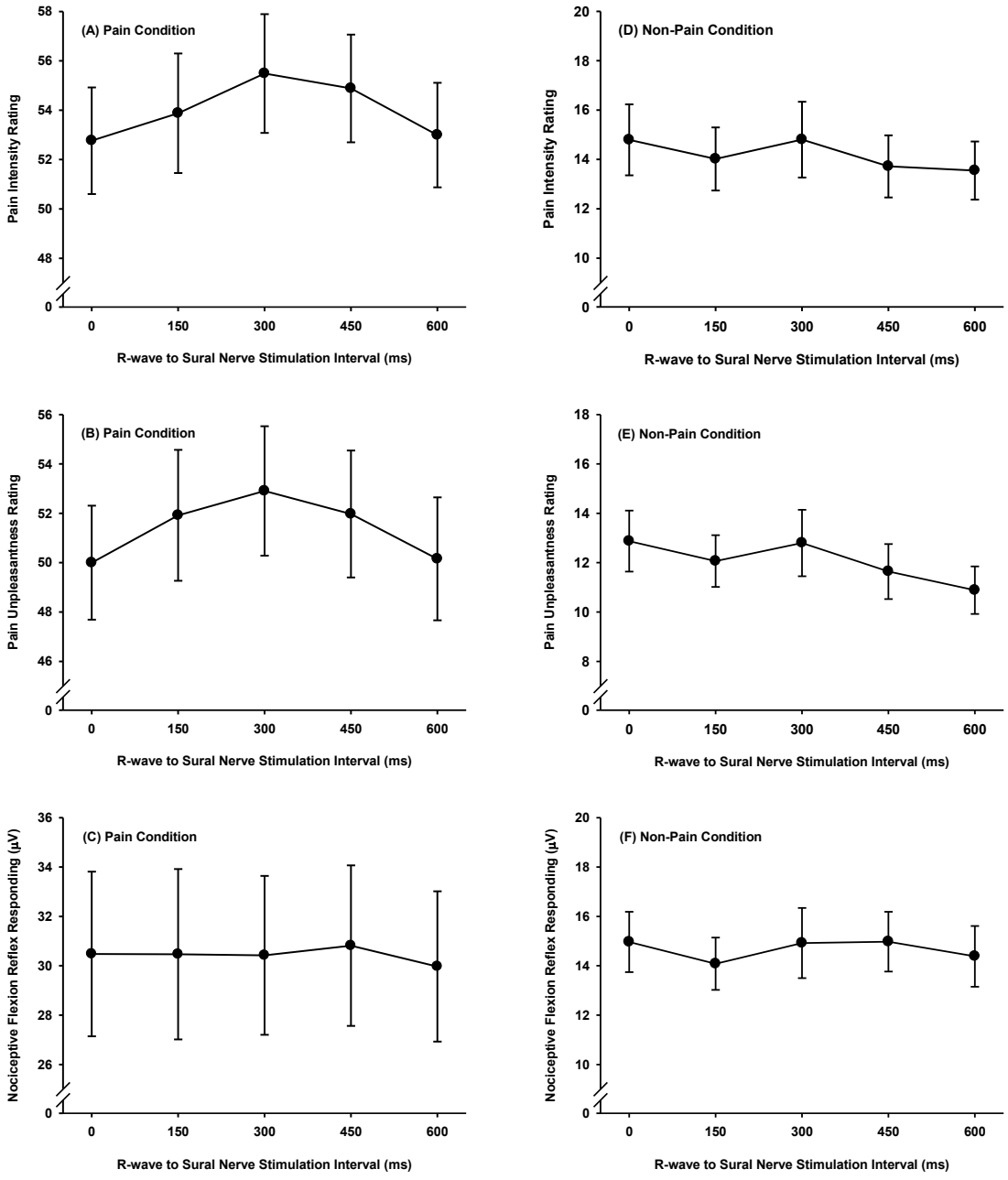

Figure 2.1. Mean (*SE*) pain intensity ratings, pain unpleasantness ratings and nociceptive flexion reflex responses as a function of the R-wave to sural nerve stimulation interval (R+0, R+150, R+300, R+450, R+600 ms) during painful stimulation (panels A, B & C, respectively) and non-painful stimulation (panels D, E & F, respectively) conditions.

Heart Rate

To examine the effect of the cycle time task on heart rate we compared the heart rates during the resting baseline with those during each block of trials. The electrocardiographic signal during the 6-second window preceding each sural nerve stimulation was used to calculate the average heart rate for each trial; these average heart rates were then used to compute the average heart rate in each 25-trial block. Heart rate data were missing for one participant, which is reflected in the reported degrees of freedom. A 2 Sex (male, female) by 5 Period (baseline, block 1, block 2, block 3, block 4) mixed-model analysis of variance (ANOVAs), with sex as a between-subject factor and period as a within-subject factor, was conducted on heart rate. This analysis yielded a significant effect for period, $F(4, 120) = 14.10$, $p = .001$, $\varepsilon = .49$, $\eta^2 = .32$. Post hoc comparisons confirmed that heart rate increased significantly from baseline to task and that heart rate did not vary significantly among the blocks of the cycle time task (see Figure 2.2). There were no main or interaction effects for sex.

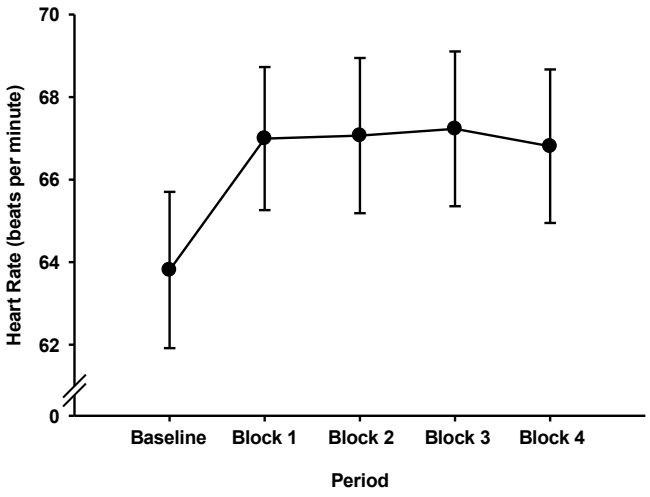

Figure 2.2. Mean (*SE*) heart rates during resting baseline and during each 25-trial block of the cycle time task.

Discussion

Overview

The present study revealed that pain was modulated across the cardiac cycle. Under conditions of high intensity electrocutaneous stimulation, both pain intensity and pain unpleasantness ratings peaked for stimuli presented at 300 ms after the R-wave of the electrocardiogram. This is the first report, to our knowledge, of a cardiac cycle time effect for electrocutaneous pain. We also replicated previous research showing that intensity ratings under conditions of non-painful electrocutaneous stimulation do not exhibit a cardiac cycle time effect (Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003). However, closer inspection of the data using polynomial trend analyses revealed that the unpleasantness and, to a lesser extent, the intensity reported by participants were lowest during the later, diastolic, phase of the cardiac cycle. It is noteworthy that nociceptive responding was unaffected by the phase of the cardiac cycle. This null finding contrasts with the results of previous studies showing that the NFR was attenuated during systole compared to diastole when the reflex was elicited while participants rested quietly (Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003). Nonetheless, our null finding for the NFR is not without precedent: an earlier study found that the pattern of attenuated nociceptive responding during systole seen at rest was abolished by psychological stress (McIntyre et al., 2006).

NFR

McIntyre and colleagues attributed the absence of systolic inhibition of the NFR to increased arousal characteristic of psychological stress (McIntyre et al., 2006). It is possible that the unpredictability of our random block design induced a state of increased

arousal or anxiety. In other words, exposure to unpredictable levels of electrocutaneous stimulation may have elicited a classic stress response (see Grillon et al., 2004). In support of this hypothesis, heart rates were faster during the cycle time task than during rest; the effect size for this task-induced cardiac acceleration response was large (Cohen, 1992). It has been demonstrated that the sympathoinhibitory effects of the baroreceptor reflex are abolished by stimulation of the hypothalamic defence area in cats (Coote et al., 1979). This defence reaction, which is centrally triggered by hypothalamic nuclei and the periaqueductal gray (Canteras, 2002), inhibits arterial baroreceptor inputs reaching the nucleus tractus solitarius (Jordan et al., 1988; Mifflin et al., 1988), thereby releasing spinal nociceptive transmission from the descending inhibitory baroreflex influence. Hence, unpredictable aversive stimulation, a novel feature of our experimental design, may have inactivated the baroreflex mechanism that inhibits nociception at rest (cf. McIntyre et al., 2006). Endogenous opioids, which have been shown to influence the NFR when the noxious stimuli are both intense and unpredictable, may be implicated in this effect (Le Bars et al., 1992; Willer et al., 1981). Indeed, evidence indicates that naloxone, an opioid antagonist, augments both arterial (Rubin et al., 1983) and cardiopulmonary (Schobel et al., 1992) baroreflex mechanisms. To establish a role for endogenous opioids in the baroreflex modulation of the NFR during stress studies are required that measure their circulating levels or block their effects.

Pain

This study is, to our knowledge, the first to demonstrate a cardiac cycle time effect for pain. Pain intensity and unpleasantness evoked by high intensity noxious stimulation were maximal during systole indicating that pain was facilitated during natural baroreceptor activation. Although this finding is contrary to what we had predicted, it is

not without precedent; some studies that used neck suction/compression procedures to manipulate carotid baroreceptor activity (Edwards et al., 2003; Elbert et al., 1988) also, unexpectedly, found that pain was minimal during artificial baroreceptor deactivation. Previous cardiac cycle time studies have never observed a baroreceptor-mediated modulation of pain intensity ratings (Edwards et al., 2001; Edwards et al., 2003; Edwards et al., 2008a). Methodological differences among the studies may help explain this discrepancy. First, the present study employed a mixed block design with a wide range of stimulus intensities presented randomly whereas the previous studies employed fixed block designs with the same constant intensity throughout (Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003; Edwards et al., 2008a). In this latter design, it is likely that participants learn that the stimuli do not vary from trial to trial and therefore disengage from the task and simply give the same ratings, preventing any cardiac cycle effect from emerging. Second, the present study delivered much higher stimulation intensities than previously, suggesting that the influence of natural baroreceptor activation on perception is confined to pain. However, this explanation is incompatible with evidence that cutaneous detection thresholds are modulated across the cardiac cycle, with cutaneous sensibility being greatest during systole (Edwards et al., 2008). That a cardiac cycle time effect is present for very low intensity, non-painful stimuli would appear to rule out stimulus intensity as an explanation for the mixed findings. The present finding that ratings were marginally increased for non-painful stimuli presented during systole would also argue against pain specificity in this context. We acknowledge that these cycle time effects for non-painful stimuli were small, however, this might be attributed to the restricted range at the low end of the scales. Specifically, participants were confined to a 0-25 point scale (see Method section) for non-painful events. In conclusion, it appears that the stimulus

unpredictability introduced by our mixed block design is the most likely explanation for the emergence of a cardiac cycle time effect for pain.

A number of mechanisms might help explain the peak in pain ratings at 300 ms after the R-wave. First, spatial summation may have occurred. Deep dorsal horn wide dynamic range (WDR) neurons receive direct inputs from both A_δ and C fibres and their dendrites integrate A_β fibres inputs (Kandel et al., 2000). Supraspinal pain modulation centres have been shown to enhance the activity of spinal dorsal horn nociceptive neurons through the facilitation of the WDR neurons (Bruehl & Chung, 2004; Dugast et al., 2003; Lima & Almeida, 2002) and some reports suggest that this outcome (inhibition/facilitation) is dependent on the intensity of the triggering signal (Urban & Gebhart, 1997; Zhuo & Gebhart, 1990; Zhuo & Gebhart, 1997). There is also evidence that the WDR neurons encode multireceptive primary afferent impulses under endogenous opioids influence (You et al., 2003). Therefore, the unpredictable nature of our experimental design may have led to WDR neurons sensitization, possibly incorporating cutaneous afferent information into the pain ratings. It should be acknowledged that the bar electrode used to stimulate the sural nerve is likely to have activated A_β fibres as well as A_δ fibres. Given that cutaneous sensibility is greatest at R+300 ms (Edwards et al., 2008b), an augmented cutaneous sensation might have summated with the nociceptive input to produce a cardiac cycle time effect for the integrated perception. Secondly, a temporal summation phenomenon may account for the findings. Phasic pain can facilitate human tactile processing (Ploner et al., 2004) and the opposite appears to happen: a recent study demonstrated that randomized concurrent innocuous somatosensory stimulation applied at the thigh, can enhance phasic electrocutaneous pain at the volar surface of the forearm, independently of attentional processes (Lautenbacher et al., 2007). Similarly, two tactile stimuli can be combined to

create a stronger sensation, a phenomenon known as the *enhancement effect* (Sherrick & Cholewiak, 1986). Therefore, it cannot be ruled out that the interoceptive heartbeat sensation, which occurs approximately 200-300 ms after the R-wave (Brener et al., 1993; Ring et al., 1994), may have been combined with the pain sensation to produce enhanced pain ratings at R+300 ms.

Summary

In sum, this is the first report indicating that (a) pain elicited by high intensity electrocutaneous stimulation is modulated across the cardiac cycle with a characteristic systolic facilitation pattern whereas (b) NFR responding remains stable with respect to variations in natural baroreceptor activation. This study provides further support to the notion that descending supraspinal modulation differentially affects NFR and pain (see (McIntyre et al., 2008; Ring et al., 2008)). These novel findings reveal a new expression of visceral afferent feedback under specific unpredictable conditions of noxious stimulation.

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CHAPTER THREE

Effects of Predictable Stimulation on Pain and Nociception across the Cardiac Cycle

Abstract

Cardiac cycle time effects for sensorimotor processes have provided support for the visceral afferent feedback hypothesis which holds that natural variations in baroreceptor activity influence sensorimotor processing. Evidence suggests that (a) the systolic attenuation of the nociceptive flexion reflex observed under resting conditions is abolished by stress and (b) a cardiac cycle time effect for pain is only seen under stress. It is well established that stress is reduced by stimulus predictability. Accordingly, the present study employed a predictable, fixed block design to assess the effects of natural arterial baroreceptor activity on the nociceptive flexion reflex and pain ratings. Specifically, electrocutaneous stimuli were delivered to the sural nerve at one of five intensities (50% pain threshold, 75% pain threshold, pain threshold, midway between pain threshold and pain tolerance, pain tolerance) at five intervals (0, 150, 300, 450, 600 ms) after the R-wave of the electrocardiogram in either an ascending or descending order of presentation. Nociceptive responding was attenuated during systole when elicited by painful but not non-painful stimuli. Pain ratings did not differ among the R-wave to stimulation intervals regardless of stimulus intensity. The cycle time effect for nociceptive responding provides further evidence for a baroreceptor-mediated antinociception mechanism. That no cardiac cycle time effects were observed for pain suggests that predictable stimulus presentation masks sensory-perceptual modulation.

Introduction

An inverse relation between chronically-elevated blood pressure and sensitivity to noxious stimulation has long been recognised (for review see Ghione, 1996). A visceral afferent feedback hypothesis has been proposed to account for this pressure-perception relationship (France & Ditto, 1996; Koltyn & Umeda, 2006), with afferent inputs from naturally-occurring phasic baroreceptor stimulation (Angell James, 1971; Mancia & Mark, 1983) being integrated into brain stem structures involved in descending inhibition of nociception (see Edwards, Ring, McIntyre, & Carroll, 2001). To test this hypothesis, early studies artificially manipulated the carotid sinus transmural pressure by applying constant suction to the neck for several seconds (Eckberg, Cavanaugh, Mark, & Abboud, 1975). However, these constant suction manipulations produced inconsistent findings, reducing pain in individuals with borderline hypertension (Elbert et al., 1988) but increasing (Elbert et al., 1988) or not affecting pain (France, Ditto, & Adler, 1991) in participants with blood pressure in the normal range. Subsequent studies that employed phasic neck suction/compression methods also generated mixed findings: neck suction during systole reduced pain in some studies (Al'Absi et al., 2005; Brody & Rau, 1994; Dworkin et al., 1994; Edwards et al., 2003; Mini, Rau, Montoya, Palomba, & Birbaumer, 1995) but not all studies (Angrilli, Mini, Mucha, & Rau, 1997; Rau et al., 1994; Rau, Elbert, & Birbaumer, 1995). Overall, artificial baroreceptor activation methods have not produced conclusive evidence to support the visceral afferent feedback hypothesis. These inconsistencies may be explained by limitations of the artificial stimulation methodology (Rau & Elbert, 2001).

The cardiac cycle time paradigm avoids these limitations. In this paradigm, probe stimuli are delivered when baroreceptors are activated (i.e., systole) and when they are

quiescent (i.e., diastole), and the respective responses compared. Using this natural baroreceptor stimulation paradigm, studies have reported attenuated cortical activity (Koriath & Lindholm, 1986; Koriath, Lindholm, & Landers, 1987) and evoked potentials (Edwards, Inui, Ring, Wang, & Kakigi, 2008; Sandman, 1984; Walker & Sandman, 1982) during systole. A series of studies have established that the nociceptive flexion reflex of the lower limb, a polysynaptic spinal withdrawal reflex (Sandrini et al., 2005), is attenuated when elicited during the middle of the cardiac cycle (Edwards et al., 2001; Edwards, McIntyre, Carroll, Ring, & Martin, 2002; Edwards et al., 2003; McIntyre, Edwards, Ring, Parvin, & Carroll, 2006). Intriguingly, none of these studies found evidence that pain varied across the cardiac cycle. A couple of methodological features may have contributed to the null results for pain ratings. First, the studies used the same stimulation intensity on every trial (Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003; Edwards et al., 2008; McIntyre et al., 2006), with this stimulus invariance causing participants to give constant ratings. Second, the electrocutaneous stimulation was not consistently painful; on average, the intensity ratings reported by participants were below pain threshold (Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003).

We recently conducted a cardiac cycle time study to address these points (Quelhas Martins et al, 2009), in which electrocutaneous stimuli were randomly delivered to the sural nerve at one of five intensities (ranging from half pain threshold up to pain tolerance). Hence, stimulus presentation was unpredictable (i.e., the design was mixed rather than fixed) and stimulus intensity was rated as painful on most trials. Both intensity and unpleasantness ratings were modulated across the cardiac cycle, being highest when painful stimuli were delivered during systole and lowest when delivered during diastole. This is the first evidence that pain is subject to the effects of visceral afferent feedback.

However, nociceptive responses elicited by painful stimulation were not modulated by the phase of the cardiac cycle, a pattern of responding seen only once before (McIntyre et al., 2006). We interpreted these results in terms of stimulus unpredictability.

Reconciling these new data with our previous findings is not straightforward because of several novel features of the experimental design. First, the unpredictability generated by a mixed block design with random stimulus presentation. Second, the use of multiple intensities of stimulation covering a broad perceptual range (i.e., non-painful to extremely painful). The present study was designed to test our stimulus unpredictability interpretation. This aim was achieved by keeping the electrocutaneous stimulation intensity the same within blocks of trials (i.e., introducing stimulus predictability by implementing a fixed block design) while retaining the use of multiple broad-ranging intensities. In light of the visceral afferent feedback hypothesis, it was expected that the nociceptive flexion reflex would be lower during systole than diastole. Based on our latest findings (Edwards, Ring, McIntyre, Winer, & Martin, 2009; Quelhas Martins et al, 2009), we hypothesized that intensity and unpleasant ratings would be greatest during systole.

Method

Participants

Forty-three healthy normotensive adults (19 males, 24 females) with a mean age of 20.4 (*SD* = 3.8) years and a mean body mass index of 23.3 (*SD* = 2.5) kg/m^2 gave informed consent and participated in the study. They had a mean resting systolic blood pressure of 115 (*SD* = 11) mmHg, diastolic blood pressure of 65 (*SD* = 8) mmHg, and heart rate of 69 (*SD* = 11) bpm. Exclusion criteria comprised any known heart problems or chronic

illnesses, or any medication apart from birth control. Participants were asked to refrain from caffeine, alcohol, and exercise for 2 hours before the testing session. The study protocol was approved by the local research ethics committee.

Physiological Measures

A chair with an adjustable leg rest supported the participant's left leg at the ankle and kept it flexed in an angle of 35°. A Spike2 computer program ran the experiment and collected physiological data via a Power1401 (Cambridge Electronic Design). The signals were all digitised at 2500 Hz with 16-bit resolution. Electrode sites were exfoliated (Nuprep, Weaver & Co) and degreased with isopropyl alcohol swabs (Mediswab, Seton Healthcare) until contact impedance was <10 kΩ (Checktrode, UFI). An electrocardiogram (ECG) was recorded with three spot electrodes (Cleartrace, ConMed) in a modified chest configuration. The active electrodes were placed on the right clavicle and lower left rib, and the reference was placed on the left clavicle. The ECG signal was amplified and filtered (0.1–100 Hz plus 50 Hz notch filter) by an AC amplifier (P511, Grass). Baseline blood pressure and heart rate were measured with an oscillometric sphygmomanometer (Dinamap Pro100, Critikon) and a brachial cuff (Dura-cuff, Critikon) attached to the left arm. Electromyographic (EMG) activity of the left biceps femoris muscle was recorded with an active differential electrode (DE 2.1, Delsys) placed with the contact bars perpendicular to the muscle fibres and secured 12 cm above the knee crease. A reference electrode was positioned 12 cm lateral to the active one. The EMG signal was bandpass filtered (20–450 Hz) and amplified (×10000) by a Bagnoli-4 system (Delsys). The sural nerve was stimulated electrocutaneously using a constant current stimulator (DS7A, Digitimer) and a bar electrode (Nicolet) with 9 mm diameter contacts and 22 mm inter-contact spacing that was secured posterior to the ankle with the anode superior.

Self-Report Measures

Pain Tolerance. A modified visual analogue scale (Janal, Glusman, Kuhl, & Clark, 1994), with anchors of 0 (no sensation), 5 (faint pain), and 10 (maximum tolerable pain), was used to determine pain threshold and pain tolerance levels (see Quelhas Martins et al, 2009).

Pain Intensity and Pain Unpleasantness. Two modified visual analogue scales (Rainville, Feine, Bushnell, & Duncan, 1992) were used to assess both the intensity and unpleasantness dimensions of pain. Participants rated the perceived intensity / unpleasantness on scales, with anchors of 0 (NOT AT ALL painful / unpleasant), 25 (SLIGHTLY painful / unpleasant), 50 (MODERATELY painful / unpleasant), 75 (VERY painful / unpleasant), and 100 (EXTREMELY painful / unpleasant).

Procedure

Following instrumentation, participants completed a 5-minute baseline resting period. Blood pressure and heart rate readings were initiated at the start of minutes 1, 3 and 5. These readings were averaged to yield baseline systolic blood pressure, diastolic blood pressure, and heart rate.

Tolerance task. The sural nerve was stimulated electrocutaneously by five rectangular 1 ms pulses at 250 Hz, starting at 2 mA. Stimulus intensity was increased in 2 mA steps until a rating of 10 (maximum tolerable pain) was reported or a maximum intensity of 50 mA was reached. The mean (*SD*) pain threshold was 14.3 mA (5.9) for men and 10.0 mA (3.5) for women whereas the pain tolerance was 29.9 mA (8.3) for men and 22.2 mA (5.8) for women.

Cycle time task. Six seconds into each trial, Spike2 initiated a search for an R-wave of the ECG and then triggered the electrocutaneous stimulation of the sural nerve (see

above) at one of five intervals after the R-wave of the ECG (R+0, R+150, R+300, R+450, R+600 ms). Five stimulus intensities were used, namely, 50% of the pain threshold, 75% of the pain threshold, pain threshold, the mid-point between pain threshold and pain tolerance, and pain tolerance. A variable inter-stimulus interval of 20-30 s was used. Five practice trials (one for each of the five intensities) familiarised participants with the task demands. Participants then completed five blocks of 20 experimental trials, with a 5 minute rest after each block. The same (i.e., fixed) stimulation intensity was delivered in each block. Two presentation sequences were employed. In the *ascending* sequence, stimulus intensity increased from block to block: first = 50% of the pain threshold; second = 75% of the pain threshold; third = pain threshold; fourth = mid-point between pain threshold and pain tolerance; fifth = pain tolerance. In the *descending* sequence, stimulus intensity decreased from block to block (i.e., the above order was reversed). Participants were randomly assigned to complete either the ascending (9 males, 12 females) or descending (10 males, 12 females) sequence of presentation.

Data Reduction and Analysis

In each trial of the cycle time task, EMG activity from the biceps femoris was rectified and the mean activity 65 to 5 ms pre-stimulation (baseline activity) and 90 to 150 ms post stimulation (RIII, nociceptive flexion reflex responding) was calculated. The mean EMG activity and mean ratings of the four trials for each cardiac cycle interval and for each stimulus intensity were calculated. Data resulting from the lowest two intensities (50% pain threshold, 75% pain threshold) were collapsed to create average non-pain condition responses. Similarly, data from the highest three intensities (pain threshold, difference between pain threshold and pain tolerance, and pain tolerance) were collapsed to create average pain condition responses.

Preliminary analyses revealed that the presentation sequence did not moderate the effects of the cardiac cycle on the outcome measures, and, therefore, this between-subjects factor was not included in the analyses reported below. Accordingly, a series of 2 Sex (male, female) by 5 Interval (R+0, R+150, R+300, R+450, R+600 ms) mixed-model analyses of variance (ANOVAs), with sex as a between-subject factor and interval as a within-subject factor, were conducted on the key outcome variables. Based on the recommendations by Vasey and Thayer (1987), ANOVAs were corrected for the assumption of independence of data points using a Greenhouse-Geisser correction (ε). Polynomial trend analyses were also performed to investigate our hypothesised *quadratic* cardiac cycle time effects. Eta-squared (η^2), a measure of effect size, was reported. In ANOVA this equals the adjusted R^2 obtained in regression analyses; values of .02, .13 and .26 for η^2 indicate small, medium and large effect sizes respectively (Cohen, 1992).

Results[2]

Pain condition

Figure 3.1 displays the average pain ratings (panels A and B) and nociceptive flexion reflex responses (panel C) as a function of the R-wave to stimulation interval in the pain condition: pain did not vary across the cardiac cycle whereas nociceptive responding appeared attenuated during systole. A series of 2 Sex (men, women) by 5 Interval (R+0, R+150, R+300, R+450, R+600 ms) ANOVAs revealed no interval effects for pain intensity ratings, $F(4, 164) = 0.45$, $p = .75$, $\varepsilon = .88$, $\eta^2 = .01$, and pain unpleasantness ratings, $F(4, 164) = 0.31$, $p = .83$, $\varepsilon = .77$, $\eta^2 = .01$. The interval effect for nociceptive responding, $F(4, 164) = 2.14$, $p = .09$, $\varepsilon = .81$, $\eta^2 = .05$, approached significance while polynomial trend

[2] The mean (SD) intensity and unpleasantness ratings evoked by each level of stimulation were, respectively, 17.2 (14.8) and 15.3 (13.5) for level 1, 34.1 (21.7) and 30.2 (20.1) for level 2, 47.7 (25.3) and 42.8 (25.3) for level 3, 71.9 (19.7) and 64.9 (22.8) for level 4, and 87.6 (13.1) and 81.2 (17.6) for level 5. Similarly, the mean (SD) nociceptive flexion reflex responses (μV) were 10.0 (4.9), 10.6 (4.9), 12.6 (8.3), 15.6 (11.1), and 20.7 (15.3) μV, for stimulation levels 1, 2, 3, 4, and 5, respectively.

analyses confirmed a medium-sized (Cohen, 1992) quadratic effect for nociceptive responding, $F(1, 41) = 5.93$, $p = .02$, $\eta^2 = .13$. Moreover, no interval effects were detected for the baseline muscle activity recorded during the pre-stimulation period, $F(4, 164) = 0.55$, $p = .68$, $\varepsilon = .88$, $\eta^2 = .01$. Finally, no sex or sex by interval effects were found.

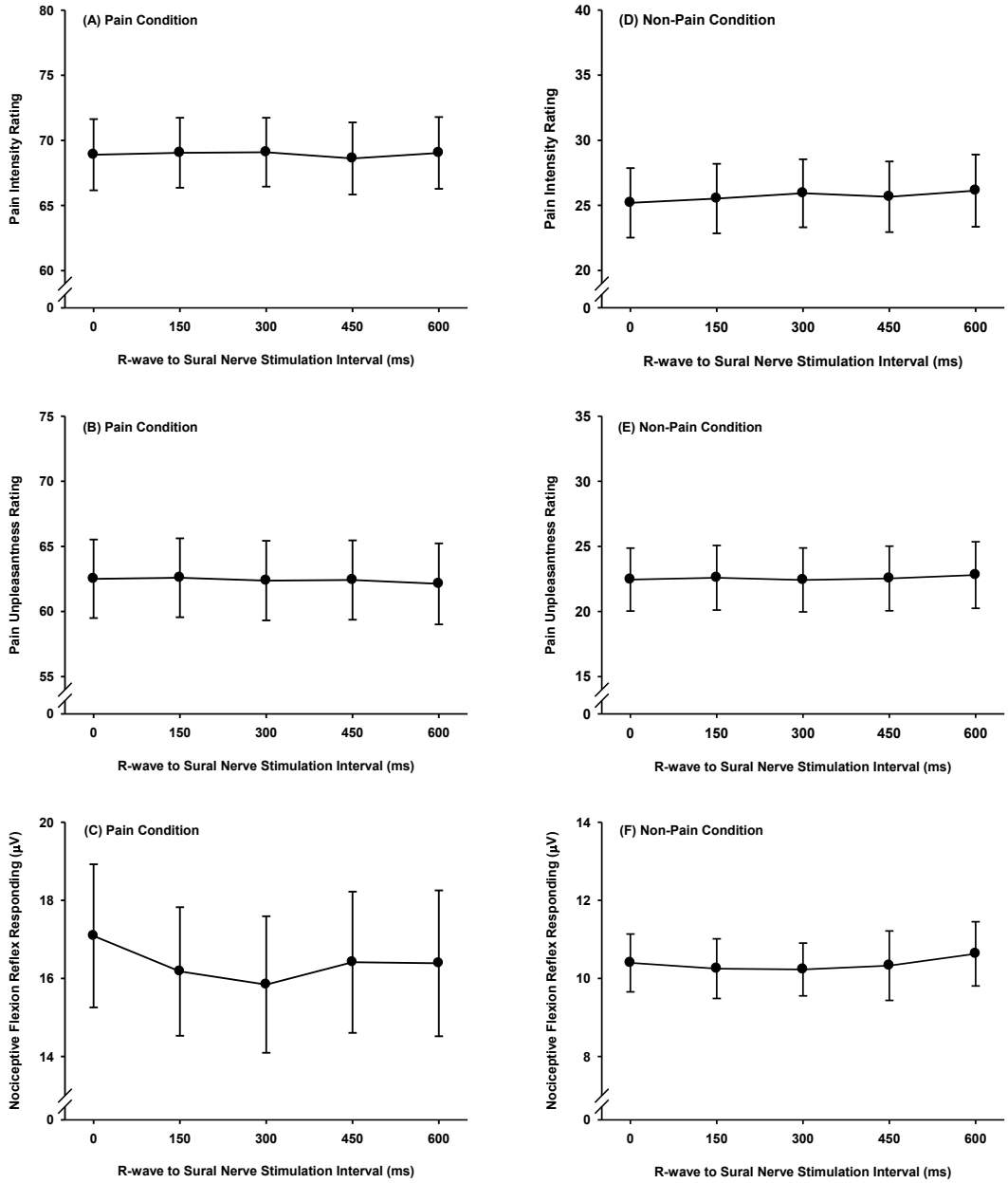

Figure 3.1. Mean (*SE*) pain intensity ratings, pain unpleasantness ratings and nociceptive flexion reflex responses as a function of the R-wave to sural nerve stimulation interval (R+0, R+150, R+300, R+450, R+600 ms) during painful stimulation (panels A, B & C, respectively) and non-painful stimulation (panels D, E & F, respectively) conditions.

Non-pain condition

Figure 3.1 (panels D, E and F) presents the summary data for the non-pain condition: no modulation effects were apparent. The 2 Sex by 5 Interval ANOVAs yielded non-significant interval effects for pain intensity ratings, $F(4, 164) = 0.96$, $p = .42$, $\varepsilon = .79$, $\eta^2 = .02$, pain unpleasantness ratings, $F(4, 164) = 0.19$, $p = .92$, $\varepsilon = .85$, $\eta^2 = .01$, and nociceptive responding, $F(4, 164) = 0.29$, $p = .79$, $\varepsilon = .60$, $\eta^2 = .01$. No trends were revealed. Further, no interval effects were detected for pre-stimulation baseline muscle activity, $F(4, 164) = 0.35$, $p = .82$, $\varepsilon = .86$, $\eta^2 = .01$. No sex effects were detected.

Heart rate

To examine the effect of the cycle time task on heart rate we compared the heart rates during the resting baseline with those during each block of trials. The ECG signal during the 6-second window preceding each sural nerve stimulation was used to calculate the average heart rate for each trial; these average heart rates were then used to compute the average heart rate in each 20-trial block. A 2 Sex (male, female) by 6 Period (baseline, block 1, block 2, block 3, block 4, block 5) ANOVA, with sex as a between-subject factor and period as a within-subject factor, was conducted on heart rate. This analysis yielded a significant effect for period, $F(5, 205) = 6.27$, $p = .001$, $\varepsilon = .70$, $\eta^2 = .13$. Post hoc comparisons confirmed that heart rate was faster in blocks 1 and 2 compared to block 3 of the task, and, moreover, that heart rate was faster in block 3 than during baseline and blocks 4 and 5 of the task (see Figure 3.2). Polynomial analyses confirmed quadratic, $F(1, 41) = 11.29$, $p = .002$, $\eta^2 = .22$, and cubic, $F(1, 41) = 14.26$, $p = .001$, $\eta^2 = .26$, trends for heart rate. In addition, a main effect for sex was found, $F(1, 41) = 6.85$, $p < .05$, but no significant interaction. Heart rates were faster for females ($M = 73.4$; $SD = 2.0$ bpm) than males ($M = 65.5$; $SD = 2.3$ bpm).

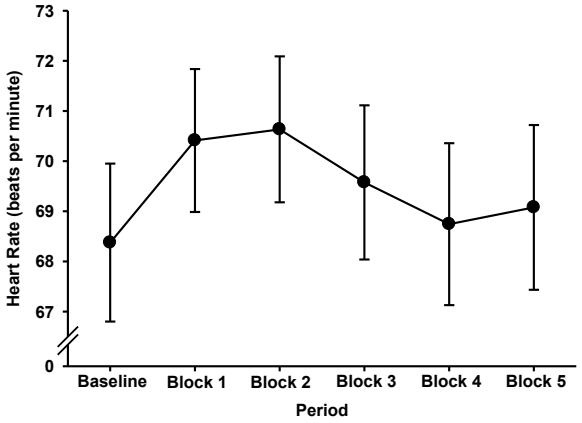

Figure 3.2. Mean (*SE*) heart rates during resting baseline and during each 20-trial block of the cycle time task.

Discussion

NFR

In support of our hypothesis, the present study demonstrated a cardiac cycle time effect for nociceptive responding elicited by painful electrocutaneous stimulation. Polynomial trend analyses confirmed the quadratic pattern for this response, typically seen under resting conditions: the nociceptive flexion reflex was attenuated during systole compared to diastole. Moreover, the size of this cycle time effect resembled the effects reported previously (Edwards et al., 2001; Edwards et al., 2003; McIntyre et al., 2006). The primary purpose of the present experiment was to investigate the influence of stimulus predictability on natural baroreceptor modulation of nociception and pain. Quelhas Martins and colleagues (2009) found that nociceptive responses elicited by painful stimulation were not modulated by the phase of the cardiac cycle. This finding was attributed to the state of increased physiological arousal generated by the unpredictability of the task. This interpretation was based on the results of a study showing that the cycle time effect for the NFR, present during rest, was abolished by an arousing mental arithmetic task that

increased heart rate by seven beats per minute, and that was presumed to deactivate the baroreflex (McIntyre et al., 2006). Quelhas Martins et al (2009) employed a mixed block design with random presentation of multiple intensities of stimulation covering a broad perceptual range. The present study employed these same multiple intensities of stimulation. The sole difference between the latter study and the present study was the blocking arrangement, i.e., mixed versus fixed intensity of stimulation in each block. A comparison of the heart rate data of the two studies suggests that the blocking arrangement had an impact on the participants' state of arousal. The predictable task elicited a small, temporary increase in heart rate (see Figure 3.2) whereas the unpredictable task elicited a larger, sustained increase in heart rate (see Quelhas Martins et al, 2009). Accordingly, the threat associated with stimulus unpredictability may elicit a defence reaction (Canteras, 2002; Coote, Hilton, & Perez-Gonzalez, 1979) which has been shown to inhibit the arterial baroreflex (Jordan, Mifflin, & Spyer, 1988; Mifflin, Spyer, & Withington-Wray, 1988).

It should also be noted that the nociceptive responding was unaffected by the phase of the cardiac cycle when elicited by non-painful electrocutaneous stimulation; a similar unmodulated pattern of responding for low intensity sural nerve stimulation was noted by Quelhas Martins et al (2009). Taken together with our previous findings for higher intensity sural nerve stimulation, these data suggest that the effects of baroreceptor activity on nociceptive transmission are only evident for painful levels of stimulation (i.e., a threshold must be exceeded for the baroreceptor mechanism to modulate spinal transmission of nociceptive afferents).

Pain

Contrary to predictions based on our latest research (Edwards et al., 2009; Quelhas Martins et al, 2009), this study found that intensity and unpleasantness ratings were not

modulated across the cardiac cycle for neither painful nor non-painful electrocutaneous stimulation. However, the current null finding is in agreement with the remainder of our previous research that also found no evidence of a cardiac cycle time effect for intensity ratings of painful electrocutaneous stimuli (Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003; Edwards et al., 2008). It is noteworthy that a fixed block arrangement of trials was used in all of these studies. In contrast, two previous studies have noted cardiac cycle time effects for psychophysical ratings of electrocutaneous stimuli: the evoked sensations were judged to be stronger when stimuli were presented during systole (i.e. R+300 ms) compared to diastole (Edwards et al., 2009; Quelhas Martins et al, 2009). Importantly, the stimuli varied in intensity from trial to trial in both instances. These data, together with other research demonstrating cycle time effects for reaction time (e.g., Edwards, Ring, McIntyre, & Carroll, 2007; McIntyre, Ring, Hamer, & Carroll, 2007; McIntyre, Ring, Edwards, & Carroll, 2008) argue that sensory processing is under the influence of natural baroreceptor activity. However, cardiac cycle time effects for ratings appear to be obscured by the use of fixed block experimental designs (cf. Coles & Duncan-Johnson, 1977). It therefore seems plausible that participants learn the stimulus intensity invariance from trial to trial with such a design, and simply give the same or similar ratings throughout. In other words, they acquire an expectation that influences their ratings (cf. Brown, Seymour, Boyle, El-Deredy, & Jones, 2008). These findings emphasise the importance of experimental design in assessing factors implicated in pain modulation.

Summary

In sum, this report indicates that predictable electrocutaneous stimulation is associated with a cardiac cycle time effect for nociceptive responding, albeit only for high intensity stimuli, but not pain ratings. Importantly, the current findings support the view

that the experimental design (i.e., mixed versus fixed) influence the expression of visceral afferent feedback on sensorimotor processing. Finally, these findings provide further evidence that nociception and pain are differentially modulated (see McIntyre, Kavussanu, & Ring, 2008; Ring, Edwards, & Kavussanu, 2008).

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CHAPTER FOUR

Implicit Learning of Aversive Event Unpredictability Causes Stress-Induced Hypoalgesia

Abstract

Temporal predictability, or knowing when a noxious stimulus will occur, has been implicated in stress-induced hypoalgesia, but the contribution of event predictability, or knowing what the stimulus will be, remains poorly understood. To address this issue, we examined the effects of event predictability on pain intensity ratings and nociceptive flexion reflex responses. Participants repeatedly experienced five intensities of electrocutaneous stimulation, ranging from non-painful to extremely painful, delivered either randomly (unpredictability group) or blocked (predictability group) with no cues provided. Unpredictable shocks produced the lowest pain ratings whilst evoking the highest nociceptive flexion reflex responses. Moreover, anticipatory heart rate data indicated that unpredictable trials were the most physiologically arousing. Our findings show that uncertainty about the upcoming stimulus intensity is stressful and causes hypoalgesia. Our findings also imply that a low event predictability schedule of stimulation could be used to ameliorate pain in clinical practice.

Introduction

The stress of noxious electrical stimulation reliably causes reduced responding to subsequent aversive stimulation in animals, a phenomenon called stress-induced hypoalgesia (SIH). Evidence for SIH in humans is scarce (Butler & Finn, 2009). One of the few positive studies showed that electrocutaneous stimulation close to pain tolerance can produce a conditioned opioid-sensitive form of SIH (Flor et al., 2002). Related research has examined the effects of stress on neurophysiological correlates of pain. Extreme stress, operationalized by threat of severe pain with and without occasional delivery of extremely noxious electrocutaneous stimulation, attenuated the nociceptive flexion reflex, an effect reversed by the opioid antagonist naloxone (Willer, 1980; Willer & Albe-Fessard, 1980; Willer et al., 1981). The available literature indicates that a necessary condition for SIH, but which is rarely satisfied in human research, is that the stressor itself is aversive.

Another necessary condition for SIH seems to be repeated noxious stimulation (Butler & Finn, 2009). Such stimulation, which is also a feature of animal SIH protocols, elicits strong emotional reactions, such as fear, in anticipation of pain. Research from aversive learning paradigms has established that the predictability of noxious stimulation influences the emotions elicited by the threat of shock. For instance, shock predictability determines the magnitude of fear-potentiated startle responses (Bradley & Lang, 2007). According to Miller (1981) two forms of predictability exist. *Temporal* predictability concerns *when* the noxious stimulus occurs whereas *event* predictability concerns *what* are its sensory properties. There is consensus that temporally predictable noxious stimulation produces fear, resulting in the potentiation of defensive reflexes and hypoalgesia, whereas

temporally unpredictable noxious stimulation induces anxiety, behavioural inhibition and hyperalgesia (Ploghaus et al., 2001). The literature concerning event predictability and pain is less clear cut. On the one hand, it has been argued that the provision of explicit information about the nature of upcoming noxious stimulation increases the experience of pain by activating pain-related schemas that facilitate sensory intake (Leventhal et al., 1979). On the other hand, such information may reduce pain by allowing a comparison between what is expected and what occurs, with a better match lessening the impact of subsequent stimuli (Rachman & Arntz, 1991).

Unfortunately, the already limited empirical evidence concerning event predictability and pain is mostly confounded by temporal uncertainty. Event unpredictability was a feature of the stress protocol that attenuated the nociceptive flexion reflex, where participants received noxious or innocuous electrocutaneous stimuli (Willer et al., 1981). Another study manipulated event predictability by administering one non-painful and two painful stimulus intensities while standardizing temporal predictability by providing a warning cue before laser stimulation (Brown et al., 2008). Event predictability was further manipulated by having the cue conveying certain information about the upcoming stimulus on half of the trials but uncertain information on the other half. Event predictability decreased pain ratings at the lowest stimulus intensity but increased pain ratings at the highest stimulus intensity. A recent study also manipulated event and temporal predictability to create overall low, moderate and high predictability (Oka et al., 2010). Low predictability was associated with increased fear, pain, brain evoked potentials and pupil dilation. Accordingly, it may be deduced that the level of perceived threat and fear associated with event predictability paradigms determines the experience of pain

(Janssen & Arntz, 2001), with paradigms employing extremely intense (Willer et al., 1981) or multiple (Brown et al., 2008) stimuli more likely to elicit SIH.

To test this hypothesis we examined the effects of event predictability – *learned implicitly by direct experience of the contingencies of multiple and intense noxious stimulation* – on pain and nociception. Specifically, participants experienced five intensities of electrocutaneous stimulation, ranging from non-painful to extremely painful, that were delivered either randomly or blocked. Temporal predictability was standardized, with no cue and a long variable interval between successive electrocutaneous stimulations of the sural nerve. It was hypothesized that event unpredictability would be associated with lower pain ratings and nociceptive flexion reflex responses, indicative of SIH, for the noxious stimuli.

Method

Participants

Seventy-six healthy adults (33 males, 43 females) gave informed consent and completed the study protocol that was approved by the local research ethics committee.

Physiological Measures

Baseline blood pressure and heart rate were measured using an oscillometric sphygmomanometer (Dinamap, Critikon). Physiological signals were digitised at 2500 Hz with 16-bit resolution (Power1401, Cambridge Electronic Design). An electrocardiogram was recorded using electrodes in a chest configuration. Electromyographic activity of the left biceps femoris muscle was recorded using an active electrode (DE-2.1, Delsys) and amplifier (Bagnoli-4, Delsys). The sural nerve was stimulated electrocutaneously by five

rectangular 1 ms pulses at 250 Hz using a stimulator (DS7A, Digitimer) and bar electrode (Nicolet).

Procedure

Participants rested for five minutes while baseline blood pressure and heart rate were recorded. Next, their sural nerve was stimulated, starting at 2 mA and increasing in 2 mA steps, to determine pain threshold and tolerance. After a brief rest, their sural nerve was stimulated at each of the five intensities to be used in the experimental task: 50% pain threshold, 75% pain threshold, pain threshold, mid-point between pain threshold and pain tolerance, and pain tolerance.

The experimental task comprised 100 trials that were completed in blocks separated by a five minute rest. Participants were assigned to one of two groups: randomly varying stimulation intensity in each block (unpredictable group) and constant stimulation intensity in each block (predictable group). In the predictable group, stimulation intensity increased or decreased across blocks, with order of stimulation counterbalanced across participants. Participants rated the intensity of pain on a scale with anchors of 0 (not at all painful), 25 (slightly painful), 50 (moderately painful), 75 (very painful), and 100 (extremely painful). A variable inter-stimulus interval of 20–30 s was used to standardize temporal predictability and reduce habituation

Data Analysis

In each trial, muscle activity was rectified and the mean activity 90–150 ms post-stimulation was calculated as a measure of nociceptive flexion reflex responding. A 2 Group by 5 Intensity ANOVA was conducted on the mean pain ratings and nociceptive flexion reflex responses that were averaged over the 20 trials at each stimulus intensity.

The multivariate solution was reported where appropriate and planned contrasts were conducted to compare groups and conditions. Heart rate reactivity elicited by the task was determined to characterise the physiological impact of the stressor. The mean heart rate during each block of trials was computed by averaging the heart rate during the six seconds prior to stimulation. Data were missing for one participant. A 2 Group by 5 Block ANOVA was performed on heart rate reactivity scores, computed as task minus baseline.

Results

Pain

Intensity ratings increased linearly with increasing stimulus intensity, $F(4, 71) = 445.80$, $p < 0.001$, $\eta^2 = .96$). Overall, the unpredictable group ($M = 38.08$, $SE = 2.35$) reported lower intensity ratings than the predictable group ($M = 51.68$, $SE = 2.06$), $F(1, 74) = 18.92$, $p < 0.001$, $\eta^2 = .20$. Furthermore, the ratings of the unpredictable group were less than those of the predictable group at each stimulus intensity level (Figure 4.1a).

Nociception

Nociceptive flexion reflex responses increased linearly with increasing stimulus intensity, $F(4, 71) = 24.50$, $p < 0.001$, $\eta^2 = .58$. Overall, electrocutaneous stimulation elicited larger responses in the unpredictable group ($M = 23.77$, $SE = 1.85$ μV) than the predictable group ($M = 13.88$, $SE = 1.62$ μV), $F(1, 74) = 16.24$, $p < 0.001$, $\eta^2 = .18$. Although the nociceptive responses of the unpredictable group were greater than those of the predictable group at each stimulus intensity level (Figure 4.1b), the discrepancy between the groups increased with increasing stimulus intensity, $F(1, 74) = 4.57$, $p < 0.002$, $\eta^2 = .21$, being most evident for the most noxious stimuli.

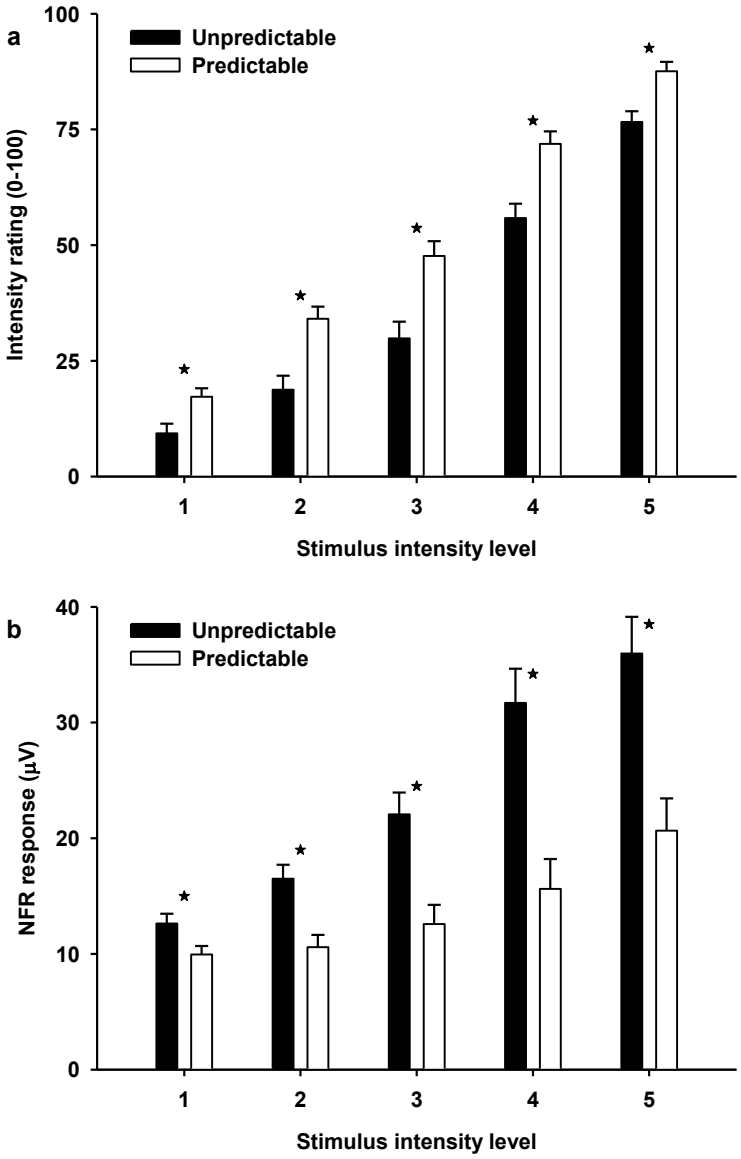

Figure 4.1. Effects of event uncertainty on pain and nociception. (**a**) Pain intensity ratings. Mean (*SE*) pain ratings for the predictable and unpredictable groups as a function of the stimulus intensity level (1 = 50% of pain threshold, 2 = 75% of pain threshold, 3 = pain threshold, 4 = mid-point between pain threshold and pain tolerance, 5 = pain tolerance). Ratings are on a 0–100 scale, with anchors of 0 (not at all painful), 25 (slightly painful), 50 (moderately painful), 75 (very painful), and 100 (extremely painful). (**b**) Nociceptive flexion reflex responses. Mean (*SE*) amplitude (μV) of the nociceptive flexion reflex responses measured as biceps femoris electromyographic activity for each group as a function of stimulus intensity level. An asterisk indicates a difference, $p < 0.05$, between groups.

Autonomic Activation

The task elicited greater overall heart rate reactions in the unpredictable group (M = 3.40, SE = 0.64 beats min^{-1}) than the predictable group (M = 1.43, SE = 0.55 beats min^{-1}), $F(1, 73) = 5.48$, $p < 0.02$, $\eta^2 = .07$. Moreover, the groups also displayed different patterns of cardiac reactivity, $F(4, 70) = 2.68$, $p < 0.04$, $\eta^2 = .13$; the unpredictable group's heart rates remained elevated throughout the task whereas the predictable group's heart rates increased at the start but declined halfway through the task (Figure 4.2). In addition, a main effect for sex was found, $F(1, 71) = 9.19$, $p < .05$, but no significant group interactions. Heart rate reactivity was higher for females (M = 3.5; SD = 0.5 bpm) than males (M = 1.0; SD = 0.6 bpm).

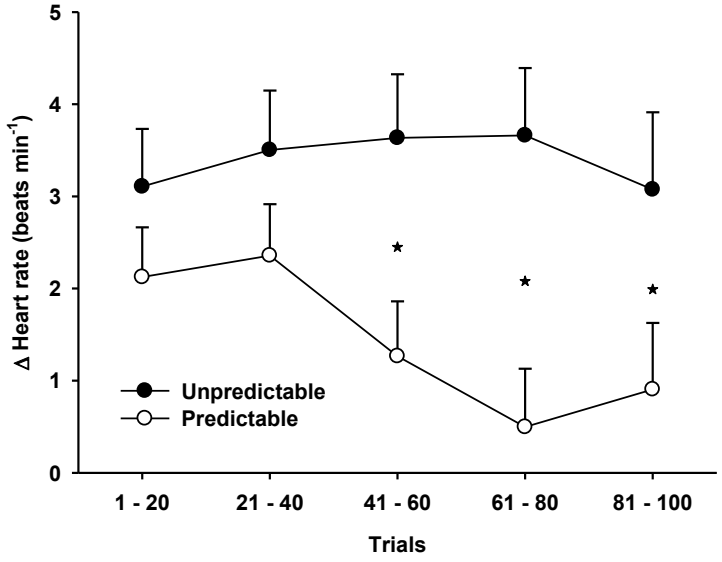

Figure 4.2. Effects of event uncertainty on an autonomic nervous system index of stress. Mean (*SE*) task-induced heart rate reactions for the predictable and unpredictable groups as a function of the trial block. An asterisk indicates a difference, $p < 0.05$, between groups.

Group Characteristics

The unpredictable and predictable groups did not differ on any demographic, cardiovascular or pain tolerance variables (Table 4.1).

Variable	*Group*		*Statistic*
	Unpredictable	Predictable	
	N = 33	*N* = 43	
Male, *N* (%)	14 (42)	19 (44)	$\chi^2(1) = 0.02$
Age (years)	19.4 (1.0)	20 (3.8)	$F(1,75) = 2.20$
Body mass index (kg m^{-2})	23.4 (2.3)	23.3 (2.5)	$F(1,75) = 0.02$
Systolic blood pressure (mmHg)	112.5 (10.8)	114.8 (10.5)	$F(1,75) = 0.89$
Diastolic blood pressure (mmHg)	64.7 (5.3)	65.1 (7.9)	$F(1,75) = 0.07$
Heart rate (beats min^{-1})	64.0 (10.4)	68.7 (10.6)	$F(1,75) = 3.73$
Pain threshold (mA)	10.8 (5.1)	11.9 (5.1)	$F(1,75) = 0.89$
Pain tolerance (mA)	26.9 (8.3)	25.6 (7.9)	$F(1,75) = 0.46$

Note: Values are group means (s.e.m.)

Table 4.1. Summary statistics comparing the unpredictable and predictable groups.

Discussion

We compared pain reports and nociceptive responses following intermittent electrocutaneous stimulation under conditions of unpredictable randomly varying stimulus intensities versus predictable constant stimulus intensities. Pain intensity ratings were lower whereas nociceptive flexion reflex responses were higher for unpredictable compared to predictable stimulation (Figure 4.1). In agreement with previous research (Brown et al., 2008; Willer et al., 1979), we found that event unpredictability reduced the pain associated with highly noxious stimulation (cf. Oka et al., 2010). We also found that event unpredictability reduced the perceived intensity of moderately noxious stimuli as well as innocuous stimuli. Fear increases heart rate whereas anxiety and orienting slow heart rate. Given that anticipatory physiological arousal is proportional to the magnitude of the danger anticipated (Miller, 1979), it is likely that our unpredictability paradigm, that employed more intensities and higher intensities than those employed by Brown et al

(2008) and Oka et al (2010), is a more potent stressor. Indeed, the anticipatory heart rate reactions indicated that unpredictable stimulation was more physiologically arousing than predictable stimulation (Figure 4.2). Our findings argue that prolonged event unpredictability surrounding the nature of the upcoming stimulus is psychologically stressful and causes SIH.

That event unpredictability also facilitated reflexive nociceptive responding reveals that the subjective experience of pain can be dissociated from the objective neurophysiological measure of nociception under specific conditions of extreme psychological stress. Previous studies have also documented that arousing secondary tasks, such as mental arithmetic, are associated with reduced pain ratings and potentiated nociception flexion reflex responses (Edwards et al., 2006; McIntyre et al., 2006). Similarly, it has been shown that mental arithmetic stress reduces pain while potentiating the nociceptive blink reflex (Koh & Drummond, 2006). It is well established that intense, unpleasant emotions, such as fear, are associated with the facilitation of reflexes, including blink (e.g., Bradley & Lang, 2007; Grillon, 2008), tendon (e.g., Bonnet et al., 1995), and nociceptive flexion (e.g., Rhudy et al., 2010) reflexes. Accordingly, our findings argue that event unpredictability generated highly arousing, negative emotions compared to event predictability. Our findings are also compatible with the view that event predictability generated low to mildly arousing, negative emotions.

Here we report data stemming from a new paradigm for studying SIH in humans that is based on implicit learning of event predictability. Ours differs from previous paradigms in important ways. First, most previous protocols rely on explicit rather than implicit learning by associating a particular cue with a particular noxious stimulus. Moreover, the unpredictability often depends on a manipulation of the validity of the

information transmitted by the cue to create a mismatch between the expected stimulus and actual stimulus (cf., Brown et al., 2008; Ploghaus et al., 2001). Our paradigm is simpler in that no cues are presented and therefore individual differences in attentional and associative learning processes are avoided (Grillon, 2008). Second, our paradigm employs prolonged (i.e., 100 experimental trials plus familiarization trials) and very intense (60% of stimuli are painful and 20% are at the maximally tolerated intensity) stimulation. These features combine to create differences in event predictability between the random and blocked schedules of stimulation. Below we tentatively outline some potential mechanisms that may underlie our paradigm.

Predictability minimizes the prediction error about subsequent stimuli (Rachman & Arntz, 1991) at the cost of imposing expectation schemas on the sensory evidence: expectancies of high intensity stimulation intensify the neural processing of pain and result in increased intensity ratings (Keltner et al., 2006), whereas expectancies of either a low intensity stimulus (Keltner et al., 2006) or an attenuated impact of it (i.e. placebo effect; Wager et al., 2004) produce reverse effects. On a trial-by-trial basis, event predictability will have led to minimal prediction errors, resulting in stable expectations and intensity ratings within each block. Ancillary evidence indicates that a block of fixed-intensity shocks produces slight sensitization of pain (Rhudy et al., 2010). In contrast, event unpredictability prevented participants from developing stable expectations. Nonetheless, over-prediction errors (i.e., expectancy of the highest intensity being followed by a lower intensity stimulus) are more likely to have happened (Rachman & Arntz, 1991). In such cases, the experience has rewarding properties (i.e., relief) that bias predictions to achieve the motivated behavior (i.e., pain relief; Seymour et al., 2005), thereby shifting the

evaluation towards the desired outcome. In plain words, pain may have been habituated as a corollary of intermittent relief.

In sum, our data demonstrate that pain following highly noxious stimulation can be alleviated by a low event predictability schedule of trials. In theory, this paradigm could be used clinically in behaviourally-based analgesic treatments. Another application could target patients with implantable cardioverter defibrillators: these individuals could benefit from the addition of occasional non-painful stimulations. Event unpredictability could therefore help to improve the quality of life of some cardiac patients whose current treatment means to be automatically given painful shocks (Baumert et al., 2006) when heart rate is abnormal. Additionally, our paradigm may also inform rehabilitation techniques (e.g., function electrical stimulation) that capitalize on the habituation and sensitization of reflexes (Nicol et al., 1998). Finally, the dissociation we report between pain ratings and nociception flexion reflex responses urges caution in the interpretation of results from analgesic quantification techniques – such as those commonly used to evaluate new drug treatments – that solely rely on reflex measures.

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CHAPTER FIVE

Moderate Intensity Exercise Facilitates Attentional Control and Working Memory

Abstract

Research concerning the effects of acute moderate exercise on cognitive performance is mixed, with opposing perspectives trying to account for disparate findings, particularly for the executive functions. Among these, working memory amenability to functional improvements induced by moderate exercise lacks inspection. We present two experiments that examined the impact of moderate intensity exercise on attention control and working memory, assessed by the paced auditory serial addition task (Experiment 1, N = 24 males) and the Sternberg paradigm (Experiment 2, N = 120 males and females). These cognitive tasks were performed at rest and/or while cycling at different graded power outputs. Experiment 1 found that moderate intensity exercise increased the number of correct responses at medium levels of task difficulty. Experiment 2 found that moderate intensity exercise lowered the response latency slopes. In conclusion, working memory and attention are improved by dynamic exercise at moderate intensities, and, moreover, this enhancement effect appears to be moderated by task difficulty.

Introduction

During the present decade, several studies examining the effects of habitual (i.e., cardiovascular fitness) or sporadic (i.e., acute bouts) aerobic exercise on cognitive functioning have emerged (see Smith et al., 2010; Hillman et al., 2008; Tomporowski, 2003 for reviews). To date, convergent behavioural (Colcombe & Kramer, 2003) and neuroimaging (Colcombe et al., 2006; Marks et al., 2007) evidence has suggested sustained benefits of regular physical exercise on cognition, possibly depending on plasticity enhancement mechanisms within frontoparietal networks. Thus, not surprisingly, the greatest improvements in performance attributed to regular aerobic exercise are commonly seen on cognitive measures of executive control (Themanson & Hillman, 2006; Themanson et al., 2008), particularly among older adults (Erickson & Kramer, 2009; Geda et al., 2010) and people with mild cognitive impairments (Baker et al., 2010).

However, the immediate effects of exercise on cognitive function are less clear-cut (see Tomporowski, 2003; Brisswalter et al., 2002 for reviews). On the one hand, improvements in the speed of responding to simple (Brisswalter et al., 1995; Davranche et al., 2006) and complex (Pesce et al., 2002; Pesce et al., 2007) reaction time tasks performed during sub-maximal aerobic exercise have been reported. From the assumption that either sub- or supra-optimal levels of cortical catecholamines impair high-order cognition (see Robbins & Arnsten, 2009 for review), these studies argued for a facilitating effect of moderate exercise-induced arousal allegedly by promoting a narrowing of attentional focus due to an optimal cortical concentration of catecholamines. In line with this view, incremental exercise paradigms have found choice reaction time performance to be related to plasmatic adrenaline and noradrenaline concentrations (Chmura et al., 1994;

Chmura et al., 1998). However, recent studies employing fractionated response times have only found support for a facilitating effect of arousal on the peripheral (motor) components of the response (Davranche et al., 2005; Chang et al., 2009), likely because the plasmatic and the cortical distribution of catecholamines are not necessarily the same. Nonetheless, a quadratic relationship between exercise-induced arousal and movement time was demonstrated for both simple and choice reaction times (Chang et al., 2009).

Conversely, other studies have failed to detect an arousal effect on basic cognitive performance (Cote et al., 1992; Travlos & Marisi, 1995). Moreover, when more complex cognitive processes are considered, rather subtle influences of moderate exercise become apparent. Among diverse cognitive tasks assessed, speed of decision-making (McMorris et al., 1999; Davranche & Audiffren, 2004) and response preparation (Arcelin et al., 1998) appear benefited by moderate exercise. However, particularly for measures of executive control, studies reported positive (Pesce et al., 2002), null (Themanson & Hillman, 2006; Coles & Tomporowski, 2008), or negative (Dietrich & Sparling, 2004; Pontifex & Hillman, 2007) effects, irrespectively of performance in other domains. For instance, in one study, performance to a Simon task during moderate cycling yielded improvements in reaction time but impairments in response inhibition (Davranche & McMorris, 2009). The same authors replicated the facilitating effect on reaction times to an Eriksen task during a similar exercise protocol, yet, detecting no impairments on cognitive control (Davranche et al., 2009).

Clearly, methodological issues (e.g., type of cognitive task, intensity / duration of the exercise protocol, individual differences in levels of fitness / expertise) may underlie such disparate findings (see Tomporowski, 2003). Nevertheless, such inconsistencies are visibly discrepant from the findings obtained by the aforementioned aerobic fitness studies.

And furthermore, they stand out in light of most studies assessing executive control *after* exercise, which provide convincing behavioural (Hogervorst et al., 1996; Sibley et al., 2006) and neurophysiological (Kamijo et al., 2004; Hillman et al., 2003; Hillman et al., 2009) evidence for its benefits, at least for moderate intensities.

An integrative view was offered by Dietrich (2003, 2006), who proposed a "transient hypofrontality" mechanism, assuming the brain functioning at constant metabolic exchanges. Accordingly, during exercise, a substantial demand upon cerebral resources occurs in order to accomplish motor, sensorimotor, and autonomic control sustaining physical activity. As a result, cortical regions not fundamental for the enduring task (as the prefrontal cortex) are deactivated and the respective resources allocated in favour of the motor and sensory cortices. This would predict decrements in executive control performance during exercise but not afterwards, once the metabolic demands of motor and sensory cortices would return to resting levels and the metabolic resources would be restored. In fact, some studies have garnered support for this perspective in recent years (Pontifex & Hillman, 2007; Del Giorno et al., 2010), mainly by demonstrating impairments in response inhibition during moderate exercise.

In this context, it is somehow surprising that only few studies have examined working memory during acute aerobic exercise. Working memory (WM) refers to the transient storage and manipulation of information for use in related cognitive processes or goal-directed behavioral guidance (Baddeley, 2003). Recent models of WM propose a "central executive" which coordinates information through subsidiary subsystems, the "visuospatial sketchpad" for manipulation of visual items, the "phonological loop" for subvocal rehearsal, and the "episodic buffer", that temporally retains and binds information from all the others (Baddeley, 2003; Rawley & Constantinidis, 2009).

One study provided evidence of impaired performance to the Wisconsin Card Sorting Task and the paced auditory serial addition test (PASAT) during periods (50 and 65 minutes, respectively) of moderate exercise (Dietrich & Sparling, 2004). Importantly, performance on tests demanding the least prefrontal cortex resources was unaffected by exercise. Similarly, another study assessed WM at 25%, 50%, 75%, and 100% of participants' VO_2 max and during the recovery period, and found that performance on a computerized WM task reduced during exercise to improve after recovery (Lo Bue-Estes et al., 2008). However, a recent study found no evidence that a 40-min period of moderate cycling could interfere with PASAT performance, either during or after the exercise period (Lambourne et al., 2010). Clearly, this matter warrants further research.

We therefore examined the effects of moderate-intensity exercise on WM performance in two related experiments. The PASAT was employed in the first experiment, as an index of attention control and working memory (Gronwall, 1977). This is a demanding task because the participant must continuously update a digit held in memory whilst consecutively summing and reporting the respective result (see Tombaugh, 2006 for review). Neuroimaging studies have confirmed numerous cerebral structures being activated during PASAT performance, including the left and right frontal and parietal regions, the anterior cingulate, among others (Lockwood et al., 2004; Audoin et al., 2005). Such profile of activations reflects the auditory perception, the attention control, the update and integration of information, and the vocalization required during the task (Audoin et al., 2005). In the second experiment, we used the Sternberg paradigm (Sternberg, 1966). To perform this task, the participant must retain a set of digits presented sequentially. After a few seconds delay, a matching or mismatching probe digit must then be compared with the set, in order for the appropriate response to be selected and executed.

In general, such task elicits increased frontal and parietal activations (Wager & Smith, 2003), with dorsal prefrontal cortex activations increasing proportionally to load demands (Wolf et al., 2006; Schon et al., 2009). Depending on the type of stimuli used, stage-specific activations can be observed in premotor regions and Broca's area (i.e., subserving subvocal rehearsal during retention; Altamura et al., 2007), in the hippocampal formation (i.e., allowing the sequential encoding and comparison of items in the "episodic buffer"; Schon et al., 2009), as well as in ventral lateral prefrontal regions (i.e., enabling the detection of mismatching probes during retrieval; Wolf et al., 2006).

From a "transient hypofrontality" perspective, we predicted that WM performance would be impaired during moderate exercise and that would be substantiated by decreased accuracy for both tasks and/or slowing of decision making (i.e., a steeper slope) for the Sternberg task; on the contrary, from an exercise-induced arousal perspective, we hypothesized that WM performance would be optimized by moderate exercise, with both tasks revealing increased accuracy and/or the Sternberg task increasing the speed of decision making (i.e., a shallower slope).

Experiment 1

Method

Participants

Participants were 24 healthy male students with a mean age of 20.5 (*SD* = 0.9) years, mean weight of 77.0 (*SD* = 8.0) kg, and mean height of 1.80 (*SD* = 0.08) m. Their mean (*SD*) resting systolic blood pressure was 128.1 (12.5) mmHg, diastolic blood pressure was 75.4 (10.1) mmHg and heart rate was 76.1 (12.4) bpm.

Apparatus

Participants sat on a cycle ergometer (814, Monark). An audiotape player and headphones (Sony) were used to present the instructions and auditory stimuli.

Physiological Measurements

Brachial blood pressure was obtained from the participant's left arm using a validated (O'Brien et al., 2001) oscillometric sphygmomanometer (HEM-705CP, Omron). Heart rate (bpm) was recorded using a heart rate monitor (Vantage NV, Polar). A coded transmitter was strapped to the participant's chest just below the xiphoid process while a coded receiver was held by the experimenter.

Paced Auditory Serial Addition Task

A version of the paced auditory serial addition task (PASAT) was used to assess working memory. The task consisted of four 2-minute blocks of trials. Participants were instructed to add two sequentially-presented single-digit numbers, while retaining the latter of the two numbers in memory for subsequent addition to the next number presented (Gronwall, 1977; Tombaugh, 2006). Numbers, which ranged from 1 to 9, were presented via an audiotape player and headphones. Participants were instructed to add each number they heard to the previous number and to state the answer out loud. If performance broke down, participants were told to continue with the next number presented. For the control group, the task consisted of four 2-min blocks of 30, 34, 40, and 48 numbers at inter-stimulus intervals of 4.0, 3.5, 3.0, and 2.5 s respectively. These inter-stimulus intervals included the duration (c. 500 ms) of each number. For the exercise group, the task consisted of four 2-min blocks of 28, 33, 37 and 47 numbers; the slight reduction in trials was due to periodic announcements of required changes in pedalling cadence. Heart rate was recorded every minute of the task.

Procedure

Participants completed a single testing session. First, they gave informed consent, provided some demographic information and had their height and weight measured. They then sat on the cycle ergometer for the remainder of the session. Following instrumentation, participants sat and relaxed for a 5-min formal rest period while three blood pressure and heart rate measurements were taken. Instructions about the task demands were then given and 10 practice trials completed. Participants were tested in a mixed multifactorial experimental design, with one between-subject factor (group) and one within-subject factor (block). Participants were randomly assigned to one of two groups to complete the paced auditory serial addition task. The control group (N = 12) completed the working memory task while sitting on the cycle ergometer whereas the exercise group (N = 12) completed the task while cycling at moderate intensity. The exercise group participants were periodically instructed to pedal at a specific number of revolutions per minute, ranging from 60 to 90 (M = 77) rpm, intended to generate a power output ranging from 60 to 180 (M = 146) Watts. Specifically, the target power outputs (and revolutions per minute) for blocks 1, 2, 3 and 4 of the memory task averaged 95 (63 rpm), 165 (83 rpm), 155 (78 rpm) and 170 (85 rpm) Watts, respectively. This exercise task was designed to simulate the changing demands associated with bicycle races.

Data Reduction and Analysis

The three resting cardiovascular measurements were averaged to yield resting systolic blood pressure, diastolic blood pressure and heart rate. In addition, the measurements were averaged to yield heart rate during each block of the task. The number of errors (omissions, incorrect responses, late responses) in each block were recorded and used to calculate the proportion of correct responses per block (Tombaugh, 2006).

Results

Paced Auditory Serial Addition Task

A 2 Group (control, exercise) by 4 Block (1, 2, 3, 4) multivariate analysis of variance (MANOVA), with group as a between-subject factor and block as a within-subject factor, was performed on the proportion of correct responses in the paced auditory serial addition task. Overall, the exercise group only tended to outperform the control group, $F(1,22) = 2.83$, $p = .11$, $\eta^2 = .11$. However, the analysis yielded multivariate effects for block, $F(3,20) = 8.06$, $p < .001$, $\eta^2 = .55$, and group by block, $F(3,20) = 5.06$, $p < .01$, $\eta^2 = .43$. The scores in each block of the paced auditory serial addition task for each group are shown in Figure 5.1. Polynomial trend analyses confirmed a significant linear trend for block, $F(1,22) = 20.74$, $p < .001$, $\eta^2 = .49$, indicating that memory function deteriorated with increasing digit presentation rates. Polynomial analyses also revealed a significant group by block quadratic trend, $F(1,22) = 7.58$, $p < .01$, $\eta^2 = .26$, with group differences in memory function most pronounced during the middle blocks of the task.

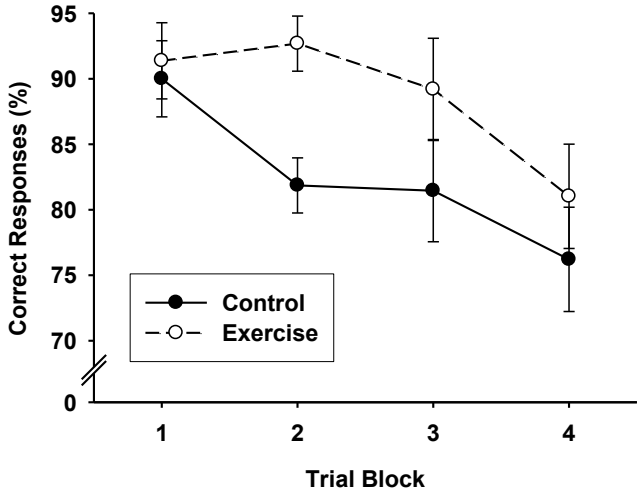

Figure 5.1. Mean (*SE*) performance accuracy scores, indexed by the proportion of correct responses, during each two minute block of the paced auditory serial addition working memory task for the non-exercising control group and the exercise group.

Cardiac Activity

A 2 Group by 5 Period (rest, block 1, block 2, block 3, block 5) MANOVA, with group as a between-subject factor and period as a within-subject factor, was performed on the heart rates. This yielded multivariate effects for group, $F(1,22) = 49.00$, $p < .001$, $\eta^2 = .69$, period, $F(4,19) = 57.49$, $p < .001$, $\eta^2 = .92$, and group by period, $F(4,19) = 27.11$, $p < .001$, $\eta^2 = .85$. The heart rates during rest and while completing each block of the memory task for the control and exercise groups are shown in Figure 5.2. Polynomial trend analyses interrogated these effects. Significant group by period linear, $F(1,22) = 112.86$, $p < .001$, $\eta^2 = .84$, and quadratic, $F(1,22) = 28.42$, $p < .001$, $\eta^2 = .56$, trends indicated that that the exercise group's heart rates increased progressively from rest to the last block of the task whereas the control group's heart rates increased from rest to the first block of the task and then remained similarly elevated throughout the remainder of the task. It is worth noting that the exercise group's heart rates during the four blocks of the task were 60 ($SD = 7$), 69 ($SD = 9$), 74 ($SD = 9$), and 77 ($SD = 10$) percent of maximum predicted heart rates, calculated as 220 minus age. In contrast, the control group's corresponding heart rates were only 47 ($SD = 6$), 46 ($SD = 6$), 47 ($SD = 6$) and 48 ($SD = 6$) percent of predicted maximum.

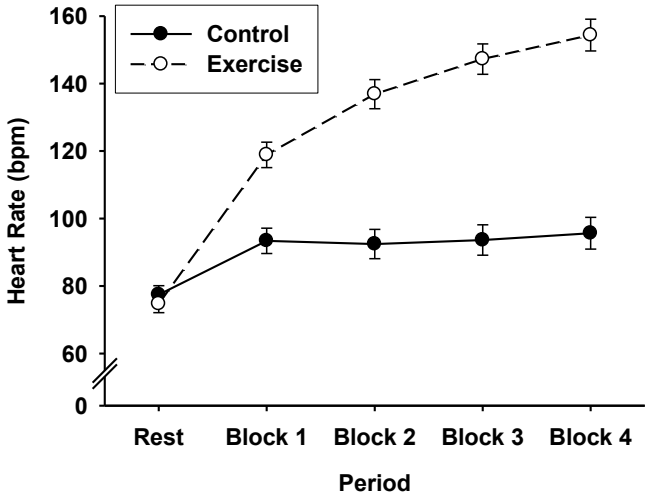

Figure 5.2. Mean (*SE*) heart rates at rest and during each two minute block of the paced auditory serial addition working memory task for the non-exercising control group and the exercise group.

Experiment 2

Method

Participants

Participants were 120 healthy right-handed students (55 males, 65 females) with a mean age of 19.6 (*SD* = 0.8) years, mean weight of 69.6 (*SD* = 12.8) kg, and mean height of 1.73 (*SD* = 0.10) m. Their mean (*SD*) resting systolic blood pressure was 121.7 (9.9) mmHg, diastolic blood pressure was 76.2 (8.7) mmHg and heart rate was 77.6 (12.4) bpm.

Apparatus

Participants sat on a cycle ergometer (824E, Monark) with a stimulus box mounted on the front of the ergometer and a response box under their dominant hand. The stimulus box contained a single 40 mm wide by 55 mm high dual-color (green, red) 7-segment light emitting diode panel that was used for presenting warning, experimental, probe and feedback stimuli. The response box contained two low force microswitch levers (D459-V3LD, Cherry).

Physiological Measurements

Blood pressure and heart rate were measured as described in Study 1.

Sternberg Task

A version of the Sternberg task was used to assess working memory. A computer was programmed in Spike2 to present stimuli and collect responses via a Power1401 (Cambridge Electronic Design). At the start of each of 96 trials, participants were required to depress the two response levers with the index and middle fingers of their dominant hand. The task waited until both response levers were depressed. Following a 250 ms delay, the program serially presented a set of either two or six green single-digit numbers ranging from 1 to 9. Each number was presented for 750 ms with a 250 ms interval between numbers. After a 3000 ms delay, a red probe number was presented for 750 ms. The participant was required to decide whether this red number was presented in the previous set of green numbers. If the red number was a match, then the participant was instructed to lift his/her middle finger whereas if the red number was not a match, then the participant was instructed to lift his/her index finger. Participants were then given performance feedback: a green U was presented if the response was correct whereas a red U was presented if the response was wrong. Participants were instructed to respond as rapidly as possible while keeping errors to a minimum. The task was divided into blocks of 48 trials, each of which lasted approximately eight minutes. Participants rested for three minutes after each block. Blood pressure and heart rate measurements were obtained during the third, fifth and seventh minute of each block.

Procedure

Participants completed a single testing session that followed a similar initial protocol to that described in Study 1, except that they completed 24 practice trials.

Participants were tested in a mixed multifactorial experimental design, with one within-subject factor (condition) and one between-subject factor (exercise intensity). All participants performed the Sternberg task under two conditions: control and exercise. In the control condition, they completed the memory task while sitting on the cycle ergometer. In the exercise condition, they completed the memory task while exercising at one of three intensities. Participants were randomly assigned to one of three exercise intensity groups. The low intensity group ($N = 40$) was instructed to pedal at 45 revolutions per minute with no added brake friction, which corresponded to a power output of approximately 5 Watts. The medium intensity group ($N = 42$) was instructed to pedal at 50 (women) and 60 (men) revolutions per minute at a power output of 50 Watts (women) and 60 Watts (men). The high intensity group ($N = 38$) was instructed to pedal at 50 (women) and 60 (men) revolutions per minute at a power output of 75 Watts (women) and 90 Watts (men). In the exercise condition, participants pedalled for two minutes to approach steady state before starting each block of trials of the memory task.

Data Reduction and Analysis

The three resting cardiovascular measurements were averaged to yield resting systolic blood pressure, diastolic blood pressure and heart rate. Similarly, the six cardiovascular measurements were averaged to yield systolic blood pressure, diastolic blood pressure and heart rate for control and exercise. Response latency (ms) was calculated as the time between the onset of the probe stimulus and the release of the switch lever. Responses were discarded if the response latency was less than 100 ms (i.e., anticipation error) or greater than 2250 ms (i.e., inattention error), or if the participant lifted both fingers concurrently (<100 ms apart). Errors (%) were calculated as the proportion of incorrect / discarded responses. The average response latencies associated

with the two-number and six-number sets were used to calculate the slope (ms/digit) and intercept (ms) using linear regression (Sternberg, 1966).

Results

Sternberg Task

A 3 Exercise Intensity Group (low, medium, high) by 2 Condition (control, exercise) multivariate analysis of variance (MANOVA), with group as a between-subject factor and condition as a within-subject factor, was performed on the slopes, intercepts and errors in the Sternberg task. This yielded multivariate effects for condition, $F(3,115) = 14.34$, $p < .001$, $\eta^2 = .27$, and group by condition, $F(6,230) = 2.90$, $p < .01$, $\eta^2 = .07$. The memory function scores under control and exercise conditions for each intensity group are shown in Figure 5.3. To interrogate these effects, a series of 2 Condition (control, exercise) analyses of variance (ANOVAs) were performed on each group. The slopes were shallower during exercise than control in the medium, $F(1,41) = 11.56$, $p < .002$, $\eta^2 = .22$, and high, $F(1,37) = 9.05$, $p < .005$, $\eta^2 = .20$, intensity groups but did not differ between conditions in the low intensity group, $F(1,39) = 0.67$, $p = .42$, $\eta^2 = .02$. To further explore this effect, we computed the change in slope (exercise minus control) and compared the change scores of the medium ($M = –12.23$ ms/digit) and high ($M = –11.13$ ms/digit) groups using a 2 Group (medium, high) ANOVA. This analysis indicated that the effect of exercise on memory performance was comparable for these two groups, $F(1,78) = 0.05$, $p = .83$, $\eta^2 = .00$. However, the intercepts did not differ between conditions for any group: low, $F(1,39) = 0.40$, $p = .84$, $\eta^2 = .00$, medium, $F(1,41) = 0.75$, $p = .39$, $\eta^2 = .02$, and high, $F(1,37) = 1.20$, $p = .28$, $\eta^2 = .03$. Finally, errors were more frequent when exercising at medium intensity, $F(1,41) = 9.85$, $p < .003$, $\eta^2 = .19$, but did not differ between conditions

at either low, $F(1,39) = 0.00$, $p = .95$, $\eta^2 = .00$, or high, $F(1,37) = 1.11$, $p = .30$, $\eta^2 = .03$, intensity exercise.

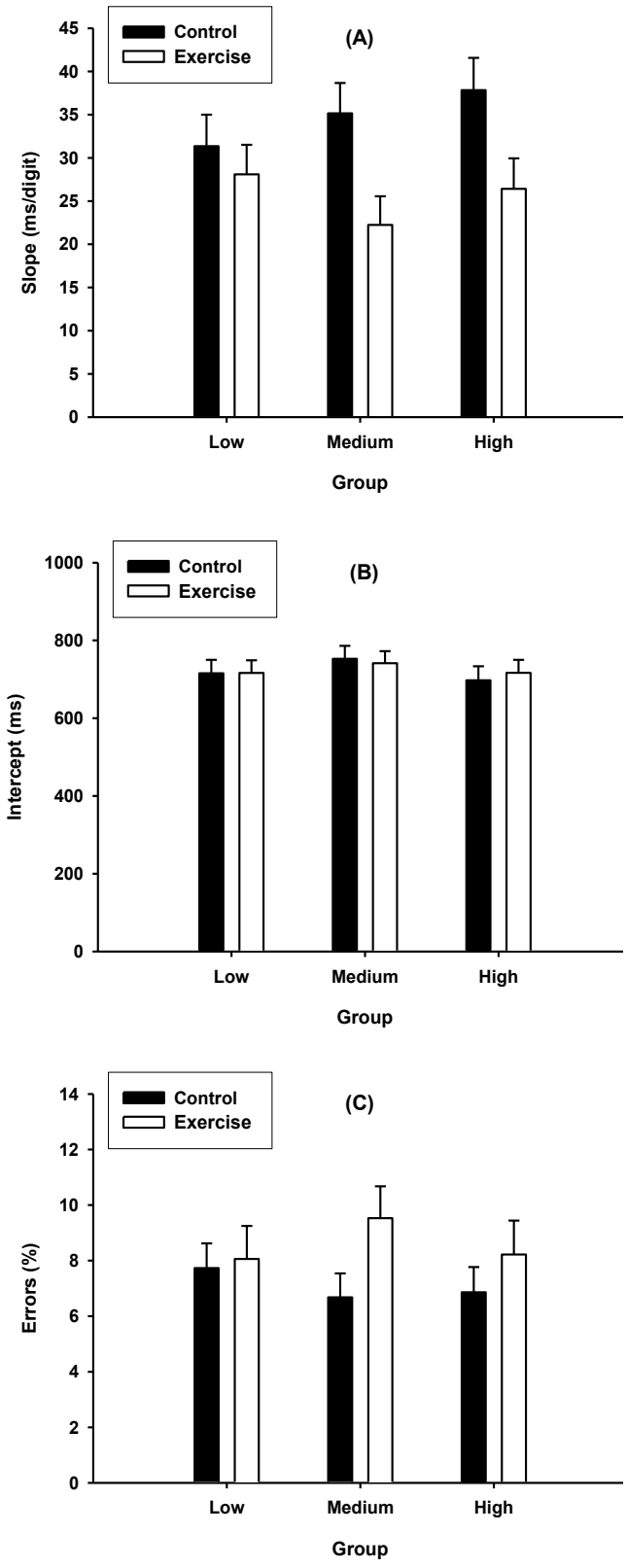

Figure 5.3. Mean (*SE*) performance scores on the Sternberg working memory task during control and exercise conditions for the low, medium and high intensity exercise groups: the slope of the response latencies (A); the intercept of the response latencies (B), and the number of errors (C).

Cardiovascular Activity

A 3 Exercise Intensity Group by 2 Condition MANOVA was performed on the heart rates, systolic blood pressures and diastolic blood pressures. This yielded multivariate effects for group, $F(6,224) = 12.56$, $p < .001$, $\eta^2 = .25$, condition, $F(3,111) = 173.78$, $p < .001$, $\eta^2 = .82$, and group by condition, $F(6,224) = 23.40$, $p < .001$, $\eta^2 = .39$. The control and exercise cardiovascular activity for each group are shown in Figure 4.4. To explore these effects, a series of 2 Condition ANOVAs were performed on each variable for each group. Heart rates increased from control to exercise in all groups: low, $F(1,38) = 6.69$, $p < .01$, $\eta^2 = .15$, medium, $F(1,40) = 263.74$, $p < .001$, $\eta^2 = .87$, and high, $F(1,35) = 184.49$, $p < .001$, $\eta^2 = .85$. A 3 Group ANOVA revealed group differences in the extent of the heart rate reactions to exercise (i.e., exercise value minus control value), $F(2,114) = 90.40$, $p < .001$, $\eta^2 = .61$: the cardiac change scores of the high (M = 47.7 bpm) and medium (M = 42.5 bpm) intensity groups were greater than those of the low intensity group (M = 3.0 bpm). Similarly, systolic blood pressure was higher during exercise than control in the low, $F(1,39) = 10.94$, $p < .002$, $\eta^2 = .22$, medium, $F(1,40) = 75.92$, $p < .001$, $\eta^2 = .66$, and high, $F(1,35) = 85.03$, $p < .001$, $\eta^2 = .71$, groups. It is noteworthy that the heart rates during the task corresponded to 41 (SD = 7), 61 (SD = 10) and 64 (SD = 13) percent of maximum predicted heart rates for the low, medium and high intensity groups, respectively. A 3 Group ANOVA highlighted group differences in the systolic pressor reactions to exercise, $F(2,114) = 17.84$, $p < .001$, $\eta^2 = .24$: the high (M = 26.6 mmHg) and medium (M = 23.6 mmHg) intensity groups exhibited greater reactivity than the low (M = 6.7 mmHg) intensity group. Finally, exercise-induced diastolic blood pressure responses were noted for the low intensity group, $F(1,39) = 21.54$, $p < .001$, $\eta^2 = .36$, but not for the medium, $F(1,40) = 0.04$, $p = .85$, $\eta^2 = .00$, and high, $F(1,35) = 1.91$, $p = .18$, $\eta^2 = .05$, intensity

groups. Diastolic blood pressure fell during low intensity exercise (M = –7.1 mmHg) but was unchanged by medium (M = 0.5 mmHg) and high (M = 3.3 mmHg) intensity exercise.

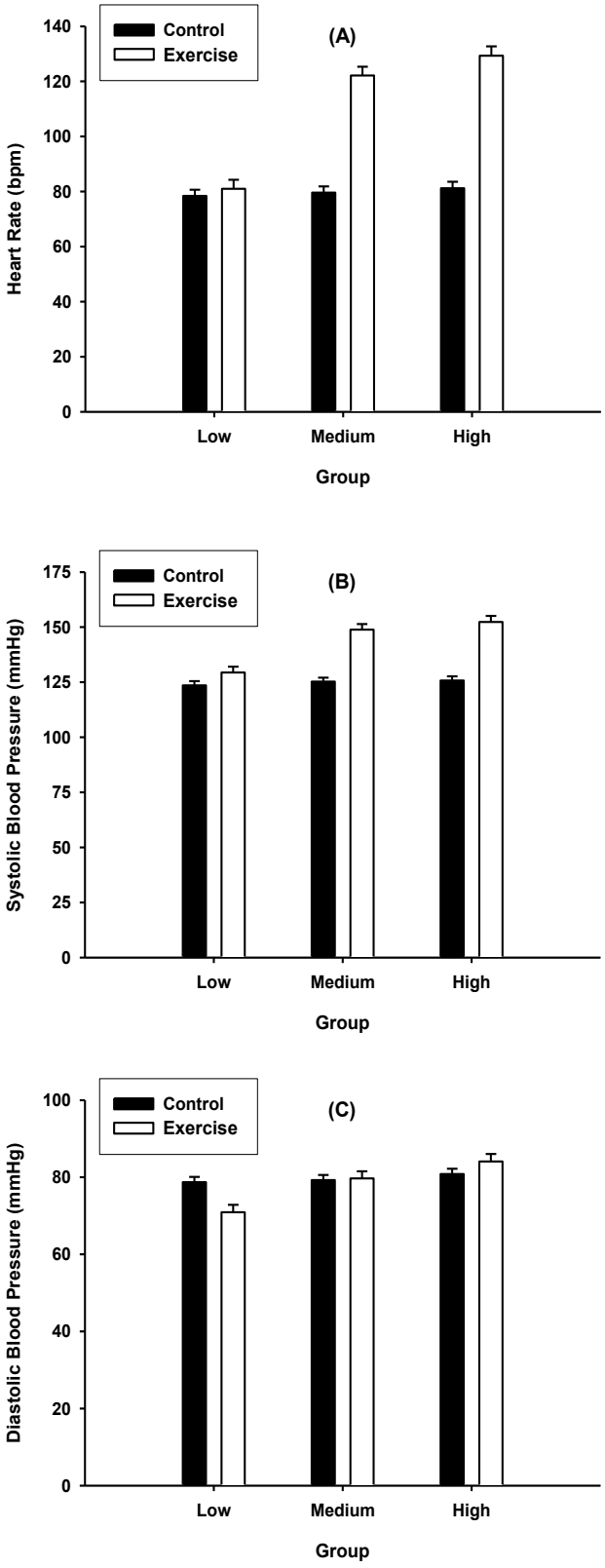

Figure 5.4. Mean (*SE*) cardiovascular activity while performing the Sternberg working memory task during control and exercise conditions for the low, medium and high intensity exercise groups: heart rate (A); systolic blood pressure (B), and diastolic blood pressure (C).

Mediation and Moderation of Condition Differences in Memory Performance by Blood Pressure

Control-to-exercise differences were noted in both systolic and diastolic blood pressures (see above), revealing them as potential mediators and moderators of the corresponding discrepancy in the key measure of working memory performance (i.e., the slope in the Sternberg task). To examine whether the difference in working memory across conditions was influenced by blood pressure, the analytic strategy described by Judd and colleagues (2001) for testing mediation/moderation in within-subjects designs was employed. Regressing the condition difference in the slopes on both the uncentred systolic blood pressure difference and the mean centred systolic blood pressure sum yielded no significant coefficients. Non-significant coefficients were also noted for analyses using diastolic blood pressure. Taken together these data indicate that neither systolic nor diastolic blood pressure mediated or moderated the improvements in working memory with exercise.

Discussion

The present study combined two experiments to assess the dual-task effects of performing attentional control and working memory tasks *during* steady-state sub-maximal exercise. Collectively, our findings argue that attention control and working memory are improved by aerobic exercise performed at moderate intensities. Specifically, the first experiment indicated that moderate exercise facilitated PASAT performance, i.e., improved response accuracy, under medium levels of task difficulty. In turn, the second experiment revealed that moderate exercise optimizes the speed of decision-taking, i.e., lowers the response latency slopes in the Sternberg task.

The results of the first experiment differ from the extant data previously reported on PASAT performance during sub-maximal aerobic exercise. Accordingly, Lambourne and co-workers (2010) found no changes in PASAT performance both during and after 40 minutes of moderate cycling, whereas we report a beneficial effect for moderate difficulty trials during a similar exercise protocol. Importantly, two differences between studies must be noted. First, Lambourne and colleagues (2010) adjusted the stimulus-presentation rate to account for "interindividual differences in processing speed". Second, the amount of practice series administered to the participants ascended to twenty-one (15 during the "familiarization" session, 3 during the "exercise" session, 3 during the "rest" session). Taken together, these procedures may have likely produced a ceiling effect on the outcome variable, decreasing the propensity for detection of performance changes (i.e., a reduced sensitivity). Supporting this view, the accuracy reported for PASAT performance was consistently above 90%, irrespectively of the time on task (before, during, or after exercise) and the condition assessed (rest / exercise; Lambourne et al, 2010). In addition, our results are contrary to those reported by Dietrich and Sparling (2004). Again, methodological differences may justify this discrepancy. First, the exercise protocol employed by Dietrich and Sparling (2004) consisted in a running session considerably long (~65 min) executed at 70–80% of maximum heart rate, with the PASAT being initiated 40 min on task. Consequently, it is possible that fatigue may have interfered with cognitive performance. Second, the version of the PASAT administered by Dietrich and Sparling (2004) included four series of 50 numbers, with inter-stimulus intervals of 2.4, 2.0, 1.6, and 1.2 s. Hence, not only the lists of stimuli were longer but also the inter-stimulus intervals were shorter than the ones comprising the version employed by the present study.

Therefore, it is possible that different levels of task difficulty were examined, producing apparently conflicting results.

The results from the second experiment indicated that WM slopes but not the intercepts can improve during sub-maximal exercise if performed at a medium intensity. These findings are compatible with studies that report faster decision-making performance during moderate exercise (McMorris et al, 1999; Davranche & Audiffren, 2004), and agree with the view that steady-state aerobic exercise can facilitate decisional processing – as indicated by changes in slopes –, but has little or no effect on simple sensorimotor processes – indexed by the zero intercept (Tomporowski, 2003). However, since this is the first report examining Sternberg performance during aerobic exercise, such similitude must be regarded with caution. Furthermore, it must be acknowledged that performance for the medium-intensity exercise group was slightly less accurate (see Figure 3). Although the precise reasons for such decrease are unclear, it is possible that it reflects a trade-off between response speed and accuracy, as occasionally reported (e.g., Pontifex & Hillman, 2007).

Our findings gather no support for a "transient hypofrontality" mechanism affecting WM performance during sub-maximal exercise. In fact, in experiment 1, the exercise group kept a very similar performance during blocks of trials 1, 2 and 3, despite the variability in exercise load (95, 165, and 155 watts, respectively) and the successive increase in task difficulty. Performance only decayed during the most difficult block, the fourth, to approach control levels. Therefore, moderate levels of exercise appear to prevent the decrements in PASAT performance resultant from increasingly difficult trials. In experiment 2, both exercise groups revealed a better efficiency of the memory updating and comparison processes needed to accomplish the task. Given that increased

frontoparietal cortical activity is associated with efficient performance during this type of task (Wager & Smith, 2003), these data also speak against the "transient hypofrontality" mechanism.

Instead, the present results combined broadly support the claims of a zone of optimal physiological arousal facilitating WM processes (see McMorris, 2009), as predicted by the arousal theories. Moreover, task-related complexity also appears to determine the successful allocation of resources, as seen in experiment 1, which is in line with Kahneman's (1973) and Oxendine's (1984) perspectives. Although the precise mechanism underlying these effects is currently unknown, some proposals have been advanced. First, some authors believe that arousal facilitation of cognitive processes results from direct or indirect action of catecholamines (see McMorris, 2009 for review). Second, central command-induced fluctuations in regional cerebral blood flow (rCBF) could also mediate changes in cognitive performance (Williamson et al., 2006; Sato et al., 2009). In fact, rCBF velocity augments proportionally to exercise intensity up until ~60% VO_{2max}, after which further increases in exercise intensity are contingent with rCBF velocity decreases (Querido & Sheel, 2007). On the other hand, cerebral auto-regulation of rCBF is multi-factorial, depending not only on muscle mechanoreceptors but also on ventilatory, metabolic, and cardiovascular adjustments (Querido & Sheel, 2007). With this in mind, it was interesting to note that neither systolic nor diastolic blood pressure were associated with changes in performance in experiment 2.

In sum, the present study provides preliminary evidence that attention control and WM can be enhanced during moderate aerobic exercise, and, moreover, that the efficiency of this effect is likely moderated by task conditions that pose medium demands upon WM capacity. Although these findings may carry implications in terms of sports and process

optimization in human resources, future research may explore the exact cardiovascular and metabolic contributions to this phenomenon and employ a fine-grain analysis of WM performance across different exercise intensities and modalities.

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CHAPTER SIX

Effects of Baroreceptor Stimulation on Performance of the Sternberg Task: A Cardiac Cycle Time Study of Working Memory

Abstract

Activation of arterial baroreceptors can affect human performance. Previous cardiac cycle time studies have established that natural variations in baroreceptor activation are associated with changes in basic sensorimotor function whereas few have investigated more complex cognitive function in this context. The present study examined performance on the Sternberg memory task as a function of the phase of the cardiac cycle. In each trial, participants were shown either two or six digits followed by a probe digit that either had or had not been presented previously and were required to press one of two response buttons to indicate a match and mismatch, respectively. Response latency per additional digit was greater for stimuli presented late compared to early in the cardiac cycle. These findings provide evidence that natural baroreceptor stimulation can interfere with complex cognitive processes, such as working memory.

Introduction

The cardiac pulse pressure wave stretches the vessel walls to activate arterial baroreceptors in the aortic arch (Angell James, 1971) and carotid sinus (Mancia & Mark, 1983). At rest, arterial baroreceptors afferents exhibit a pulsatile activity, with maximum firing synchronous with increases in blood pressure during systole. Moreover, this pressure-dependent information is transmitted to the brain, reaching brainstem sites approximately 100–400 ms after the R-wave of the electrocardiogram (for review see Edwards et al., 2001). It is also evident that this information is transmitted more widely in the brain. For instance, discharge rates of one in five amygdala and hippocampus cells are modulated by the phase of the cardiac cycle (Frysinger & Harper, 1989). Behavioral scientists have capitalised on such naturally occurring variations in baroreceptor stimulation to investigate the effects of blood pressure on task performance. In this form of the cardiac cycle time paradigm, responses to stimuli delivered when the baroreceptors are activated (i.e., systole) are compared with responses to stimuli delivered when the baroreceptors are quiescent (i.e., diastole).

Most cardiac cycle time research has investigated basic sensorimotor function. Early studies demonstrated that auditory (Saxon, 1970) and visual (Requin & Brouchon, 1964) stimuli were detected less accurately when presented during the QRS complex of the electrocardiogram, and, moreover, that simple reaction times to auditory (Birren et al., 1963) and visual (Callaway, III & Layne, 1964) stimuli were slowest when presented at the start of the cardiac cycle. These promising findings were interpreted in terms of interference caused by afferent baroreceptor inputs being integrated into medullary and cortical structures (Lacey & Lacey, 1974). However, doubts were raised over the

robustness of the phenomenon when other researchers were unable to replicate the reported cardiac cycle time effects (Delfini & Campos, 1972; Elliott & Graf, 1972; Salzman & Jaques, 1976; Thompson & Botwinick, 1970; Weisz & Ádám, 1996). It now seems likely that these null findings were due to the use of small sample sizes, insufficient sampling across the cardiac cycle and primitive equipment (cf. Carroll & Anastasiades, 1978) as recent large studies have repeatedly documented that simple reaction times are slowest for stimuli presented early in the cardiac cycle (Edwards et al., 2007; McIntyre et al., 2007; McIntyre et al., 2008; cf. Stewart et al., 2006). Other cardiac cycle time studies have generated neurophysiological evidence for pressor-related cortical interference. For instance, systole is associated with reduced auditory, visual and pain evoked potentials (Edwards et al., 2008; Sandman et al., 1982; Walker & Sandman, 1979; Walker & Sandman, 1982). Systole is also characterized by lower frequency electroencephalographic oscillations measured in the alpha band (Walker & Walker, 1983).

This evidence is supplemented by neuroscientific research that has employed other baroreceptor stimulation protocols. For example, stimulation using phasic neck suction is associated with increased contingent negative variation (Elbert & Rau, 1995; Rau et al., 1993) while stimulation using body tilt is characterised by increased theta and delta power (Vaitl & Gruppe, 1990; Vaitl & Gruppe, 1995). Furthermore, recent neuroimaging studies have implicated insular, somatosensory and anterior cingulate cortices in the processing of baroreceptor afferents (Critchley et al., 2004; Khalsa et al., 2009; Kimmerly et al., 2005; Kimmerly et al., 2007; Wong et al., 2007). Given this wealth of information it is somewhat surprising that we do not know whether higher order cognitive functioning is susceptible to variations in arterial baroreceptor activity.

The available evidence is extremely limited and confined to choice reaction time paradigms. One study presented an auditory or visual stimulus randomly during the cardiac cycle and required participants to indicate the sensory modality (Saari & Pappas, 1976). Responses were retrospectively classified as occurring during one of nine bins that were derived by dividing the R-R interval into nine equal periods. Reaction times were slower during the second bin compared to the fourth, sixth, and ninth bins. A second study (McIntyre et al., 2007) examined 1, 2 and 4 choice reaction times to visual stimuli presented at one of six intervals (0, 150, 300, 450, 600, 750 ms) after the R-wave of the electrocardiogram. The intercept, a measure of the speed of basic sensorimotor processing, varied across the cardiac cycle whereas the slope, a measure of the speed of decision making, did not. These findings suggest that basic sensory processing rather than complex cognitive operations are susceptible to baroreceptor-related interference.

The purpose of the present study was to investigate variations in working memory performance as a function of the phase of the cardiac cycle. Working memory refers to the transient storage and manipulation of information; functional models propose that a central executive coordinates information via subsidiary subsystems, including a visuospatial sketchpad for manipulating visual items, a phonological loop for subvocal rehearsal, and an episodic buffer for retaining and combining information (Baddeley, 2003; Rawley & Constantinidis, 2009). Working memory was assessed in the present study using a Sternberg task which requires storage and rehearsal of a set of digits that are presented sequentially, a brief maintenance period, the evaluation of a probe digit that might have been presented in the previous set followed by a binary response (Sternberg, 1966). Based on the extant literature reviewed above, we hypothesized that naturally-occurring variations in baroreceptor stimulation would interfere with (and therefore slow) the

cognitive processing needed for data comparison for probes presented during systole (i.e., when arterial baroreceptor activation is maximal) compared to probes presented earlier and later in the cardiac cycle.

Method

Participants

One-hundred (45 males, 55 females) healthy right-handed students (M = 19.6, SD = 1.0 years of age) gave informed consent and volunteered to participate. They had a mean resting systolic blood pressure of 122 (SD = 10) mmHg, diastolic blood pressure of 76 (SD = 9) mmHg, and heart rate of 78 (SD = 13) bpm. Exclusion criteria comprised any known heart disease and any medication except birth control. Participants were asked to refrain from caffeine, alcohol, and exercise for 2 hours before testing. A local research ethics committee approved the study protocol.

Apparatus

Participants sat quietly facing a stimulus box that was located 1 m in front of them and kept a response box under their dominant hand. The stimulus box contained a single 40 mm wide by 55 mm high dual-color (green, red) 7-segment light emitting diode panel that was used for presenting warning, experimental, probe and feedback stimuli. The response box contained two low force microswitch levers (D459-V3LD, Cherry).

Physiological Measurements

A Spike2 (Cambridge Electronic Design) computer program ran the experiment and collected physiological data via a Power1401 (Cambridge Electronic Design). An electrocardiogram was recorded continuously with three spot electrodes (Cleartrace, ConMed) in a modified chest configuration; the active electrodes were placed on the right

clavicle and lower left rib and a reference electrode was placed on the left clavicle. The electrocardiographic signal was amplified and filtered (0.1–100 Hz plus 50Hz notch filter) by an AC amplifier (P511, Grass) and then digitised at 2500 Hz with 16-bit resolution. Resting blood pressure and pulse rate were measured with a validated (O'Brien et al., 2001) oscillometric sphygmomanometer (HEM-705CP, Omron) attached to the participant's left arm.

Procedure

Participants completed a single session. Demographic data were collected at the start, and following instrumentation, participants rested for 5 minutes. During this period, blood pressure and heart rate readings were initiated at minutes 1, 3 and 5. Participants were then instructed about the task demands and performed 24 practice trials.

Sternberg Task. A Sternberg task (Sternberg, 1966) was used to assess working memory. Two blocks of 48 trials separated by a 3-min rest period were completed. Participants were required to depress the two levers on the response box with the index and middle fingers of their dominant hand to initiate each trial. The computer program waited until both response levers were depressed before starting a trial. The trial started with a 500 ms fixation stimulus followed by a preparatory 500 ms delay. A sequence of either two or six green single-digit numbers, ranging from 1 to 9, was then serially presented. Each number was visible for 500 ms with a 750 ms interval between numbers. After a 3000 ms delay, a red probe number was presented for 500 ms. Participants were required to decide whether the probe was included in the previous set of green numbers by lifting the index finger for matching probes and the middle finger for mismatching probes. Performance feedback concluded the trial: correct decisions were followed by a green U

whereas a red U was presented after incorrect responses. Participants were instructed to respond as rapidly as possible while keeping errors to a minimum.

Data Reduction and Analysis

The three resting cardiovascular readings were averaged to yield mean systolic blood pressure, diastolic blood pressure, and heart rate. Response latency (ms) was calculated as the time between the onset of the probe stimulus and the release of the switch lever. A trial was discarded if the latency was less than 100 ms (i.e., anticipation error) or greater than 2250 ms (i.e., inattention error), or if the participant lifted both fingers concurrently (< 100 ms apart). Only correct responses were included in the analysis. The R-wave latency relative to probe onset (ms) was measured in each trial. Trials were then sorted retrospectively into one of six 100 ms wide intervals (with each interval labelled by its midpoint), whose minimum and maximum indicated the timing of probe onset after the R-wave: 0–99 ms (R+50 ms), 100–199 ms (R+150 ms), 200–299 ms (R+250 ms), 300–399 ms (R+350 ms), 400–499 ms (R+450 ms), and 500–599 ms (R+550 ms). The slope (ms per digit), a measure of the time required to process one additional digit in memory, and the zero intercept (ms), a measure of sensorimotor processing time, were computed for each interval (Sternberg, 1966).

Results

Memory processing per additional digit was faster during the early compared to the later phase of the cardiac cycle. A repeated measures ANOVA, with R-wave to probe interval (R+50, R+150, R+250, R+350, R+450, R+550 ms) as a within subjects factor, conducted on the slopes confirmed a main effect for interval, $F(5, 95) = 2.33$, $p < .05$, $\eta^2 = .11$, and a cubic trend, $F(1, 99) = 5.83$, $p < .05$, $\eta^2 = .06$. The respective mean (*SD*) slopes

for probe stimuli presented 50, 150, 250, 350, 450 and 550 ms after the R-wave were 35.19 (51.89), 30.17 (39.96), 30.89 (44.87), 45.76 (36.91), 41.81 (42.09), 38.56 (40.47) ms per digit (see Figure 6.1).

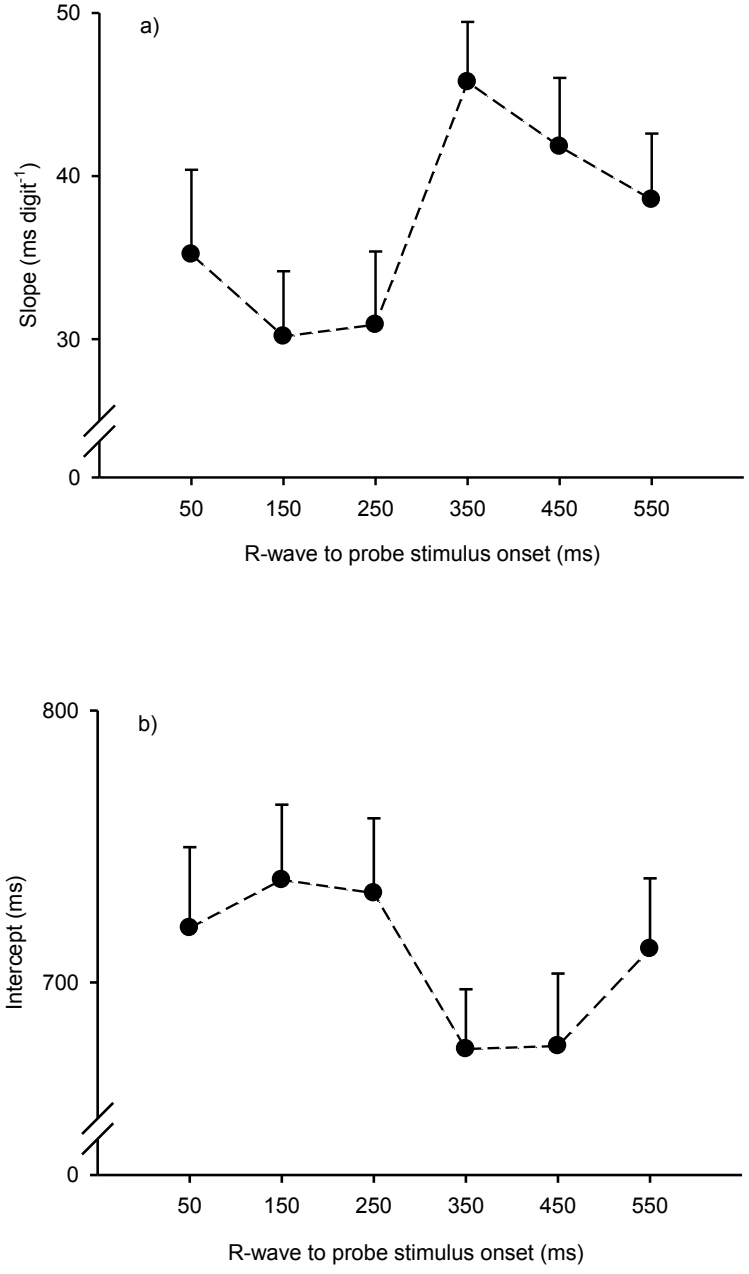

Figure 6.1. Mean (*SE*) slopes (top panel, a) and intercept latencies (bottom panel, b) for imperative probes delivered at six intervals within the cardiac cycle (50, 150, 250, 350, 450, and 550 ms after the R-wave of the electrocardiogram). Error bars indicate s.e.m.

Basic sensorimotor processing and responding were slower during the early phase of the cardiac cycle compared to later. A repeated measures ANOVA (6 intervals) conducted on the zero intercepts found a main effect for interval, $F(5, 95) = 2.86$, $p < .05$, $\eta^2 = .13$, and a cubic trend, $F(1, 99) = 8.16$, $p < .005$, $\eta^2 = .08$. The respective mean (*SD*) zero intercepts for probe stimuli presented across the cardiac cycle were 720 (297), 738 (277), 733 (275), 675 (220), 677 (267) and 712 (260) ms (see Figure 6.1).

Discussion

The present study investigated cardiac cycle time effects for working memory. In the context of the Sternberg task, memory processing was slowed for comparison stimuli presented 300-600 ms after the R-wave of the electrocardiogram compared to those presented earlier in the cardiac cycle. In contrast to the results of previous choice reaction time studies (McIntyre et al., 2007; Saari & Pappas, 1976), the current finding provides the first evidence that natural variations in arterial baroreceptor activity can influence complex cognitive function. Although the mechanism responsible for such an effect has yet to be established, the observed modulation favours the suggested cortical impact of arterial baroreceptor afference (Birren et al., 1963) that has become known as the visceral afferent feedback hypothesis (Lacey & Lacey, 1974), whereby the transmission of information about the state of the cardiovascular system may have interfered with working memory processing.

In agreement with research showing that simple reaction times are slower for imperative stimuli presented early in the cardiac cycle (Birren et al., 1963; Callaway, III & Layne, 1964; Edwards et al., 2007; McIntyre et al., 2007; McIntyre et al., 2008), the

present study also found that the zero intercept, a measure of basic sensorimotor processing, was greater for probe stimuli presented temporally proximal to the R-wave of the electrocardiogram. Accordingly, the current findings indicate that the phase of the cycle time effect differs for simple sensorimotor processes and complex memory scanning processes. These data add to our appreciation of the cardiac-cortical relationship, whereby the patterning of the effect varies with the complexity and/or duration of the response in question (cf., Edwards et al., 2001; Edwards et al., 2008).

The findings of the current study, which were based on a large sample using a well validated methodology, revealed medium-sized (Cohen, 1992) cardiac cycle time effects for both memory and sensorimotor processes. Nevertheless, they need to be interpreted in light of some potential shortcomings. First, to standardize the retention period of the task at three seconds for every trial we opted to score the data by retrospectively determining the timing of probe onset relative to the R-R interval. This feature that ensured task difficulty was standardized provided no control over the timing of stimulation within the cardiac cycle. Second, performance was only analysed up to 600 ms after the R-wave. Although some participants had slower heart rates that would have permitted examination of performance later in the cycle this was not possible for many others, and, accordingly, we restricted the window to R+0 ms to R+600 ms. Finally, the findings were collected using only one task, and, therefore studies are required that test the generalizabilty of the effect to other high order cognitive functions using other paradigms. Evidence that sensory evoked potentials (e.g., Sandman et al., 1982; Walker & Sandman, 1979; Walker & Sandman, 1982) and cortical oscillations (Walker & Walker, 1983) vary as a function of the phase of the cardiac cycle, will hopefully encourage researchers to explore cardiac cycle time effects using the classic paradigms developed by cognitive neuroscientists.

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CHAPTER SEVEN

General Discussion

The main purpose of the present thesis was to expand the current knowledge on the eventual psychological and cognitive influences that may be brought about by arterial baroreceptor functioning. Although being "hidden" physiological processes to the common observer, the mechanisms of baroreflex regulation have become increasingly important to the understanding of numerous clinical conditions, ranging from hypertension and orthostatic hypotension to congestive heart failure or even the metabolic syndrome (Skrapari et al., 2006; Benarroch, 2008). However, only in recent years had the scientific community thrived in the investigation of the cognitive impact of pressor-related mechanisms (e.g., Edwards et al., 2007; Thayer et al., 2009; see Waldstein & Wendell, 2010 for review). We hope our modest contribution would also encourage the upcoming behavioural research on the subject.

This closing chapter intends to summarise the experimental findings obtained, and to outline some theoretical implications of the present research for the coming ones. Finally, it concludes by acknowledging the main limitations of this series of studies.

Summary of Findings

Study One. The first experiment of this thesis was designed to test some core limitations of previous cardiac cycle time studies that have shown an attenuation of neurophysiological measures of pain during systole (e.g., the nociceptive flexion reflex, Edwards et al, 2001; pain-related evoked potentials, Edwards et al, 2008), but

simultaneously have been unable to find a similar systolic pattern of modulation for pain ratings, i.e., an index of the subjective evaluation of the participant. By employing a mixed block design with randomly presented stimuli, this study created the contextual demands and variability that are proximal to the experience of pain "in the real world". As such, it was possible to confirm that the cognitive-affective processing of the experience was indeed influenced by afferent baroreceptor input. Specifically, intensity and unpleasantness ratings for painful stimuli were highest at R+300 ms and lowest at R+0 and R+600 ms after the R-wave of the EKG. However, nociceptive responses did not differ among the R-wave to stimulation intervals for both painful and non-painful intensities. This lack of modulation of a specific nociceptive defensive reflex had only rarely been observed in arousing situations (e.g., McIntyre et al., 2006). This may serve an evolutionary purpose, since the visceral afferent inhibition of the nociceptive withdrawal reflex does not occur when a fight or flight response is required. We interpreted these findings accordingly, particularly because neuroanatomical evidence supports the notion that there are neural systems at a spinal and medullary level that interconnect with limbic structures to blunt the afferent limb of the baroreflex during conditions of heightened arousal. In practical terms, baroreceptor afferents can be attenuated at structures (e.g., the nucleus tractus solitarius) belonging to the baroreflex circuit. Finally, these findings together with preliminary evidence that cutaneous sensibility is greatest during systole (Edwards et al., 2009), suggest that still unveiled patterns of visceral afferent feedback may modulate behaviour.

Study Two. This study followed up the results obtained previously with the aim of "restoring the lost modulation" of the nociceptive flexion reflex. Clearly, the novelty of the experimental design (i.e., the unpredictability introduced by the range of electrocutaneous intensities of stimulation, and by the random presentation) was contributing to the

unexpected findings. Putting this suspicion to test, the same properties of electrocutaneous stimulation and exactly the same intervals after the R-wave of the electrocardiogram were kept. Yet, the schedule of stimuli presentation was this time blocked, i.e., predictable. Not surprisingly, the nociceptive flexion reflex was attenuated during systole when elicited by painful but not non-painful stimuli. It should be acknowledged that this null finding is not uncommon: for the nociceptive flexion reflex to be elicited, it is usually required an intensity of electrocutaneous stimulation close to the pain threshold (see Sandrini et al., 2005 for review). In parallel, pain ratings were now unaffected by the phase of the cardiac cycle, regardless of stimulus intensity. This null finding for pain replicated the "old recipe": equal stimuli tend to elicit equal perceptions if the context is unchanged (e.g., Edwards et al., 2001). Therefore, the findings were interpreted as suggesting that sensorimotor processing is more amenable to the influence of natural baroreceptor activity than pain perception when predictable conditions are met. In addition, the results also warned to the habituation of criteria in experimental designs with overt assessment: participants learn when a stimulus is invariant, and simply give the same or similar ratings. Such process may result from the development of an expectation, to which the participant feels obliged to sustain some degree of self-consistency.

Study Three. This study was the methodological consequence from the two previous ones. It intended to delimit (a) what were the precise effects of each schedule of electrocutaneous stimulation employed on the measures of nociception and pain assessed; and (b) if any of the schedules would be associated with a stress-induced hypoalgesic response. As such, the data from the two previous studies was collapsed across the cardiac cycle time intervals and compared. Anticipatory heart rate data collected during each trial provided the manipulation check for stress. Overall, this study yielded evidence that the

event unpredictability generated by the random schedule of stimulation could elicit a stress-induced hypoalgesic response. Moreover, the random schedule of stimulation evoked the highest nociceptive flexion reflex responses. This facilitation of the withdrawal reflex under stressful (as confirmed by the heart rate data) and unpredictable conditions was interpreted as an (negative) emotional-driven mechanism that promotes the general facilitation of reflexes under threat (e.g., startle potentiation; Bradley & Lang, 2007). The findings for pain were interpreted in light of (a) stimulus-comparator cognitive theories (e.g., Rachman & Arntz, 1991), stating that predictability imposes expectation schemas on the sensory evidence of noxious experiences; and (b) recent neuroimaging evidence suggesting that unpredictable pain can habituate due to the reinforcing properties of intermittent relief. Importantly, this study underscores the latent influences that the experimental designs may carry into the outcomes assessed.

Study Four. This study combined two experiments that tested predictions derived from (a) the "transient hypofrontality" hypothesis; and (b) the exercise-induced arousal theories, two opposing perspectives regarding human executive function performance during moderate aerobic exercise. Experiment 1 examined the performance of participants randomly assigned to a moderate cycling or a no-exercise condition in the paced auditory serial addition task (PASAT), and revealed that moderate intensity exercise was not detrimental on performance. On the contrary, exercise improved the performance accuracy, particularly at medium levels of task difficulty. However, despite the random assignment of the participants, it must be acknowledged the lack of a baseline measurement for the moderate exercise group. As such, Experiment 2 extended these findings by employing a mixed multifactorial experimental design, in which each participant performed a Sternberg task under control and exercise conditions, and furthermore, was randomly assigned to one

of three exercise intensities (low, medium, high). This second experiment demonstrated that moderate intensity exercise lowers the response latency slopes, i.e., improves the speed with which the probe is compared to the retained set. However, participants' performance in the medium-intensity exercise condition was less accurate, an effect interpreted as a trade-off between response speed and accuracy. In all, the evidence from this study refutes the "transient hypofrontality" hypothesis and gives some support to exercise-induced arousal perspectives. However, the influence that task demands may introduce in experimental designs examining cognitive performance during steady-state exercise (as detected by the first experiment) suggests that other variables (e.g., the type of exercise protocol, the type of cognitive task) may also be implicated.

Study Five. The rationale for this study resulted from two facts. On the one hand, cardiac cycle time data available on high-order cognitive processing is very limited: only a few studies that examined choice reaction times were conducted employing a cardiac cycle time paradigm. As such, is broadly unknown whether pressor-related afferences impact human cognition. On the other hand, there is now research indicating that vagal tone influences performance on tasks involving executive function (Thayer et al, 2009). Apparently, participants with high heart rate variability yield better cognitive performance on this type of task. Therefore, this study investigated whether natural baroreceptor stimulation could impact performance on a task depending on executive functions, the Sternberg working memory task. In order to standardize the retention period for every trial, response latencies were scored retrospectively according to the timing of probe onset after the R-wave into one of six intervals across the cardiac cycle. Interestingly, the results revealed that the zero intercept, a measure of sensorimotor processing time, was greater for probes presented early in the cardiac cycle whereas the slope, an index of working memory

processing efficiency, was steeper for probes presented later in the cycle. In other words, whereas basic sensorimotor processing appears to be delayed early in the cardiac cycle, memory processing per additional digit appears to be delayed on a later phase. These findings (a) constitute the first evidence that afferent cardiovascular input (i.e., natural variations in arterial baroreceptor activity) can interfere on complex cognitive function; (b) provide further support for the visceral afferent feedback hypothesis; and (c) suggest that baroreceptor input differentially affects the transmission of basic sensorimotor processing and high-order cognitive information.

Future Directions – Towards an Integrated Model of Afferent Interference on Cognition

Task Demands and Processing Strategies

Many studies employing a cardiac cycle time paradigm assess human performance on tasks implicating binary decision, and some degree of response monitoring and inhibition. Consequently, cognitive paradigms like choice reaction time tasks, the go/no-go task or the Sternberg task require the participant to perform to matching or mismatching stimuli under time constraint. As so, the task demands are central to this discussion, and may have been slightly overlooked by previous research, and consequently left "out from experimental control". The Sternberg task clearly exemplifies that, by the fact that different retention strategies can be used by the participants depending on the length of the set size (i.e., the memory load). Accordingly, studies examining the performance to the Sternberg task commonly report very similar (if not equal) response latencies to both matching and mismatching probes but only for high memory load conditions (e.g., Altamura et al, 2007; Schon et al, 2009). For short set sizes (i.e., 2 or less) response latencies tend to be faster for matching than mismatching probes. Such delay may reflect the sensory registration into the

"visuospatial sketchpad" (as opposed to matching probes, already registered) to allow subsequent comparisons with the previous series (Baddeley, 2003; Rawley & Constantinidis, 2009). Conversely, given that performance for high memory load sets tends not to reveal differences between the probe types, it is likely that another strategy for on-line probe comparison with the set is taking place. Precisely, "subvocal rehearsal" may well be the preferential maintenance strategy for the immediate retention of a six-digit string, for instance. In such a case, both types of probe would involve reading followed by the phonologic comparison of the stimulus to the rehearsed auditory string registered into the phonological short-term store (Baddeley, 2003).

Maybe trivial at first sight, but this detail would be crucial if considered in the context of a cardiac cycle time study. Not only would "subvocal rehearsal" produce similar response latencies for different types of probe but would probably result in a null cardiac cycle time effect on performance to high memory load sets. Specifically, studies exploring hemispheric lateralization of neural activity during working memory processing stages have consistently shown right prefrontal and premotor cortical activation during visuospatial memory updating and comparison, and enhanced left hemisphere activity depicted in the sensorimotor cortex, Broca's and supplementary motor areas during "subvocal rehearsal" (Wager & Smith, 2003). In parallel, neuroimaging (Kimmerly et al., 2005; Kimmerly et al., 2007; Wong et al., 2007), and neurophysiological studies (Weisz et al., 2001; Pollatos & Schandry, 2004) have accumulated evidence for a right-hemisphere preponderance in the processing of visceral sensory information arising from baroreceptors. Hence, a visuospatial working memory strategy would be amenable to afferent baroreceptor interference whilst it would not be reasonable to expect the same from a phonological-based maintenance process.

Therefore, future cardiac cycle time studies investigating cognitive processing may wish to examine stimuli across different content (non-verbal *versus* verbal). Moreover, factorial designs to manipulate the several processing stages implicated in the task to be assessed (encoding, probe comparison, response selection and execution), may also be employed to specify the necessary conditions that allow afferent baroreceptor information to interfere with cognitive processes.

Patterns of Cardiac Cycle Modulation

It has been previously argued that any cardiac cycle time modulatory effect on performance should conform to the quadratic pattern of activity exhibited by the arterial baroreceptors for it to be unequivocal (see Edwards et al, 2007).

In light of this, the evidence resulting from the fifth study is not only a novel finding of a cardiac cycle time effect on working memory. It can be combined with that from previous cardiac cycle time studies yielding patterns of modulation that differ from the "ideally" quadratic pattern (and particularly with the pain facilitation observed at R+300 ms after the R-wave of the EKG, reported in the first study) to contradict such assumption. In our view, it cannot be assumed that the latency of behavioural responses depending on cognitive or even basic sensorimotor processes (i.e., simple reaction time), known to implicate a myriad of neural pathways, should mimic the same firing pattern revealed by some nerve terminals to be regarded as evidence of afferent input interference. On the contrary, each behavioural response to be assessed has a specific neural organization, which may not generalize to other responses.

Supporting this perspective, previous studies have noticed a quadratic modulation pattern solely for neurophysiological indexes, such as the nociceptive flexion reflex

(Edwards et al., 2001; McIntyre et al., 2006) and the N2 amplitudes of pain-evoked potentials (Edwards et al., 2008), whilst most other studies have found linear patterns of cardiac cycle modulation for simple (McIntyre et al., 2007; McIntyre et al., 2008; Edwards et al., 2007) and choice (McIntyre et al., 2007) reaction time paradigms. Two considerations result from these data. First, in comparison to the short latency of neurophysiological responses, the latency of behavioural responses is hardly coincident with the integrated firing pattern of the arterial baroreceptors. Second, baroreceptors nerve traffic, the nociceptive flexion reflex, and pain-evoked potentials all share common neural substrates at brain stem and medullary levels (e.g., the nucleus tractus solitarius), whereas most behavioural responses assessed in cardiac cycle time studies do not.

Limitations

It is acknowledgeable that the present series of experiments has suffered from a few limitations. Considering the first two studies, it would have been desirable (from the scientist viewpoint) that every participant could experience each schedule of electrocutaneous stimulation during different experimental sessions. However, ethical constraints precluded such procedure.

Baroreflex sensitivity was not measured across the several studies. Although baroreflex sensitivity measured at rest has been reported to be unrelated to sensitivity to pain in adults with blood pressure in the normotensive range (France et al., 1991), it could have provided ancillary data on the parasympathetic/sympathetic balance of the participants, particularly during the most arousing experimental conditions.

In addition, other measures of cardiovascular functioning (e.g., portable finapres) could have informed the present cardiac cycle time studies. Yet, such devices could

interfere with the experimental setup, by increasing the awareness of the heartbeat timing, for instance.

In human participants, it is still not possible to assess the precise timings of the arrival of afferent input at medullary, thalamic and limbic structures in the brain, centres that may operate to produce the patterns of cardiac cycle modulation on behaviour. However, the combination of imaging techniques such as the transcranial doppler ultrasonography may contribute to this quantification.

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