HIGHER CENTRE AND AUTONOMIC CONTROL OF CARDIORESPIRATORY FUNCTION

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ABSTRACT

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Higher Centre and autonomic control of cardiorespiratory function

This thesis is concerned with the role of 'central command' in cardiorespiratory control during exercise and the peripheral autonomic modulation of cardiac excitability.

Chapter One reviews the background to the work in the thesis. The cardiorespiratory and autonomic changes occurring during exercise and the ways in which they are brought about are discussed. This is followed by a review of some pathological changes in autonomic activity and the ways in which they might be arrhythmogenic.

Chapter Two details the experimental techniques used in the thesis and discusses their theoretical background.

Chapter Three studies the cardiorespiratory responses to imagination of exercise under hypnosis. This results in hyperventilation, hypocapnia and an increase in heart rate. In contrast, no cardiorespiratory changes are seen when the same protocol is repeated in the awake state. The response to imagined exercise under hypnosis is not affected by maintaining isocapnia. A powerful drive to breathe arising from higher centres, that is independent of peripheral muscular feedback, is elicited when 'exercise' is imagined under hypnosis.

Chapter Four investigates the neural correlates of imagined exercise under hypnosis using positron emission tomography to see if they are the same as those classically implicated in 'central command' during exercise. Activation of 'motor' areas occurs, including the supplementary motor area, primary motor cortex for breathing, premotor area, thalamus, basal ganglia and cerebellum. The insular cortex, right dorsolateral prefrontal cortex and posterior parietal cortices are also activated. These activations are similar to those previously reported during actual exercise, but occur in the absence of peripheral feedback that accompanies actual exercise and may therefore reflect 'central command'.

Chapter Five studies the responses to altered perception of work rate during actual exercise using hypnotic suggestions. If exercising hypnotised subjects are told that the work rate has increased (when it hasn't), they hyperventilate, become hypocapnic and heart rate increases, suggesting a partial uncoupling of 'central command' from peripheral feedback.

Chapter Six investigates the cardiovascular effects of electrical stimulation of the thalamus and midbrain in awake man to determine the role of subcortical areas in cardiovascular control. High-frequency stimulation of the thalamus, subthalamic nucleus and substantia nigra results in increases in heart rate and blood pressure, whereas stimulation of the globus pallidus has no effect. In patients with implanted stimulating electrodes, chronic high-frequency electrical stimulation does not affect baroreflex sensitivity, heart rate variability or blood pressure variability.

Chapter Seven characterises the cardiac electrophysiological consequences of sympathetic imbalance using multi-electrode array mapping techniques in the pig heart *in vivo*. A novel potential arrythmogenic mechanism of noradrenaline is also investigated in two *in vitro* preparations.

Chapter Eight presents a brief summary of the findings in the thesis and discusses future research directions.

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The experiments described in Chapter Six were performed with the help of the neurosurgical team at the Radcliffe Infirmary, I would particularly like to thank Tipu Aziz, David Schlugman, Carol Joint, Simon Parkin and Nicky Maartens. Thanks also to Graham Pluck of the Institute of Neurology, Queens Square, who originally told me about these patients and encouraged me to study them.

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Finally I would like to thank my family and friends, who provided encouragement, support and wider perspectives.

Selected abbreviations

Neurological terms

Abbreviation	
BET	Benign essential tremor
CBF	Cerebral blood flow (r, regional; g, global)
CCHS	Central congenital hypoventilation syndrome
CT	Computerised tomography
DBS	Deep Brain Stimulation
DLPFC	dorsolateral prefrontal cortex
fMRI	functional magnetic resonance imaging
FWHM	Full-width-at-half-maximum
GLM	General Linear Model
GP	Globus pallidus or pallidum (i, internal; e, external)
MLR	Mesencephalic locomotor region
NTS	Nucleus of the solitary tract
PD	Parkinson's Disease
PET	positron emisson tomography
PMA	premotor area
PPn	Pedunculopontine nucleus
SMA	supplementary motor area
SNc	Substantia nigra par compacta
SNr	Substantia nigra par reticulata
SPECT	single photon emission computerised tomography
SPM	Statistical Parametric Mapping
STN	Subthalamic nucleus
VA/VL	Ventral anterior/ventral lateral nuclei of the thalamus
VIM	Ventralis intermedius nucleus of the thalamus
VOP	Ventralis oralis posterior nucleus of the thalamus

Cardiorespiratory terms

BPV	Blood pressure variability
BRS	Baroreflex sensitivity (ms/mmHg)
cAMP	Cyclic adenosine monophosphate
CICR	Ca^{2+} induced Ca^{2+} release
DBP/SBP/MAP	Diastolic/systolic/mean arterial blood pressure (mmHg)
f	Breathing frequency (breaths/min)
HR	Heart rate
HRV	Heart rate variability
LF/HF	Low frequency/high frequency
LVP	Left ventricular pressure (mmHg)
MVC	Maximum voluntary contraction (kgW)
NA	Noradrenaline
$P_{ET}CO_2$	End-tidal partial pressure of CO_2 (Torr)
SR	Sarcoplasmic reticulum
TVEAT	Total ventricular epicardial activation time
$\dot{V}_{CO_2}$	CO_2 output (ml/min)
$\dot{V}_I$	Ventilation (inspired, l/min)
$\dot{V}_{O_2}$	O_2 consumption (ml/min)
VACR	Voltage-activated Ca^{2+} release
VT	Ventricular Tachycardia
V_T	Tidal volume (litres)

Chapter One

Introduction

The great tragedy of Science – the slaying of
a beautiful hypothesis by an ugly fact.
TH Huxley.

This thesis is primarily concerned with cardiorespiratory control during exercise, particularly the role of 'central command', but it also investigates some aspects of cardiac autonomic control and its role in arrhythmogenesis. Chapter One therefore reviews the cardiorespiratory and autonomic changes occurring during exercise, followed by the mechanisms by which these changes may be brought about. It then moves on to deal with pathological changes in autonomic activity and the ways in which they may be arrhythmogenic.

1.1 Cardiorespiratory control and autonomic function

The basic pattern of breathing is initiated by a neuronal network in the pons and medulla known as the 'respiratory rhythm generator' e.g. Koshiya & Smith (1999), reviewed by von Euler (1983). This region has descending bulbo-spinal projections that synapse in the anterior horn of the spinal cord (C3-5, T1-12) onto neurones projecting to the respiratory muscles (figure 1.1). The pattern of breathing produced by the rhythm generator is modulated by inputs from other brain regions and from the periphery. Brain regions modulating $\dot{V}_I$ include the motor (Colebatch *et al.* 1991) and limbic (Corfield *et al.* 1995) cortices, the central chemoreceptors of the medulla (Comroe 1943), the hypothalamus (Smith *et al.* 1960) and the midbrain (Kabat 1936). The peripheral influences on $\dot{V}_I$ include the chemoreceptors of the carotid bodies (Cunningham 1987), C fibres from exercising muscles (McCloskey & Mitchell 1972) and pulmonary afferents (Phillipson *et al.* 1981a; Phillipson *et al.* 1981b) which synapse in the nucleus tractus solitaris (NTS) in the dorsomedial medulla from where they project to the respiratory rhythm generator. It has also been proposed that increases in venous return can drive breathing, so called 'cardiodynamic hyperpnoea' (Wasserman *et al.* 1974).

Cardiovascular regulation via the autonomic nervous system is coordinated in medullary nuclei, including the ventrolateral medulla, the parabrachial nucleus and the NTS. The NTS contains the first synapse for virtually all cardiorespiratory afferents (figure 1.2) and has reciprocal connections with brainstem and forebrain nuclei (including the raphe nuclei, central grey, and paraventricular and lateral hypothalamus). In addition to medullary nuclei the hypothalamus, amygdala, cerebellum and the insular and medial prefrontal cortices also play important roles in cardiovascular regulation, reviewed by Verberne & Owens, 1998.

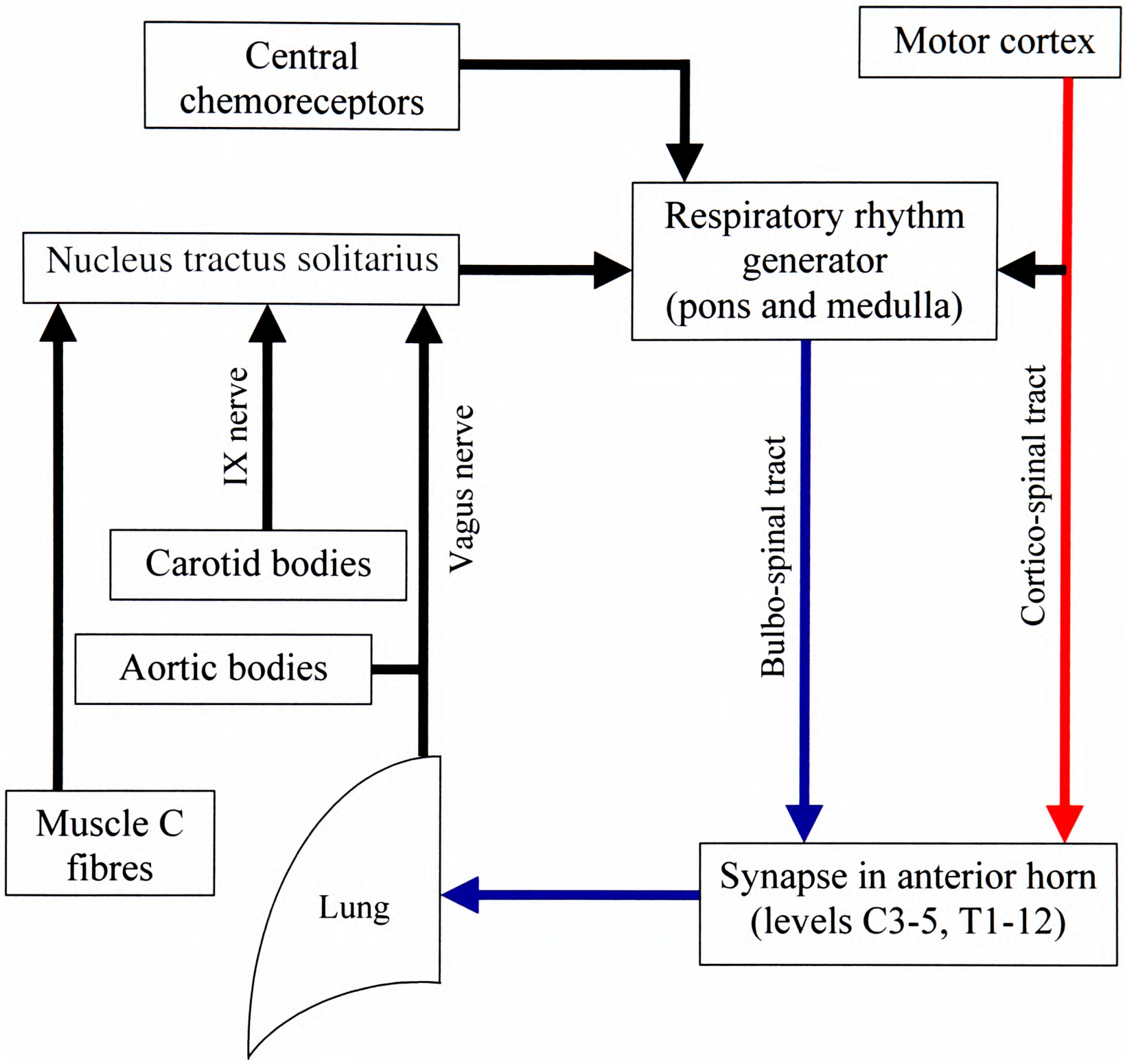


Figure 1.1. The neurophysiology of breathing. The blue arrows indicate the pathway for automatic breathing. The red arrows indicate a pathway by which the cortex may influence breathing, although this may also occur via the respiratory rhythm generator.

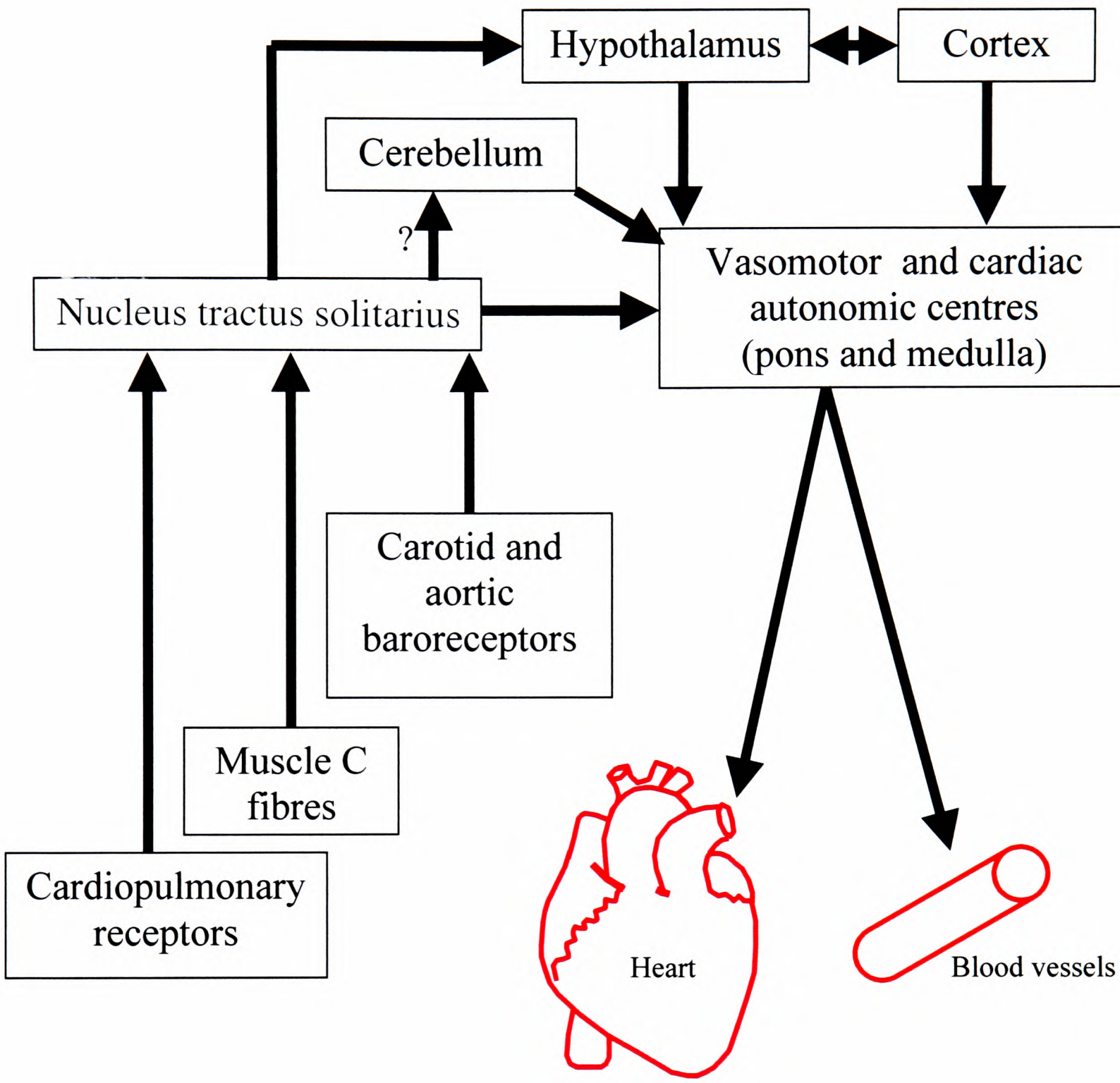


Figure 1.2. Autonomic control of the cardiovascular system.

1.2 Exercise and autonomic function

Dynamic exercise is associated with a rapid increase in $\dot{V}_I$ occurring within the first seconds (or in anticipation) of exercise (phase I). This is followed by a more gradual rise (phase II) to a steady state (phase III) and is well represented by a single-phase exponential (figure 1.3). Total $\dot{V}_I$ in the steady state is linearly related to $\dot{V}_{O_2}$, and arterial PCO_2 and PO_2 remain fairly constant unless work rate increases above *ca.* 60% of maximum exercise capacity. These changes are illustrated in figure 1.4. The cardiovascular changes occurring with exercise include a rapid increase in cardiac output with the onset of exercise and a redistribution of blood flow. Mean arterial pressure is maintained or may increase. Despite a century of study, the way in which these changes are brought about is unclear. There are two general schools of thought, 'peripheral feedback' and 'central command' (table 1.1) and many studies have found that a normal or near-normal response is possible when many drives are removed, indicating that a high degree of redundancy exists (Cunningham 1987).

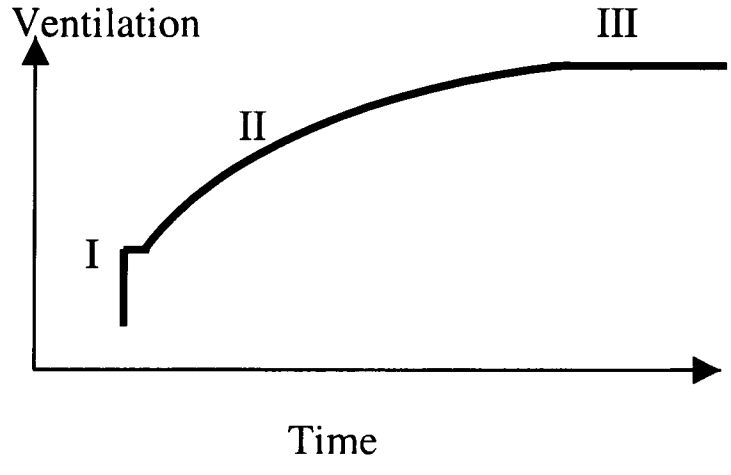


Figure 1.3. Phases of the ventilatory response to exercise.

1.2.1 Peripheral feedback

The importance of peripheral feedback in contributing to the cardiorespiratory response to exercise was first recognized by Zuntz & Geppert (1886) following experiments in dogs in which electrical stimulation of the hindlimbs resulted in hyperpnoea and hypocapnia. After section of the vagus and spinal cord $\dot{V}_I$ still increased, leading the authors to conclude that some acid-like substance released from muscle was responsible for the increased ventilation.

1.2.1.1 Reflexes arising from exercising muscle

The exercise pressor reflex was first demonstrated in man by Alam & Smirk (1937) who found that blood pressure remained elevated following exercise if the circulation to the

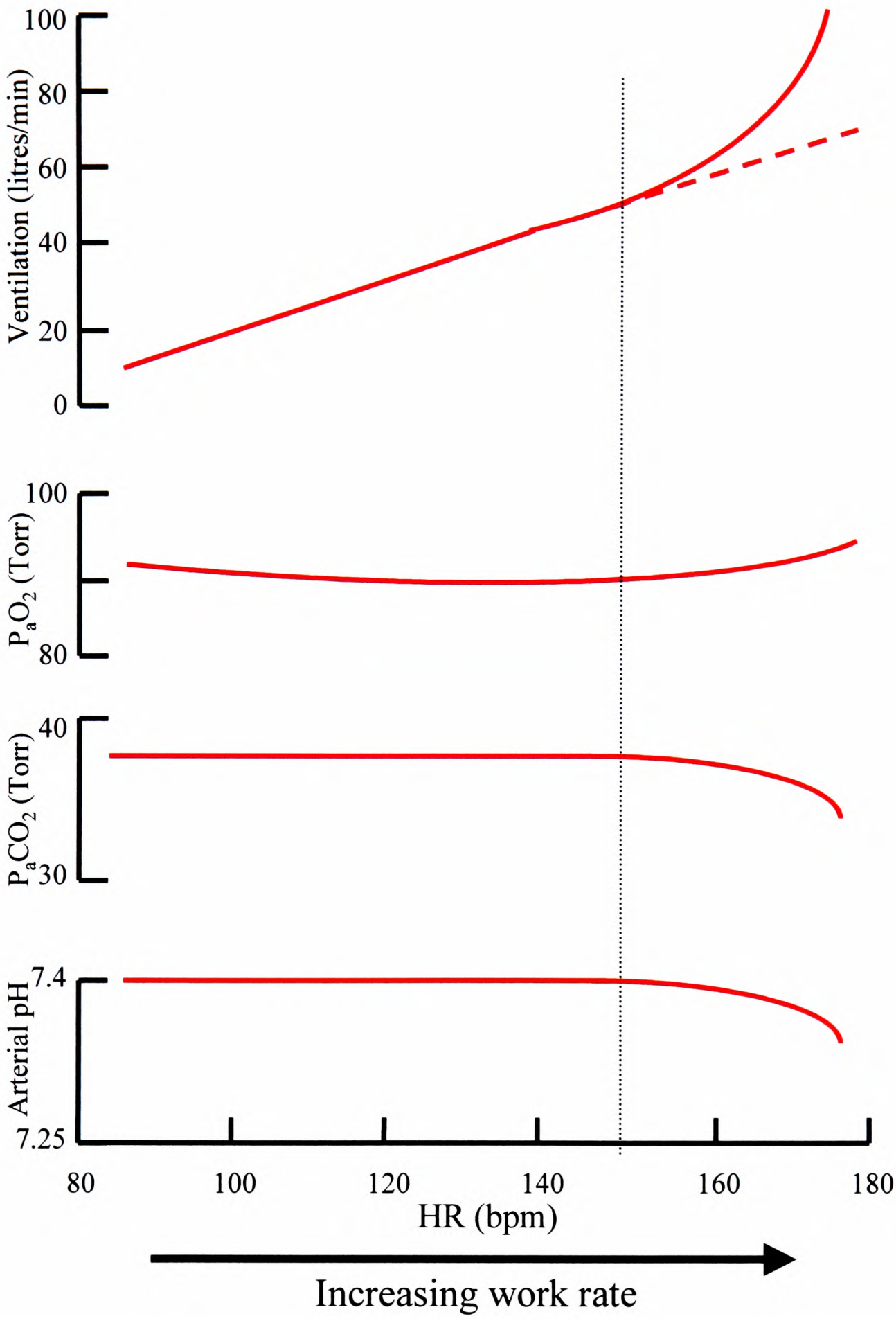


Figure 1.4. Ventilatory changes during exercise. Ventilation increases in direct proportion to work rate during mild and moderate exercise and arterial blood gases and pH remain constant. During heavy exercise above the so-called 'anaerobic threshold' (indicated by the dotted line), ventilation increases out of proportion to the work rate.

Drive	*Method of study*	*References*
Peripheral drives		Reviewed by Kaufman & Forster (1996)
Carotid bodies		Cunningham (1987)
CO_2, H^+, lactate	Isolated chemoreceptor cells	Buckler *et al.* (1993)
K^+	McArdle's Syndrome	Paterson (1992)
catecholamines	Efferent blockade	Eisele *et al.* (1967)
blood gas oscillations	Arterial cannulation	Band *et al.* (1980)
Increased gain	Hypoxic sensitivity tests	Weil *et al.* (1972)
Muscle chemoreflex	Occlusion of exercising muscle	Alam & Smirk (1937)
Muscle mechanoreflex	Electrically evoked exercise	Hollander & Bouman (1975)
Pulmonary afferents	Venous CO_2 loading in sheep	Phillipson *et al.* (1981)
Respiratory muscle afferents	Denervation in ponies	Forster *et al.* (1984)
Cardiac afferents	Heart transplant patients	Banner *et al.* (1988)
Central drives		Reviewed by Waldrop *et al.* (1996)
Concept of central drive:	Electrically evoked exercise	Krogh & Lindhard (1917)
	CCHS	Shea *et al.* (1993)
	GTO stimulation	Goodwin *et al.* (1972)
	Paralysis	Gandevia & McCloskey (1977)
Specific brain regions:		
Motor cortex, PMA, SMA	PET	Fink *et al.* (1995)
Insular cortex	SPECT	Williamson *et al.* (1997)
Cerebellum	PET	Fink *et al.* (1995)
Hypothalamus	Electrical stimulation in decorticate cats	Eldridge *et al.* (1981)
Basal ganglia	Electrical stimulation in chronically instrumented cats	Angyan (1996)
Mesencephalic locomotor region	Electrical stimulation in anaesthetised and decorticate rats	Bedford *et al.* (1992), Chong & Bedford (1997)
Brainstem	c-*fos* immunocytochemistry in rats	Iwamoto *et al.* (1996)

Table 1.1. Summary of the some of the major studies on the cardiorespiratory responses to exercise, showing the diversity of methodologies. Experiments are in man unless otherwise stated. Abbreviations; CCHS congenital hypoventilation syndrome, GTO golgi tendon organ, PET positron emission tomography, SPECT single photon emission computerised tomography, PMA premotor area, SMA supplementary motor area.

exercising muscle was arrested, indicating that the pressor response was caused by chemicals trapped in the exercising muscle. However, HR and $\dot{V}_I$ fall rapidly on cessation of handgrip exercise even in the presence of occlusion, indicating that chemicals from exercising muscle do not contribute to increases in HR and $\dot{V}_I$ during exercise. Since these early observations, numerous studies have indicated the importance of this reflex in mediating the redistribution of blood flow and changes in regional perfusion pressures occurring in exercise. Patients with McArdle's Syndrome[1] have smaller muscle sympathetic nerve activity and pressor responses to exercise than normals (Pryor *et al.* 1990), so it seems likely that H^+ is partially responsible. Indeed alterations in muscle H^+ (measured by ^{31}P NMR) parallel the changes in arterial blood pressure during static exercise (Victor *et al.* 1988; Sinoway *et al.* 1993). However, nerve recordings from muscle afferents in the cat show an initial burst of activity when H^+ is injected intramuscularly, but this increase in firing is not maintained (Mense & Stahnke 1983). Taken together with the fact that McArdle's Syndrome patients do have a pressor response, albeit reduced, it seems that other metabolites in addition to H^+ must play a role. K^+ has been implicated and the increases in plasma K^+ parallel those of MAP during static exercise (Fallentin *et al.* 1992). Interestingly, whilst McArdle's Syndrome patients show a reduced pressor response compared to normals, their HR responses are identical, indicating a difference in mechanism between HR and pressor responses. A mechanoreflex from exercising muscle also exists. Direct recordings from muscle afferents in anaesthetised cats (Kaufman *et al.* 1983) have shown that group III afferents (small myelinated fibres) are more sensitive to the mechanical aspect of contraction (discharge frequency increases immediately at the onset of contraction), whereas group IV afferents (unmyelinated) are preferentially stimulated by metabolites (and had a longer latency than group III afferents).

The role of muscle reflexes in exercise hyperpnoea has been studied using electrically evoked exercise in both anaesthetised animals and in man. Comroe (1943) found that hyperventilation in response to electrical stimulation of the hindlimbs in anaesthetised dogs was abolished by spinal cord transection. Adams *et al.* (1984a) showed that electrically induced exercise in man produced a similar ventilatory response compared to voluntary exercise, with the exception of

[1] These patients lack skeletal muscle myophosphorylase, hence cannot convert glycogen to glucose and do not produce lactic acid during exercise.

a slower ventilatory transient. However, proving a complete absence of 'central command' in human studies of electrically induced exercise is problematic. Moreover, the role of muscle afferents in mediating exercise hyperpnoea during electrically induced exercise is questionable since a similar hyperpnoea can be evoked during electrically induced exercise in paraplegics (Adams *et al.* 1984a).

1.2.1.2 Role of the carotid bodies

As the major peripheral arterial chemoreceptors in man, the carotid bodies have been studied extensively as a drive for exercise hyperpnoea despite the fact that end-tidal PCO_2 and PO_2 do not change significantly during moderate exercise (Douglas & Haldane 1909)[1]. A number of other factors may increase carotid body drive during exercise, including exercise-induced hyperkalemia (Paterson 1992), circulating catecholamines (Folgering *et al.* 1982; Milsom & Sadig 1983), sympathetic drive (Petersen *et al.* 1983), increased oscillations in blood gases (Band *et al.* 1980) and chemicals from exercising muscles (Innes *et al.* 1989). Carotid body gain may be increased during exercise, as indicated by an increase in hypoxic sensitivity (Weil *et al.* 1972). It has been proposed that an increased gain might allow the carotid bodies to respond to much smaller changes in blood gases than they do at rest. However, denervation of the carotid bodies in man (Wasserman *et al.* 1975) or in ponies (Pan *et al.* 1995) does not alter the steady-state response to moderate exercise. In addition, children with central congenital hypoventilation syndrome (CCHS) show a normal response to moderate exercise despite having no functional chemoreception, so it seems that the carotid bodies do not play an obligatory role in the steady-state ventilatory response, rather they may 'fine-tune' the ventilatory response to exercise. Consistent with this modulatory role, CCHS patients have more labile $P_{ET}CO_2$ both during exercise and at rest than control subjects (Shea *et al.* 1993). The importance of the carotid bodies in mediating the hyperpnoea of heavy exercise is better established; neither carotid body resected patients (Wasserman *et al.* 1975) or CCHS patients (Shea *et al.* 1993) hyperventilate during exercise above the 'anaerobic threshold'. Both H^+ and K^+ have been implicated as the carotid body stimulants responsible (Paterson 1992).

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[1] Man is fairly unique in this respect since most animals become hypocapnic during exercise.

1.2.1.3 Respiratory muscle, pulmonary and cardiac afferents

There is little evidence to suggest that type III and IV afferents of the intercostals and diaphragm provide a major drive to exercise hyperpnoea, since denervation does not affect the total exercise response in either goats (Mitchell 1990) or ponies (Forster *et al.* 1994a). However, these afferents do affect the *pattern* of breathing in ponies (Forster *et al.* 1994a; Forster *et al.* 1994b). Breathing frequency was lower at rest and during exercise in diaphragm deafferented ponies than in sham operated ponies and this was compensated for by increases in V_T, the total $\dot{V}_I$ was unchanged.

The pulmonary vagus may also play a role in the pattern of exercise hyperpnoea; airway anaesthesia caused by bupivicaine inhalation resulted in slower deeper breathing during exercise in normal man, although there was no change in resting breathing (Winning *et al.* 1985). Similar conclusions were reached in studies on ponies using hilar nerve (pulmonary vagus) denervation (Forster *et al.* 1994b).

'Cardiodynamic hyperpnoea' (the theory that an increase in $\dot{V}_I$ can be secondary to an increase in cardiac output) has been proposed to provide a drive to breathe during exercise (Wasserman *et al.* 1974). The basis for this idea was that the increase in $\dot{V}_I$ at the onset of exercise occurs abruptly without a change in $P_{ET}CO_2$, indicating that it is precisely matched to an increase in perfusion. Mechanisms increasing cardiac output (intravenous isoprenaline infusion or cardiac pacing) resulted in hyperpnoea with a maintained $P_{ET}CO_2$ in dogs (Wasserman *et al.* 1974), indicating that increases in cardiac output *could* drive increases in breathing. However, since heart and heart-lung transplant patients have a normal ventilatory response to both voluntary exercise and electrically induced exercise despite a blunted HR response (Banner *et al.* 1988), 'cardiodynamic hyperpnoea' is clearly not an obligatory drive.

1.2.2 Central command

The importance of 'central command' in exercise was recognised by Johansson in 1893, who showed in the rabbit that voluntary movements produced increases in heart rate, but passive movements or electrical stimulation of muscle did not. He concluded that the motor impulses to the muscles 'irradiate the centres governing the heart'. Krogh & Lindhard (1913, 1917)

drew similar conclusions regarding exercise hyperpnoea when they demonstrated in man that the ventilatory response to normal leg exercise began simultaneously with the onset of exercise whereas electrically induced leg exercise was associated with a slower onset of the ventilatory response. An enormous number of studies with widely differing methodologies have since confirmed the existence of 'central command' as a mechanism for the increase in cardiorespiratory variables during exercise, reviewed by Waldrop *et al.* (1996). The following discussion concentrates on studies conducted in man.

1.2.2.1 Muscle spindle vibration studies in man

Goodwin *et al.* (1972) used vibration of muscle spindles in man to increase or decrease the amount of effort required to maintain an isometric force. Subjects were asked to maintain a constant tension with either the biceps or triceps. By stimulating the muscle spindle afferents of the biceps during a voluntary contraction of the biceps, contraction is assisted and the amount of 'central command' required to generate a given tension is reduced. Conversely if the biceps are stimulated whilst the subject is voluntarily contracting the *triceps*, the contraction is inhibited and so the amount of 'central command' required to maintain tension is increased. Blood pressure, HR and $\dot{V}_I$ all increased during contraction *in proportion to the effort required*, thus indicating that the cardiorespiratory responses were governed by 'central command.' This study has been repeated with similar results in awake baboons (Hobbs 1982), providing further evidence of a relationship between 'central command', motor outflow and effort.

1.2.2.2 Paralysis and muscle weakness in man

Cardiorespiratory responses to exercise in man during partial muscular paralysis were first studied by Asmussen *et al.* (1965). The hypothesis was that with paralysis more 'central command' would be required to elicit the same work rate. Ventilatory responses were greater during partial paralysis than without, consistent with this hypothesis. Innes *et al.* (1992) reached similar conclusions in studies on subjects with unilateral leg weakness; the ventilatory and HR responses at a set work rate were greater with the weak leg than with the normal leg.

1.2.2.3 Central congenital hypoventilation syndrome

Central congenital hypoventilation syndrome (CCHS) is associated with ineffective chemoreceptor regulation of breathing and severe hypoventilation during sleep and may be due to a lesion in the brainstem respiratory complex. As already discussed, CCHS patients have a normal ventilatory response to exercise (Shea *et al.* 1993). The only difference between CCHS patients and normals is a higher variability in $P_{ET}CO_2$ of the CCHS group, and an absence of hyperventilation during heavy exercise above the 'anaerobic threshold'. Interestingly Paton *et al.* (1993) found that the hyperpnoea of exercise during treadmill running in these patients was via an increase in f rather than in V_T and proposed that locomotor-respiratory coupling might drive $\dot{V}_I$ in these patients. An alternative hypothesis is that these patients rely to a greater extent on 'central command' than normals (and that 'central command' is predominantly a frequency-based drive to breathe). Consistent with this is the finding that these patients hypoventilate at rest; despite normal f they have an abnormally low V_T.

Taken together, these studies overwhelmingly suggest that 'central command' can produce a large proportion of the cardiorespiratory response to exercise. However few of these studies have provided insight into the precise *origin, time-course and magnitude* of 'central command' in normal intact man.

1.3 The origins and neuroanatomy of 'central command'

Since exercise is generally a voluntary motor act, any brain region involved in initiation or control of movement is potentially implicated as a contributor to 'central command'. The basic circuitry of motor control is illustrated in figure 1.5, from which it can be seen that possible 'central command' areas include a) the cortex, b) the thalamus and basal ganglia, c) the brainstem and d) the cerebellum.

The pathways by which 'central command' acts are also unclear; in addition to an action on the cardiorespiratory centres of the pons and medulla it has been proposed that corticospinal projections from the primary motor cortex may bypass the respiratory rhythm generator and elicit exercise hyperpnoea directly as illustrated by the red arrow in figure 1.1 (Krogh &

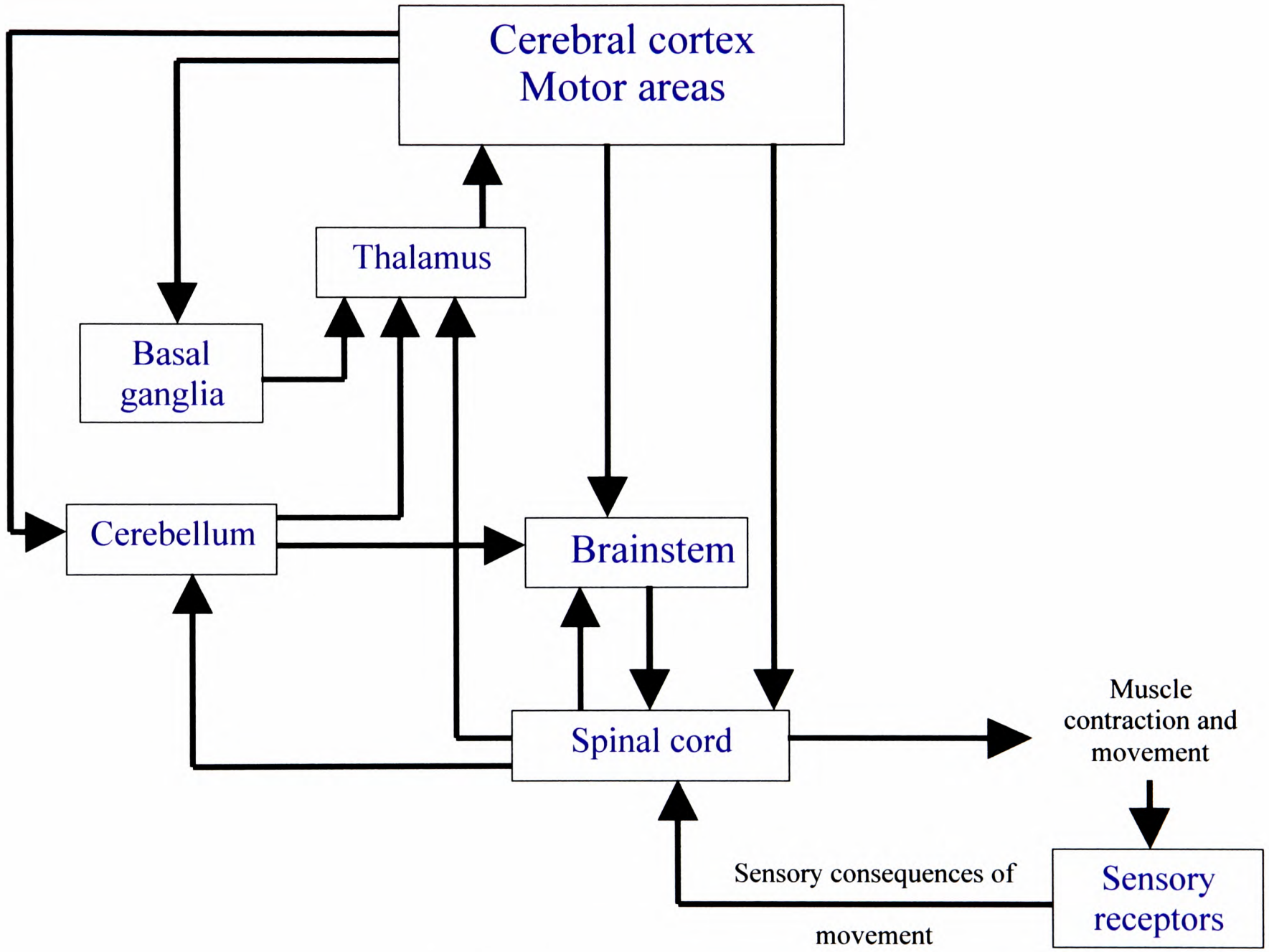


Figure 1.5. The neural circuitry of motor control, there are three hierarchical levels; the spinal cord, the brainstem and the cortex. The motor areas of the cerebral cortex can influence the spinal cord directly and via the brainstem. The motor system is also under the influence of two independent subcortical systems; the basal ganglia and the cerebellum. Both act via relay nuclei in the thalamus.

Lindhard 1913). The existence of neurosurgically induced 'Ondine's Curse' where high cervical cord damage abolishes automatic breathing whilst voluntary control is maintained (Severinghaus & Mitchell 1962) provides evidence for a pathway that can drive breathing independently of the ponto-medullary oscillator. Similar abnormalities result from pontine tumors (Kuhn *et al.* 1999). In addition, trans-cranial electrical stimulation can result in diaphragm activation with a latency similar to that seen for other muscles (Gandevia & Rothwell 1987), indicating that a rapidly conducting oligo-synaptic pathway exists for diaphragmatic activation. Taken together, these studies suggest that there is a separation of drives to breathe (as illustrated in figure 1.1) with a cortico-spinal tract that bypasses the respiratory rhythm generator allowing volitional and 'higher centre' control, and a bulbospinal tract from the respiratory rhythm generator in the pons and medulla allowing automatic control. The interactions between these pathways are discussed in Chapter Five.

1.3.1 Cortical contribution to 'central command'

Johannson (1893) and Krogh & Lindhard (1913, 1917) were the first to recognise that the cortex could drive the cardiorespiratory response to exercise and coined the phrase 'cortical irradiation'. A number of positron emission tomography (PET) studies (Colebatch *et al.* 1991; Ramsay *et al.* 1993; Fink *et al.* 1996) have suggested that the primary motor cortical representation of the diaphragm is located just lateral to the vertex. This is consistent with early neurosurgical evidence (Foerster 1936) that electrical stimulation of this region caused contraction of the diaphragm, although there was no mention of breathing in Penfield's description of sensory and motor homunculi (Penfield & Rasmussen 1950). In addition to primary motor cortical activations, increased activation of the ventral premotor areas, the supplementary motor area, the thalamus and the cerebellum has also been demonstrated in PET studies of volitional breathing (Colebatch *et al.* 1991; Ramsay *et al.* 1993). However, the relationship between the areas involved in volitional breathing and those involved in exercise hyperpnoea has been more difficult to establish, due mainly to the difficulty in constraining the head sufficiently during exercise to allow neuroimaging. Fink *et al.* (1995) PET scanned subjects during mild single-leg exercise or immediately following heavier exercise and found activation in very similar areas to those seen in studies of volitional breathing, i.e. the primary motor cortex just lateral to the vertex. In addition, activation of the supplementary and

premotor areas, the cingulate gyrus and the thalamus occurred in both the exercise study and the post-exercise state; these areas are all implicated in motor control and are potential sources of drive for cardiorespiratory variables. In addition to the areas involved in the motor activity of exercise, it might be expected that brain areas involved in will, motivation and emotion may also provide central drive for cardiorespiratory changes during exercise, for example the limbic system and prefrontal cortex. Arousal, emotion and learning all affect breathing (reviewed by Shea, 1996) but the role of these regions in exercise hyperpnoea remains unclear.

1.3.2 Subcortical regions in 'central command'

Thalamus and brainstem

As illustrated in figure 1.5, the thalamus and basal ganglia are both important for motor control, although the thalamus also has a more general role in autonomic responses and arousal. Other parts of the diencephalon and brainstem including the hypothalamus and many of the midbrain nuclei play a role in arousal, emotion and motivation, consequently all these areas potentially have the neural circuitry to participate in 'central command' and integration of the cardiorespiratory response to exercise. Generalised activations of the brainstem have been observed during ventilatory tasks (Gozal *et al.* 1994; Gozal *et al.* 1995; Gozal *et al.* 1996) using fMRI and thalamic activations have been shown during exercise (Fink *et al.* 1995). However, neuroimaging has not yet successfully identified *specific* thalamic or brainstem nuclei during exercise in man. Since the pons and medulla contain the 'respiratory rhythm generator' and integrate cardiorespiratory drives it seems likely that the brainstem will be involved in cardiorespiratory responses to exercise. However, until recently (Corfield *et al.*, 1999) neuroimaging the brainstem had not been performed with any convincing degree of accuracy during tasks involving cardiorespiratory changes since brain tissue moves in time with the cardiac and respiratory cycles (Poncelet *et al.* 1992) and unless corrected for this leads to task-related signal artefacts. Consequently most of what is known about the role of the thalamus and brainstem in exercise comes from animal studies.

1.3.2.1 Electrical or chemical stimulation of the midbrain in decerebrate animals

Studies in chronically instrumented awake dogs (Smith *et al.* 1960) have shown that electrical stimulation of the hypothalamus results in locomotion together with a cardiorespiratory response. When the animal was paralysed and electrical stimulation repeated, cardiorespiratory variables still increased, indicating that the cardiorespiratory response was not dependent on peripheral feedback. Similar studies have been performed in anaesthetised and decorticated cats (Eldridge *et al.* 1981; Eldridge *et al.* 1985). Stimulation of the hypothalamus results in increases in blood pressure, HR, V_T, f, ventricular contractility and sympathetic discharge (reviewed by Horn *et al.* (1998) and results in an 'exercise-like' redistribution of blood flow, with vasodilation of skeletal muscle beds and vasoconstriction in the skin and intestines (Waldrop *et al.* 1986).

Electrical stimulation of regions other than the hypothalamus has similar effects. Stimulation of the mesencephalic locomotor region (MLR)[1] in decerebrate or anaesthetised rats (Bedford *et al.* 1992; Chong & Bedford 1997) resulted in locomotion and increases in HR and ABP. Whilst the blood pressure responses remained intact after paralysis, the HR response did not; this may indicate an obligatory role for peripheral feedback in the HR response to MLR stimulation. Electrical and chemical stimulation of the substantia nigra in acutely decorticated cats results in increases in cardiorespiratory variables (Angyan 1991) as did stimulation of the mediodorsal thalamus, caudate, putamen and substantia nigra in chronically instrumented cats (Angyan 1994; Angyan 1996). Unfortunately some of these studies on the basal ganglia did not include paralysis controls, making interpretation of the results difficult. Nevertheless, it seems reasonable to hypothesise that the basal ganglia, as a region involved in fine-tuning and co-ordinating motor responses may be involved in the cardiorespiratory response to exercise.

1.3.2.2 Labelling studies in exercising rats

A study of the exercise-induced changes in local cerebral glucose uptake in the rat brain (Vissing *et al.* 1996) found increases in uptake in almost all brain regions, notable exceptions being the prefrontal cortex and cingulate. C-*fos* immunocytochemistry (c-*fos* is a transcription factor and so indicates increased genomic activity) has been used to show that exercised rats

show increased staining above control rats in a number of areas implicated in cardiorespiratory control during exercise (Iwamoto *et al.* 1996). These included the hypothalamic locomotor region, the cuneiform nucleus, periaqueductal grey, rostral ventrolateral medulla and the ventral midline medulla.

1.3.2.3 Lesioning studies

Hobbs (1982) trained baboons to perform static exercise and found that following bilateral 'subthalamic' lesions the baboons lost their exercise pressor response, but had a maintained pressor response to startle. This indicates that the pressor response to exercise may be integrated in the subthalamic region, although the 'subthalamic' region lesioned in this study may be distinct from the 'hypothalamic locomotor region', since lesions here do not abolish the cardiorespiratory responses to exercise in beagles (Ordway *et al.* 1989). A study of patients with brainstem lesions (Morrell *et al.* 1999) demonstrated reduced ventilatory sensitivity to CO_2 in patients with unilateral rostrolateral medullary lesions but not in patients with lesions in the pons, cerebellum or medial medulla. These lesions did not affect breathing at rest or during exercise.

Cerebellum

Cerebellar activation has been shown using PET during exercise in man (Fink *et al.*, 1995). This was attributed to hyperpnoea, since cerebellar activations occur during other breathing tasks (Ramsay *et al.* 1993; Fink *et al.* 1996). However the cerebellum was not activated in the post-exercise state whilst $\dot{V}_I$ was still increased. The cerebellum is also implicated in cardiovascular control including a modulatory role in the baroreflex; decerebrate rabbits showed a smaller reflex bradycardia in response to aortic nerve stimulation following removal of cerebellar nuclei (La Noce *et al.* 1991). Electrical stimulation of cerebellar nuclei in decerebrate or anaesthetised cats also results in a pressor response (Achari & Downman 1970; Miura & Reis 1970).

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[1] Defined in this study as the pedunculopontine and cuneiform nuclei.

1.3.3 Methods for studying the origin of 'central command' in man

There are a number of problems with studying the neuroanatomical origins of 'central command' in exercise. First, it is necessary to isolate 'central command' from peripheral feedback, since the areas involved in integration of higher and peripheral drives may differ from those generating 'central command'. Additionally, separating 'movement' from 'exercise' is required; the areas involved in planning and co-ordinating a motor task that does not involve significant increases in $\dot{V}_{O_2}$ may differ from those that generate exercise hyperpnoea. However, the major difficulty with neuroimaging 'central command' in exercise is the extent to which movement of the head limits the resolution of the technique.

Given these difficulties, the approach taken in this thesis was to study *imagination of exercise whilst at rest under hypnosis*. HR and $\dot{V}_I$ increase during mental imagery of treadmill exercise (Decety *et al.* 1991), although more recent work (Wuyam *et al.* 1995) has only seen this response in athletes. Imagination of exercise *whilst under hypnosis* has been studied (Levin & Egolinsky 1936; Nemtzova & Shatenstein 1936; Dudley *et al.* 1964; Agosti & Camerota 1965; Daly & Overley 1966) with somewhat more consistent increases in cardiorespiratory variables. It seems that the hypnotic state improves the robustness of the response to imagination of exercise, perhaps due to the more directed imagination that occurs under hypnosis compared to the awake state (Coe *et al.* 1980). The studies described in Chapter Three characterised the response to imagination of exercise under hypnosis, along with a number of additional 'control' tasks. The studies described in Chapter Four then used the technique of PET scanning to investigate the neural correlates of this response to see if they were the same as those implicated in 'central command'.

Studying the role of subcortical structures in man

As discussed above, electrical and chemical stimulation of a number of subcortical regions has been performed in awake, anaesthetised or decorticated animals, although the relevance of these results to 'central command' in intact man is unclear. Owing to a novel surgical treatment for movement disorders involving the basal ganglia (including Parkinson's Disease and dystonia) it is now possible to study the effects of electrical stimulation of the midbrain in awake man and this is the focus of the studies described in Chapter Six.

Parkinson's Disease is a progressive, degenerative movement disorder, the hallmark symptoms of which are tremor, rigidity, akinesia and bradykinesia. As originally described:

> *"Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace; the senses and intellect being uninjured"*
>
> James Parkinson, 1817.

This description remains apt, although cognitive and autonomic defects also occur. The cause of Parkinson's Disease is unclear; genetic (Gasser 1998) and environmental (Tanner *et al.* 1994) factors are both implicated and an epidemic of Parkinson's occurred in the 1920's following an outbreak of encephalitis lethargica (Dimsdale 1946). The pathology of Parkinson's Disease is characterised by loss of the dopamine containing neurones of the nigrostriatal pathway and the presence of Lewy bodies[1] (Greenfield 1997). Similar lesions may be present in other regions, including the cortex, spinal cord, hypothalamus and amygdala. The most common drug treatment for Parkinson's is levodopa (a dopamine precursor), but this has unfortunate side effects including dyskinesia (involuntary movements) and rapid 'on-off' fluctuations (patients fluctuate between almost total symptom relief ('on') and complete disablement ('off') within a matter of minutes). Consequently stereotaxic lesioning of the thalamus or basal ganglia is also used to treat intractable Parkinson's Disease (Limousin *et al.* 1995) and on occasion is also used for dystonia and benign essential tremor. A recent alternative is to implant electrodes in the basal ganglia and perform chronic high frequency electrical stimulation. This results in a reversible inactivation, in contrast to a permanent electrolytic lesion. The mechanism responsible for the beneficial effects on movement of these interventions is believed to be a decrease in the output of the basal ganglia (which are pathologically overactive in these movement disorders). During the surgical procedure electrical stimulation of the ventralis interomedius thalamus (VIM), ventralis oralis posterior thalamus (VOP), subthalamic nucleus (STN), substantia nigra (SN) and pallidum (GP) is performed in awake patients, thus providing a human model in which to study electrical stimulation of the midbrain and the hypothesis that the midbrain can activate locomotion and the cardiorespiratory systems in parallel.

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[1] Eosinophilic hyaline cytoplasmic inclusions with a central core and a surrounding pale peripheral rim.

In addition to performing the 'parallel activation' type experiment of the acute effects of electrical stimulation of the midbrain, studying the long-term effects of electrical stimulation and its interaction with the autonomic nervous system in patients with implanted stimulators is also of interest since the midbrain seems to play a role in control of blood pressure and the baroreflex. Electrical stimulation of the substantia nigra in rats causes tachycardia and hypertension (Lin & Yang 1994) and 'subthalamic' lesions in baboons (Hobbs 1982) result in loss of the exercise pressor reflex. It is therefore of great interest to see if *chronic* electrical stimulation of the basal ganglia has an effect on the baroreflex, exercise pressor responses, or on cardiovascular autonomic function in these patients and these questions are investigated in Chapter Six.

1.4 The contribution of 'central command' to the exercise response

> *"Practically any afferent system you care to mention relays far more information to the central nervous system than actually reaches consciousness, or is actually acted upon; in fact, there is a great redundancy of information. Why should the respiratory system be an exception?"*
>
> D. J. C. Cunningham, 1987

A number of human studies have shown that the 'whole' cardiorespiratory response to exercise can occur in the absence of many of the potential drives (e.g. Adams *et al.* 1984a; Adams *et al.* 1984b; Shea *et al.* 1993) and have led to the concept of redundant mechanisms. Results from animal studies support this conclusion, for example the cross-circulation studies of Pan *et al.* (1995). Given such redundancy, there is a need for integration of the various drives and this may occur in the hypothalamus. For example, the discharge rate of hypothalamic neurones increases with peripheral muscle stimulation (Waldrop & Stremel 1989) and stimulation of this region evokes locomotion and a cardiorespiratory response. In addition, 'central command' and feedback from contracting muscles seem to exert different respiratory and cardiovascular effects when activated simultaneously than when activated individually (Waldrop *et al.* 1986). These studies highlight the role of the hypothalamic locomotor region as an integrating site for 'central command' and peripheral feedback, however there is no good reason to suppose that this integration does not happen at a number of different levels, particularly given the fact that lesioning the hypothalamic locomotor region does not impair exercise responses in dogs (Ordway *et al.* 1989).

A consequence of redundancy is that the magnitude of any response to 'central command' will differ according to the level of peripheral feedback and therefore on the work rate. In the absence of an objective measure of either 'central command' or peripheral feedback in man, delineating their roles requires a study in which one of these factors remains constant and the other is changing. The approach taken in this thesis was to use hypnosis as a tool with which to manipulate perception (and hopefully 'central command'). This forms the basis of the studies described in Chapter Five. Two types of study were performed, both under hypnosis. First, *constant load* exercise tasks with *altered suggestion* (both increase and decrease), secondly *alterations in load, without informing the subject*. In the former the intention was to keep peripheral feedback constant and increase or decrease 'central command' independently. In the latter, the aim was to maintain 'central command' constant and manipulate peripheral feedback. Such a combination of experiments allows one to test what happens in circumstances in which 'central command' and peripheral feedback are generating conflicting signals. These protocols also allowed an investigation of the *time-course* over which central and peripheral mechanisms are important since there is a good deal of evidence that whilst there is a high degree of redundancy in the steady state, transient responses may depend upon specific drives (e.g. the initial phase I response, (Adams *et al.* 1984b).

1.5 Pathological autonomic nervous system activity

As discussed above, autonomic function is dramatically altered during exercise, but it also changes in a number of pathologies including Parkinson's Disease. The autonomic failure associated with Parkinson's involves impairments in both sympathetic and parasympathetic nervous systems (Netten *et al.* 1995) and whilst it can result in autonomic symptoms of urinary incontinence, postural hypotension and sweating disturbances, it is rarely associated with arrhythmias. However, imbalance between the two limbs of the autonomic nervous system, such as an increase in the sympathetic drive to a localised region of myocardium can be profoundly arrhythmogenic. High adrenergic tone occurs around regions of ischemia (Schomig *et al.* 1984) and during right ventricular outflow tract dysfunction (Palileo *et al.* 1982) and may facilitate spontaneous activity in the ventricle. This can alter the normal activation sequence of the ventricle and so compromise cardiac output (Lister *et al.* 1964; Grover & Glantz 1983; Burkhoff *et al.* 1986). Studies on the *in-vivo* non-ischemic pig heart

have shown that a localised subepicardial infusion of noradrenaline (NA) is effective in causing sustained ventricular arrhythmia with a deleterious effect on haemodynamics (Podzuweit 1980; Podzuweit *et al.* 1989). Characterising the global electrical changes associated with this arrhythmia using multi-electrode array mapping form the basis of the pig studies described in Chapter Seven.

1.5.1 Cellular basis of arrhythmias induced by sympathetic stimulation

The cellular electrophysiological mechanisms underlying the arrhythmic effects of NA and sympathetic stimulation are not fully understood but several seem to exist. NA leads to increases in cAMP and protein kinase A phosphorylation via its action on β receptors. This leads to increases in intracellular Ca^{2+} via a number of mechanisms, including phosphorylation of the L-type Ca^{2+} channel and increases in sarcoplasmic reticulum Ca^{2+} content. Sympathetically evoked arrhythmias can be prevented or terminated by β- blockade (Sung *et al.* 1983; Lerman 1993) Ca^{2+} channel antagonists and acetylcholine (Wit & Cranefield 1977) and the final common mechanism is believed to be Ca^{2+} overload (Lubbe *et al.* 1992).

The Ca^{2+} required for cardiac contraction comes mainly from the sarcoplasmic reticulum and enters the cytoplasm through the ryanodine receptor (RYR) which incorporates a Ca^{2+} channel. The trigger for opening the RYR is generally accepted to be Ca^{2+} entry from the extracellular space, so called Ca^{2+}-induced Ca^{2+} release (CICR), (Fabiato 1983). Recently however, Hobai *et al.* (1997) reported that when Ca^{2+} entry is blocked by 5mM nickel (Ni^{2+}), membrane depolarisation *alone* triggered *ca.* 80% of the Ca^{2+} transient in the guinea-pig ventricular myocyte at 37°C. This only occurred when cells were prestimulated with cAMP. They termed this mechanism 'voltage-activated Ca^{2+} release (VACR). Since the arrhythmic effects of NA in the anaesthetised pig are mimicked by cAMP analogues (Podzuweit 1980) it is of interest to see if 'voltage-activated Ca^{2+} release' is functionally important in eliciting contraction in multicellular cardiac preparations. If this is shown to be the case, an increase in VACR during sympathetic activity may provide an alternative arrhythmic mechanism in addition to the established mechanisms by which cAMP causes Ca^{2+} overload. Two preparations were used to study VACR; the isolated guinea pig papillary muscle and the isolated perfused frog heart. These studies are described in Chapter Seven.

1.6 Specific aims of the thesis

1) To establish whether imagination of exercise at rest under hypnosis can mimic the cardiorespiratory responses to actual exercise.
2) To image the brain during imagination of exercise at rest under hypnosis, with no exercise-related peripheral feedback and establish whether the areas activated are similar to 'central command' areas identified during actual exercise.
3) Determine whether altered perception of exercise load during actual exercise can uncouple peripheral feedback from 'central command' in cardiorespiratory control.
4) To establish whether subcortical command centres can drive the cardiovascular system in awake man during electrical stimulation of midbrain nuclei.
5) To determine whether chronic electrical stimulation of the basal ganglia alters cardiovascular autonomic reactivity in man.
6) To characterise cardiac electrophysiological events caused by sympathetic imbalance and identify potential cellular mechanisms underlying it.

Chapter Two

General Methods

Science moves, but slowly slowly,
creeping on from point to point.
Tennyson.

This chapter describes the general methods used in the thesis and theoretical basis behind each technique. Four techniques were applied to humans:

- Hypnosis (2.1).
- Cardiorespiratory recordings and exercise physiology techniques (2.2).
- Positron emission tomography (PET) scanning (2.3).
- Stereotaxic neurosurgery for movement disorders (2.4).

Electrophysiological and cardiac function techniques are presented in Chapter Seven.

2.1 Hypnosis

Gibson & Heap (1991) describe the hypnotic state as having the following characteristics:

- Selective attention to ideas and events brought to attention by the hypnotist.
- Subjects are less likely to initiate action than when in the awake state.
- Reduced logical consistency and a decrease in reality testing.
- Increased suggestibility.
- Enhanced imagery.
- Amnesia (independent of any suggestions of amnesia made by the hypnotist).

2.1.1 Neurophysiology of hypnosis

Whilst the psychology of hypnosis has been studied extensively, the neurophysiological basis is poorly defined (Gruzelier 1998) and this may be a limitation when using this technique as a tool to investigate physiology. It has been suggested that hypnosis is associated with a shift in dominance from the left hemisphere towards the right (Frumkin *et al.* 1978). There is limited behavioural data in support of this theory, including a stronger responsiveness to hypnotic suggestions on the left than on the right side of the body (Sackeim 1982), and shifts towards the left hand in haptic discrimination (Gruzelier *et al.* 1984) and the left ear in dichotic listening tasks (Frumkin *et al.* 1978).

Evidence from EEG studies

EEG studies of hypnosis have shown widely differing results (reviewed by Crawford & Gruzelier (1992). The most consistent observation is an increase in θ (3-7 Hz) waves under

hypnosis compared with the awake state (Sabourin *et al.* 1990). This increase in θ is topographically widespread and there is no consensus that hemispheric differences exist. θ waves have been associated with sleep and drowsiness, the production of imagery and attentive performance of cognitive tasks (Pedley & Traub 1990).

Evidence from PET scanning

Recent PET scanning studies (Rainville *et al.* 1999) demonstrated a number of differences in regional cerebral blood flow between the resting awake and hypnotic states. The most striking difference was a bilateral increase in blood flow in the occipital cortex under hypnosis, suggesting an objective neural correlate associated with the enhanced visual imagery that occurs under hypnosis. In addition there were increases around the right Sylvian fissure, the left insular cortex, the left dorsolateral frontal cortex and the right anterior cingulate. There are a number of methodological difficulties with this study, particularly the non-randomized scan order and the use of a resting control state with no suggestion (and thus no control over what the subject was thinking about). Nevertheless, as the first neuroimaging study on the neural basis of hypnosis it is of considerable interest.

2.1.2 The hypnotic procedure

Volunteers were healthy and experimentally naïve[1] (active but not athletically trained). Experiments were carried out with local ethics committee approval (COREC 96.164). On their first visit to the laboratory, subjects were examined by a clinician and then assessed for their hypnotic susceptibility by experienced medical hypnotists. The hypnotic induction used in the studies described in this thesis was a combination of 'visual fixation' and 'arm heaviness' suggestions, described by Waxman (1989). The subject was asked to hold their non-dominant arm outstretched in the air and to stare at their thumbnail. Suggestions of relaxation, and arm and eyelid heaviness are made. The natural physical tendencies for the arm to feel heavier and for the eyes to become tired are self-reinforcing; the arm begins to fall, the eyes close and the subject becomes completely relaxed and focussed on the hypnotist's instructions.

[1]Subjects were undergraduate and graduate students. Students studying medical or related disciplines were excluded.

Hypnotic susceptibility is defined as the potential a subject has for achieving the hypnotic state or producing hypnotic phenomena (i.e. it is a predictor of performance). It can be quantified according to one of various scales, the most common of which is the Stanford Scale (Weitzenhoffer & Hilgard 1959) which is graded from 0 to 12 with 12 being the highest. It consists of a defined script that is read to the subject, starting with a standard hypnotic induction, followed by a number of suggested experiences of increasing difficulty including visual, olfactory and auditory suggestions. The subject is graded according to his physical responses and his own reported experience on waking. Whilst such scales are only semi-quantitative they are the most objective measure of hypnotic susceptibility available. Subjects participating in the current study were scored according to a modified version of this scale; those scoring 6 or more proceeded to the rest of the study. These subjects showed features associated with deep hypnosis (Davis & Husband 1931), including pupil dilation, limb catalepsy[1], partial amnesia, automatic responding, an altered perception of the passage of time and rapid responses to suggestions of visual, auditory and olfactory imagery.

2.2 Cardiorespiratory measurements and exercise physiology techniques

2.2.1 Assigning exercise levels

Subjects performed an incremental exercise test in the semi-reclined position on an electromagnetically braked cycle ergometer (Elema-Schonander, Sweden) at a pedalling frequency of 60 rpm achieved with the aid of a metronome. Work rate started at 16 Watts and increased in 16 Watt increments every minute until the subjects' HR was 75% of the predicted maximum above their resting level (HR_{max} taken to be 220 – age, (Astrand & Rodahl 1986). Subsequently, three exercise loads were assigned to each subject; unloaded pedalling ('light') and two loads corresponding to HR's 30% ('moderate') and 50% ('heavy') of the predicted maximum.

[1] As defined by Charcot (1882); "the limbs and all parts of the body maintain, often for a very long time, the positions, the postures, even the most difficult to hold, when these are communicated [by manipulation]. They seem extremely light when they are raised and moved about, no resistance being felt when they are flexed or stretched out".

2.2.2 Cardiorespiratory measurements

Except during the assessment of hypnotic susceptibility, subjects breathed via a facemask (one-way valves, mouth-only breathing, dead-space ~100ml, resistance at 50 l/min of 0.8cm H_2O, Hans Rudolph). A pneumotachometer (Fleisch size 4, unheated) was fixed to the inspired side of the facemask; the pressure across it was measured with a differential pressure transducer (Anodyne), the signal was then amplified and integrated (transducer, amplifier and integrator, P.K. Morgan, UK) for analysis of tidal volume (V_T) and breathing frequency (*f*). Calibration of the flow signal was performed prior to each experimental session using a 3-litre syringe. Where indicated, CO_2 was added to the inspired air via a line attached to the inspired air tube and the amount added was manually controlled using a rotameter (0-1100cc min^{-1}, Platon). Expired gases were analysed for O_2 and CO_2 (Servomex 570A, Servomex 1400) and minute averages of O_2 uptake ($\dot{V}_{O_2}$) and CO_2 output ($\dot{V}_{CO_2}$) were obtained. All gas volumes were corrected for barometric pressure and temperature. $P_{ET}CO_2$ was recorded (Datex Normocap 200) from a sampling line attached to the facemask. Beat-by-beat finger arterial blood pressure was obtained using a Finapres at heart level (2300, Ohmeda). HR was measured from lead II of the ECG (Minimon 7136, Kontron Instruments, UK). The experimental setup is illustrated in figure 2.1.

2.2.2.1 Baroreceptor sensitivity calculation using the spontaneous sequence technique

The baroreflex response can be characterised by observing the HR response to the injection of vasoactive drugs (Hartikainen *et al.* 1995) or the stimulation of the carotid baroreceptors by neck suction, but these methods cannot *continually* measure baroreflex function. Systolic blood pressure (SBP) and R-R interval show spontaneous fluctuations which can provide a non-invasive continuous measure of baroreceptor sensitivity (BRS, in mmHg/ms) as first described by Bertinieri *et al* (1988). A computer program (Kardos *et al.* 1997) was used to scan 15 minutes of baseline recordings of systolic blood pressure and R-R interval for sequences in which SBP and the subsequent R-R interval progressively increased (up-slopes) or decreased (down-slopes) over at least three consecutive beats (figure 2.2). Linear regressions relating the R-R interval to SBP were plotted for each sequence and the slope of the function used as an estimate of the BRS. The minimum change that was accepted for a spontaneous rise or fall in SBP was 1 mmHg. As recommended by Blaber *et al.* (1995), only regression lines with a correlation coefficient greater than 0.85 were used.

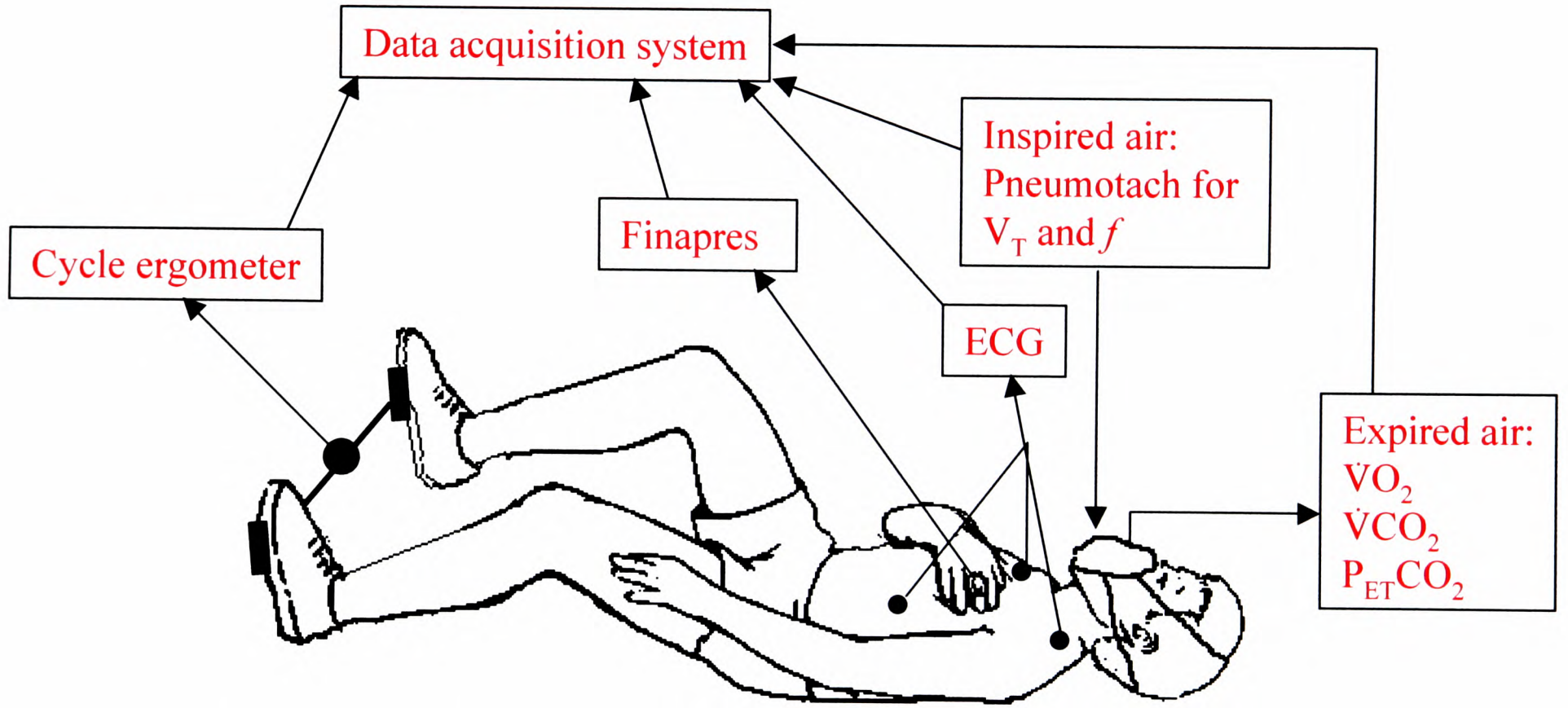


Figure 2.1. Experimental setup for cardiorespiratory recordings during exercise under hypnosis. The subject wears an ECG and facemask and has his feet strapped to the pedals of a cycle ergometer.

2.2.2.2 Spectral analysis of heart rate and blood pressure

The interval between heart beats continually oscillates and this is believed to reflect cardiovascular autonomic activity. This has led to the use of heart rate variability (HRV) and blood pressure variability (BPV) as markers of autonomic activity. Cardiovascular fluctuations can be studied through beat-to-beat blood pressure and HR monitoring and calculation of the variance of their mean values (Mancia *et al.* 1983). Frequency domain analysis has also been used to subdivide BPV and HRV into different frequency components and to quantify the variance or "power" at each specific frequency (Jenkins & Watts, 1968). Two frequency bandwidths were considered in the present study, low frequency (LF) (0.04-0.15 Hz) and high frequency (HF) (0.15-0.4 Hz). Although the interpretation of HRV and BPV is still a matter of some debate, it is generally believed that HF HR power is closely linked with respiratory sinus arrhythmia and is a reasonable measure of cardiac vagal control. LF HR power is less specific and is affected by both sympathetic and vagal influences (Parati *et al.* 1995), but seems to be a marker of baroreflex control of sympathetic outflow to the vasculature (Di Rienzo *et al.* 1989; Parati *et al.* 1990). HF BP power is suggested to be a result of the mechanical exertion of respiration on the pressure gradients and size of the heart and large thoracic vessels (Parati *et al.* 1995). LF BP power is predominantly caused by fluctuations in vasomotor tone and systemic vascular resistance.

In order to perform spectral analysis, ECG and blood pressure data of 5-15 minutes duration were used for calculating variances. An autoregressive technique was used to evaluate the power spectral density of the time series. The spectral power, in ms^2, was computed for the high frequency (0.15-0.40 Hz) and low frequency (0.04-0.14 Hz) components (Kardos *et al.* 1997). Typical spectra and the data they are derived from are illustrated in figure 2.3.

2.2.3 Data acquisition

Analogue signals were sampled at 200 Hz[1] (Biopac MP100WS) and recorded on a Power Macintosh computer using Acqknowledge software for subsequent analysis. Breath-by-breath

[1] Whilst 500Hz or above is normally recommended for acquiring data that is to be used for HR/BPV calculations, preliminary analysis showed that identical results were seen with data acquired at 200Hz.

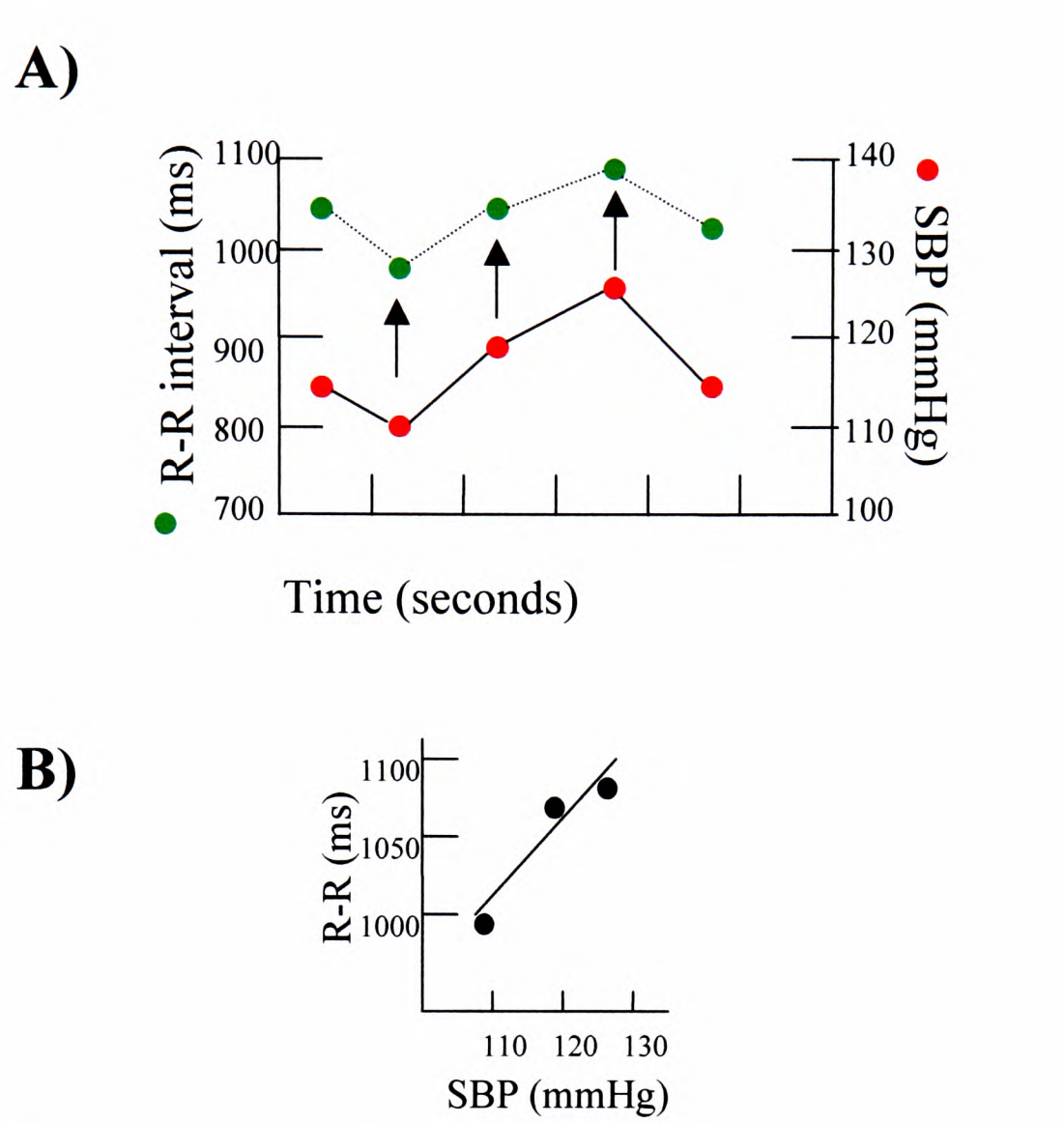


Figure 2.2. Analysis of baroreflex sensitivity using the spontaneous sequence technique. A) Example of a sequence in which SBP (red) and R-R (green) change in the same direction (i.e. a baroreflex) with a lag of 0 (i.e. R-R changes on the same beat as the SBP). B) Linear regressions relating R-R to SBP are plotted for each sequence and the slopes of these regressions are used as an estimate of the baroreflex sensitivity.

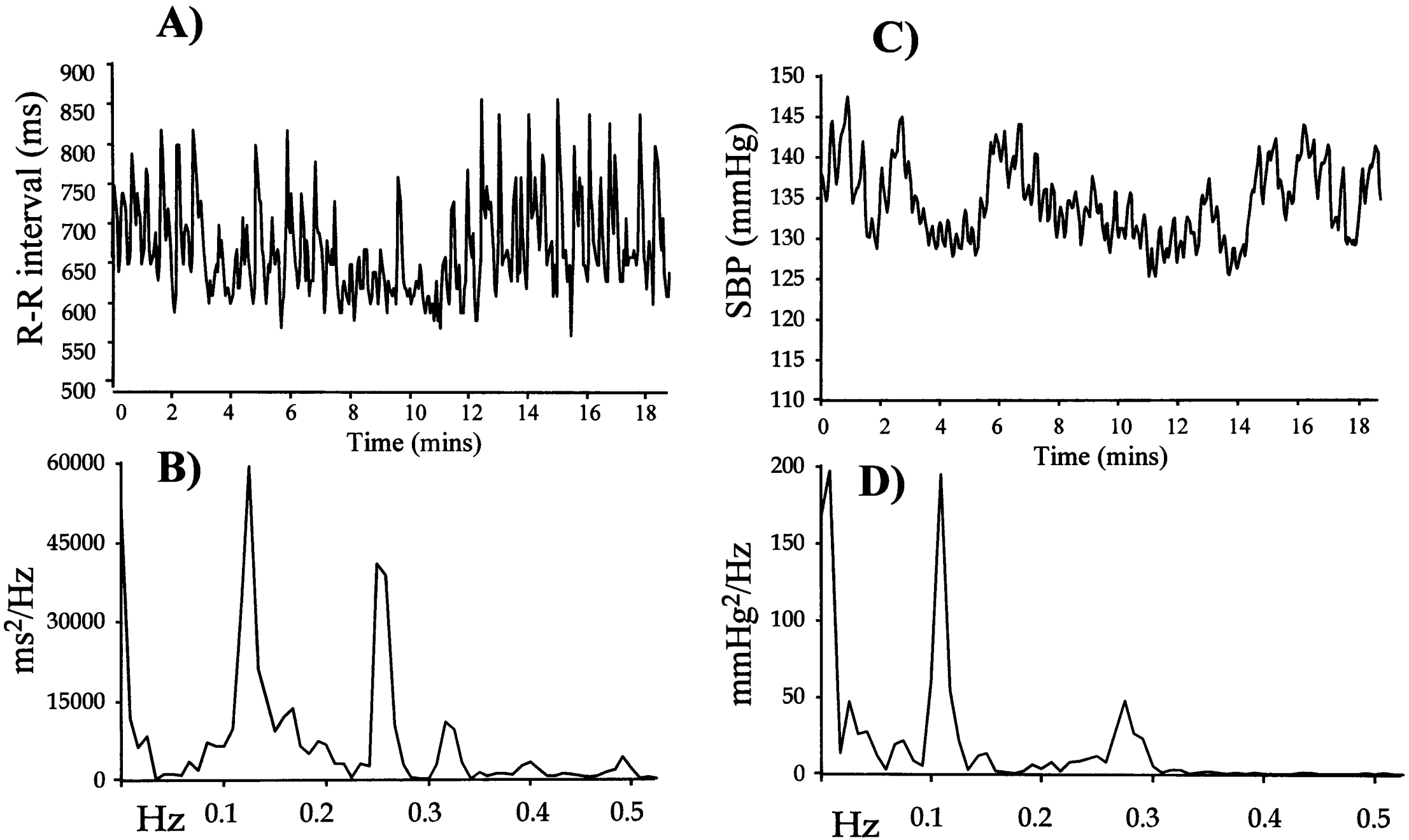


Figure 2.3. Typical data obtained during analysis of heart rate and blood pressure spectra. A) R-R intervals recorded over 20 minutes. B) Frequency spectrum derived from the data in A. C) Systolic blood pressure recor ded over 20 minutes. D) Frequency spectrum derived from the SBP in C.

values of V_T, *f*, $P_{ET}CO_2$ and HR were extracted from the raw data by triggering from the pneumotach signal.

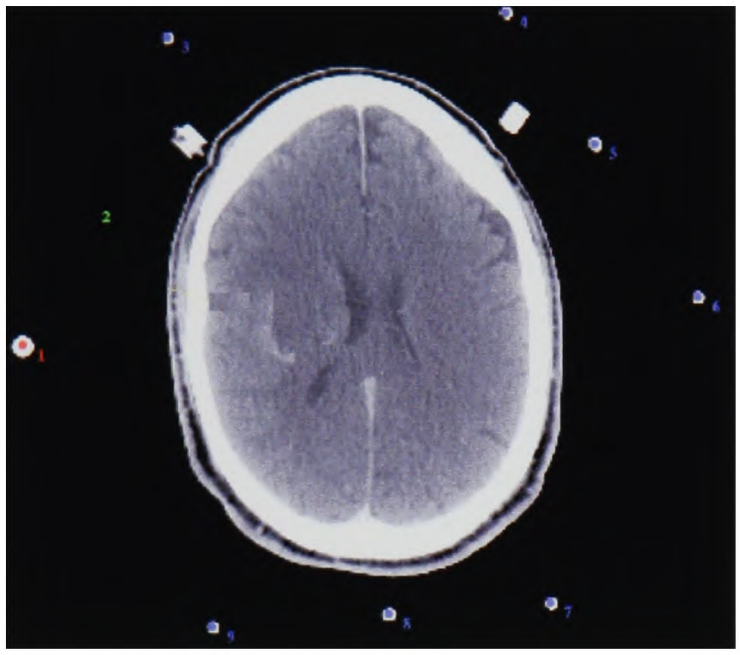
2.3 Neuroimaging

The studies described in Chapter Four used the techniques of positron emission tomography (PET) and magnetic resonance imaging (MRI). Those described in Chapter Six required MRI and computerized tomography (CT).

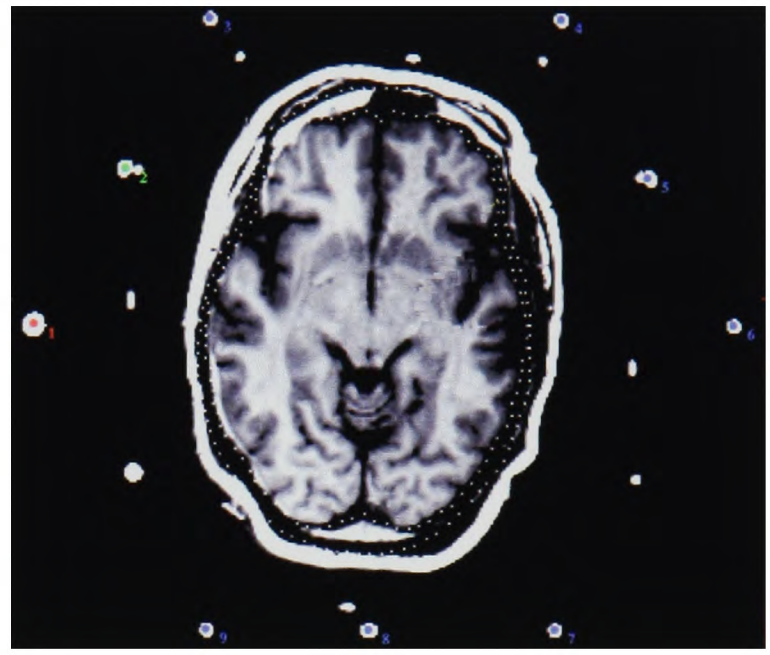
Positron emission tomography (PET): Small quantities of positron emitters such as ^{11}C, ^{18}F and ^{15}O are injected in to the bloodstream and circulate throughout the body. When a positron collides with an electron, they annihilate and form two γ-rays emitted at 180° to each other. The scintillation crystals in the PET scanner rely on this 'coincident detection' of γ-rays from the subjects brain placed in the scanner to generate an image (figure 2.5). Many studies use radiolabelled water ($H_2{}^{(15)}O$) as a measure of regional blood flow; such studies rely on the relationship between brain blood flow and neural activity; areas of the brain that are most active in a given circumstance will receive more blood and hence more radioactivity. Alternatively, radiolabelled ligands for specific receptors or neurotransmitters can be used, for example ^{18}F labelled dopa, which is taken up by nigrostriatal nerve terminals and stored as ^{18}F-dopamine and its metabolites and has been used to study Parkinson's Disease (Brooks *et al.* 1990).

Magnetic resonance imaging (MRI): The subjects head is positioned in a large static magnetic field (e.g. 1.5 Tesla; 25,000 times stronger than the earth's magnetic field), causing the H^+ of water molecules in the brain to line up in this field (since water molecules behave as dipoles). A second, much smaller (0.02 Tesla) pulsed magnetic field (radiofrequency, rf pulse) is applied perpendicular to the static magnetic field which causes the H^+ to momentarily realign to the rf field. The process of the H^+ returning to their original alignment causes a small electrical signal, dependent upon the strength of the local magnetic field (in turn dependent upon the amount of water in the region). The time taken for this realignment is known as the longitudinal relaxation time (T1) and is used to generate an image with structural resolution based on the differing amounts of water in grey and white matter, CSF and bone, figure 2.4B.

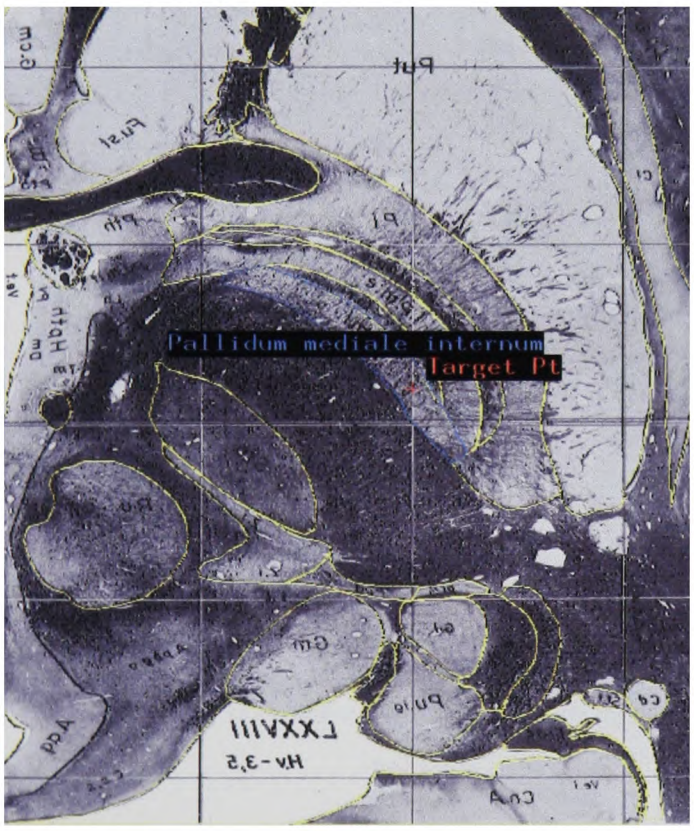
A)



B)



C)



D)

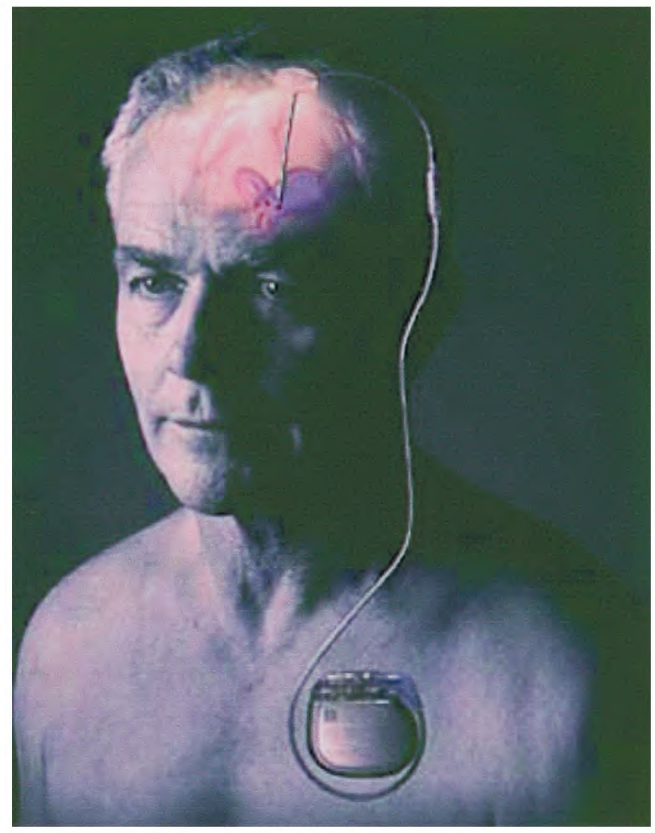


Figure 2.4. A) CT scan showing the fiducials of the stereotaxic localizer. Note the poor tissue definition. B) CT scan fused with an MRI scan, enabling good tissue definition in conjunction with stereotaxic localisation. C) MRI image warped into normalised stereotactic co-ordinates and overlayed on the Schaltenbrand-Wahren Atlas to aid anatomical localisation. D) Artists impression of stimulator implantation in the left basal ganglia and placement of the stimulation unit in the left sub-clavicular region. All from Medtronic web site with permission.

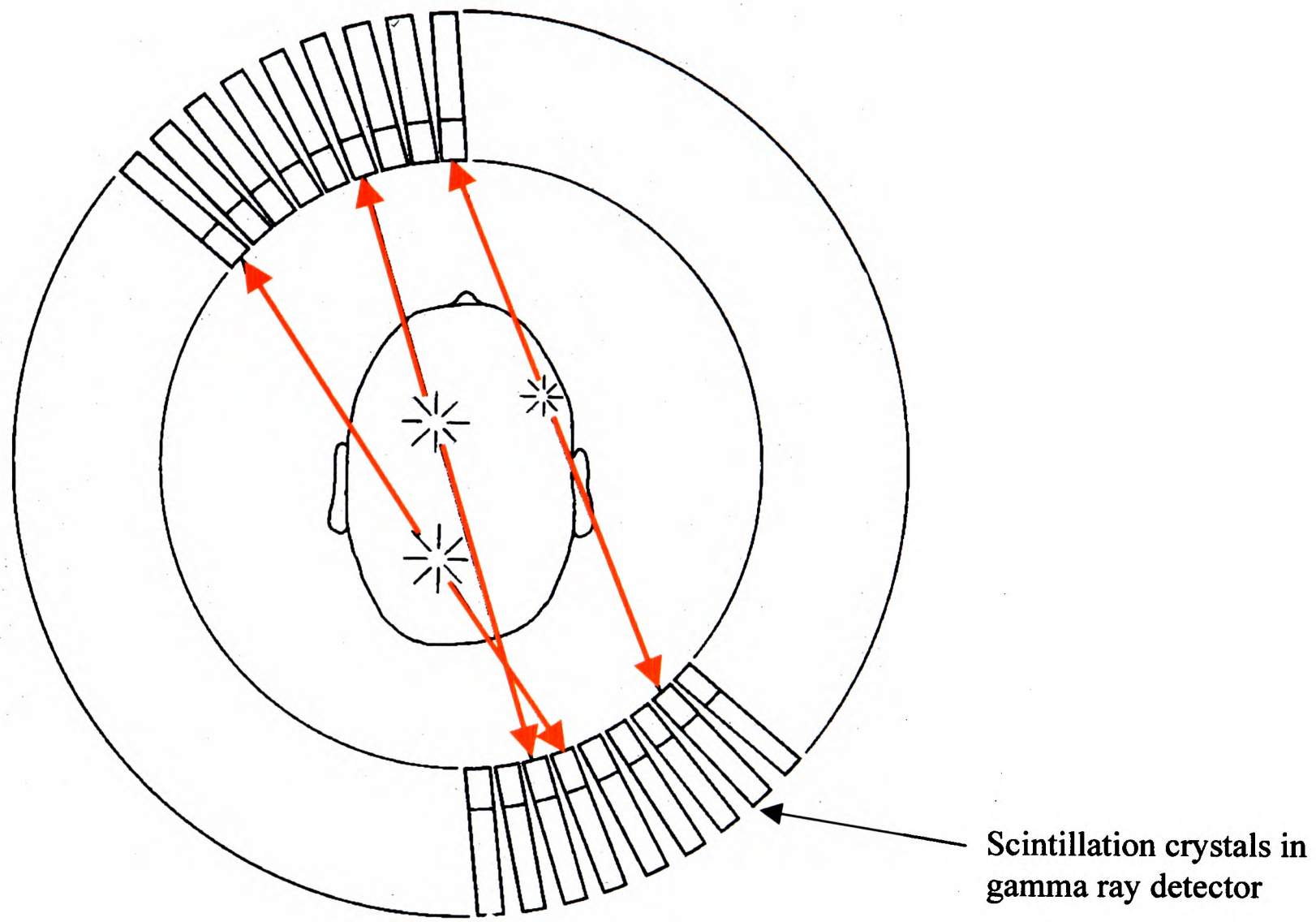


Figure 2.5. The principles of positron emission tomography. Small quantities of positron emitters are injected into the bloodstream and circulate through the body. When a positron collides with an electron, they annihilate and form two γ rays emitted at 180° to each other (red arrows). The scintillation crystals in the PET scanner rely on 'coincident detection' of γ rays from the subjects brain to generate an image.

Computerized tomography scanning: An X-ray source is rotated around the head whilst an X-ray detector is rotated around the other side. This allows a 3 dimensional representation of attenuation coefficients (a numerical value relating to the proportion of X rays passing through the tissue compared to that absorbed or reflected) to be generated. CT can resolve grey and white matter, blood, CSF and bone, despite differences in radiodensity of less than 2% but takes several minutes to scan the whole brain (figure 2.4A).

2.3.1 Brain imaging for Stereotaxic Neurosurgery

MRI provides superior anatomical detail and tissue definition compared to CT (figure 2.4B), but has the disadvantage that metallic stereotaxic localizers cannot be used within the confines of the magnet. MRI is also subject to image distortions due to susceptibility artefacts and peripheral magnetic field warping. These image distortions can translate into a 3-5mm offset when performing MRI guided stereotaxic neurosurgery (Alexander *et al.* 1995), conferring considerable risk to the patient. CT scanning does not suffer from these image distortions and use of a stereotaxic localiser is possible so the techniques have been combined to improve neurosurgical accuracy. The patient is MRI scanned several days preoperatively to determine anatomical detail. Immediately prior to surgery general anaesthesia is introduced, a stereotaxic localizer is placed on the head and the CT scan performed. The stereotaxic localising rods (fiducials) can be located by eye on the CT scans. The CT and MRI images are then fused (ImageFusion™, Radionics), thus combining the 3D accuracy of CT with the anatomical detail of MRI (figure 2.4). The fused image can then be warped (according to the anterior commisure-posterior commissure distance) and overlaid on images from the Schaltenbrand-Wahren Atlas (Schaltenbrand *et al.* 1977) to aid anatomical identification (figure 2.4C).

2.3.2 Physiology of brain imaging

Since PET measures changes in regional brain blood flow (rCBF) rather than neural activity its usefulness depends upon how closely blood flow changes in relation to local neural activity. This relationship provides a fundamental limit to spatial and temporal resolution and is *ca.* 5mm and 3 seconds (Malonek & Grinvald 1996). Blood flow changes correlate with the activity of energy dependent sodium pump activity (Mata *et al.* 1980), leading to the suggestion that

synaptic activity is the main determinant of neuronal metabolism. Since both excitatory and inhibitory synapses produce increases in rCBF whilst having opposite functional effects this provides a limitation to interpretation of imaging data.

Technique	*Spatial resolution*	*Temporal resolution*
PET	~10mm	Minutes (dependent on isotope)
SPECT	~25mm	Hours
fMRI	~5mm	~3sec

Table 2.1. Temporal and spatial resolution limitations of brain imaging techniques. Reviewed by Frackowiak *et al.* (1997).

2.3.3 Experimental design for functional neuroimaging

The multitude of neural processes occurring in the brain means that neuroimaging studies must be designed to allow simplification and isolation of the neural processes of interest. Three main design types are used; subtraction, parametric and factorial (reviewed by Friston in Frackowiak *et al.* 1997).

a) *Subtraction.* This involves imaging the brain during a 'control' state and an 'experimental' state and subtracting one image from the other. This has limitations, the most serious of which is that it assumes that the activation or brain state of interest can be purely inserted (i.e. can be treated algebraically) and does not interact with other aspects of brain function. Whilst this may be a reasonable working hypothesis to study simple processes, it is clearly inappropriate for the study of complex phenomenon such as mechanisms of consciousness, emotion or memory. This is the major analysis technique used in the study described in Chapter Four.

b) *Parametric.* This approach compares images obtained with several levels of the variable of interest, for example task difficulty. The assumption is that regional CBF will vary systematically with the degree of activation, i.e. the neural process is being treated not as a categorical invariant (as in subtractive designs), but as an attribute that can be expressed to a greater or lesser extent. This analysis requires that the variable of interest has a robust dose-

dependence, i.e. can be studied repeatably over a wide range of values. This technique was also used in Chapter Four.

c) *Factorial.* This type of design combines two or more factors of interest in the same experiment. The effect of one factor on the effect of another factor is studied, for example task performance (one factor) before and after drug administration (second factor).

2.3.4 Theoretical basis of data analysis using SPM

Analysis of the PET data in this thesis was performed using statistical parametric mapping (SPM96/99). Detailed mathematical theory behind this technique can be found in Frackowiak *et al* (1997); a 'physiologist-friendly' description follows.

Images are reconstructed as axial planes from the raw head counts (taking account of the background scan and the attenuation of the head calculated from the transmission scan). Analysis using SPM then consists of 4 stages:

1) Realignment
2) Spatial Normalisation
3) Smoothing
4) Statistical analysis

2.3.4.1 Realignment

If images from repeat runs within an experimental session are not realigned then the mean intensity dervied from any given voxel will be derived from different locations in the brain. The algorithm used in SPM to correct for motion is a six parameter rigid body model and incorporates a least-squares approach (Friston *et al.* 1995). In brief, the image from each scan (e.g. figure 2.6A) can be assumed to be a rigid body (the brain inside the skull) which can be moved along three orthogonal axes (x, y and z)[1] and rotated around each of these 3 axes, giving a total of six parameters describing the transformation. SPM divides each image into voxels and then subtracts each image in turn from the first image. If the head has moved between image 1

[1] x, y and z refer to standard neuroanatomical convention (x increases from left to right, y increases from posterior to anterior, z increases from inferior to superior). The origin (0, 0, 0) is the anterior commissure (a band of tissue between the two thalami at the anterior end of the third ventricle).

and image 2, subtracting the intensity value of a voxel from the edge of the head in scan 2 from the same voxel in scan 1 will result in a large difference in voxel intensity. The six parameter transformation required to minimise these differences in voxel intensity between scan B and scan A is computed. The same procedure is performed on all scans in the series and an output report is generated consisting of a plot of the translational and rotational parameters used to correct for misalignments within the image series (figure 2.6B, C).

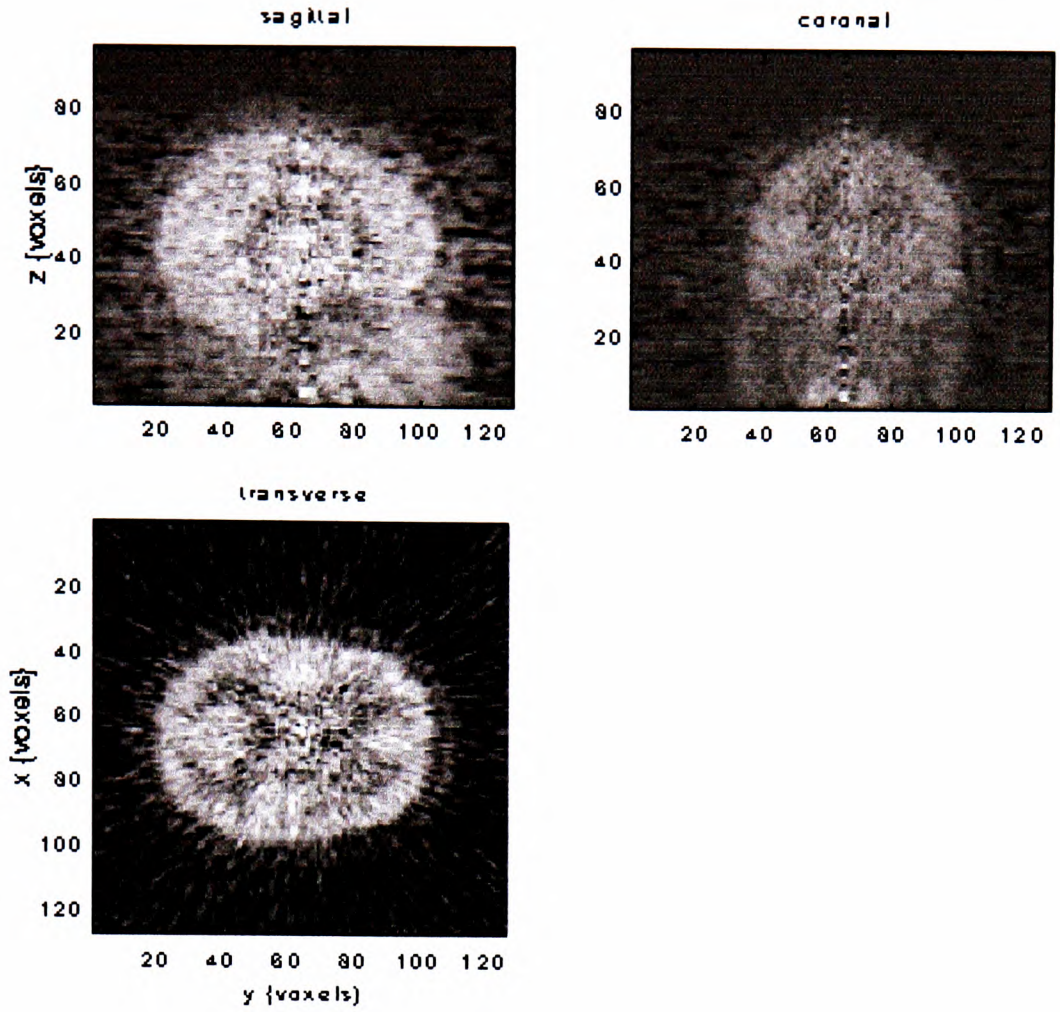
2.3.4.2 Spatial normalisation

This stage transforms the realigned images into a standard reference space, thus allowing image sets from different subjects to be combined in a single analysis and facilitating comparison of data from different studies. The standard template brain used in SPM96 is based on analysis of brain structure at the Montreal Neurological Institute (ICBM, NIH P-20 project). The template is a computer generated average of 305 brains and adopts the co-ordinate system of Talairach & Tournoux (1988) in which the anterior commissure is the origin. The process of spatial normalisation is described by Friston *et al* (1995).

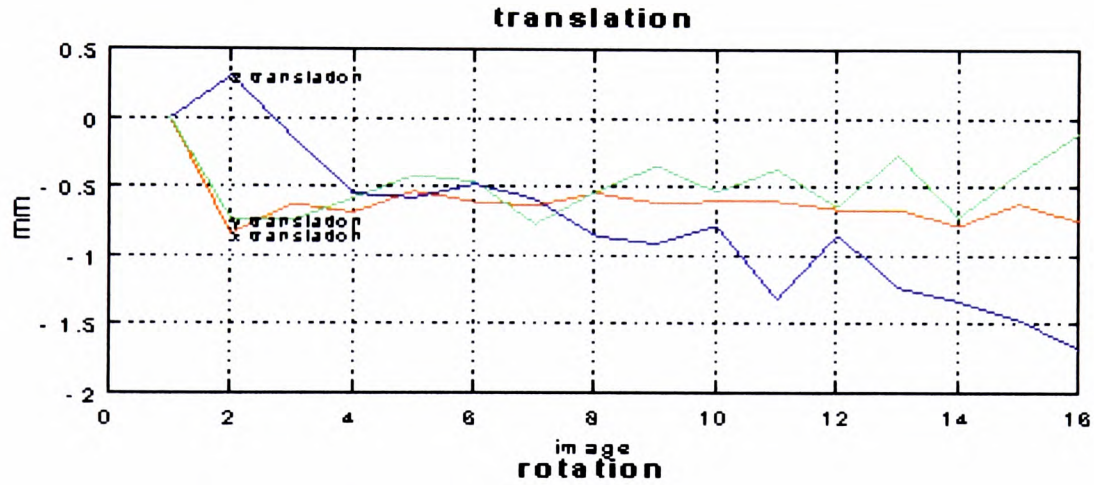
2.3.4.3 Smoothing

Smoothing PET data prior to statistical analysis has 3 purposes. First, smoothing with a Gaussian kernel ensures that the data conforms to a normal distribution; a requirement if a parametric statistical test is to be used. Secondly, it improves the signal:noise since in PET scanning, noise is of a higher spatial frequency (in the order of sub mm) than effects of interest, thus by smoothing the data the influence of noise is minimized. Thirdly, smoothing de-emphasizes individual differences in gyral anatomy (which can be as much as 1.5cm, (Steinmetz & Seitz 1991). Smoothing is performed by convolving images with a Gaussian kernel specified by the full-width-at-half-maximum (FWHM) of the kernel (in mm). As the width of the kernel increases, so does the degree of smoothing. The basic idea is that the differences in intensity between individual voxels are made to fit a normal distribution with the degree of smoothing (i.e. the width of the Gaussian curve) being defined by the FWHM. The FWHM chosen is influenced by the spatial resolution of the scanning method and as a general rule should not be less than twice the voxel size. In this study images were smoothed with a kernel of FWHM 10mm (see figure 2.7A).

A)



B)



C)

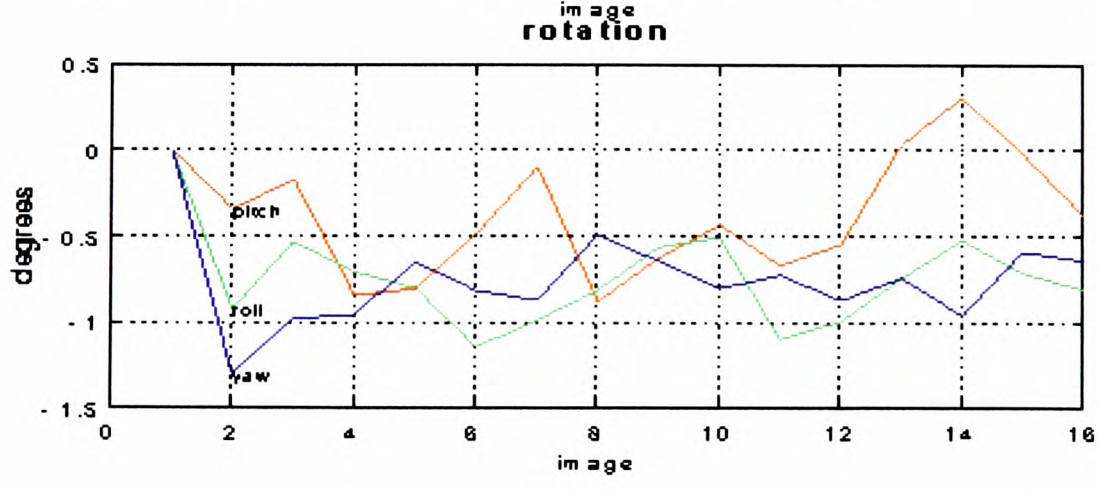
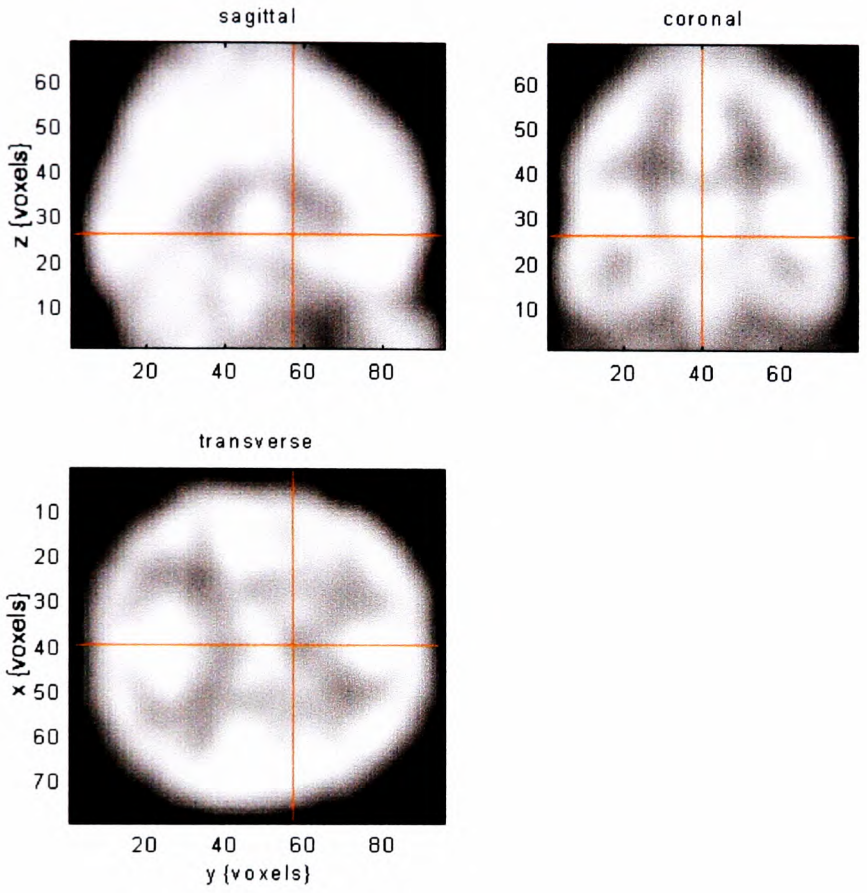


Figure 2.6. A) Raw PET images in the saggital, coronal and transverse planes. B) and C) Example of the summary output after realignment of scans from one subject. Numbers along the x axis refer to the scan number (16 per subject). B) Translations applied in **x**, **y** and **z** planes, C) Rotations applied; **roll**, **pitch** and **yaw**.

A)



B)

$$Y = X * B + E$$

$$\begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ \dots \end{pmatrix} = \begin{pmatrix} x_1 & 1 \\ x_2 & 1 \\ x_3 & 1 \\ x_4 & 1 \\ \dots & 1 \end{pmatrix} x \begin{pmatrix} \beta \\ C \end{pmatrix} + \begin{pmatrix} E_1 \\ E_2 \\ E_3 \\ E_4 \\ \dots \end{pmatrix}$$

Data matrix | Design matrix | Parameter matrix | Error matrix

Figure 2.7. A) Smoothed PET image in saggital, coronal and transverse planes. B) Matrix representation of the statistical model used to analyse voxel data. The data matrix (Y) consists of the voxel intensity (i.e. response) for each scan (4 scans in this example). The design matrix (X) consists of a predictor value and a column of 1's (this allows the constant C to be represented in matrix form). According to matrix multiplication, this is the same as equation 1 on page 29. See text for further explanation.

2.3.4.4 Statistical analysis

The aim of the statistical analysis is to find voxels in the brain whose intensity is significantly correlated with experimental variables in the study and an example of the statistical output is shown in figure 2.8. The analysis consists of 4 stages:

1) Analysis of variance, separately at each voxel
2) Calculation of the t statistics from the results of this analysis, separately at each voxel
3) Conversion of the t statistic to a z score
4) Application of a correction factor to take account of multiple comparisons

Testing for significance of a covariate

In SPM the statistical hypothesis is expressed in matrix form using the General Linear Model (GLM) to describe the variability of the data in terms of experimental and confounding effects, and residual variability (Friston *et al.* 1995). Effects that influence voxel intensity can be subdivided into covariates and confounds. Covariates are effects of interest, i.e. experimental factors that may influence the response. Confounds are covariates of no interest (eg gCBF); identifying confounds and including them in the model helps to improve the statistical power by reducing the variance that isn't modelled. Considering a single covariate and a single brain voxel Y:

$$y(j) = \beta * x(j) + C + error(j) \qquad (1)$$

j indexes the scans performed, y is the data for each scan at this voxel (i.e. the response), x is the predictor variable (the covariate we are hypothesising affects voxel intensity) and β is a scaling factor (yet to be worked out). In other words the data at a voxel for each scan (j) is represented by a straight line relationship with the predictor variable (x), with a slope of β and a y intercept of C. When a number of scans have been performed, this is conveniently rephrased as a matrix (figure 2.7B). A least squares fit is then performed to obtain β and C. The remaining error (E) is then the distances of the voxel data values from the values predicted by the least squares fitting line. This is an analysis of variance and is repeated for every voxel in the brain, resulting in a separate β for each voxel.

The next stage is to assess whether or not there is a correlation between the predictor variable x and the voxel scores y using a t-test. If the null hypothesis (that there is no relationship between x and y) is correct, the slope of the line between x and y (i.e. β) will not be significantly different from 0. This can be tested with a t statistic of β/(SE of slope). The SE of the slope is calculated from the error matrix E. The numerator of the t statistic is calculated using a contrast which is a matrix that when multiplied by B gives you the numerator of the t statistic. In this case the matrix is (1, 0) since:

$$(1, 0) * \begin{bmatrix} \beta \\ C \end{bmatrix} = \beta$$

The t statistics are converted into Z scores (allowing p values to be calculated) and displayed for each voxel of the brain (figure 2.8).

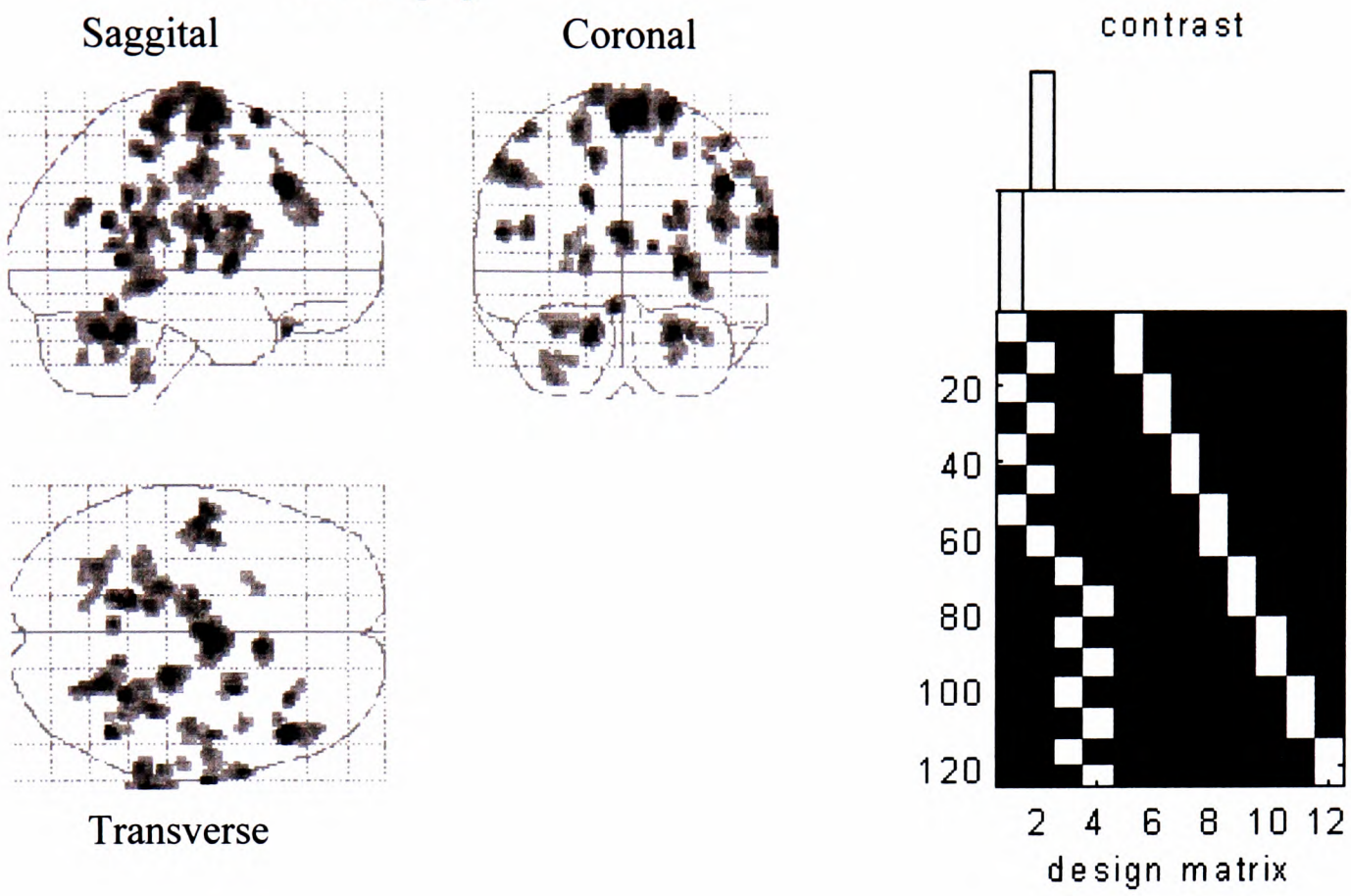
Whilst the covariate analysis described is appropriate for certain study designs it forms a secondary part of the analysis used for the study described in Chapter Four. A more conventional approach used with subtractive study designs is to analyse the data in terms of the experimental conditions.

Testing for the effect of a condition

The general linear model allows one to perform ANOVA with a multiple regression. For example if we have two conditions, **F** and **G**, each performed several times:

$$Y(j) = \beta(\mathbf{F}) * x(\mathbf{F})(j) + \beta(\mathbf{G}) * x(\mathbf{G})(j) + e(j) + C \quad (1)$$

β(**F**) and β(**G**) are constants to be worked out, x(**F**) and x(**G**) are 'dummy' variables. The variable x(**F**) is a variable that takes the value of 1 if the scan is condition **F** and a value of 0 if the scan is condition **G**. Similarly the variable x(**G**) takes a value of 0 if the scan is condition **F** and 1 if the scan is condition **G**. Consequently β(**F**) is the mean of the voxel values in condition **F**, and β(**G**) is the mean of the voxel values in condition **G**. Once β(**F**) and β(**F**) are calculated and an F map is obtained, two contrasts are then used to give t statistics and p values. (–1, 1) will be the contrast for voxels having significantly greater values in G than in F, and (1, -1) is the contrast for voxels greater in F than in G.



P values & statistics: /usr/paterson/joode/PET

set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z	x,y,z {mm}
0.180 (37)	0.248 (182, 4.73)	0.084 (4.73)	0.005	0.000	46 28 40
		1.000 (3.16)		0.001	40 24 52
	0.014 (770, 4.73)	0.087 (4.73)	0.000	0.000	6 -6 74
		0.261 (4.44)		0.000	-2 -12 68
		0.999 (3.51)		0.000	-20 -30 66
	0.658 (176, 4.24)	0.469 (4.24)	0.006	0.000	24 -58 -26
		1.000 (3.07)		0.001	24 -52 -38
	0.750 (123, 4.16)	0.583 (4.16)	0.018	0.000	68 -8 24
		0.960 (3.78)		0.000	64 8 22
		1.000 (2.82)		0.002	64 0 26
	0.802 (32, 4.10)	0.657 (4.10)	0.194	0.000	-16 -42 34
	0.858 (102, 4.04)	0.739 (4.04)	0.029	0.000	0 -6 18
	0.876 (122, 4.02)	0.769 (4.02)	0.018	0.000	-14 -48 -30
	0.889 (50, 4.00)	0.789 (4.00)	0.110	0.000	46 -54 22
	0.900 (76, 3.98)	0.807 (3.98)	0.054	0.000	26 4 6
		1.000 (2.59)		0.005	28 -2 12
	0.582 (191, 3.91)	0.874 (3.91)	0.005	0.000	20 -26 72
		1.000 (2.72)		0.003	18 -28 60

Height threshold {u} = 2.33, p = 0.010
Extent threshold {k} = 20 voxels, p = 0.302
Expected voxels per cluster, E{n} = 20.3
Expected number of clusters, E{m} = 31.4
Volume {S} = 211000 voxels or 2460.4 Resels
Degrees of freedom due to error = 115.0
Smoothness FWHM {mm} = 7.8 9.0 9.7
{voxels} = 3.9 4.5 4.9

Number of activated clusters and the P value for obtaining that many clusters

P value (corrected) for the cluster, it's spatial extent (k) and the Z score of that cluster.

P value (corrected) for the voxel and the Z score of that voxel.

Uncorrected p value (cluster)

Uncorrected p value (voxel)

Coordinates of the activation (in Talairach space)

Figure 2.8. Example statistical output from SPM. The top part consists of saggital, coronal and transverse 'glass brains' showing all areas activated with p<0.01 uncorrected. On the right is the 'design matrix'; a pictorial representation of the order of scan entry and the effects included in the statistical model.

Correction for multiple comparisons

The images of t statistics contain a large number of non-independent voxels. This is caused by the initial resolution of the image, physiological non-independence (the idea that the fundamental spatial resolution of blood flow responses occur over several mm) and the use of smoothing. The significance of activations should therefore be corrected for multiple comparisons unless an *a priori* hypothesis exists for a given voxel. There are various ways in which this can be performed, discussed in Chapter Four.

2.4 Cardiorespiratory recordings in patients with movement disorders

Electrolytic lesioning or chronic electrical stimulation of the thalamus and basal ganglia is an increasingly common treatment for Parkinson's disease and other movement disorders that fail to respond to drug therapy (Limousin *et al.* 1995). During surgery, electrical stimulation of the thalamus, subthalamic nucleus, substantia nigra and pallidum is performed in awake patients. This surgical procedure therefore provides the ideal setting to study the cardiorespiratory effects of electrical stimulation of subcortical structures in awake man. When studied post-operatively, patients with implanted stimulators provide the opportunity to study the effects of chronic stimulation of these brain regions in a more controlled circumstance than is possible intraoperatively.

2.4.1 Surgical procedures

Patients were given an MRI scan 2 days prior to surgery (T1 volume scan, 64 axial slices, 3mm slice thickness, Siemens Vision 1.5T scanner). Anti-Parkinsonian medication was withdrawn 12 hours prior to surgery. Patients were given a general anaesthetic, an endotracheal tube inserted and artificial ventilation commenced. Venous and arterial cannulas were inserted for fluid and drug administration and recording of ABP. A stereotactic localizer was attached to the patients head and a CT scan performed (3mm axial slices using a Siemens ART scanner). Stereotaxic localisation of the surgical target was determined by fusing and spatially correcting the previously acquired MRI images with the CT images (Image Fusion™ and Stereoplan™, Radionics, as described in section 2.3.1). Patients were then woken up and following scalp incision a posterior frontal twist drill (14 mm burr hole) was performed under local anaesthesia (lignocaine). To minimise risk of cerebral haemorrhage during electrode placement anti-

hypertensive drugs (nifedipine or clonidine) were administered if ABP exceeded *ca.* 140/80. A Radionics electrode, 2.5mm in diameter was introduced into the brain using the pillar of the stereotactic localizer to ensure the correct trajectory (figure 2.9, 2.10). The microelectrode was connected to a combined stimulator/lesion generator (RFG 3CF, Radionics). Electrical stimulation began 5 mm above the target and was repeated whilst proceeding along the electrode trajectory in steps of 3 mm. Motor function was assessed during electrical stimulation (rapid finger tapping to test for bradykinesia, arm flexion to test for power). Pulse width, frequency and voltage were varied within the range of 0.1-1msec, 2-200 Hz and 0-7 Volts. Optimal electrode site was chosen according to the site where motor symptoms were maximally reduced with minimum side effects.

Chronic electrical stimulation (group A)

These patients had a lead for chronic stimulation (Medtronic 3389 DBS)[1] inserted at the point where the greatest beneficial effect on motor symptoms was observed with the temporary electrode. Stimulation was repeated to affirm optimal localisation. The electrode was secured using a burr hole ring and bone screws. A subgaleal pocket was prepared in the skull to hold excess lead wire and the connector. General anaesthesia was reintroduced and an implantable pulse generator was placed subcutaneously in the subclavicular area (Itrel 2/3 IPG, Medtronic). The electrode was connected to the pulse generator via a wire tunnelled between the scalp and the skull (figure 2.4D). The pulse generator could be programmed by telemetry to change voltage, frequency and pulse width within the range 0-10Volts, 2-130Hz, 60-450μs pulse width. 3 days post-operatively, patients were given a CT scan which was fused with the preoperative MRI to confirm electrode location.

Electrolytic lesions (group B)

These patients were stimulated with the temporary electrode as described above until an optimal location was found. Electrolytic lesions were then made a) 3mm beyond the optimum site for 30s at 75°C, b) on the optimum site for 60s at 75°C, and c) 2mm above optimum site for 90s at

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[1] The electrode consists of a flexible wire (1.27mm diameter, 28cm long) with 4 electrical contacts, each spaced 2 mm apart and each with a surface area of $6mm^2$. Any combination of the 4 contacts can be used as anode and cathode for stimulation.

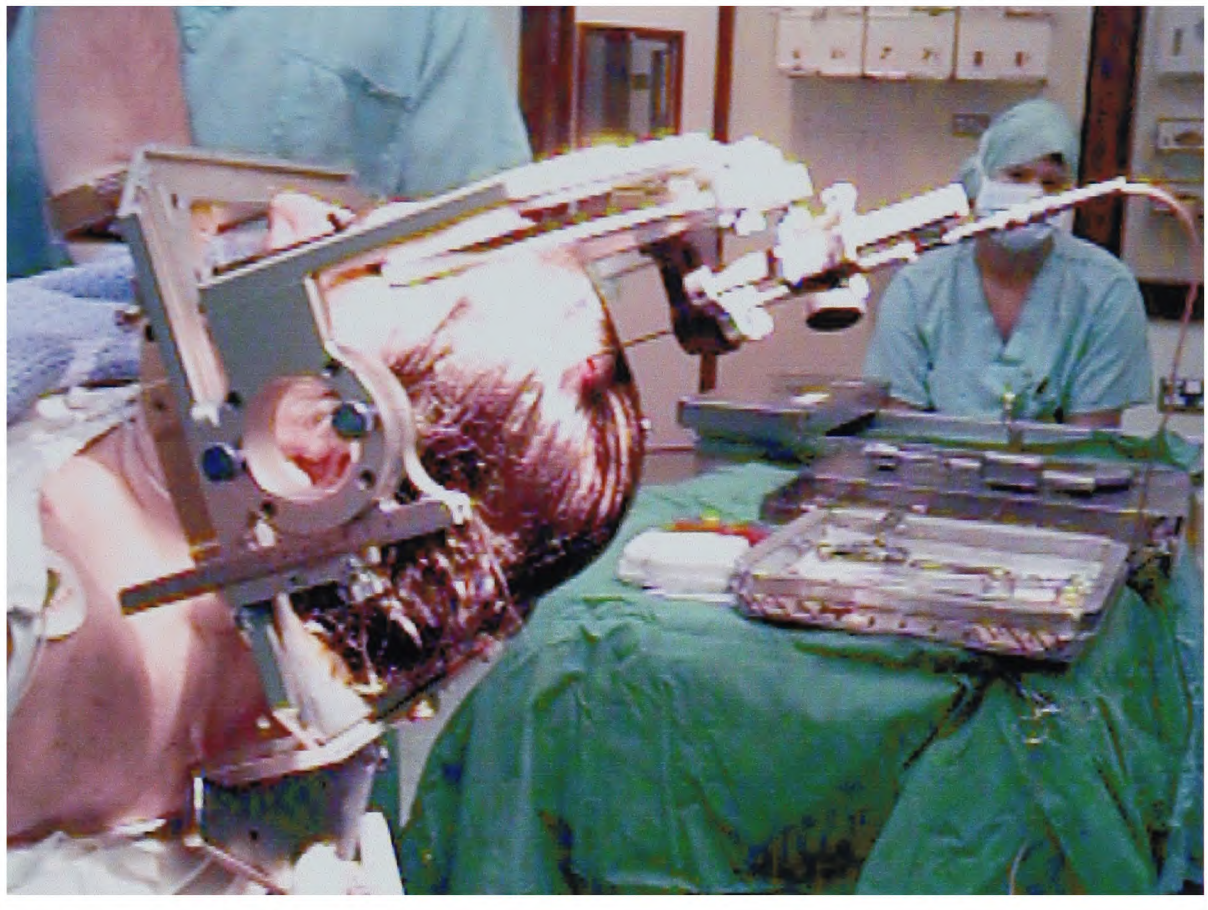


Figure 2.9. Stereotaxic neurosurgery for Parkinson's Disease. The patient is awake and is wearing a stereotaxic frame. The temporary stimulating electrode is passed into the basal ganglia using the co-ordinates determined from the MR and CT scans.

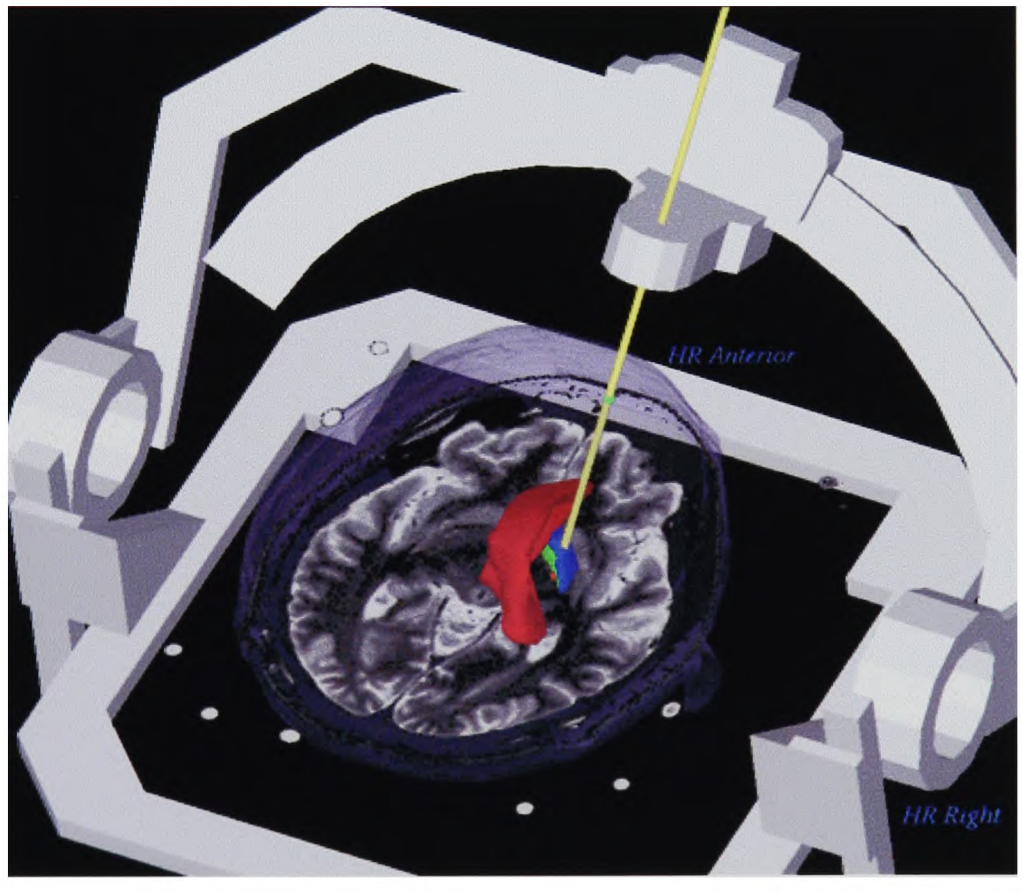


Figure 2.10. Computer generated image of the use of a stereotaxic frame to guide the electrode into the midbrain. The red structure is the left ventricle which must be avoided! From Medtronic website with permission.

75°C. 3 days post-operatively, patients had an MRI (96 x 1mm axial slices) to confirm lesion site.

Chapter Three

Cardiorespiratory responses to imagination of exercise under hypnosis

Whenever people agree with me, I always feel I must be wrong.

Oscar Wilde.

3.1 Introduction

Major difficulties exist in unravelling the complexities of mechanisms underlying the cardiorespiratory response to exercise in man. In particular, the significance of 'central command' to increase ventilation and circulation *pari passu* with the 'command' to exercise has been a subject for research ever since Johansson (1893) proposed this mechanism. He suggested from studies in rabbits that motor impulses to the exercising muscles simultaneously activated the neural centres governing heart rate, whereas passive movements or electrical stimulation of the muscle did not. Similarly, Krogh & Lindhard (1913, 1917) demonstrated in man that the ventilatory response to normal leg exercise began simultaneously with the onset of exercise whereas it was slower in onset during electrically induced leg exercise. Whilst these and many subsequent studies have provided evidence for central command as a mechanism, it is difficult to determine the relative magnitude of central command and the circumstances in which it operates in normal exercising man.

Mental imagery of treadmill exercise at rest (Decety *et al.* 1991) results in an increase in HR and $\dot{V}_I$; however in more recent work (Wuyam *et al.* 1995) this response was only seen in athletes. Imagination of exercise has also been studied under hypnosis (Levin & Egolinsky 1936; Nemtzova & Shatenstein 1936; Agosti & Camerota 1965; Daly & Overley 1966; Arvidsson *et al.* 1970; Wang & Morgan 1992) with all studies observing increases in $\dot{V}_I$ and one (Arvidsson *et al.* 1970) observing increases in cardiac output. However, interpretation of a number of these studies has been complicated by the use of explicit cardiorespiratory cues that may entrain breathing. The hypnotic state allows isolation from the environment and a more directed imagination than is possible awake (Coe *et al.* 1980), so the aim of this chapter was to see if imagination of exercise without any suggestions regarding cardiorespiratory variables could be used reliably to study cardiorespiratory control during exercise in experimentally naïve subjects.

3.2 Methods

Initial assessments of hypnotic susceptibility were performed on twenty-seven subjects as described in Chapter Two. Seven subjects were rejected after assessment owing to low susceptibility scores and the study group then consisted of 18 men and 2 women, age 26±1 years, weight 75±2 kg, height 1.78±0.01 m, body mass index 23.6±0.5 kg/m^2 (mean ± SEM). All performed an incremental exercise test (described in Chapter Two) and were assigned three exercise loads; unloaded pedalling ('light') and two loads corresponding to HR's 30% ('moderate') and 50% ('heavy') of their predicted maximum.

3.2.1 Protocols

Protocols were all performed on a subsequent visit to the laboratory. Subjects were instrumented as described in Chapter Two, familiarised with the three exercise loads that had been assigned to them (by exercising at each load for 2 minutes) and were asked to remember them as 'light', 'moderate' and 'heavy'. Hypnotic induction was then performed and was followed by manoeuvres designed to increase the depth of the hypnotic state (arm levitation, sensory imagery). These manoeuvres were repeated in between experimental protocols if hypnotic depth was judged to have decreased (assessed by pupil dilation, relaxation of facial muscles; e.g. (Waxman 1989). The time taken from the beginning of the induction to starting the protocols was approximately 20 minutes. The subjects performed four protocols whilst under hypnosis, and another two protocols whilst awake, the order of which was randomised, with the exception of volitional hyperventilation, which was always performed last in order not to alert the subjects to our interest in breathing. Great care was taken to avoid mentioning cardiorespiratory variables at any stage during the investigations except during instructions for volitional hyperventilation. All protocols were performed without use of a metronome and the subjects breathed room air except where indicated.

Protocol 1. Imagination of exercise, awake and under hypnosis

Seventeen hypnotised subjects imagined themselves exercising for 2 minutes at the 'heavy' work rate (*ca.* 50% of predicted HR_{max}) with which they had been familiarised. It was suggested

to the subject that they were cycling up a steep hill with a grade equivalent to the heavy work rate. The instructions given to the subjects were as follows:

> *'...In a few moments I'm going to ask you to imagine yourself exercising at the heavy work rate...we'll do this for 2 minutes... So I'd like you to imagine yourself getting on to a bicycle at the beginning of a steep hill ... imagine the colour of the bicycle and exactly what it looks like...imagine the road and look at it stretching up the hill in front of you...when you can imagine yourself there on the bicycle at the bottom of the hill just raise the index finger of your right hand...that's very good...so in 5 seconds I'd like you to start pedalling up the hill...5, 4, 3, 2, 1 and go, start imagining yourself pedalling up the hill...that's very good ... keep it up...as you can feel it's very hard to push against the weight of the pedals... that's one minute... you're half way up the hill... just one more minute to go... it's getting really hard now...keep going... you can see the top of the hill now as you approach it...in 10 seconds you're going to reach the top of the hill and I'm going to ask you to stop pedalling and become totally relaxed... 5, 4, 3, 2, 1 and stop...that was very good, absolutely excellent ... just relax totally...'*

Six of these subjects repeated the imagination of exercise with the suggestion that they were exercising at the 'moderate' work rate (*ca.* 30% of predicted HR_{max}) for 2 minutes, during which suggestions were similar to those described above, but the subject was asked to imagine cycling along a flat road instead of up a hill. Imagination of 'heavy' exercise was also repeated in eight subjects in the awake state. In 5 subjects electromyograms (EMGs) were obtained during imagination of exercise under hypnosis from left and right quadriceps and hamstrings (Neurolog System, NL820, 135, 824, Digitmer, UK).

Protocol 2. Volitional hyperventilation under hypnosis

Hypnotised subjects were told to 'breathe faster' for 2 minutes. Feedback on the breathing frequency required was provided by the hypnotist and was aimed to be the same as seen during imagination of exercise. No suggestions were made regarding V_T. This protocol was performed in order to determine whether any increases in $\dot{V}O_2$ and HR during imagination of exercise could be attributed to an increased work of breathing. Instructions given by the hypnotist were as follows:

> *'Now I'd like you to imagine yourself out in the countryside... the sun is shining and it's a beautiful day...you can see a bench a few yards away... imagine what colour it is and what it's made from...now just walk over and sit on the bench...when you're sitting on the bench just raise the index finger of your right hand...that's very good indeed...admire the view over the surrounding fields...now*

I'd just like you to concentrate on your breathing...in a moment I'd like you to start breathing a little faster than you are currently...I'm going to ask you to do this for 2 minutes ...keep admiring the view...I'll tell you when to start breathing faster...5, 4, 3, 2, 1 and go...that's excellent...not quite so fast...very good...that's 30 seconds...just breathing a little faster...excellent...keep up that rate, that's 1 minute, just 1 more minute to go...just speed it up a little...very good... keep breathing at that rate...30 seconds to go now and then I'd like you just to breathe naturally and relax, still sitting on the bench... 5, 4, 3, 2, 1 and stop...that's excellent just breathe naturally...'

In order to examine whether the magnitude of any hyperventilation was limited by hypocapnia, seven subjects repeated protocols 1 and 2 with CO_2 added to the inspired air to maintain isocapnia.

Protocol 3. Imagination of freewheeling downhill under hypnosis

In order to examine the cardiorespiratory effects of cognitive effort *per se* hypnotised subjects were asked to imagine themselves on a bicycle freewheeling down a hill for 2 minutes, whilst applying the brakes gently. Instructions given were as follows:

'...I'd like you to imagine a scene...it's a beautiful summers day and you're out in the countryside...you can see the fields and hedges stretching out towards the horizon...the sun is shining... you're sitting on a bicycle, imagine the colour and exactly what it looks like... you are on a country lane at the top of a very gentle hill...it's a very quiet lane...In a moment I'm going to ask you to freewheel gently down the hill...I don't want you to go too fast so you'll need to brake slightly all the way down...it's perfectly safe, the brakes work perfectly so you will be able to relax as you coast down the hill, just braking slightly so you can admire the view on the way down...it's going to take 2 minutes to freewheel down the hill... So I'd like you to imagine yourself getting onto the bicycle and just pushing off gently to get yourself moving...just raise the index finger of your right hand when you've done that...that's very good...so you're now freewheeling down the hill...no need to turn the pedals at all... you've built up a little momentum now so in 5 seconds I'd like you just to put the brakes on very gently...5, 4, 3, 2, 1 and put the brakes on gently...that's very good... just carry on gently down the hill and admire the view...that's 30 seconds...that's very good...coasting gently down the hill, not turning the pedals...braking slightly so you don't go too fast...that's 1 minute, you're halfway down the hill...have a look at what's in the fields along the side of the road...just freewheeling gently along...no need to go any faster than that...just braking gently...30 seconds to go now...you've still got your fingers gently resting on the brakes...in 10 seconds you're going to reach the bottom of the hill and get off the bicycle and sit down on the grass and relax totally... 5, 4, 3, 2, 1 and stop...that was very good, absolutely excellent ... just sit down on the grass and relax totally...'

Protocol 4. Assessment of CO_2 sensitivity awake and under hypnosis

In order to test chemosensitivity awake and under hypnosis, CO_2 was added to the inspired air in order to maintain $P_{ET}CO_2$ at 3 levels above eucapnia. For these experiments the pneumotachometer was on the expired side of the mask, with a T-piece on the inspired side. A continuous flow of room air and varying amounts of CO_2 across the T-piece allowed rapid (*ca.* 2 seconds) changes of inspired gas concentrations. Each increased $P_{ET}CO_2$ level was maintained for 6 minutes, with 6 minutes rest in between each level. The order of hypercapnic steps was randomised. This protocol was performed in both the awake and hypnotised states in the same subjects. No specific instructions were given by the hypnotist during this protocol.

3.2.2 Analysis

Baroreflex sensitivity and spectral analysis of HR and blood pressure

15 minutes of baseline recordings of blood pressure and R-R interval awake and under hypnosis were analyzed for sequences in which systolic blood pressure and the R-R interval progressively increased or decreased to calculate baroreflex sensitivity. Values were compared using a paired t-test. HR and BP variance in both the awake and hypnotized states were calculated as described in Chapter Two. Values of HF and LF HR/BPV were not normally distributed (as shown by the Kolmogorov-Smirnov test). Data were therefore log transformed and were then compared using a paired t-test. Values quoted in table 3.3 are the log transformed values.

Statistics

Breath-by-breath data from each subject was divided into 15 second epochs (protocols 1, 2 and 3) and averages were calculated for each epoch. Where deltas are indicated (figures 3.2-3, 3.6-8), the average value from 30 to 15 seconds prior to the onset of the instruction of the condition was taken as the control value which was then subtracted from each average. Deltas were then averaged across the group and all data were expressed as mean ± SEM. For assessment of CO_2 sensitivity (protocol 4) average values of $\dot{V}_I$ and $P_{ET}CO_2$ were calculated for the last minute at each level of hypercapnia and graphs of $\dot{V}_I$ versus $P_{ET}CO_2$ were drawn (figure 3.9). Paired t-tests were performed on all values quoted in tables 3.1 and 3.4, comparison of imagined exercise ±

inspired CO_2 (figure 3.3) was performed using a repeated measures ANOVA and post-hoc Tukey test.

The ventilatory and HR responses to steady state exercise generally conform well to single phase exponentials (Casaburi *et al.* 1989). Consequently, monophasic exponentials were fitted to the group data in protocol 1 using a standard statistical package (SigmaPlot 4). A 2-parameter exponential rise to maximum [$y = a(1-e^{-bx})$] was applied using the program's in-built initial parameter estimates followed by 100 iterations. If the goodness of fit regression coefficient r^2 was greater than 0.9, the fit was deemed reasonable and no further fitting was performed.

3.3 Results

The peak work rate attained during the incremental exercise test was 172±8 Watts, corresponding to a peak $\dot{V}O_2$ of 2332±187 ml/min. The 'light', 'moderate' and 'heavy' work rates assigned to each subject were 8 Watts ($\dot{V}O_2$ 431±39 ml/min), 55±4 Watts ($\dot{V}O_2$ 1105±79 ml/min) and 103±5 Watts ($\dot{V}O_2$ 1356±83 ml/min) respectively.

3.3.1 Rest under hypnosis versus rest awake

Hypnosis resulted in small but significant decreases in $\dot{V}_I$ and *f* and an increase in $P_{ET}CO_2$, with no change in V_T (n=20, table 3.1). There was a significant decrease in $\dot{V}O_2$ but no change in $\dot{V}CO_2$. There was no significant change in baroreflex sensitivity (table 3.2), in HR, or in HRV or BPV (table 3.3).

Subjects' comments on the hypnotic state included descriptions of intense relaxation and altered self-perception

"*...I felt all warm and tingly and it was like I wasn't actually anywhere at all...*"

Subjects consistently underestimated for how long they had been in the hypnotic state; they also had periods of amnesia

> *"...I would just suddenly realise that I had no idea what had been happening for the last half an hour..."*

3.3.2 Imagination of exercise under hypnosis

Imagination of 'heavy' exercise

In seventeen resting hypnotised subjects, imagination of a previously performed heavy exercise task (at a work rate predicted to be 50% of their HR_{max}) resulted in hyperventilation and hypocapnia and an increase in HR (table 3.4, figures 3.1, 3.2). This was reproducible within subjects when performed on different days (22 observations in 17 subjects). The dynamics of the $\dot{V}_I$ and HR responses over the 2 minute period of imagination were well fitted by single-phase exponentials (group data; $\dot{V}_I$: $t_{(50\%)}$ 16s, goodness of fit of regression coefficient r^2=0.978, HR: $t_{(50\%)}$ 5s, r^2=0.960). The magnitude of the hyperventilation was *ca.* 30% of that seen during actual performance of the exercise task (table 3.4) and occurred via an increase in *f* with no change in V_T. Both $\dot{V}O_2$ and $\dot{V}CO_2$ increased (table 3.4). The magnitude of the ventilatory response to imagination of exercise was not affected by the addition of CO_2 to the inspired air to maintain isocapnia, (n=7, repeated measures ANOVA, p=0.345, figure 3.3). The typical ventilatory pattern following the instruction to stop imagining exercise is shown in figure 3.5; slow, deep breathing ensued whether the $P_{ET}CO_2$ was controlled (B) or uncontrolled (A) during the imagination period.

In the four subjects who repeated the protocol eight times, the magnitude of the ventilatory responses were similar between repeats. In this subgroup, the coefficient of variation of the steady state ventilatory response was 9%. There was no marked increase in quadriceps or hamstring EMG activity in the 5 subjects in which it was measured (e.g. figure 3.4), although 4 subjects did move slightly during imagination of exercise; this consisted of twitching of the toes and fingers (3 subjects) and rhythmical contractions of alternate quadriceps (1 subject).

When asked about the experience, subjects gave vivid accounts

> *"...I was really into it, I felt like I had to give the pedals a real shove to get them to go round..."*

and reported sensations that included tiredness in the legs and sweating

> *"... I knew that my legs weren't moving, but they felt really tired and I was battling against myself to keep going...my legs started tingling as if I was about to break out into a sweat, it was like I was blushing or something..."*

Seven subjects mentioned that they noticed their breathing had increased

> *"...I felt like I was breathing harder than when I was actually exercising..."*

Imagination of 'moderate' exercise

Six subjects performed imagination of 'moderate' exercise. Whilst $\dot{V}_I$ did not differ between the two protocols, $P_{ET}CO_2$ reached significantly lower levels during imagination of 'heavy' exercise than during imagination of 'moderate' exercise (figure 3.6) as judged by a repeated measures ANOVA ($p<0.001$). There was a significantly larger increase in *f* when subjects imagined 'heavy' exercise compared to when they imagined 'moderate' exercise (repeated measures ANOVA, $p<0.001$).

Imagination of exercise in awake subjects

Imagination of heavy exercise in awake subjects resulted in small increases in *f* and $\dot{V}_I$ in two of the eight subjects in whom this protocol was performed; this was not significant when averaged across the group (table 3.1, figure 3.7).

3.3.3 Imagination of freewheeling downhill under hypnosis

Imagination of freewheeling downhill under hypnosis did not result in any changes in cardiorespiratory variables (table 3.4).

3.3.4 Volitional hyperventilation under hypnosis

The instruction to breathe faster resulted in an immediate and sustained increase in ventilation (figure 3.8). The magnitude of volitional hyperventilation under hypnosis was not affected by maintaining isocapnia (n=6, table 3.4, figure 3.8) and resulted in a doubling of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. There was no significant increase in HR during volitional hyperventilation.

	V_I ($l\ min^{-1}$)	f ($breaths\ min^{-1}$)	V_T (litres)	$P_{ET}CO_2$ (Torr)	HR (bpm)	$\dot{V}_{O_2}$ ($ml\ min^{-1}$)	$\dot{V}_{CO_2}$ ($ml\ min^{-1}$)
Awake rest	10.5±0.6	16±0.6	0.7±0.1	40.7±0.7	68±3	298±22	230±18
Hypnosis rest (n=20)	9±0.6 **	14.5±0.7 *	0.7±0.1	41.8±0.7 *	66±3	266±21 * (n=13)	204±12 (n=12)
Awake rest	10±1	16±1	0.6±0	40.1±0.6	67±2		
Imagined 'heavy' exercise (n=8)	10±1	19±2	0.5±0	39.1±0.8	67±2		

Table 3.1. Cardiorespiratory data (mean ± S.E.M) for protocols performed in awake subjects. ** indicates $p<0.001$, * indicates $p<0.05$, both compared to awake rest, paired t-test.

	Awake (16)	*Hypnosis (17)*
Total (ms $mmHg^{-1}$)	18.39±2.53	15.05±1.92
Down-slope	17.83±2.58	14.43±1.84
Up-slope	18.54±2.69	14.43±2.17

Table 3.2. Baroreflex sensitivity (lag 0) assessed by the spontaneous sequence method awake and under hypnosis. Numbers in brackets refer to the number of subjects. There was no difference in baroreflex sensitivity awake compared to under hypnosis as determined by a paired t test. Up-slope, slope of the correlation lines between R-R interval lengthening in response to spontaneous increases in systolic blood pressure; Down-slope, slope of the correlation lines between R-R interval shortening in response to spontaneous decreases in systolic blood pressure.

	Awake (16)	***Hypnosis (17)***
HR variability		
RR mean	899.84±30.05	942.86±31.55
RR variance (SD)	82.52±10.7	83.47±10.9
ln LF power	7.67±0.22	7.43±0.25
ln HFpower	7.87±0.29	8.24±0.31
BP variability		
Variance		
ln Systolic BP	7.17±0.42	7.18±0.47
ln Diastolic BP	3.72±0.18	3.72±0.22
LF power		
ln Systolic	2.31±0.19	2.76±0.21
ln Diastolic	1.35±0.15	1.63±0.15
HF power		
ln Systolic	1.33±0.16	1.88±0.2
ln Diastolic	0.22±0.19	0.54±0.23

Table 3.3. Heart rate and blood pressure variability awake and under hypnosis. Numbers in brackets refer to the number of subjects. HF, high frequency (0.15-0.40 Hz); LF, low frequency (0.04-0.15 Hz). There was no significant difference in any parameter between awake and hypnosis (paired t-test). N.B. Spectral data were not normally distributed and so a natural log transformation was performed prior to statistical analysis (indicated by ln).

	$\dot{V}_I$ ($l\ min^{-1}$)	f ($breaths\ min^{-1}$)	V_T ($litres$)	$P_{ET}CO_2$ ($Torr$)	HR (bpm)	$\dot{V}_{O_2}$ ($ml\ min^{-1}$)	$\dot{V}_{CO_2}$ ($ml\ min^{-1}$)
Hypnosis rest	8.6±0.5	15.6±0.9	0.6±0.1	40.9±0.7	67±3	266±21	219±19
Imagined 'heavy' exercise (n=17, protocol 1)	15.5±1.2 **	26.7±2.1 **	0.6±0.1	35.2±0.8 **	75±2 **	431±52 ** (n=13)	388±58 ** (n=13)
Actual heavy exercise	34.8±1.9 **	23.7±0.9 **	1.5±0.1 **	45.8±0.4 **	128±4 **		
Hypnosis rest	9.9±0.4	15.5±1.0	0.7±0.1	43.0±1.0	61±3		
Imagined freewheeling downhill (n=8, protocol 3)	9.8±0.4	15.5±1.3	0.7±0.1	43.3±0.9	61±3		
Hypnosis rest	8.3±1.2	16.6±1.3	0.5±0.1	42.5±1.3	62±6	281±29	215±24
Volitional hyperventilation (n=6, protocol 2)	12.9±1.1 *	30.4±2.7 *	0.4±0.1	37.1±1.3 *	65±2	531±67 * (n=6)	503±52 * (n=6)
Hypnosis rest	9.2±1.2	16.2±0.7	0.6±0.1	43.6±0.9	62±6		
Volitional hyperventilation + CO_2 (n=6, protocol 2)	12.2±0.7 *	26.0±1.4 *	0.5±0.1	41.7±1.0 *	63±1		

Table 3.4: Cardiorespiratory data for protocols 1-3 in hypnotized subjects. ** indicates $p<0.001$, * indicates $p<0.05$ (all compared to rest, paired t-test). 'Heavy' refers to a work rate that the subjects had previously been familiarized to, designed to produce 50% of HR_{max}. Resting values are taken as the mean ± S.E.M for the period 30s to 15s before starting the protocol. All values are mean ± S.E.M for 1.75 to 2 minutes of the procedure.

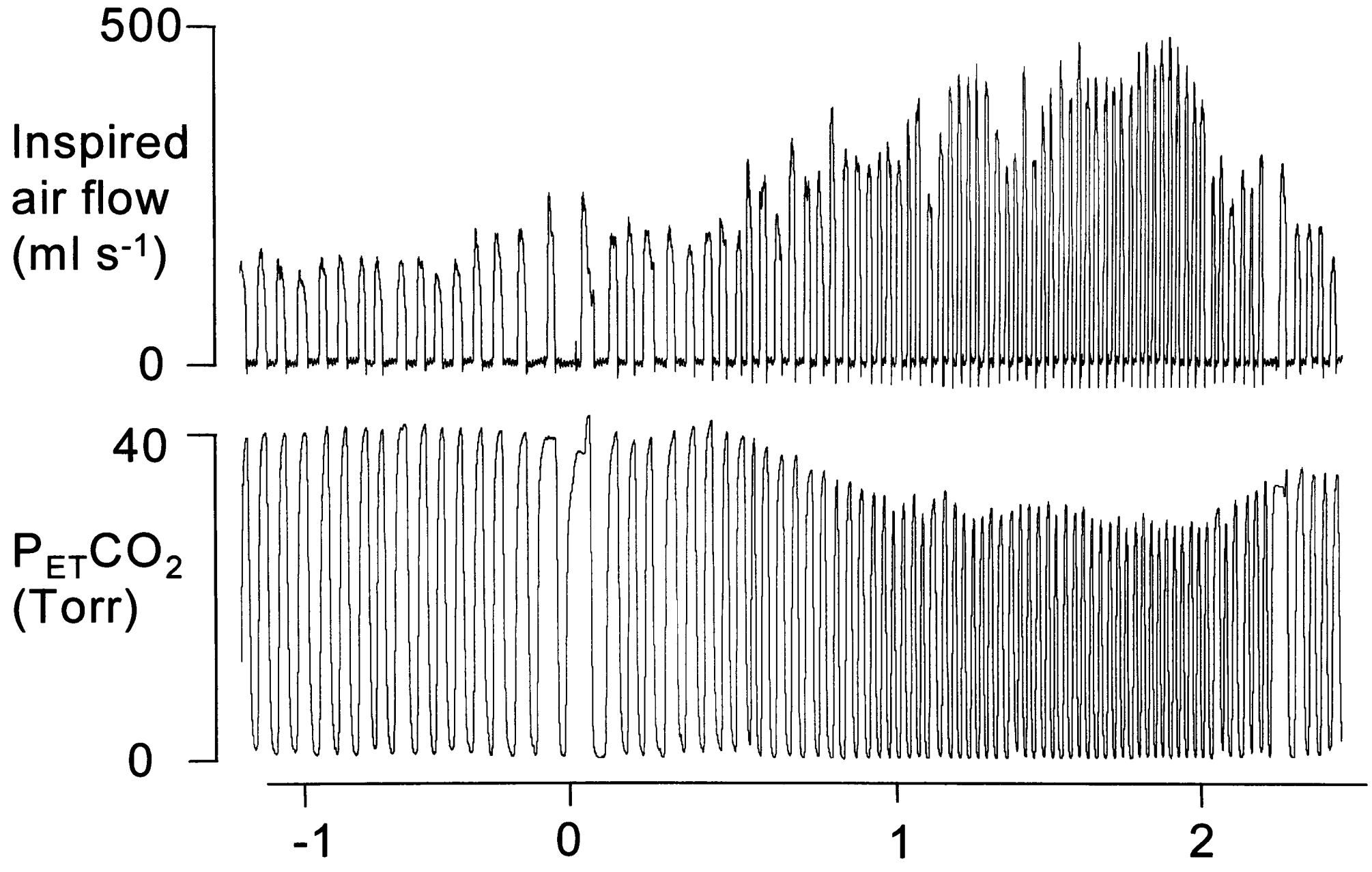
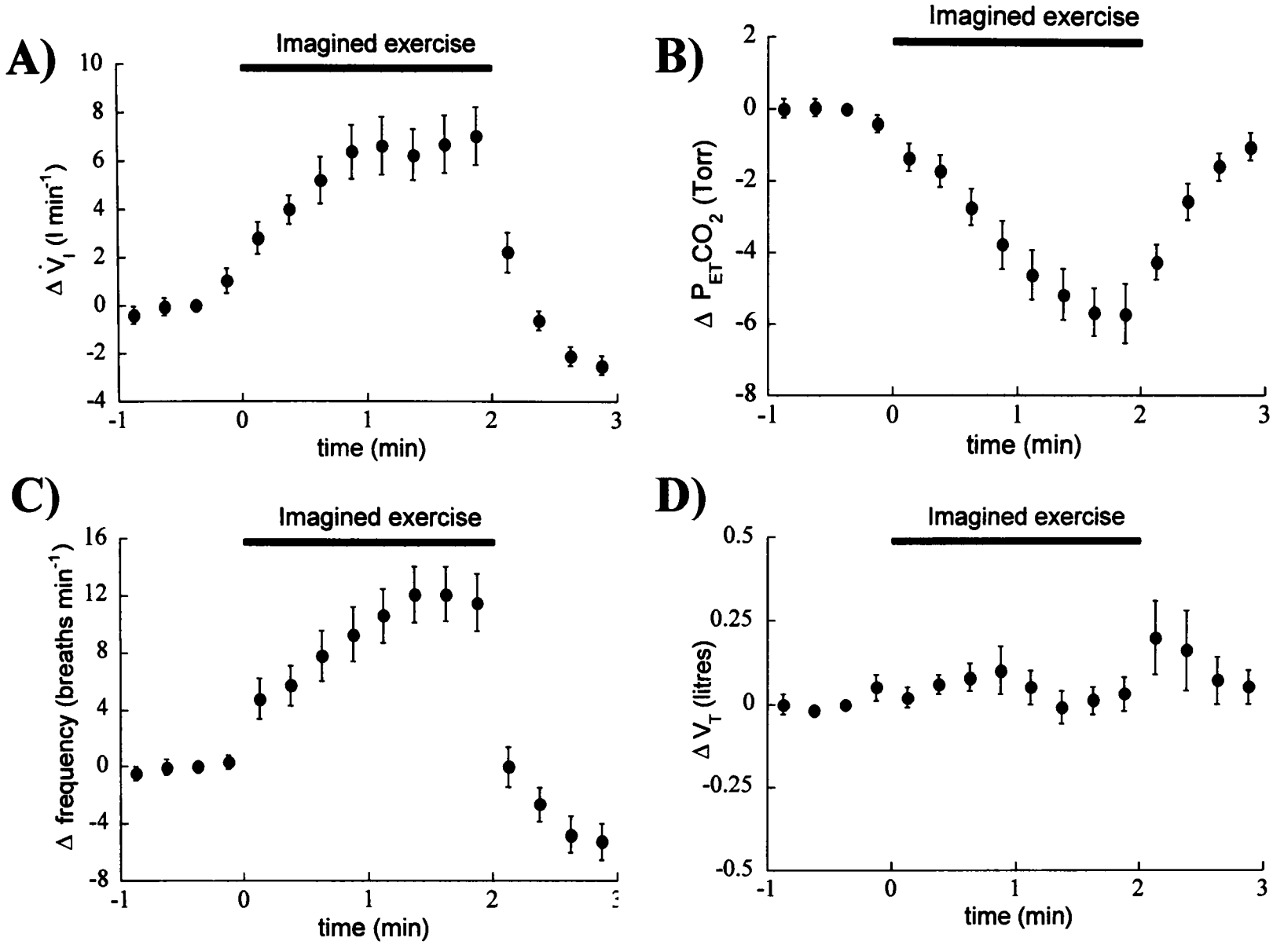


Figure 3.1. Imagination of heavy exercise for 2 minutes whilst under hypnosis (protocol 1) resulted in an increase in inspired air flow (top panel) and a decrease in $P_{ET}CO_2$ (bottom panel). Raw data from one subject.



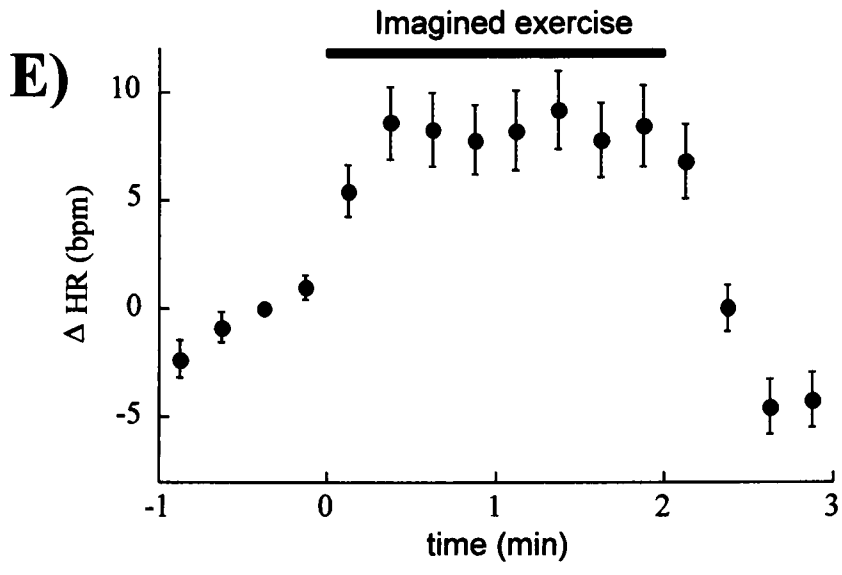
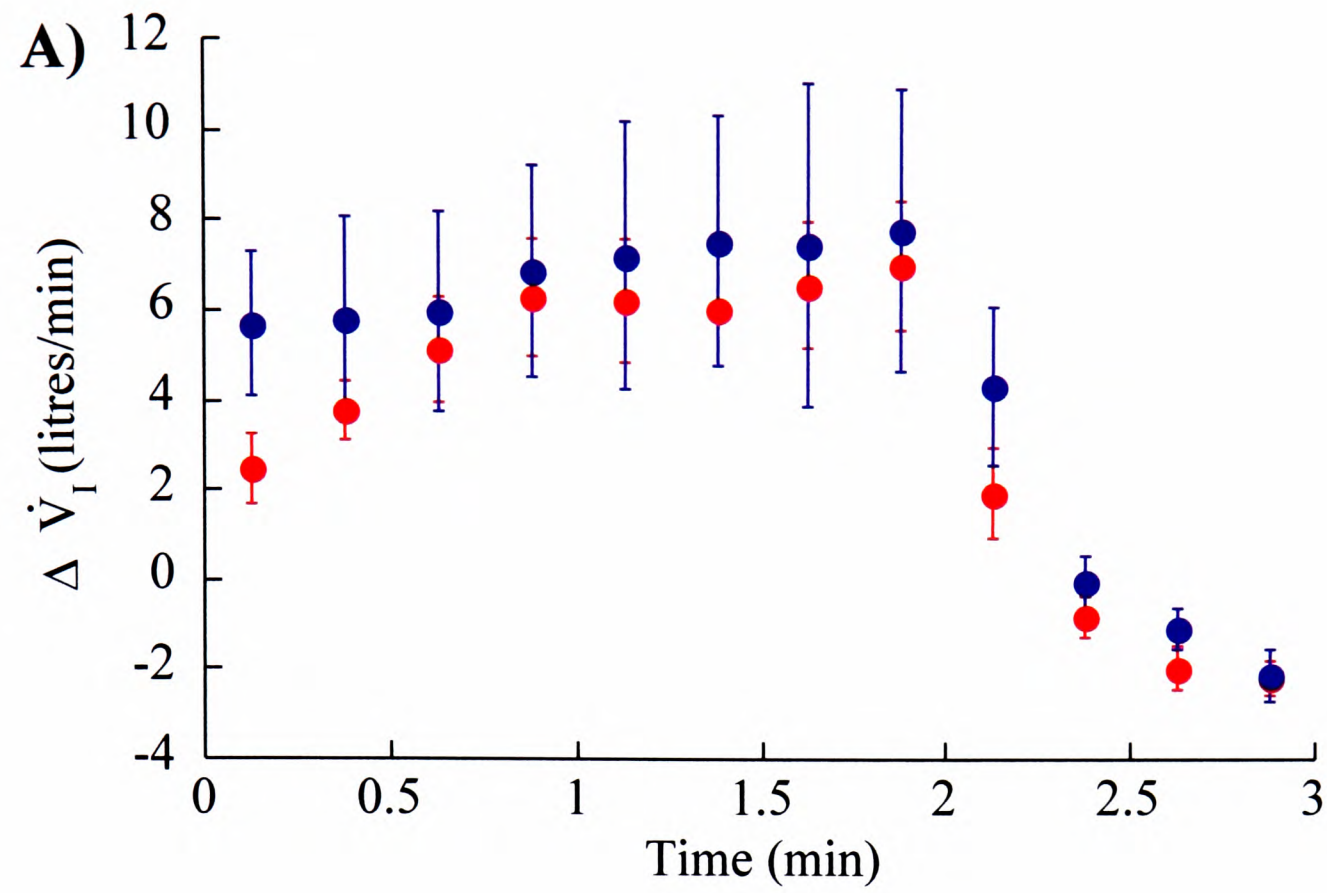


Figure 3.2: Group data for the imagination of 'heavy' exercise **under hypnosis** (protocol 1, n=17). Each data point represents mean ± S.E.M of a 15 second epoch, with the resting value (taken as the mean of the variable between 30 and 15 seconds prior to the onset of imagined exercise) subtracted from it. A) $\Delta\dot{V}_I$, B) $\Delta P_{ET}CO_2$, C) Δf, D) ΔV_T, E) Δ HR.



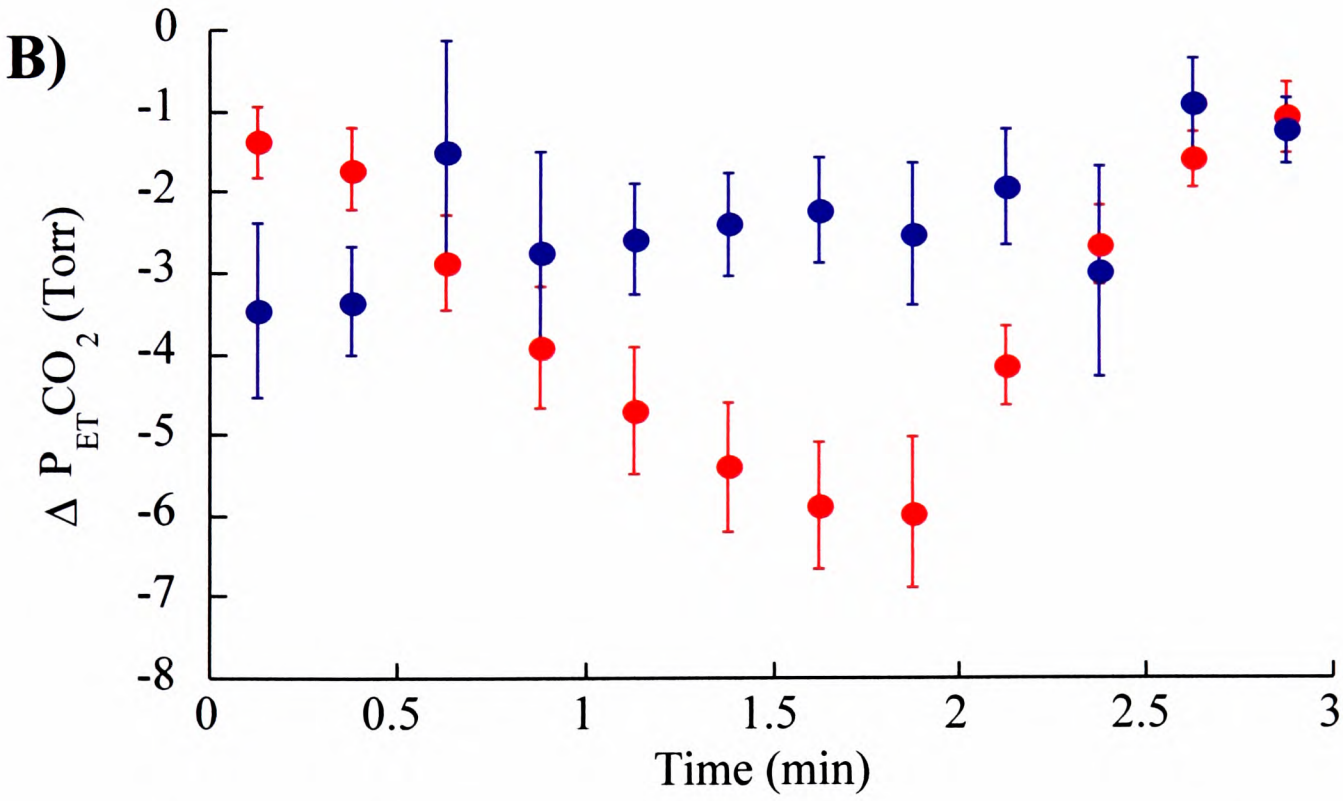


Figure 3.3: Addition of CO_2 to the inspired air to maintain isocapnia did not alter the magnitude of the ventilatory response to the imagination of exercise under hypnosis. Each data point represents mean ± S.E.M of a 15 second epoch, with the resting value (taken as the mean of the variable between 30 and 15 seconds prior to the onset of imagined exercise) subtracted from it, n=7. A) $\Delta \dot{V}_I$, B) $\Delta P_{ET}CO_2$ (●control, ●isocapnic).

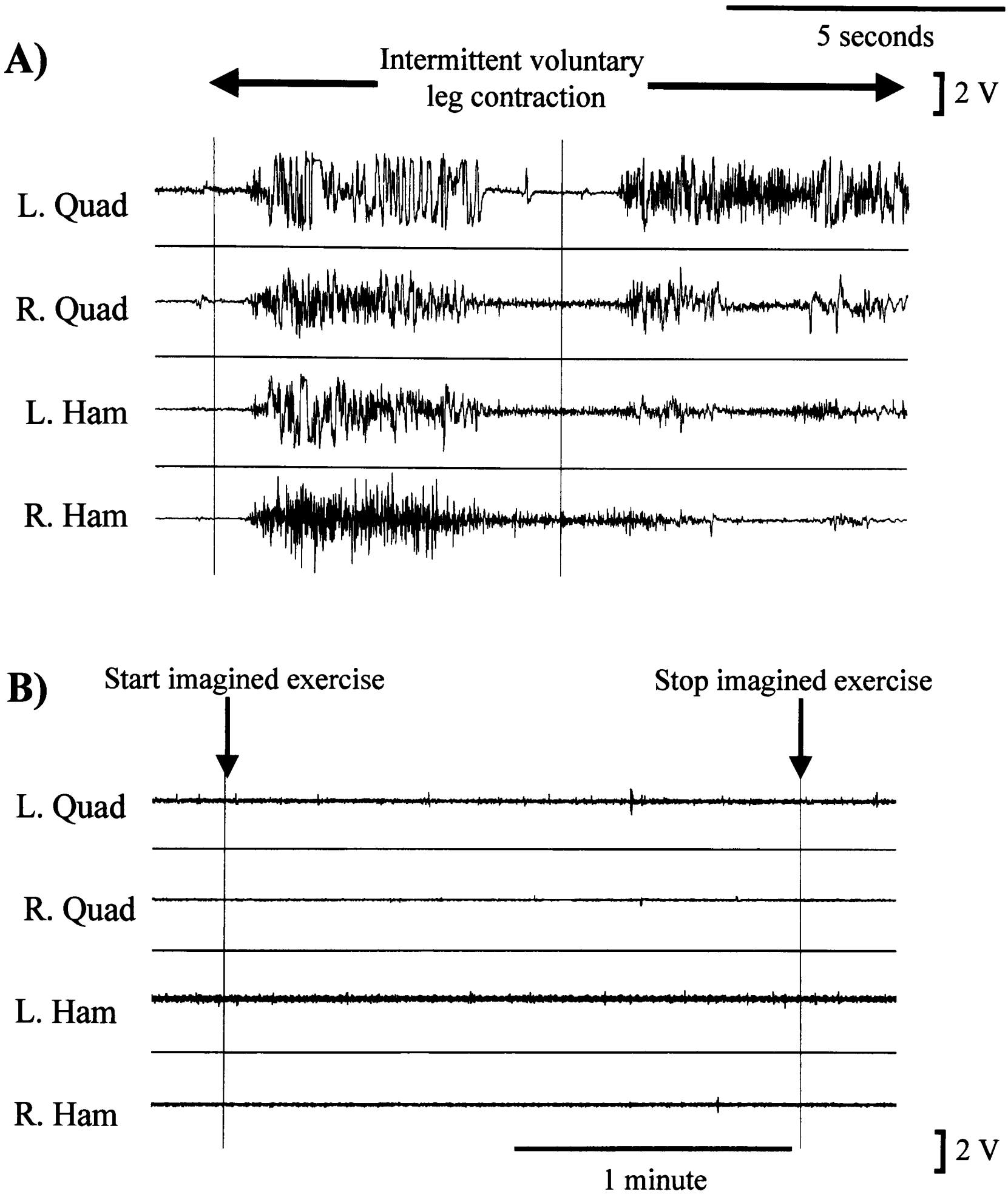


Figure 3.4. A comparison of the electrical activity induced by voluntary leg movement (A) with that during imagination of exercise (B) in one subject. EMG recordings were made from left and right quadriceps and hamstrings. The vertical scale is the same in both panels; the increase in EMG activity during imagination of exercise is minor compared to that elicited by voluntary contraction.

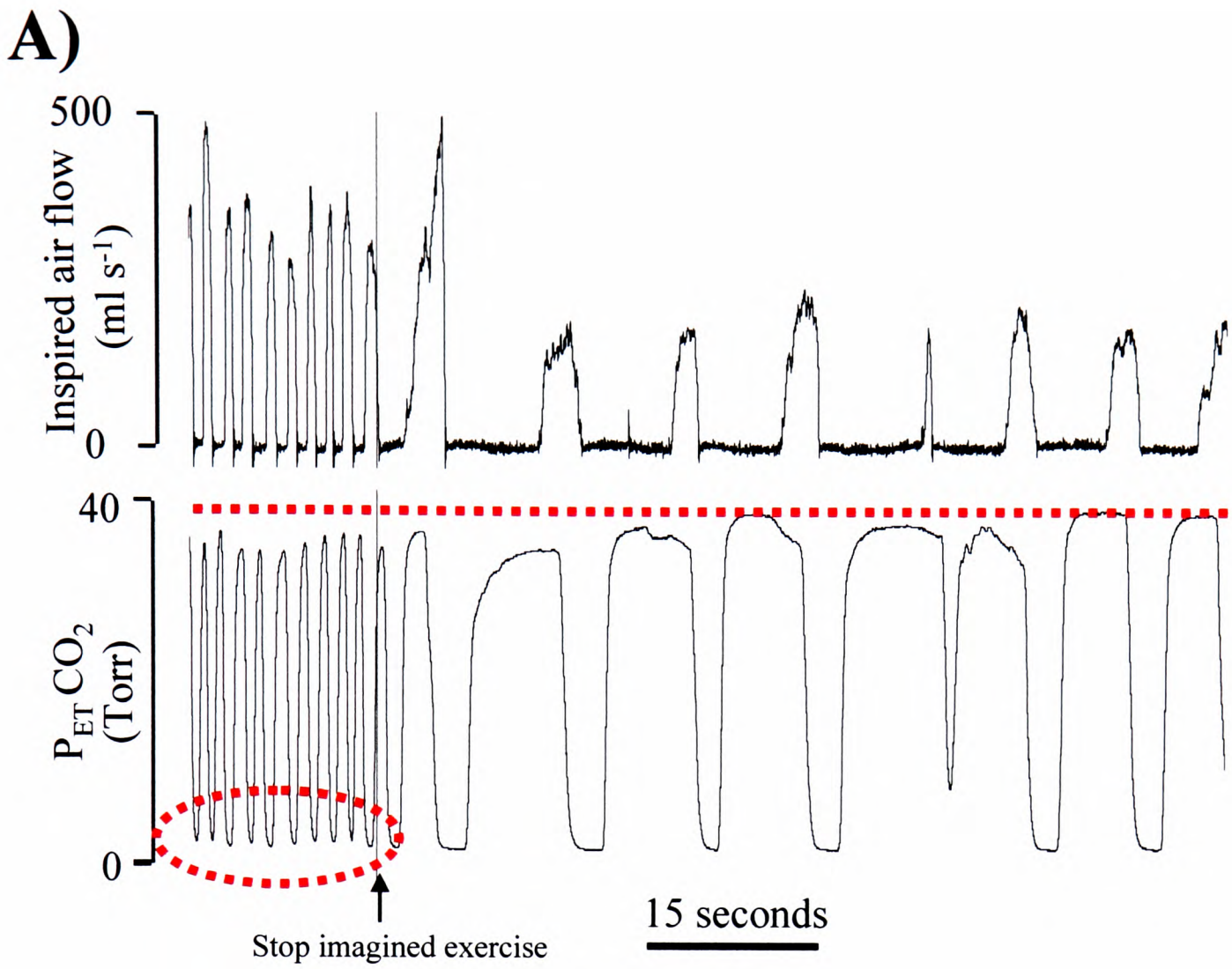


Figure 3.5. A) Raw data from one subject showing the expiratory pauses occurring following imagined exercise, B) despite maintaining isocapnia during the period of imagined exercise, apnoeas still occurred when imagination of exercise ceased. Red dotted lines indicate the main difference between the two repeats; A) the subject becomes hypocapnic, B) the subject remains isocapnic during imagination of exercise owing to the addition of CO_2 to the inspired air.

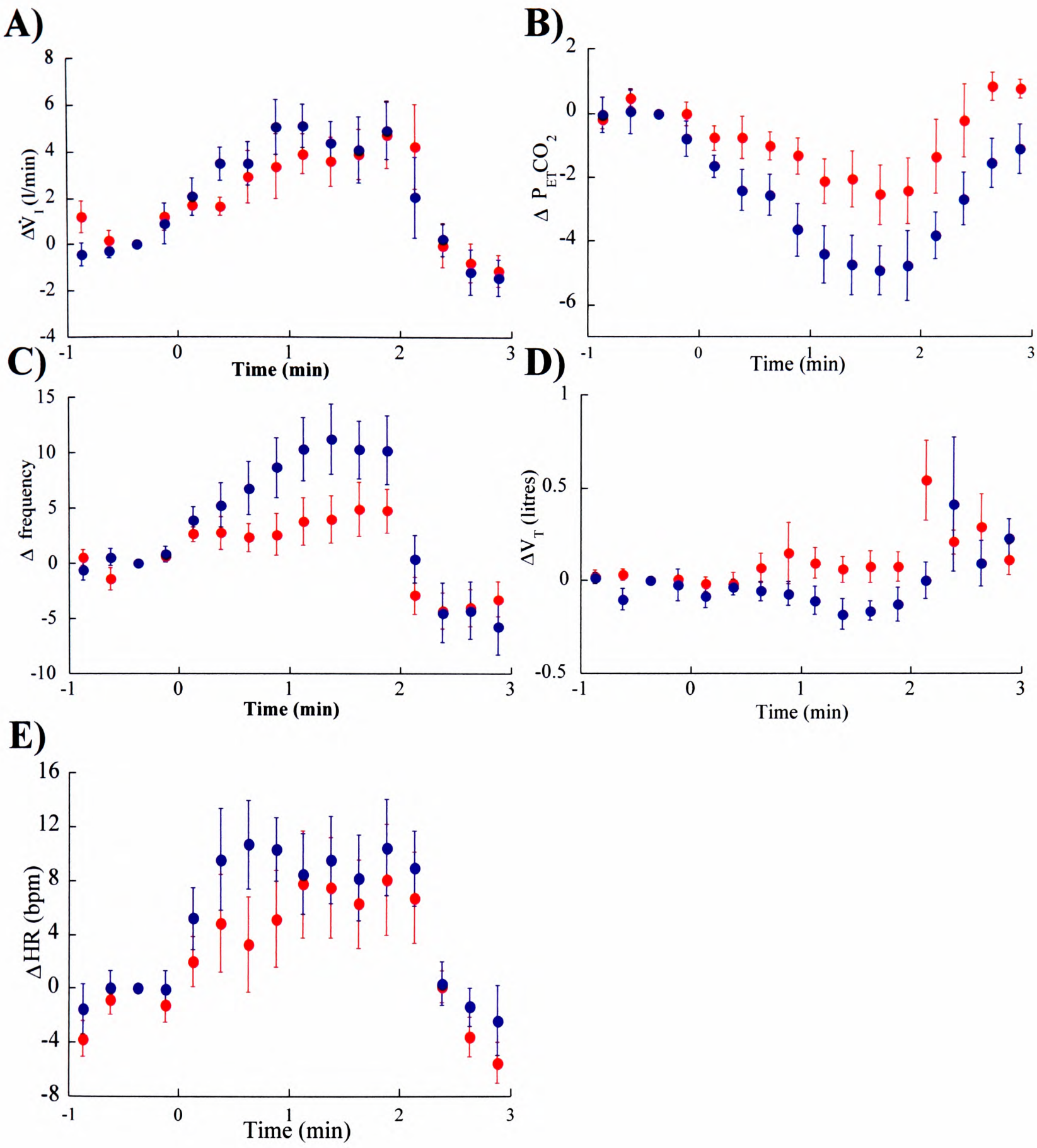


Figure 3.6. Group data for the imagination of 'heavy' exercise (●) compared to imagination of 'moderate' exercise (●) under hypnosis. Each data point represents mean ± S.E.M of a 15 second epoch, with the resting value (taken as the mean of the variable between 30 and 15 seconds prior to the onset of imagined exercise) subtracted from it. A) $\Delta\dot{V}_I$, B) $\Delta P_{ET}CO_2$, C) Δf, D) ΔV_T, E) Δ HR.

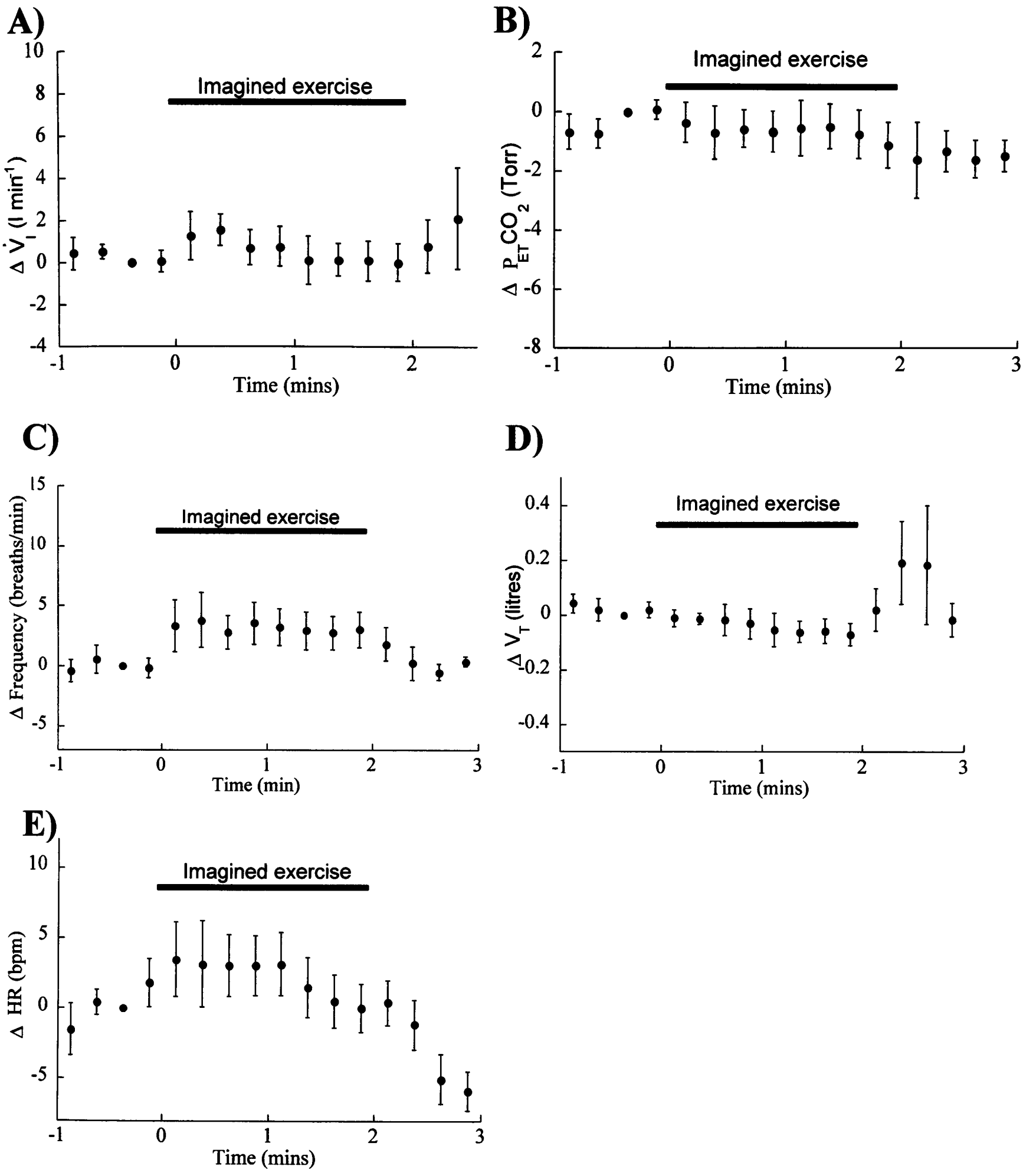
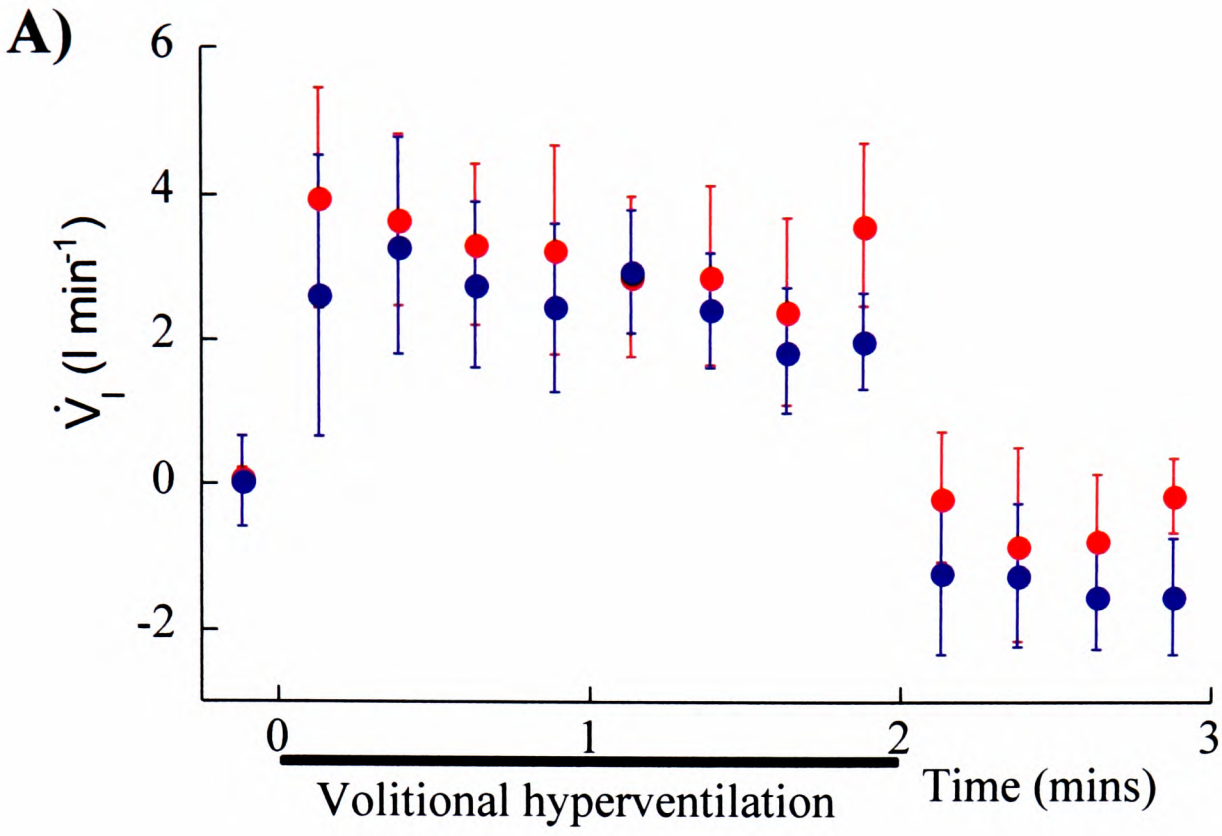


Figure 3.7: Group data for the imagination of 'heavy' exercise **whilst awake** (n=8). Each data point represents mean ± S.E.M of a 15 second epoch, with the resting value (taken as the mean of the variable between 30 and 15 seconds prior to the onset of imagined exercise) subtracted from it. A) $\Delta\dot{V}_I$, B) $\Delta P_{ET}CO_2$, C) Δf, D) ΔV_T, E) Δ HR.



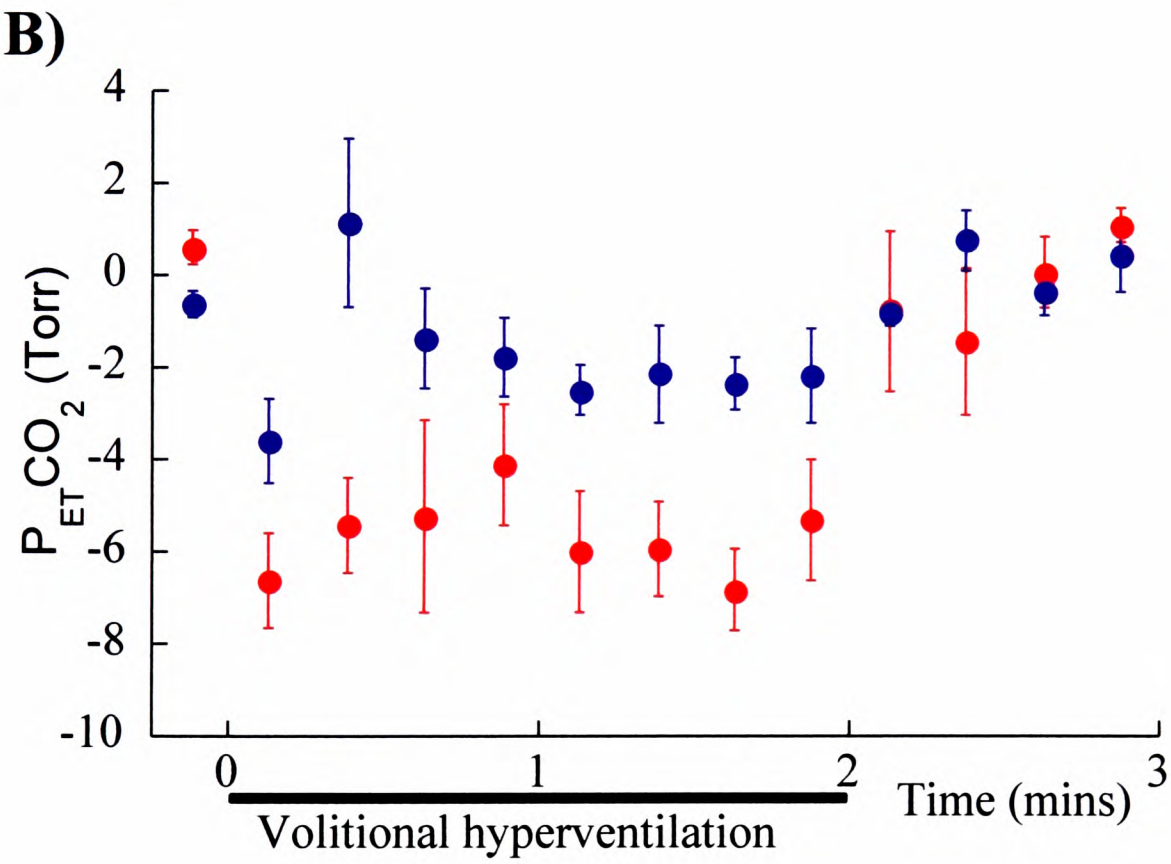


Figure 3.8: Addition of CO_2 to the inspired air did not alter the magnitude of volitional hyperventilation under hypnosis. Each data point represents mean ± S.E.M of a 15 second epoch, with the resting value (taken as the mean of the variable between 30 and 15 seconds prior to the onset of imagined exercise) subtracted from it, n=6. A) $\Delta \dot{V}_I$, B) $\Delta P_{ET}CO_2$ (● control, ● isocapnic).

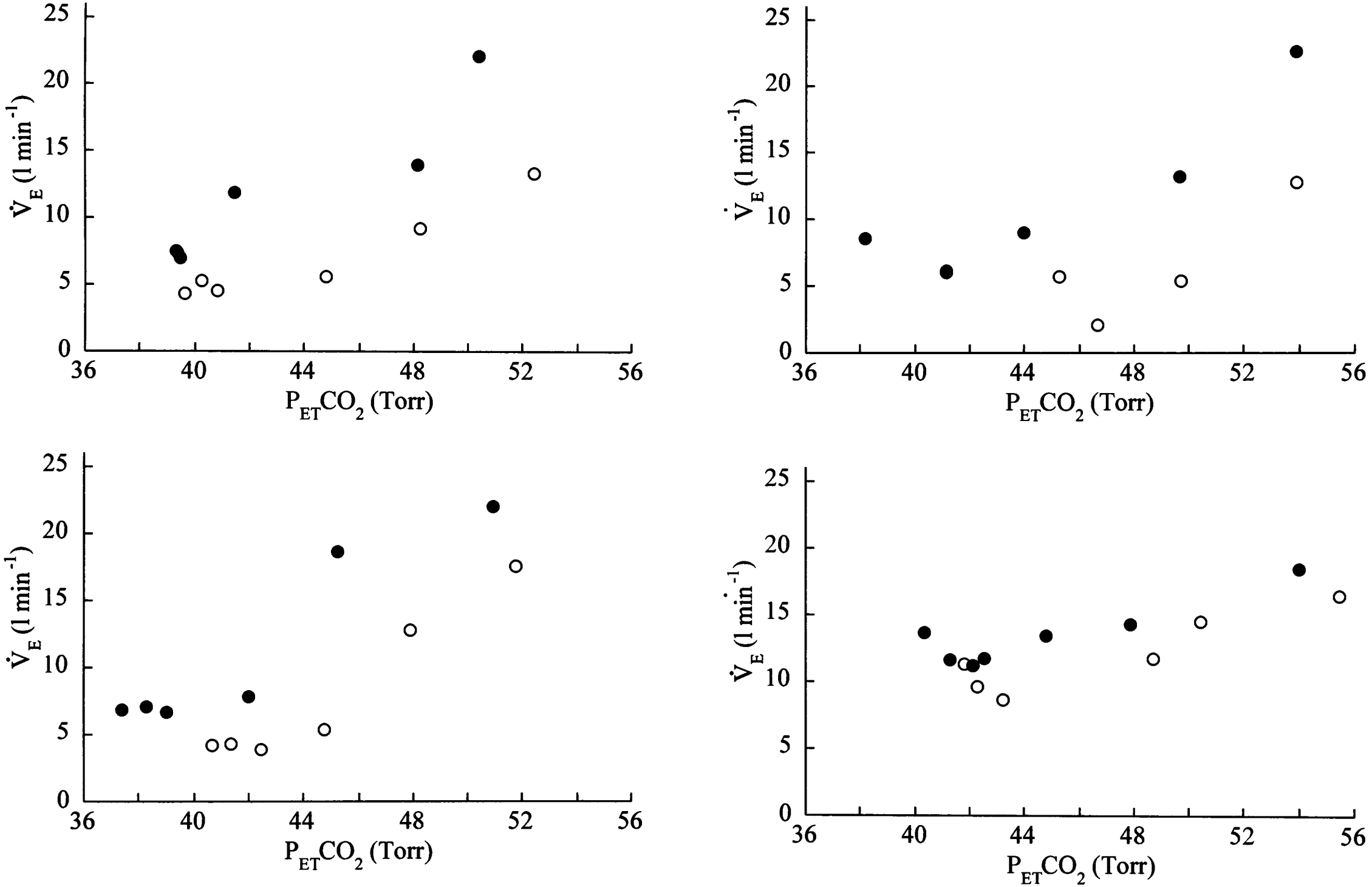


Figure 3.9. Individual data from four subjects showing the steady-state ventilatory responses to increases in $P_{ET}CO_2$ (● awake,○ hypnosis). In three of the four subjects the relationship between $\dot{V}_E$ and $P_{ET}CO_2$ shifted to higher values of $P_{ET}CO_2$ under hypnosis compared to awake.

3.3.5 Ventilatory responses to inspired CO_2 awake and under hypnosis

Hypnosis did not affect the slope of the $\dot{V}_E$-$P_{ET}CO_2$ relationship; however in 3 subjects the relationship was shifted to the right under hypnosis compared to the awake state (figure 3.9).

3.4 Discussion

The main findings of this study are 1) Imagination of exercise whilst under hypnosis results in hyperventilation, hypocapnia and a modest increase in HR, 2) immediately on cessation of imagination of exercise $\dot{V}_I$ decreases below control levels for *ca.* 2 minutes and is characterised by slow deep breathing with expiratory pauses, 3) the magnitude and time course of hyperventilation during imagined exercise is not affected by maintaining isocapnia, 4) the slope of the $\dot{V}_E$-$P_{ET}CO_2$ curve is not altered under hypnosis, but the curve may shift to higher $P_{ET}CO_2$ values.

3.4.1 Resting cardiorespiratory variables

The small decreases in $\dot{V}_I$, $\dot{V}_{O_2}$ and *f* and the increase in $P_{ET}CO_2$ during hypnosis compared with the awake state in this study are in agreement with previous studies (Dudley *et al.* 1964; Daly & Overley 1966). Others however, have seen no change in cardiorespiratory variables (Jana 1967; Arvidsson *et al.* 1970), or increases in $\dot{V}_I$ and HR during hypnosis (Agosti & Camerota 1965). These inconsistencies may be caused by differences in the degree of arousal or the emotional state of the subjects. Transient increases in $\dot{V}_I$, hypocapnia and increases in HR were observed during the hypnotic induction in this study; this may reflect a requirement for focussed attention (Gruzelier 1998). Some previous studies of hypnosis have found decreases in HR and blood pressure under hypnosis compared to the awake state (Daly & Overley 1966), in addition several authors have observed an increase in the high-frequency power of HR variability under hypnosis (Harris *et al.* 1993; DeBenedittis *et al.* 1994). Together, these findings suggest that there may be an increased vagal drive under hypnosis compared to the awake state. However, I

did not observe any change in HR, blood pressure, baroreflex sensitivity or HR/blood pressure variability between the awake and hypnotic states.

3.4.2 Imagination of exercise in awake subjects

There was no significant change in cardiorespiratory variables during imagination of exercise in *awake* subjects. This is in contrast to Decety *et al* (1991) who found increases in HR and $\dot{V}_I$ (predominantly via *f* not V_T) in response to imagination of treadmill exercise. However recent findings (Wuyam *et al.* 1995) were more equivocal; increases in HR and $\dot{V}_I$ during imagination of exercise occurred in athletes but not in sedentary subjects. This may reflect visualisation and mental rehearsal strategies that are commonly used by athletes. Only two of the subjects participating in the current study regarded themselves as athletes and there were no audio cues related to exercise such as the sound of a treadmill or exercise bike. The type of exercise used (semi-supine cycling) is also unfamiliar to subjects and this may make visual imagery more difficult. The differences in subjects and protocol between this study and those of other authors may account for the absence of cardiorespiratory responses during imagined exercise whilst awake in this study.

3.4.3 Imagination of cycling downhill

There was no effect of imagination of cycling downhill on cardiorespiratory variables. This protocol was performed as a control for cognitive effort since other authors have found that cognitive tasks such as visualising letters and mental arithmetic increase HR and $\dot{V}_I$ (Shea *et al.* 1988; Wuyam *et al.* 1995). The lack of effect seen in this study may reflect the ease of performing visual imagery in the hypnotised state compared to the awake state, direct evidence for this would require subjects to perform imagination of freewheeling downhill whilst awake.

3.4.4 Imagination of exercise under hypnosis at rest

Imagination of exercise under hypnosis was associated with an increase in HR, $\dot{V}_I$ and hypocapnia (table 3.4, figures 3.1-3.3). Whilst this has been observed previously (Daly & Overley 1966; Wang & Morgan 1992), this response now appears to be robust and repeatable in 17 experimentally naïve subjects *in the absence of direct suggestions regarding cardiorespiratory variables*. This is clearly not an increase in breathing in response to cognitive effort (for reasons discussed above). Neither is it an increase in breathing associated with metabolic demands of movement; whilst 4 subjects did start to move slightly during imagination of exercise $\dot{V}_{O_2}$ remained relatively low compared to that occurring in exercise (table 3.4). The onset dynamics of the response to imagined exercise followed a monophasic exponential, albeit faster than that described for real exercise (Whipp & Wasserman 1991). This could be due to the fact that imagined exercise was being studied, rather than exercise *per se*. An interesting finding in this study was the observation that $\dot{V}_I$ decreased below control levels immediately following imagination of exercise at rest. It seems unlikely that the post-hyperventilation pauses seen at rest are related to hypocapnia, since they occurred even when isocapnia was maintained.

The frequency-tidal volume relationship during imagination of exercise under hypnosis

The increases in $\dot{V}_I$ seen during imagination of exercise under hypnosis in this study were via increases in f with no steady-state alterations in V_T. This is consistent with awake data (Decety *et al.* 1991; Wuyam *et al.* 1995) and with one previous hypnosis study (Agosti & Camerota 1965), although another study reported hyperventilation with increases in both f and V_T (Arvidsson *et al.* 1970). I have no good explanation for the fact that V_T did not change during imagination of exercise in the present study but there are several possible hypotheses.

Although subjects denied it when debriefed, it is difficult to exclude the possibility that breathing frequency was used as a cognitive strategy to enhance imagination or was entrained with imagined movement. This is difficult to test, but in the four subjects who did move slightly during imagination of exercise there was no evidence for entrainment of their breathing frequency with their rhythm of movement. A metronome was not used except during

performance of the incremental exercise test and since the increase in *f* during imagination of exercise followed phase kinetics it seems unlikely that entrainment can explain the *f*-V_T pattern observed. Subjects also denied using breathing frequency as a cognitive strategy.

The relationship between *f* and V_T is generally considered in terms of minimising the work of breathing and was described by Hey *et al.* (1966) as an exponential rise to maximum V_T and a linear increase in frequency with increasing $\dot{V}_I$. A number of situations exist in which *f* and V_T have been separated experimentally. Ventilatory responses to exercise in children with congenital central hypoventilation syndrome (CCHS) are via *f* with little V_T component (Paton *et al.* 1993). Indeed, CCHS patients hypoventilate at rest due to a low V_T, patients have a normal breathing frequency. Whilst Paton interpreted this as a contribution for limb afferents and ventilatory entrainment in the hyperpnoea, could it alternatively mean that 'central command' provides a frequency-based drive to breathe? Experiments in cats have shown that chemical stimulation of discrete regions of the ventrolateral medulla can alter *f* and V_T independently (Tolentino Silva *et al.* 1993). Transient responses to ventilatory stimuli were originally described by Haldane (1919)

> *"The effect of want of oxygen is to increase the rate of the respiration rather than the depth, while CO_2 increases the depth rather than the rate"*

However, this general finding has not been confirmed; most authors (e.g. Hey, 1966) find the V_T-*f* relationship to be independent of alveolar partial pressures.

The absence of an increase in V_T during imagination of exercise whilst at rest, both in this study under hypnosis and in studies awake might indicate that the increase in V_T during exercise is dependent upon some type of peripheral feedback occurring during exercise that is not occurring at rest. The most obvious is the absence of locomotor activity itself. Locomotor activity may alter V_T in several ways; peripheral feedback from Type III and IV muscle afferents is clearly important for the exercise pressor response (Alam & Smirk 1937) and may play a role in exercise hyperpnoea (Kao 1963). Locomotor-respiratory coupling confers mechanical advantages (Bramble & Carrier 1983) and so may act to minimise the work of breathing and therefore may alter the f-V_T relationship. In addition to the effects of locomotor activity on V_T, it is possible that there may be a link between cardiac output increases and the *f*-

V_T relationship. In the current study and in previous studies of volitional hyperventilation (Cummin *et al.* 1986) hyperventilation via *f* alone does not increase HR, whereas hyperventilation including a tidal volume component does increase HR. Such a link would be consistent with the only study of imagination of exercise under hypnosis in which V_T increased (Arvidsson *et al.* 1970), in that study cardiac output also increased during imagination of exercise. Changes in vagal afferent activity also alter the relationship between *f* and V_T in animals (Eldridge *et al.* 1985) and the same has been shown in man (Winning *et al.* 1985).

Taken together, these data suggest that *f* and V_T may be under differential control during exercise compared to at rest and this could potentially explain why imagination of exercise in man, both under hypnosis and awake results in a frequency-based ventilatory response.

HR response to imagination of exercise under hypnosis

The changes in HR observed were small compared to those observed during real exercise. However they were larger than those predicted solely due to the increased work of breathing (reported to be 1% of total $\dot{V}_{O_2}$ at rest, rising to a maximum of 10% during exercise; (Aaron *et al.* 1992) as evidenced by the fact that HR did not increase during volitional hyperventilation despite the increases in $\dot{V}_{O_2}$ during volitional hyperventilation being greater than that during imagination of exercise.

Imagination of 'moderate' exercise, compared to imagination of 'heavy' exercise

Whilst $\dot{V}_I$ did not differ between imagination of 'moderate' exercise compared to imagination of 'heavy' exercise, there did appear to be a degree of 'dose-dependence' in the response since the $P_{ET}CO_2$ reached lower levels during imagination of 'heavy' exercise[1] and there was a striking difference in *f* between imagination of 'moderate' and 'heavy' exercise (figure 3.6). An interesting feature of the response is that during the first few seconds it is the same for both imagined work rates and that it is only after *c.a.* 20 seconds that the *f* response differs between imagined moderate exercise and imagined heavy exercise. This is similar to phase I of the ventilatory response seen during actual exercise, which is of the

same magnitude regardless of the work rate (Whipp & Wasserman 1991). This grading of the response may suggest that the aspects of 'central command' mimicked in this study is not merely an alerting, all-or-nothing event, but reflects a genuine drive proportional to 'effort.'

The effect of maintaining isocapnia on ventilatory responses

A new finding of this study is that the magnitude of the hyperventilation during imagined exercise under hypnosis was not affected by maintaining isocapnia (figure 3.3). Likewise, the magnitude of volitional hyperventilation was also not limited by hypocapnia (table 3.2, figure 3.8). It seems unlikely that the time periods over which the imagined exercise/volitional hyperventilation and CO_2 administration was occurring (2 minutes) were too short for modulation of central chemoreceptors. Having erred on the side of caution when adding CO_2 to the inspired air (there was a small but significant decrease in $P_{ET}CO_2$ during the supposed 'isocapnic' control during protocols 1 and 2, figures 3.3 and 3.8), it is possible that if true isocapnia had been maintained the magnitudes of ventilatory responses to imagination of exercise or volitional hyperventilation may have been altered. More likely is that the rightward shift of the $\dot{V}_E$-$P_{ET}CO_2$ under hypnosis could be a factor underlying the lack of effect of CO_2 on the magnitude of hyperventilation; if during imagination of exercise under hypnosis or volitional hyperventilation $P_{ET}CO_2$ remains on the flat portion of the $\dot{V}_E$ response curve, then adding CO_2 to the inspired air will have no effect on $\dot{V}_E$. Regardless of the mechanism, the lack of effect of maintaining isocapnia in this study suggests that under the circumstances of this experiment, breathing may be driven by mechanisms that are not chemosensitive.

3.4.5 Limitations of the study

The use of a technique such as hypnosis to study cardiorespiratory variables raises concerns regarding conformity in subjects who are eager to please. However, I believe that the responses observed are NOT merely volitional for a number of reasons. Subjects were experimentally naïve with no medical or physiological training and no mention was made of cardiorespiratory

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[1] $P_{ET}CO_2$ is a more sensitive indicator of change than $\dot{V}_I$ owing to the relationship between alveolar ventilation and alveolar PCO_2 given by the alveolar gas equation.

variables during exercise suggestions. Whilst studying volitional hyperventilation might alert subjects to an increase in breathing, this was always the final protocol performed and was only done in six of the seventeen subjects. When specifically asked at the end of the study the subjects denied using their breathing as a cognitive strategy to aid imagination. This contrasts with some previous studies under hypnosis (Wang & Morgan 1992) in which subjects were specifically told that their breathing during imagination of exercise was "*getting faster and deeper*". Whilst the increase in HR observed during imagination of exercise was small compared with that occurring during actual exercise, it does not seem to reflect an increased work of breathing since volitional hyperventilation with comparable increases in *f* and a greater increase in $\dot{V}_{O_2}$ was not associated with an increase in HR (table 3.4). There is no convincing evidence that HR is under voluntary control; thus it is unlikely that subjects were being deliberately compliant. Additionally, the response to imagination of exercise in this study is different from that seen in actual exercise in that increases in $\dot{V}_I$ occurred via increases in *f* with no change in V_T. If subjects attempted to mimic an exercise response there is no obvious reason why they would choose to do this without altering their tidal volume. Moreover, both ventilatory and HR responses to imagination of exercise in this study were well fitted by a monophasic exponential. It is unlikely that experimentally naïve subjects could mimic such kinetics, particularly with the high degree of repeatability observed when subjects performed multiple repeats of the same protocol.

An obvious limitation of this study is that the neural basis of hypnosis is poorly understood and that there is no satisfactory objective measure of depth of the hypnotic state (discussed in Chapter Two). Given the behaviour of the subjects (immobility, automatic responding) and the vividness of the subjects descriptions of their experience (they spontaneously reported having been in an altered state of consciousness, describing the state in terms of deep relaxation, automatic responding and an altered perception of the passage of time) experienced medical hypnotists were confident that the subjects were in a deep hypnotic state. There was also no evidence that the depth of hypnosis affected the magnitude of results as the subjects exhibiting the largest responses were not those who were classed as being in the deepest hypnotic state.

3.4.6 Implications for cardiorespiratory control

Numerous studies in animals and man have supported the significance of 'central command' indirectly since patients with CCHS breathe normally during exercise (Shea *et al.* 1993) and that denervation of the carotid bodies (Wasserman *et al.* 1975), heart and lungs (Banner *et al.* 1988) in man and also denervation of carotid bodies, heart, lungs and spinal afferents in ponies (Pan *et al.* 1995) have relatively normal steady-state ventilatory responses to exercise, although onset dynamics are slowed. However, doubt has been cast on the role of 'central command' by results from electrically stimulated exercise in man in which the steady-state ventilatory response was identical to that seen during voluntary exercise (Adams *et al.* 1984). It therefore seems that multiple mechanisms exist to maintain cardiorespiratory performance during exercise. It is tempting to speculate that the ventilatory response to imagination of exercise and altered perception of exercise load in this study may be the result of a higher centre motor programme governing control of $\dot{V}_I$ during exercise (Somjen 1992). The absence of large HR changes in this study argues against a similar motor programme dominating in cardiovascular control during exercise.

In conclusion, the evidence presented supports the idea that a powerful drive to breathe originating from higher centres arises when exercise or increased work rate is imagined under hypnosis. Hypnosis may be a useful tool with which to investigate 'central command' in humans as it seems to allow partial uncoupling of central mechanisms from peripheral ones. The extent of this uncoupling is difficult to gauge owing to the difficulty in equating 'central command' with parameters that can be measured objectively in man. Whether the neural structures underlying the imagination of exercise are the same as those believed to underlie 'central command' remains to be determined.

3.5 Summary

The main findings in this chapter are:

1) Imagination of exercise whilst under hypnosis resulted in hyperventilation, hypocapnia, and a modest increase in HR. The response followed a monophasic exponential and was not affected by maintaining isocapnia.

2) On cessation of imagined exercise slow deep breathing ensued and $\dot{V}_I$ remained below control levels for *ca.* 2 minutes. A similar breathing pattern was also seen following imagination of exercise when near-isocapnia is maintained.
3) The slope of the $\dot{V}_E$-$P_{ET}CO_2$ curve is not altered under hypnosis, but the curve may shift to higher $P_{ET}CO_2$ values.
4) In conclusion, hypnosis allows partial uncoupling of central mechanisms from peripheral feedback. Higher centre activity can contribute significantly to 'exercise' hyperpnoea, possibly through activation of a respiratory motor programme.

Chapter Four

Neuroimaging 'central command'

Brevis esse laboro, obscurus fio

Horace.

4.1 Introduction

Numerous studies have demonstrated the *existence* of 'central command' and its power to increase cardiorespiratory variables during exercise (reviewed by Waldrop *et al.* 1996). However, few studies have investigated the *origins* of 'central command'. Animal studies (Smith *et al.* 1960; Angyan 1978; Eldridge *et al.* 1981; Waldrop *et al.* 1986; Bedford *et al.* 1992; Chong & Bedford 1997) have shown that electrical stimulation of subcortical sites (including the hypothalamus, basal ganglia, thalamus and mesencephalic locomotor region) causes locomotion and a cardiorespiratory response even when peripheral feedback is abolished. The extent to which these areas are involved in generating 'central command' in humans is unclear since constraining the head sufficiently to allow neuroimaging has proved difficult in exercise studies and even if this could be achieved, imaging the brainstem is complicated by movement related to cardiac and respiratory cycles. The role of the cortex is somewhat better established. Mild exercise and the post-exercise state have been studied using PET (Fink *et al.* 1995), and SPECT (Williamson *et al.* 1997). Fink *et al.* (1995) demonstrated superolateral motor cortical activation in regions previously described to be involved in tasks involving increased respiratory muscle activity (Colebatch *et al.* 1991; Ramsay *et al.* 1993; Fink *et al.* 1996). Activations were also observed in the SMA, thalamus and cerebellum, all of which are involved in motor control (figure 1.5) and could therefore form part of the neural circuitry for 'central command'. Activation of the left insular cortex in the SPECT study of Williamson *et al.* (1997) is consistent with studies in which electrical stimulation of the insular cortices elicits cardiovascular responses (Oppenheimer & Cechetto 1990). The insular cortex projects to the lateral hypothalamus and the ventrolateral medulla (Yasui *et al.* 1991) and also to the nucleus of the solitary tract (Saper 1982), all of which are implicated in cardiovascular control.

Given the difficulties, imaging *imagined* exercise may prove to be a useful avenue of research. Having mimicked 'central command' using imagination of exercise under hypnosis (described in Chapter Three), it is of interest to consider the neural correlates of the response, to see if they are the same as those implicated in 'central command' during exercise. Positron Emission Tomography (PET) scanning was used with the simplest form of experimental design, that of

subtraction (discussed in Chapter Two). In order to separate the neural correlates of imagined exercise from those involved in more general imagination and visualisation of motor activity, the control task was imagination of freewheeling downhill whilst gently applying the brakes. In the study described in Chapter Three this had been found not to affect $\dot{V}_I$ or HR. Because of concern about the potential for voluntary changes in breathing affecting the responses to imagination of exercise, it was important not to alert subjects to our interest in breathing, but it was also necessary to compare the brain areas activated during volitional hyperventilation with those activated during imagination of exercise; i.e. those involved in an increase in $\dot{V}_I$, as opposed to those involved in an increase in $\dot{V}_I$ associated with imagination of exercise. Consequently two independent groups of subjects were studied, one group performing imagination of exercise and imagination of freewheeling downhill, the other group performing volitional hyperventilation and imagination of freewheeling downhill. In this way no subject performing imagination of exercise had previously been asked to voluntarily hyperventilate.

Given previous studies of the neural activations associated with exercise (Fink *et al.* 1995; Williamson *et al.* 1997) and tasks involving increased respiratory muscle activity (Colebatch *et al.* 1991; Ramsay *et al.* 1993; Fink *et al.* 1995), there were several *a priori* hypotheses regarding the neural correlates of imagination of exercise. It was predicted that superolateral motor cortex would be activated, also the SMA, cerebellum, thalamus, midbrain and insular cortex.

4.2 Methods

This study was carried out with local ethical committee approval (COREC 96.164, IC 98.5311). Permission to administer radioactivity was given by the Administration of Radioactive Substances Advisory Committee, Department of Health (ARSAC RPC 262-425, 10680).

4.2.1 Subject assessment and familiarisation

Subjects participating in this study were assessed for hypnotic susceptibility and given an incremental exercise test as described in Chapter Two. There were nine subjects (age 28±1 years, weight 74±1kg, height 1.79±0.02m, all male). Subjects were assigned to perform either protocols 1 and 3 (n=4, group A), or protocols 2 and 3 (n=5, group B), see below. On a subsequent visit to the laboratory subjects were instrumented with a facemask and ECG (as described below), hypnotised and then performed 3 repeats of the protocols that had been assigned to them. All protocols lasted for 130 seconds and were performed at 6 minute intervals (relative timings illustrated in figure 4.1). Protocols used were protocols 1, 2 and 3 from the study described in Chapter Three:

Protocol 1: Imagination of 'heavy' exercise' (cycling uphill)

Protocol 2: Volitional hyperventilation (tachypnea, no suggestions regarding V_T)

Protocol 3: Imagination of freewheeling downhill whilst putting the brakes on.

The main contrasts of interest would therefore be:

***Group A**, imagination of 'heavy' exercise minus imagination of freewheeling downhill.* Assuming a 'perfect' subtraction, this would result in activations of areas associated with imagination of exercise and the associated increase in $\dot{V}_I$. Imagination of a motor task, the hypnotic state and auditory input should be occurring equally between the two tasks, so no activations should be apparent due to these aspects of the tasks.

***Group B**, volitional hyperventilation minus imagination of freewheeling downhill.* With a perfect subtraction this should result in activation of areas involved with a volitional increase in $\dot{V}_I$ (resulting from an increase in *f* with no change in V_T). Since during volitional hyperventilation the subject is asked to imagine a countryside scene as well as to hyperventilate, the areas involved with imagination *per se* should not be apparent and the same should be true of the hypnotic state and auditory input.

4.2.2 PET scanning

Subjects were instrumented for cardiorespiratory measurements (described in Chapter Two) and a cannula inserted into the left antecubital vein for repeated intravenous injections of

radiotracer. Subjects were hypnotised on the scanning table and the table was moved into the body of the scanner (Siemens CTI-966). The head was positioned to minimise movement and was supported in a stable position. A transmission scan was performed using an external $^{68}Ge/^{68}Ga$ positron source to determine the field distortion occurring due to the subjects head. This was repeated if the subject had moved more than 5 mm during the course of the protocols (one subject), judged according to the localising lines projected over the subject's face (figure 4.2).

Each subject underwent sixteen 155s scans at 6 minute intervals. During these scans subjects performed *either* protocols 1 and 3 (n=4), *or* protocols 2 and 3 (n=5) as described above. Each protocol was performed for 130 seconds, with the $H_2{}^{(15)}0$ infusion (5mCi of $H_2{}^{(15)}0$ *i.v.*) occurring for 20 seconds at the start of the protocol. The scans comprised a 30s background scan, a 35s delay and a 90s scan after tracer arrival in the brain (activation scan). The activation scan was timed to coincide with the steady-state of protocol performance (figure 4.1). Integrated counts for the activation scan were corrected for background activity (from the background scan) giving an estimate of rCBF. No arterial blood samples were taken so no absolute quantification of CBF was possible.

Cardiorespiratory measurements

A facemask with pneumotachometer and an ECG were used to measure breathing and HR respectively (described in Chapter Two). In order to minimise the potential confounding effects of hypocapnia on global cerebral blood flow (Kety & Schmidt 1948), CO_2 was added to the inspired air to maintain isocapnia during protocols 1 and 2. This had been shown (Chapter Three) not to alter the magnitude of responses to either imagination of exercise or volitional hyperventilation. Cardiorespiratory data were acquired continuously at 200 Hz (Biopac MP100WS) and recorded on a Macintosh computer using Acqknowledge software for subsequent analysis.

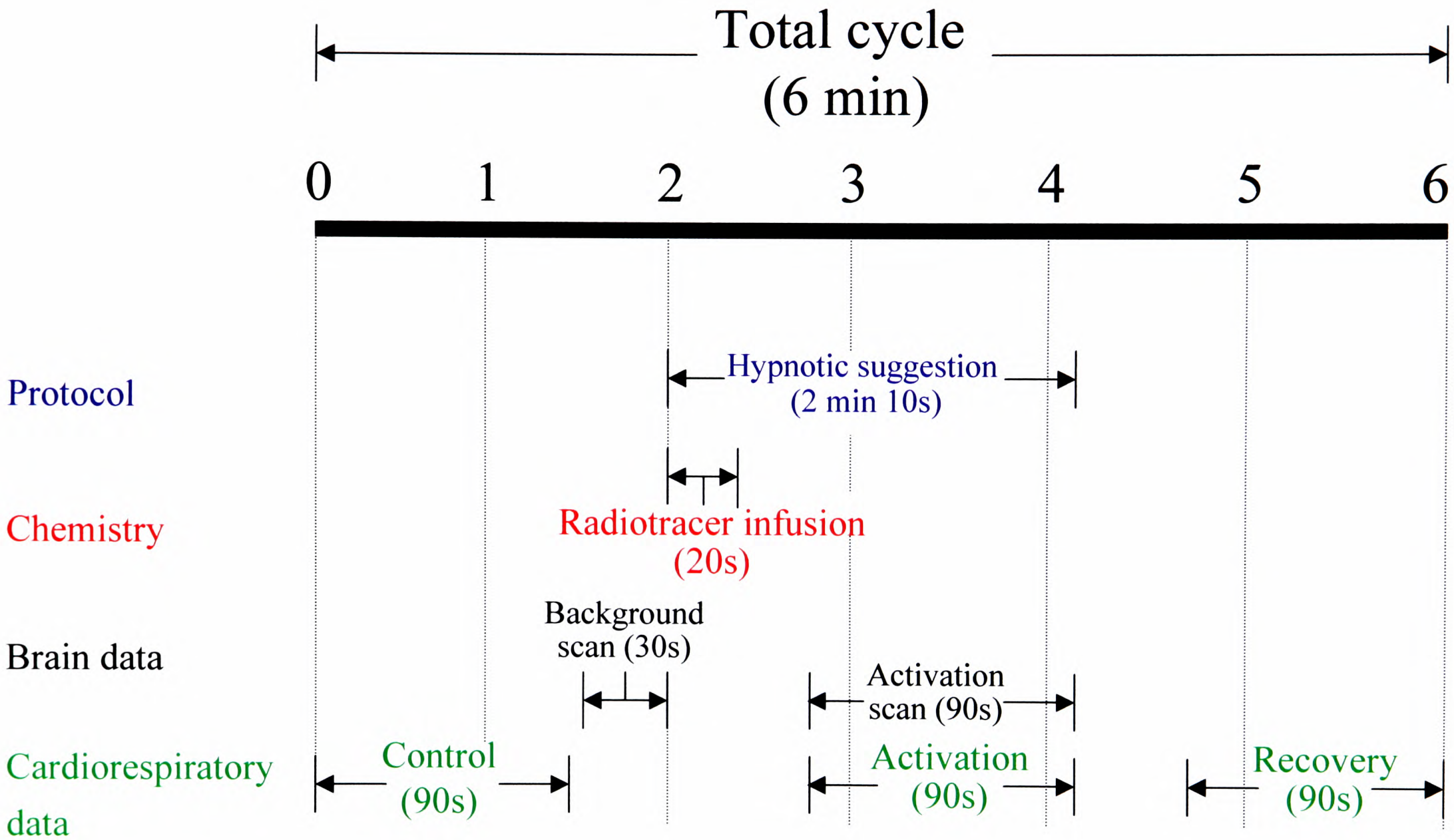
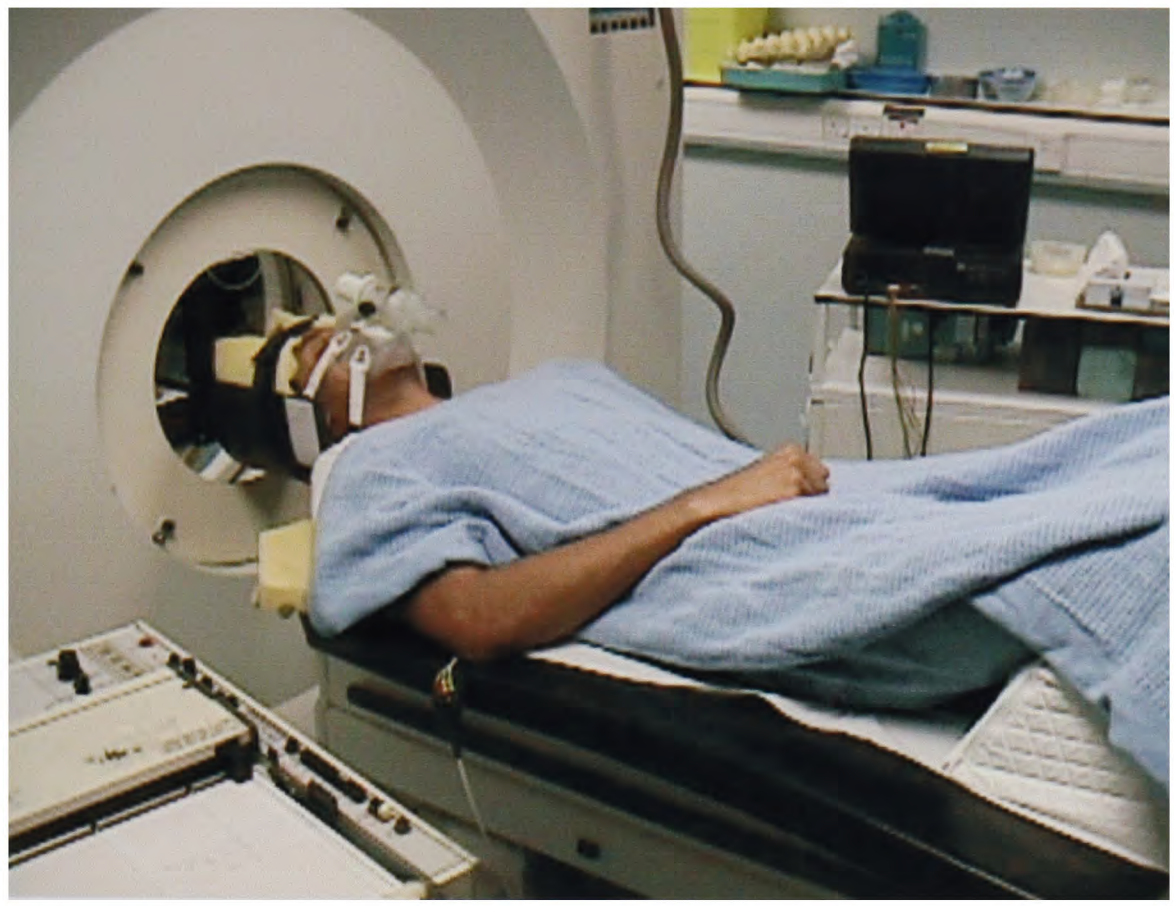


Figure 4.1. Schematic showing the co-ordination between the infusions of radioacative water, the hypnotic suggestion, the brain data and the cardiorespiratory data. The background scan is used to determine the baseline radioactivity in the brain. This is subtracted from the activation scan which is timed to occur when protocol performance has reached steady-state.

A)



B)

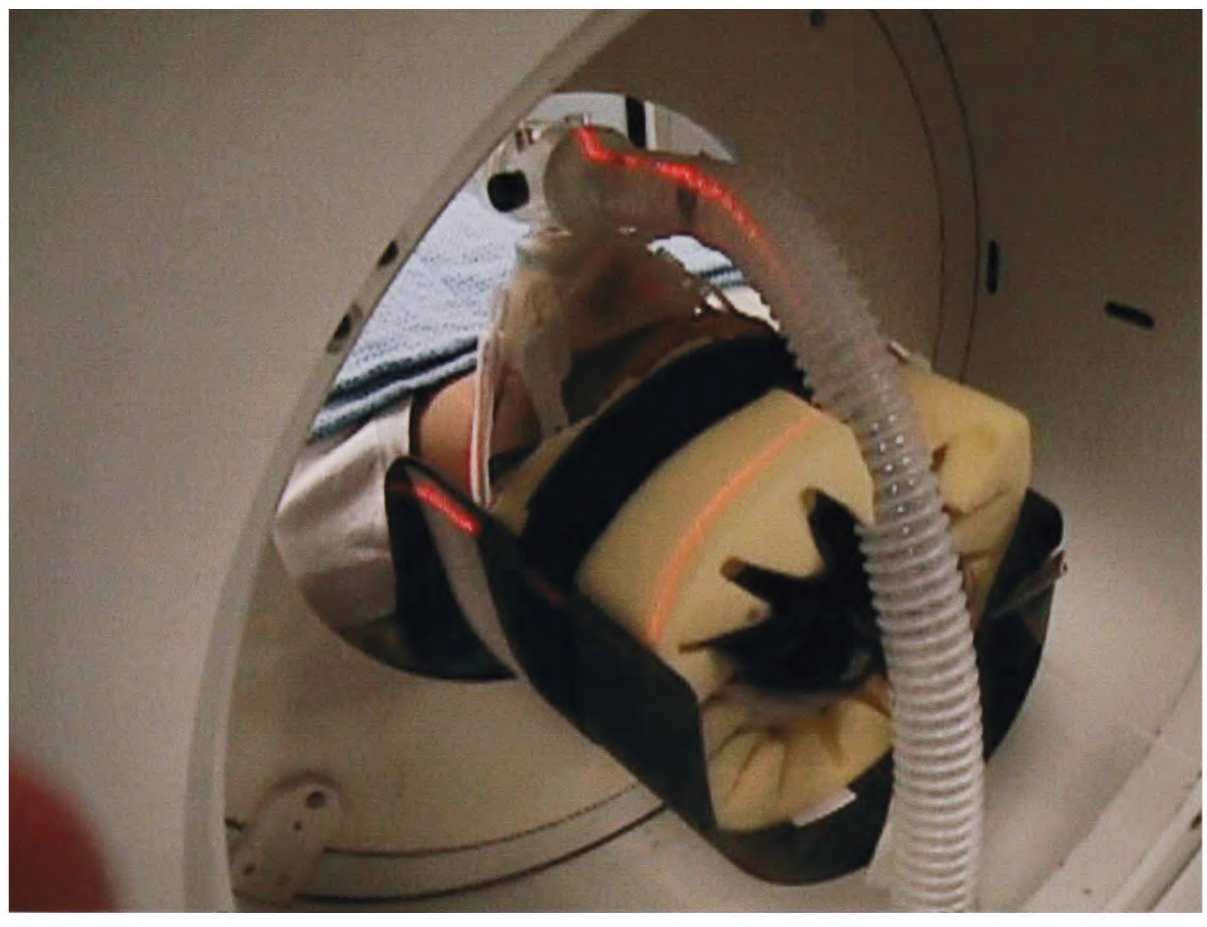


Figure 4.2. A) Subject lying hypnotised on the scanning table. B) Volunteer in PET scanner (instrumented with a facemask and inspired air tube) with head restraints. The red lines projecting over the subjects head are to aid localisation; lines are drawn on the subjects head corresponding with these lines, enabling rapid assessment of any head movement. The metal ring bolted to the scanner (visible in both A and B) is a lead screen to minimise the effects of radiotracer from the rest of the body influencing the head counts.

4.2.3 Statistical analysis

Cardiorespiratory data

Mean ± S.E.M of $\dot{V}_I$, *f*, V_T, $P_{ET}CO_2$ and HR were averaged from 120 to 30 s prior to the protocol onset (control), 40 to 130 s (activation), 270 to 360 s (recovery) as indicated in figure 4.1. Data for each protocol were pooled and control, activation and recovery compared using repeated measures ANOVA and a *post-hoc* Tukey test.

Brain images

Brain data were analysed using statistical parametric mapping (SPM96/99), the theoretical basis of which is described in Chapter Two. In brief, images from each subject were reconstructed, realigned, normalised into standard stereotaxic space and filtered in three dimensions with a 10mm FWHM isotropic Gaussian kernel filter. ANOVA were performed independently at each voxel using proportional scaling to correct for changes in global CBF (discussed in the Appendix). F maps were constructed and Z scores and t tests calculated. Data are presented with p values both corrected and uncorrected for multiple comparisons. The template for normalisation was the 'MNI brain' (an average of 305 brains from the Montreal Neurological Institute) which uses the stereotaxic convention of Talairach & Tournoux (1988). Coordinates were therefore corrected (Meyer-Lindenberg 1998) to the 'Talairach' brain (which is the template used in SPM95) in order to facilitate comparison with previous studies. Coordinates in 'MNI space' are given in the Appendix.

Statistical significance is a contentious issue in neuroimaging research. Areas of activation surviving correction for multiple comparisons are indicated and the convention in this thesis is to consider any activation with $p<0.001$ (uncorrected) ($Z>3.09$) as significant (Friston *et al.* 1996). Areas with $0.001<p<0.01$ (Z 2.33-3.09) are also considered, since if an *a priori* hypothesis exists about the region these activations might be considered significant. Statistical issues are discussed further in section 4.4.3.1. Activations surviving correction for multiple

comparisons at the cluster level (as opposed to the voxel level) are also indicated where appropriate[1]. Results are presented in neurological convention (right is right).

4.3 Results

One subject in group B became anxious during scanning so did not complete the study and was excluded from the analysis.

4.3.1 Subjects' descriptions

Subjects' general descriptions of the experience were similar to those documented in Chapter Three. Four subjects felt 'less hypnotised' than when performing the protocols in the lab, two subjects felt 'deeper' and two didn't notice any difference. There was no consistent change in subjects' feelings of hypnotic 'depth' over the course of the scanning procedure, although four subjects reported boredom and a lack of concentration as the experiment progressed. One subject experienced pain related to infusion of the radiotracer.

4.3.2 Cardiorespiratory data

4.3.2.1 Imagination of exercise (protocol 1)

Imagination of exercise under hypnosis resulted in hyperventilation and an increase in HR (figure 4.3, 4.4, table 4.1). The magnitude of this hyperventilation occurred via an increase in *f*; there was a small but significant fall in V_T. Despite addition of CO_2 to the inspired air, $P_{ET}CO_2$ fell slightly during imagination of exercise. These findings were repeatable within an individual subject across the 8 repeats (figure 4.3); the coefficient of variation within an individual during imagination of exercise was 9%.

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[1] Areas of increased blood flow are considered to be significant because of their intensity and spatial extent (Friston *et al.* 1994). Significance at the voxel level implies that the voxel intensity is higher than would be predicted by chance. Significance at the cluster level implies that whilst the voxel intensity is not so high, more voxels in the region show increased activity than would be expected by chance.

4.3.2.2 Volitional hyperventilation (protocol 2)

As intended during volitional hyperventilation the increase in *f* was similar (p=0.08, unpaired t-test) to that seen during imagination of exercise (table 4.1). There was no significant change in HR, $P_{ET}CO_2$ or V_T. The increases in $\dot{V}_I$ were significantly smaller than those seen during imagination of exercise (figure 4.5, 4.6).

4.3.2.3 Imagination of freewheeling downhill (protocol 3)

Cardiorespiratory variables did not change during imagination of freewheeling down a hill in either group, with the exception of a slight increase in $P_{ET}CO_2$ in group B subjects (figures 4.3-4.6, table 4.1).

4.3.3 rCBF data

The last 3 scans from one of the group B subjects in which radiotracer infusion was not synchronised with the activation period were excluded from the analysis. The remaining 128 scans (16 for each subject, 8 for each condition, with the exception of one of the group B subjects) were entered into a single design matrix using a 'multistudy, conditions only' design. Groups A and B were considered as two separate studies within this matrix (the design matrix is illustrated in the Appendix), there were two conditions within each study (corresponding to the 2 protocols).

4.3.3.1 Imagination of exercise (compared to imagined cycling downhill)

The areas of significant increase in relative rCBF ($p<0.01$ uncorrected) associated with imagination of exercise in 4 subjects are shown in figure 4.7 as through-projections on a 'glass' brain and the coordinates of the maximal activations within these regions are given in table 4.2. Two groups of activations remained significant at the *cluster* level after multiple comparisons; a region including the superolateral motor cortex and SMA (p=0.045 corrected) and a region incorporating the right lateral sensorimotor cortex, superior temporal and premotor area (p=0.039 corrected). Two activations were significant at the *voxel* level after correction for multiple comparisons; one in the right SMA (Z=5) and one in the right middle frontal gyrus (Z=4.8). The supplementary motor area (SMA) bilaterally, temporal-parietal

junction (right side), premotor area (PMA), cerebellum (bilaterally), insular cortex (left), lateral sensorimotor areas, thalamus, superolateral somatomotor cortex bilaterally, parietal cortex and the parahippocampal gyrus were also activated. These activations are illustrated in figures 4.8 and 4.9 on a rendered[1] brain. Individual responses in some of these areas are given in the adjusted response plots of figure 4.14. Given the fall in $P_{ET}CO_2$ during imagination of exercise the relationship between $P_{ET}CO_2$ and the estimated global CBF was examined (figure 4.10). Correlation between the two was poor (average r^2=0.18), although it was significantly different from 0 (p=0.03, paired t-test).

A second analysis was performed on the data in Group A subjects investigating the correlates between total $\dot{V}_I$ and rCBF. This was a 'covariates only' analysis, in which both imagination of exercise and imagination of freewheeling downhill were included and activations associated with an increase in $\dot{V}_I$ were plotted without regard for the condition (i.e. no distinction was made between imagination of exercise and imagination of freewheeling downhill). This was therefore a parametric design with the parameter of interest being $\dot{V}_I$. This allowed a plot to be constructed of the correlation between the intensity of a given voxel and $\dot{V}_I$. These are illustrated in figures 4.11 and 4.12 for the activations that were significantly correlated to $\dot{V}_I$ when corrected for multiple comparisons. These were the right PMA (Z=5.8), the right middle frontal gyrus (Z=5.3), the right temporal pole (Z=5.2) and the left posterior cerebellum (Z=4.8). Coordinates of these activations are given in the figure legends.

4.3.3.2 Volitional hyperventilation (compared to imagined cycling downhill)

The areas of significant increase in relative rCBF (p<0.01 uncorrected) associated with volitional hyperventilation averaged across the group are shown in figures 4.13-4.15 and table 4.3. Two regions remained significantly activated at the *cluster* level after correction for multiple comparisons; the left (p=0.001) and right (p=0.022) lateral sensorimotor cortices.

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[1] These views are of a 'representative' brain from the dataset of 305 brains provided by the Montreal Neurological Institute. Any activation within 2cm of the brain surface is represented, this can lead to misleading 'surface' activations, so the figures should be considered in parallel with the table detailing coordinates.

	$\dot{V}_I$ (l min^{-1})	*f* (breaths min^{-1})	V_T (litres)	$P_{ET}CO_2$ (Torr)	HR (bpm)
Group A					
Control	10±0.8	15±0.5	0.7±0.1	42.2±1.3	63±2
Imagined downhill (protocol 2)	10.4±0.7	15.1±0.5	0.7±0.1	42.6±1.3	62±2
Control	10.8±0.5	15.1±0.6	0.8±0.1	42.2±1.4	64±3
Imagined exercise (protocol 1)	19.3±2 *	28.5±2.8 *	0.7±0.1 *	38.9±1.8 *	71±5 *
Group B					
Control	9±0.3	14.7±2.1	0.7±0.1	42.8±1.3	59±5
Imagined downhill (protocol 2)	9.2±0.3	15.8±2.7	0.7±0.1	44±1.3 *	59±5
Control	8.8±0.2	14±2.2	0.7±0.1	42.9±1.4	59±5
Volitional hyperventilation (protocol 3)	13.6±1.5 * †	26.2±3.6 *	0.6±0.1 †	41.8±1.1 †	60±5 †

Table 4.1. Cardiorespiratory data for protocols 1, 2 and 3 performed under hypnosis. * signifies $p<0.01$ compared to control value, repeated measures ANOVA, followed by *post hoc* Tukey test. † indicates $p<0.01$ compared to value during imagined exercise, unpaired t-test.

Table 4.2. Coordinates (in standard stereotactic space; Talairach & Tournoux, 1988) refer to maximally activated foci during **imagination of exercise** under hypnosis (compared to imagination of freewheeling downhill) as indicated by the highest Z value within an area of activation. x, distance (mm) to right (+) or left (-) of the mid-saggital line; y, distance anterior (+) or posterior (-) to a vertical plane drawn through the anterior commissure; z, distance above (+) or below (-) the intercommissural (AC-PC) line. Numbers in brackets refer to Brodmann Areas where activations could be clearly attributed. P values both corrected for multiple comparisons (C) and uncorrected (NC) are indicated. To aid statistical interpretation the 'traffic light' convention has been used; **green** = survives correction for multiple comparisons, **amber** = $p<0.001$ uncorrected ($Z>3.1$), **red** = $p<0.01$ uncorrected ($3.1>Z>2.3$). Anatomical locations were assigned to coordinates using the Talairach Daemon and confirmed with the assistance of a neuroanatomist using the canonical MRI template supplied with SPM96.

Region	*Left*						*Right*					
	x	y	z	Z	P (C)	P (NC)	x	y	z	Z	P (C)	P (NC)
Supplementary motor area (6)	-8	-21	69	3.4	0.991	0.000	**4**	**-9**	**64**	**5**	0.011	0.000
							8	12	62	3.6	0.933	0.000
Ventral premotor area (6)							59	3	19	4.4	0.192	0.000
Middle frontal gyrus (9/46)							**43**	**24**	**36**	**4.8**	0.039	0.000
							43	35	24	3.4	0.989	0.000
							38	22	45	3.3	0.999	0.001
Inferior frontal gyrus/temporal pole							27	26	-22	3.2	1	0.001
Lateral sensorimotor							61	-11	20	4.1	0.368	0.000
Sensorimotor	-43	-15	39	3.4	0.992	0.000	43	-9	52	3.3	0.999	0.001
	-41	-13	54	3.4	0.992	0.000						
Cerebellum: Anterior/lateral	-24	-60	-24	3.5	0.976	0.000	22	-60	-26	4.2	0.301	0.000
Anterior/medial	-12	-50	-27	4.2	0.311	0.000						
Posterior/medial	**-15**	**-62**	**-28**	**3**	1	0.001	**15**	**-46**	**-36**	**2.5**	1	0.007
	-13	**-38**	**-46**	**2.9**	1	0.002						
Posterior/lateral	-29	-38	-44	3.2	0.98	0.001						
	-26	**-48**	**-34**	**2.6**	1	0.005						
Superolateral sensorimotor	-17	-34	56	3.5	0.985	0.000	18	-29	63	4	0.517	0.000
Parietal lobe (precuneus) (7)	-10	-36	45	3.4	0.991	0.000						
Left parietal cortex	-13	-46	26	3.6	0.961	0.000						
Temporal/parietal junction							62	-40	19	4.6	0.068	0.000
Parahippocampal gyrus							32	-38	-8	3.7	0.84	0.000
							27	-48	3	3.7	0.889	0.000
Insular cortex	-47	-11	17	3.4	1	0.001						
	-38	-15	17	3.2	1	0.000						
Thalamus/fornix							1	-9	15	4.1	0.44	0.000
Tail of caudate/fornix	**-20**	**-40**	**8**	**3**	1	0.001						
Putamen							24	1	5	3.2	1	0.001
Body of caudate							20	-1	19	3.2	1	0.001
							18	**-9**	**24**	**2.9**	1	0.002

Region	*Left*						*Right*					
	x	y	z	Z	P (C)	P (NC)	x	y	z	Z	P (C)	P (NC)
Supplementary motor area (6)	-4	-11	54	4.7	0.057	0.000						
Superolateral sensorimotor	**-4**	**-25**	**57**	**2.6**	1	0.005						
Posterior temporal/parietal	-45	-34	12	4.7	0.06	0.000						
Lateral sensorimotor	**-48**	**-5**	**19**	**7**	0.001	0.000	43	-19	36	3.9	0.658	0.000
	-56	**-15**	**15**	**4.8**	0.028	0.000	**55**	**-5**	**21**	**5.1**	0.008	0.000
Cerebellum	-38	-63	-41	3.9	0.71	0.000	11	-69	-48	3.2	1	0.001
	-29	**-73**	**-48**	**2.4**	1	0.008						
	-8	-63	-28	3.4	0.989	0.000						
Paracentral gyrus	-10	-13	45	3.5	0.98	0.000						
Insular cortex/operculum							47	-7	6	3.6	0.955	0.000
							27	-23	21	3.2	1	0.001
Occipital cortex (lingual gyrus)	**-19**	**-77**	**3**	**3**	1	0.001						
Thalamus	-8	-9	10	3.5	0.987	0.000						
Putamen							22	3	9	3.8	0.765	0.000
							25	-5	8	3.5	0.986	0.000
							34	-15	8	3.2	1	0.001
Pallidum	-22	-9	-4	3.2	1	0.001						
Pons/medulla	-8	-32	-34	3.9	0.693	0.000						

Table 4.3. Coordinates (standard stereotactic space; Talairach & Tournoux, 1988) refer to maximally activated foci during **volitional hyperventilation** (tachypnea) under hypnosis (compared to imagination of freewheeling downhill), indicated by the highest Z value within an area of activation. Details as for table 4.2.

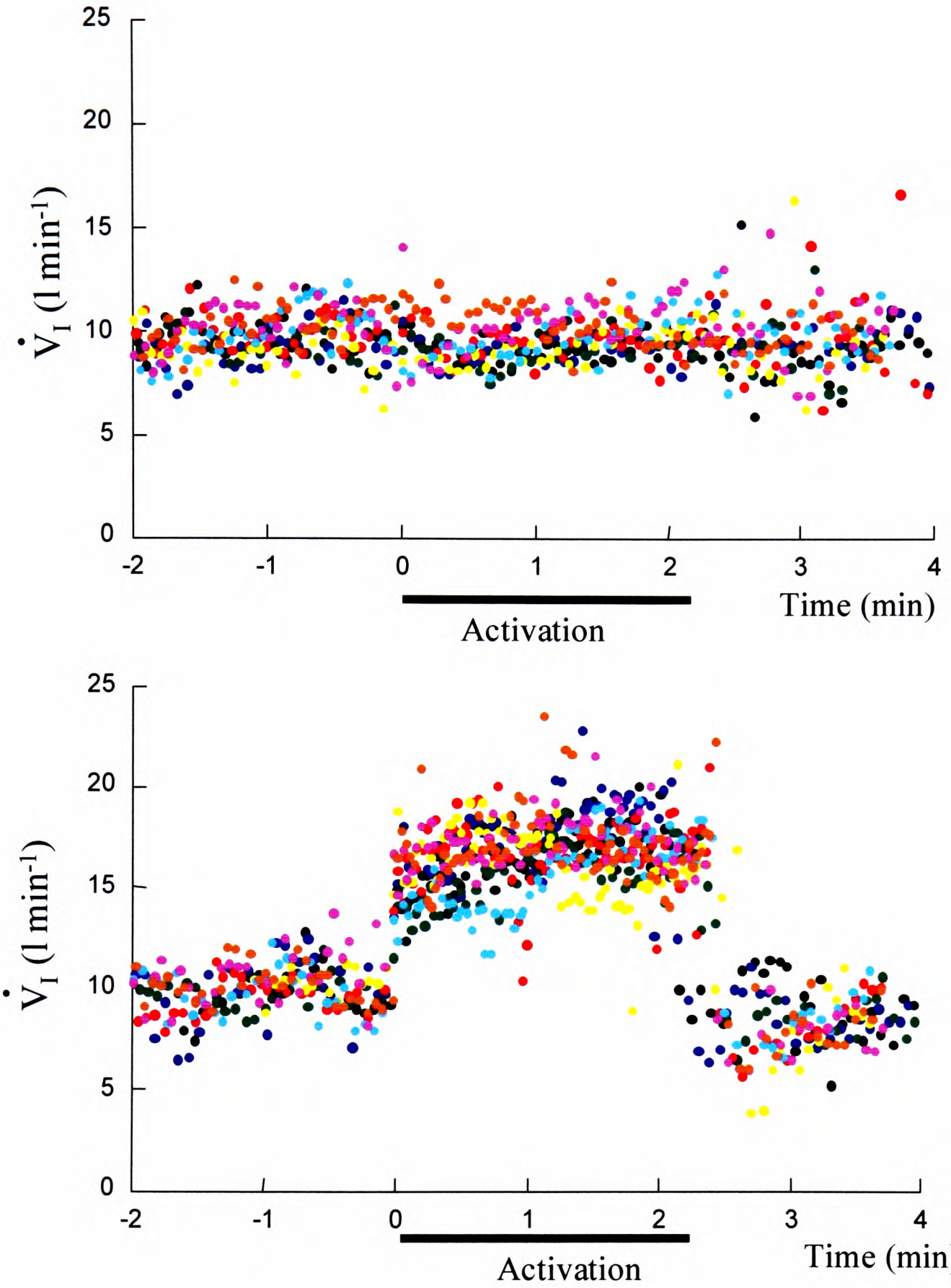


Figure 4.3. Breath-by-breath data for imagination of freewheeling downhill (top panel) and imagination of exercise (bottom panel) in one subject. Each point represents a single breath. The 8 colours represent each repeat of the protocol.

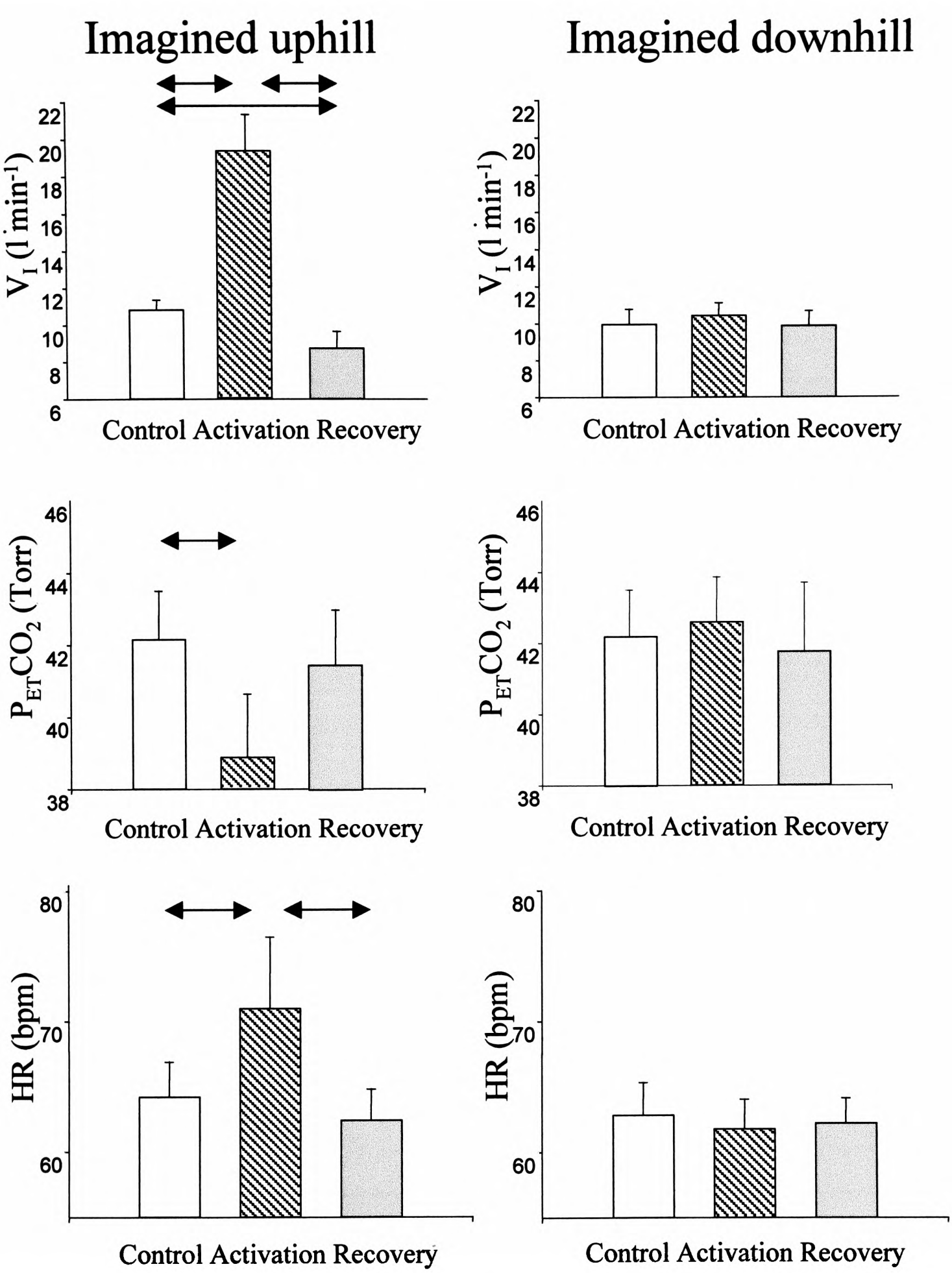


Figure 4.4: Cardiorespiratory responses to imagination of exercise and imagined freewheeling downhill in group A subjects. $\dot{V}_I$ (top), $P_{ET}CO_2$ (middle) and HR (bottom) during the control period (-120 to -30s), the scanning period (40 to 130s) and the recovery period (270 to 360s) during protocol 1 (left) and protocol 3 (right). Arrows indicate significance (repeated measures ANOVA, followed by *post hoc* Tukey test, $p<0.01$). Mean ± SEM. n=4.

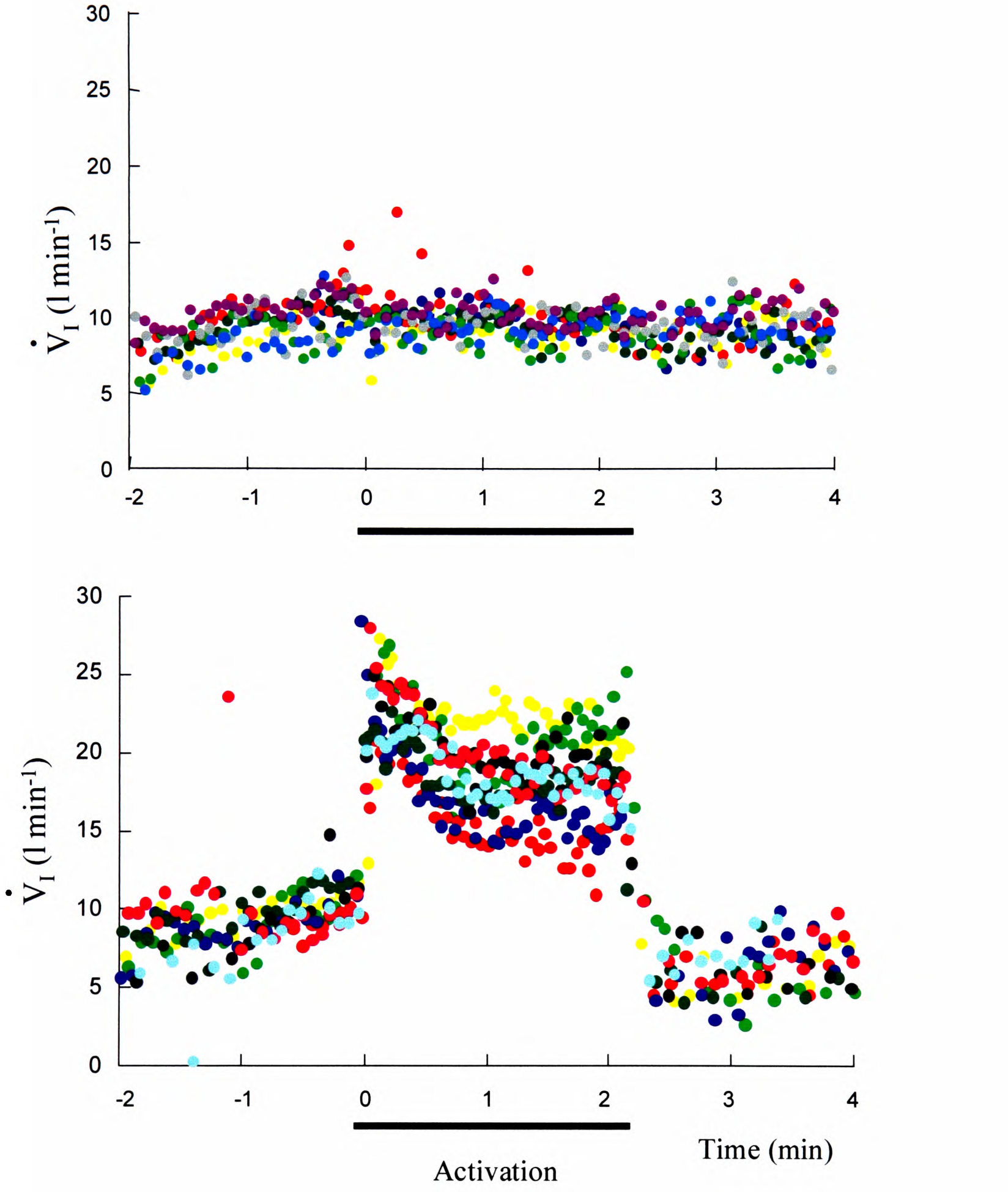


Figure 4.5. Breath-by-breath data for imagination of freewheeling downhill (top panel) and volitional hyperventilation (bottom panel) in one subject. Each point represents a single breath. The 8 colours represent each repeat of the protocol.

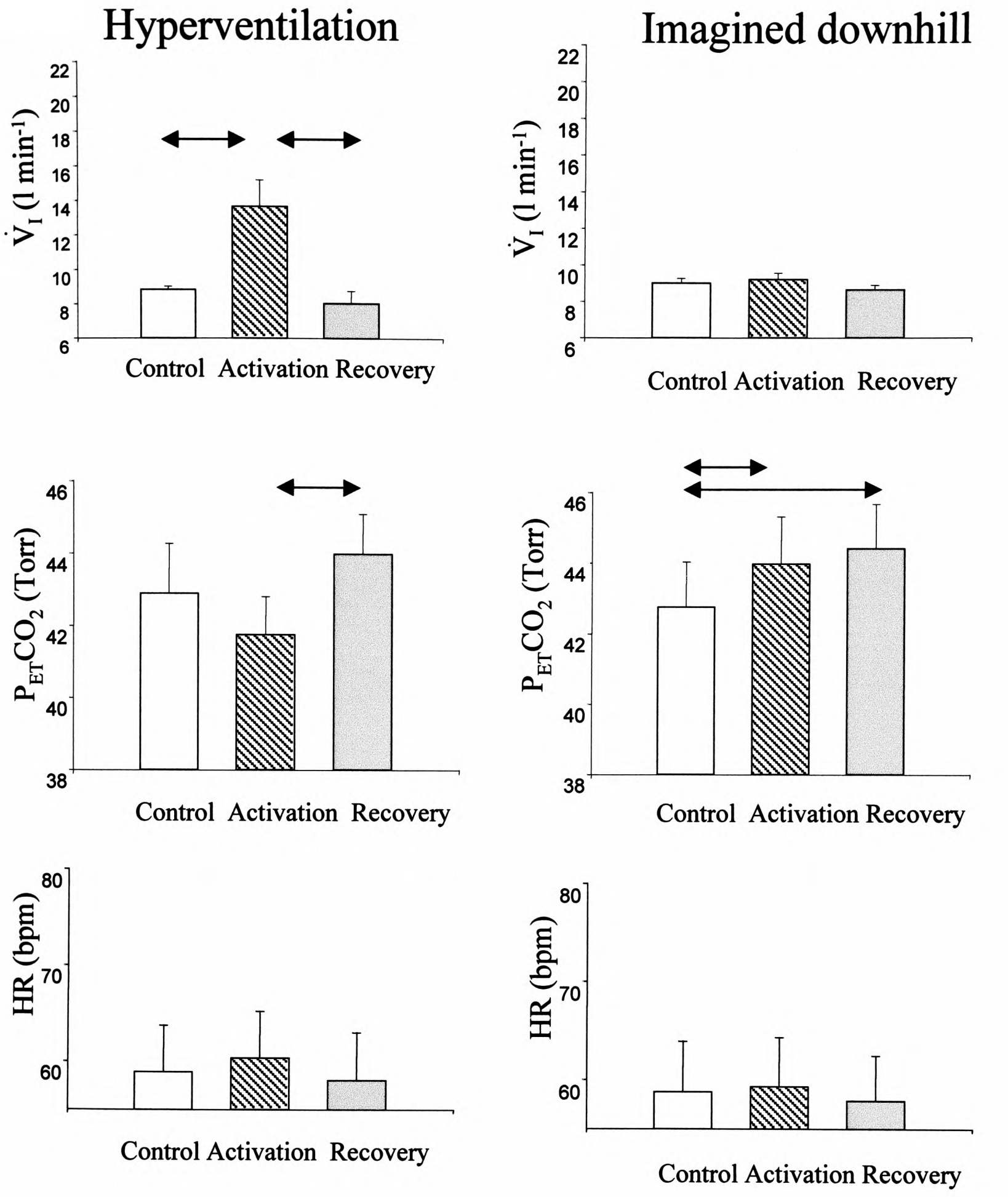


Figure 4.6. Cardiorespiratory responses to volitional hyperventilation and imagined freewheeling downhill in group B subjects. $\dot{V}_I$ (top), $P_{ET}CO_2$ (middle) and HR (bottom) during the control period (-120 to -30s), the scanning period (40 to 130s) and the recovery period (270 to 360s) during protocol 2 (left) and protocol 3 (right). Arrows indicate significance (repeated measures ANOVA, followed by *post hoc* Tukey test, $p<0.01$). Mean ± SEM. n=4.

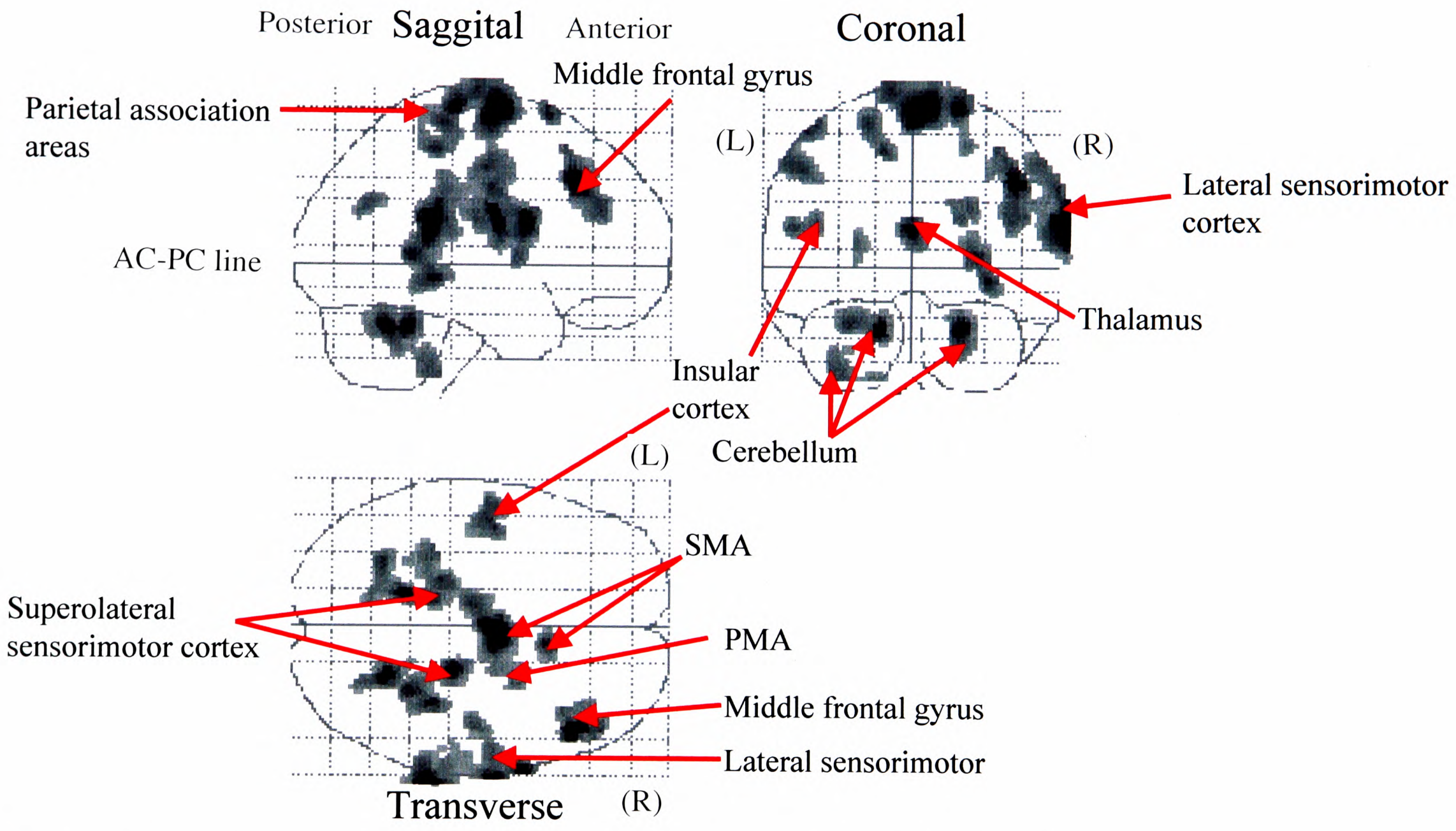


Figure 4.7. Main activations during imagination of exercise compared to imagined freewheeling downhill under hypnosis shown as through projections in standard stereotactic (MNI) space. Abbreviations; L left, R right, AC anterior commissure, PC posterior commissure.

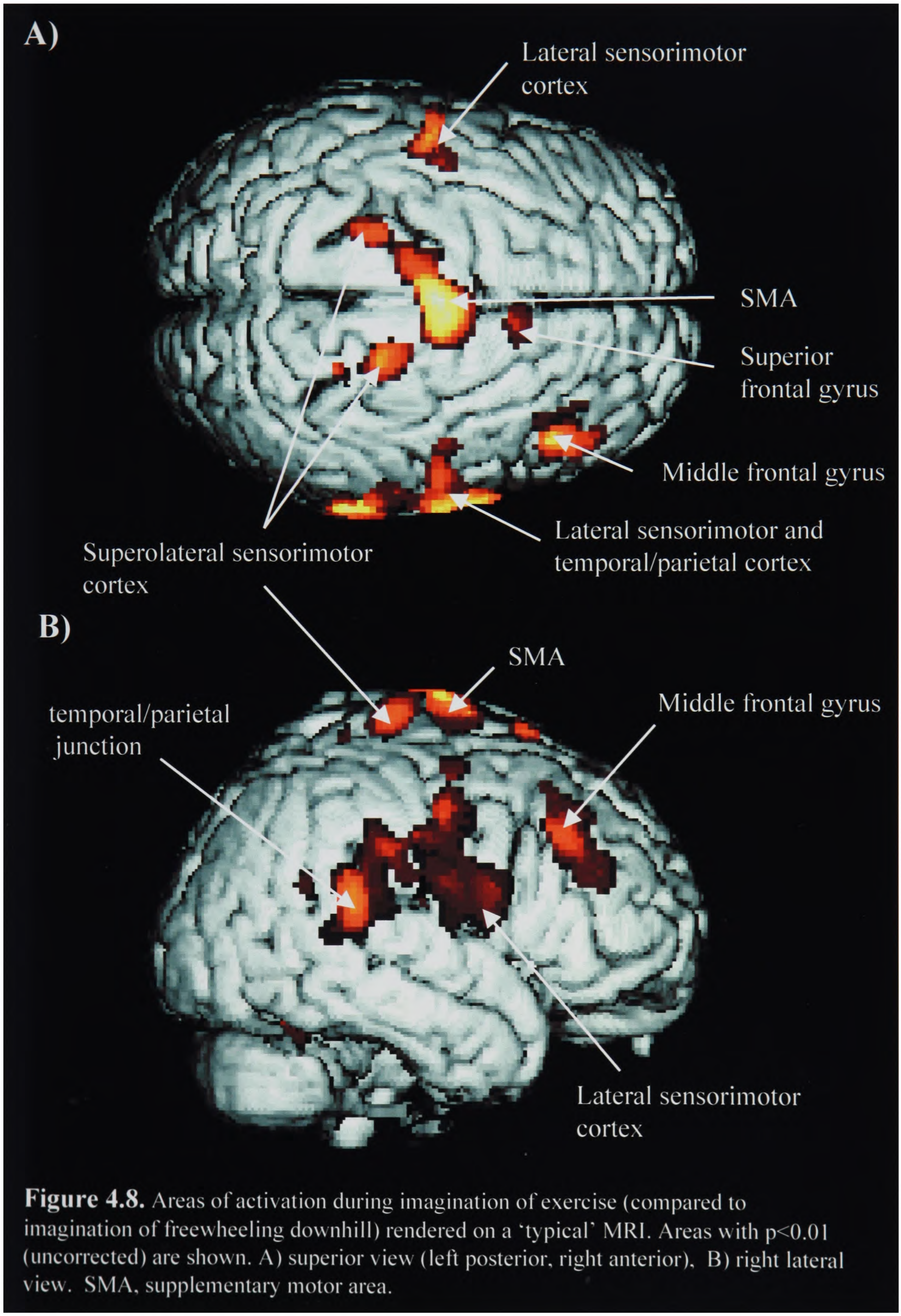


Figure 4.8. Areas of activation during imagination of exercise (compared to imagination of freewheeling downhill) rendered on a 'typical' MRI. Areas with $p<0.01$ (uncorrected) are shown. A) superior view (left posterior, right anterior), B) right lateral view. SMA, supplementary motor area.

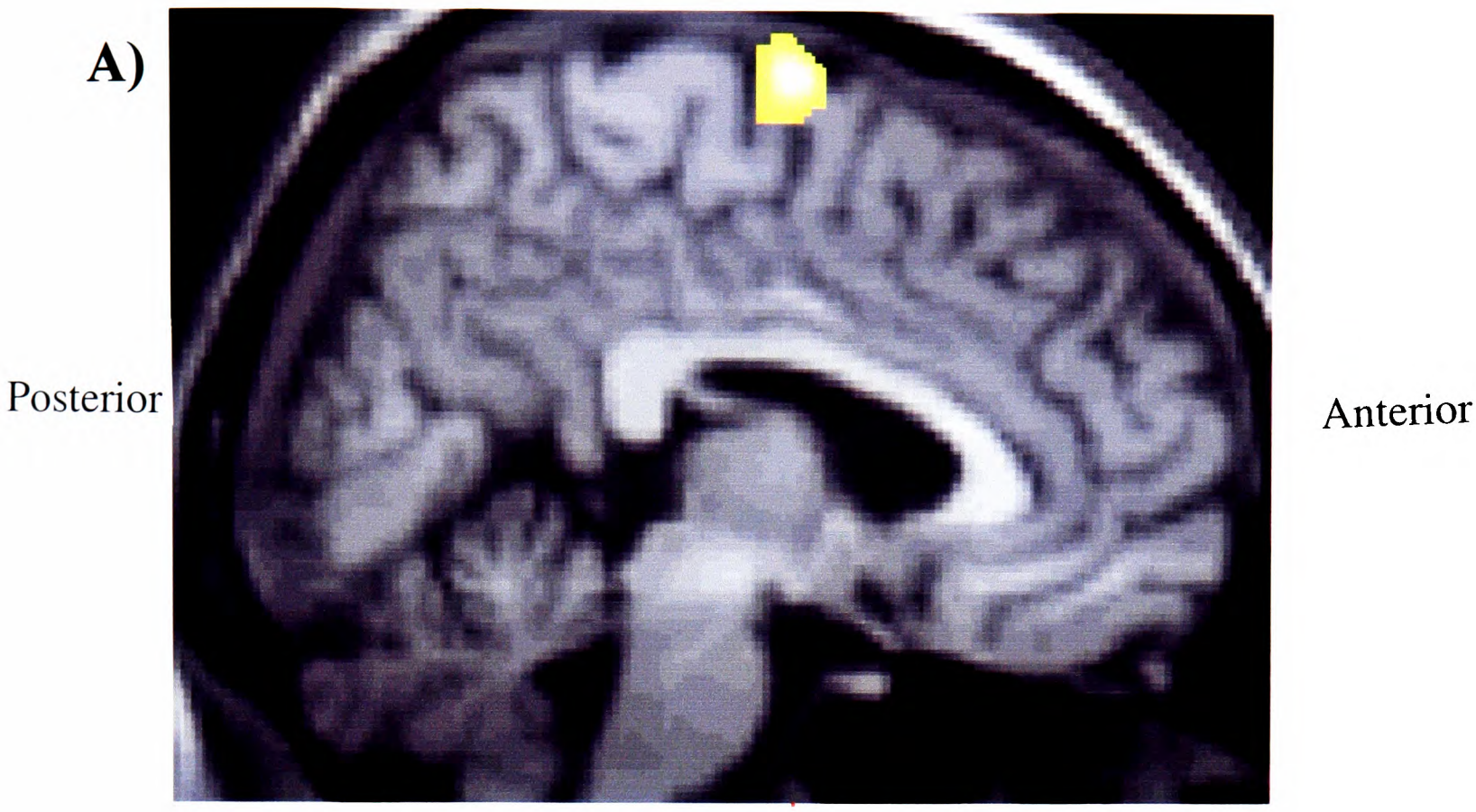


Figure 4.9. Areas of activation during imagination of exercise (compared to imagination of freewheeling downhill) overlayed on a 'typical' MRI. Areas with $p<0.01$ (uncorrected) are shown. A) saggital section (x=-8) showing activation of the SMA. B) transverse slice (z=-28) showing bilateral activation of the cerebellum.

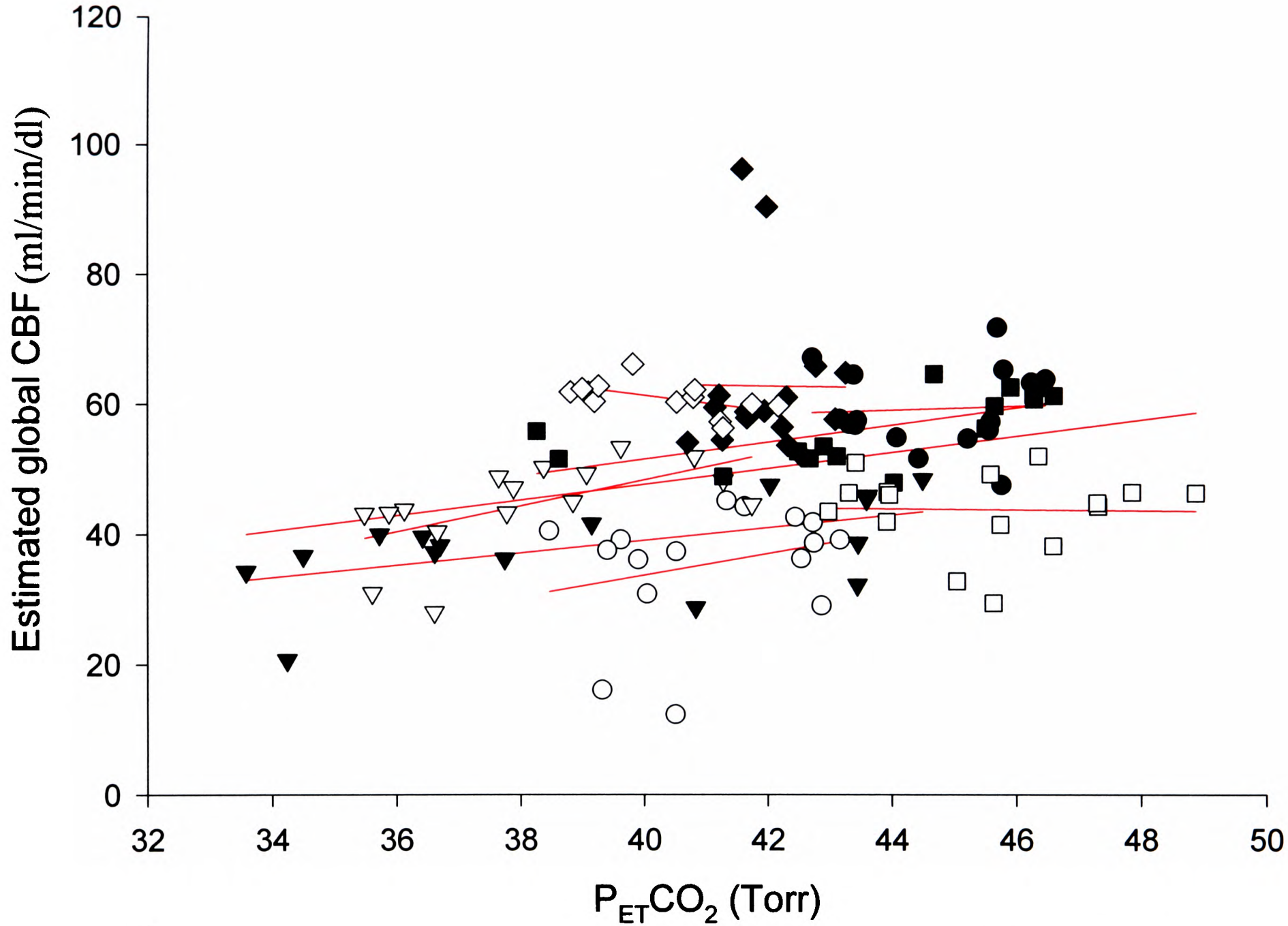
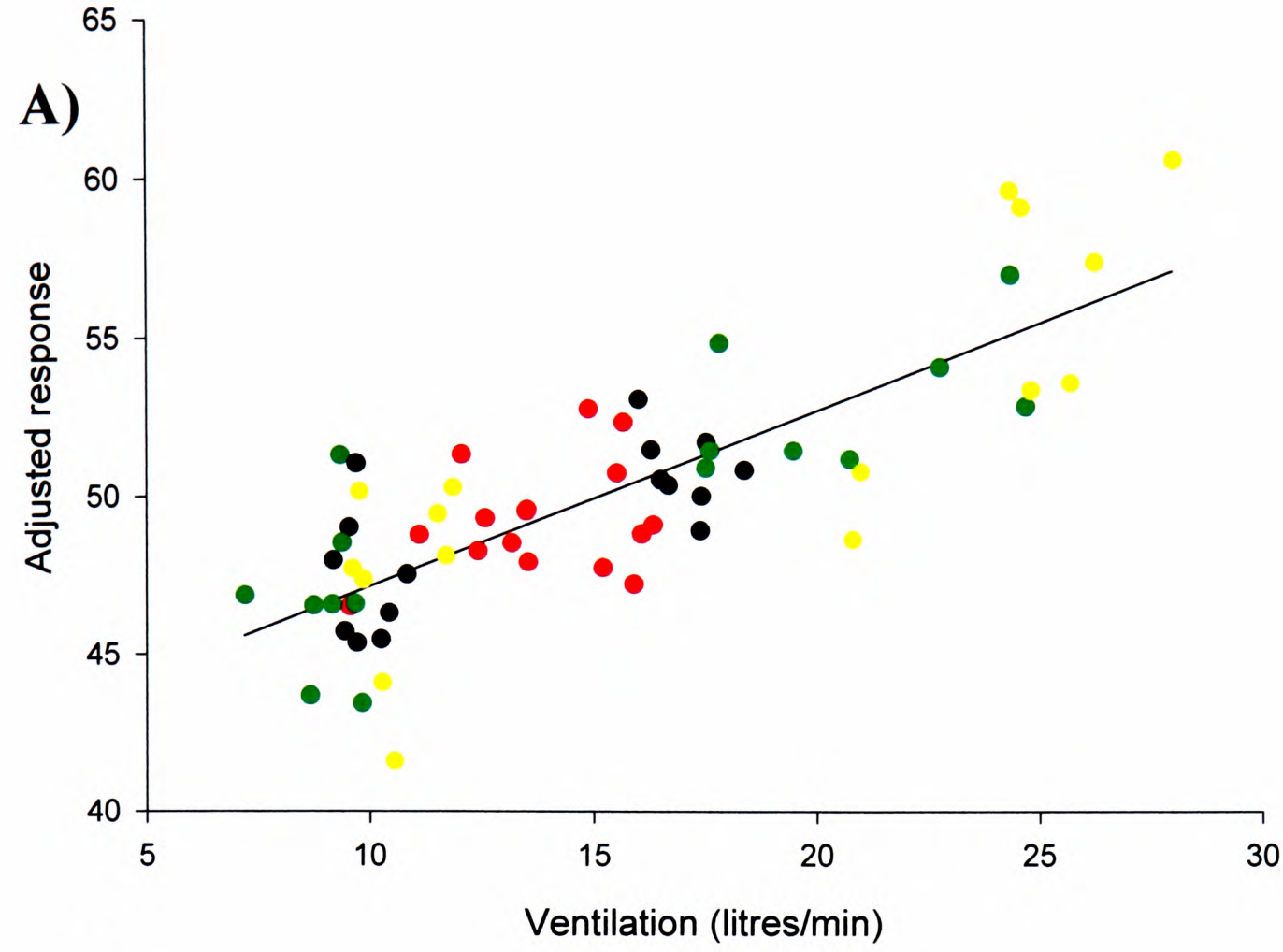


Figure 4.10 The relationship between $P_{ET}CO_2$ and estimated global CBF in the 4 subjects in group A. There were significant falls in $P_{ET}CO_2$ during imagination of exercise, but there was no clear correlation between $P_{ET}CO_2$ and blood flow (goodness or fit of regression coefficient, r^2 averaged 0.18). Regression lines are drawn through the values of each set of 8 repeats for each of the 4 subjects (ie. 8 repeats of protocol 1 and 8 repeats of protocol 3 for each subject).



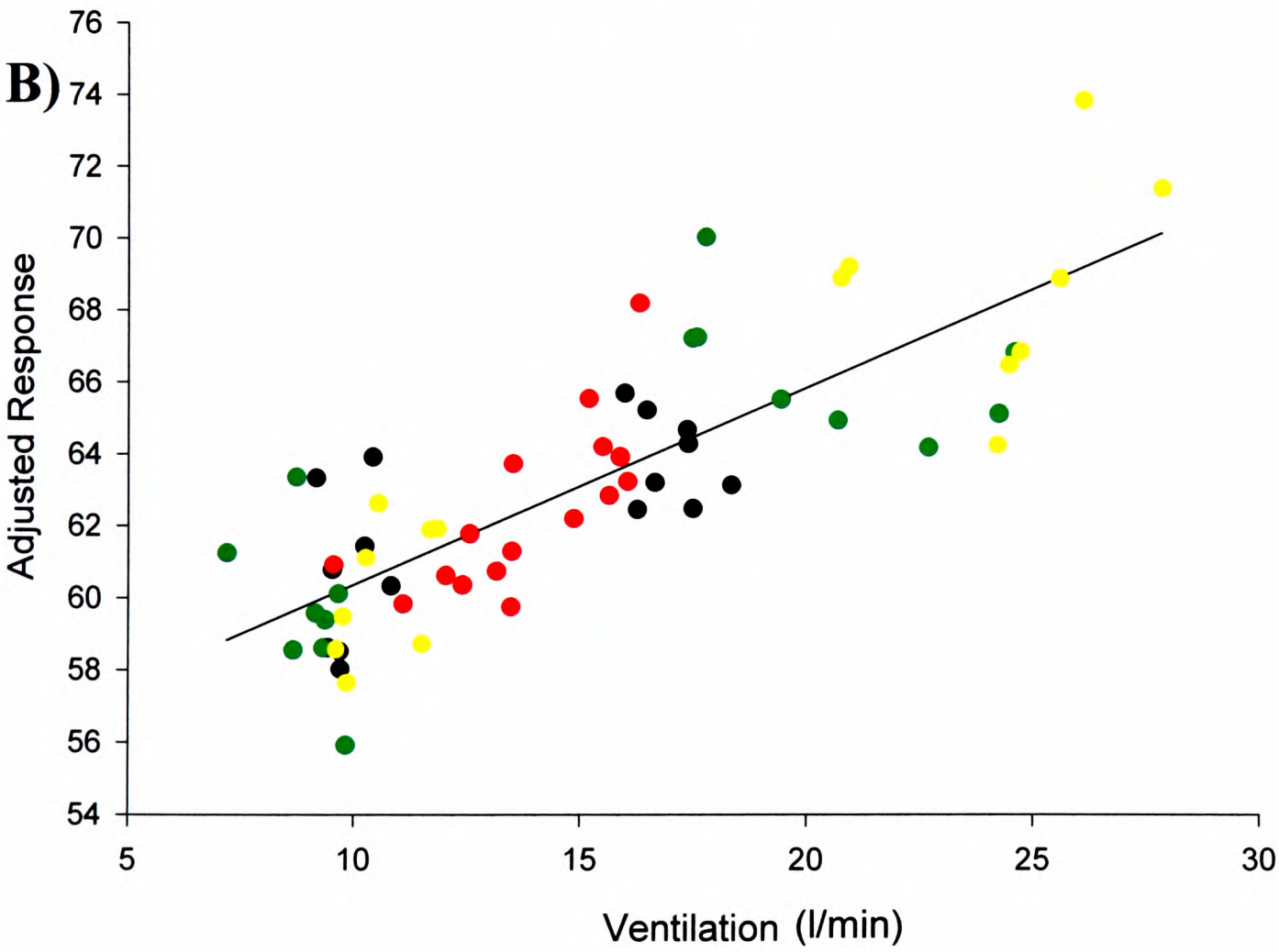
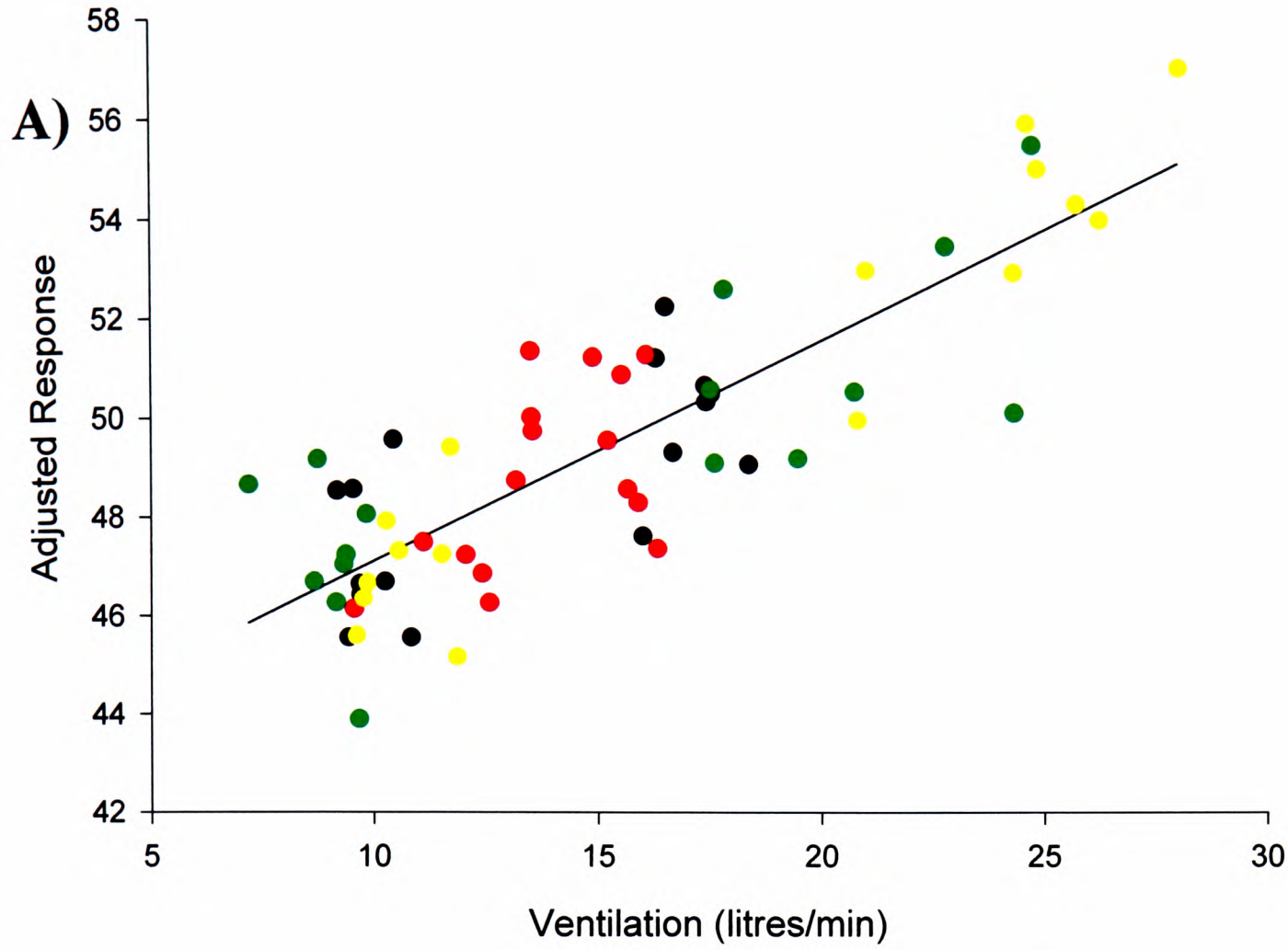


Figure 4.11. Correlation between $\dot{V}_I$ and voxel intensity (adjusted to a global mean CBF of 50ml/min/dl) across all group A subjects for both protocols (imagination of exercise and imagination of freewheeling downhill). Each colour represents a different subject. The comparison was performed for the voxel that was maximally activated in the region. A) temporal pole (Z=5.2, coordinates 50 14 -22, r^2 =0.7), B) dorsolateral prefrontal cortex (Z=5.3, coordinates 43 35 19, r^2=0.7).



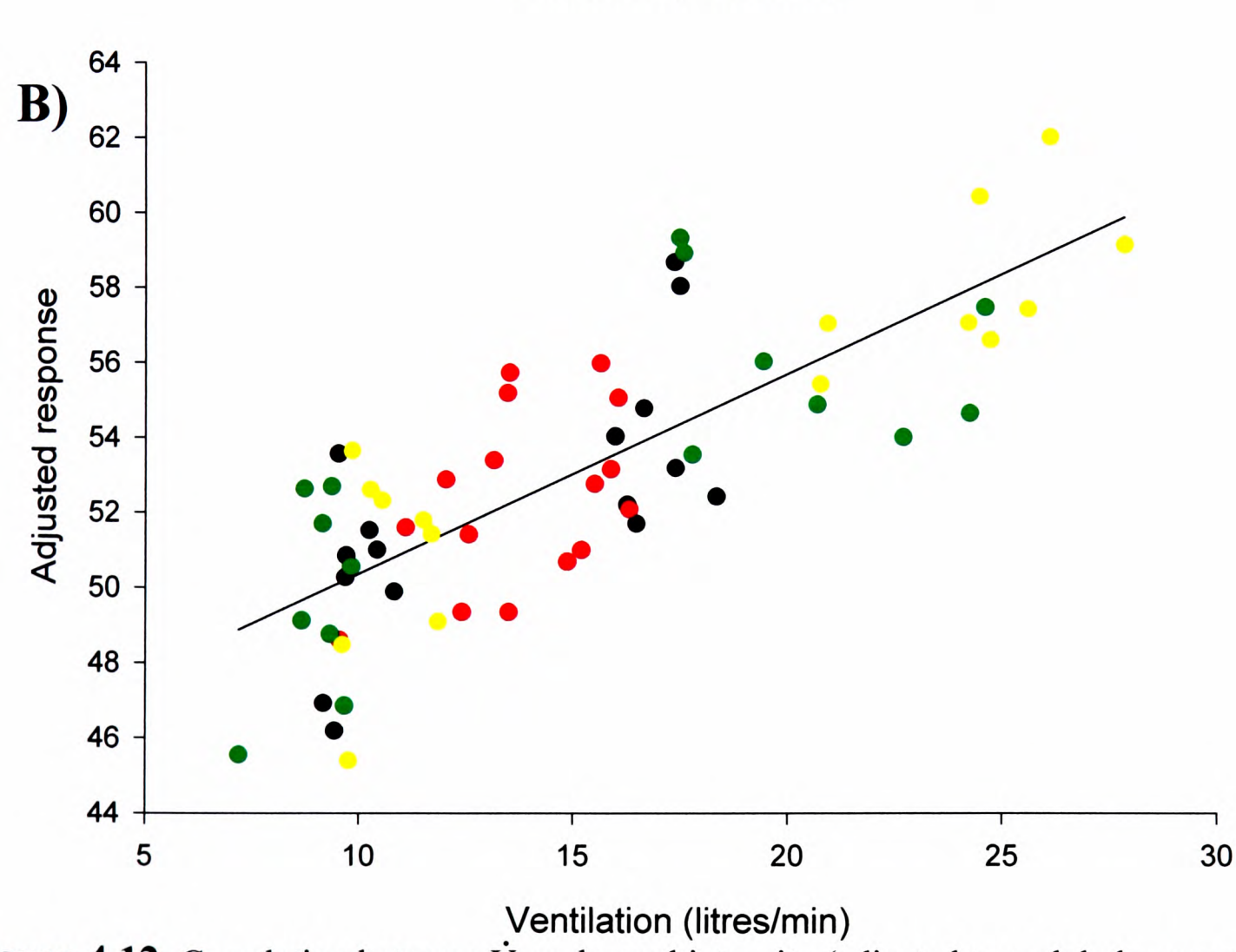


Figure 4.12. Correlation between $\dot{V}_I$ and voxel intensity (adjusted to a global mean of 50ml/min/dl) across all group A subjects for both protocols (imagination of exercise and imagination of freewheeling downhill). Each colour represents a different subject. The comparison was performed for the voxel that was maximally activated in the region. A) PMA (Z=5.8, coordinates 59 3 29, r^2 =0.7), B) the cerebellum (Z=4.8, coordinates -40 -42 -43, r^2=0.6).

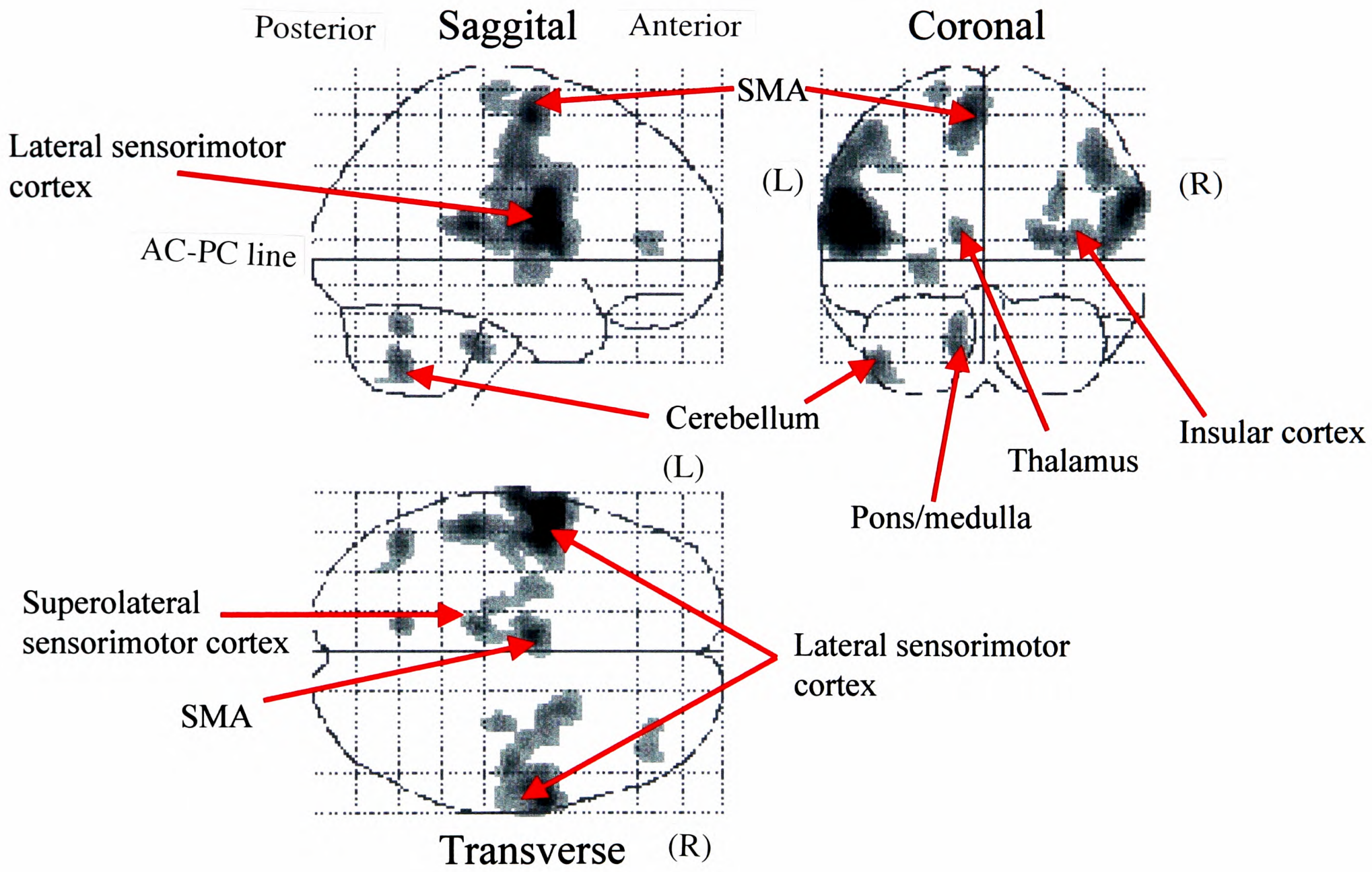


Figure 4.13. Main activations during volitional hyperventilation compared to imagined freewheeling downhill under hypnosis shown as projections in standard stereotactic space (MNI space). Abbreviations; L left, R right, AC anterior commissure, PC posterior commissure.

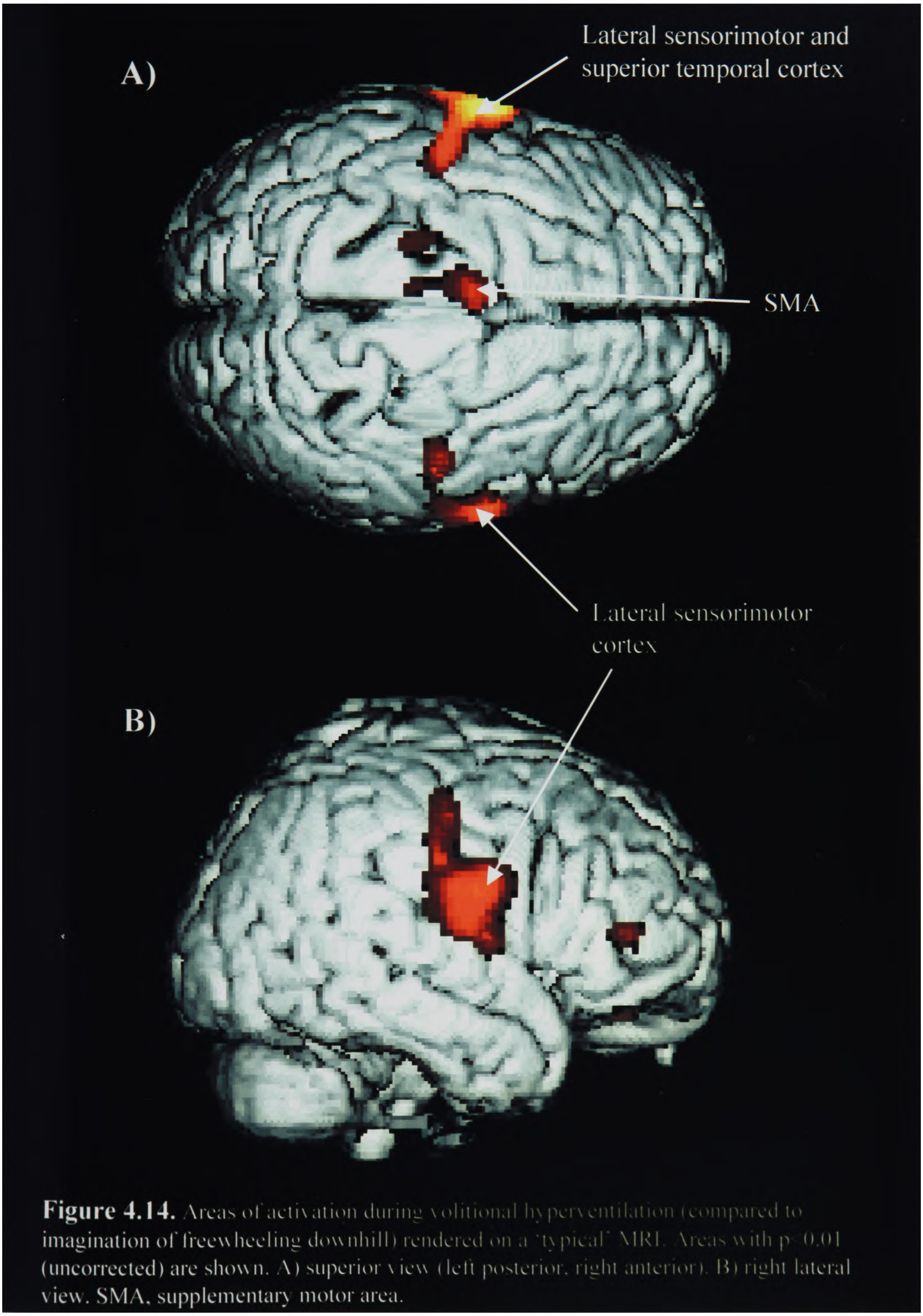


Figure 4.14. Areas of activation during volitional hyperventilation (compared to imagination of freewheeling downhill) rendered on a 'typical' MRI. Areas with $p < 0.01$ (uncorrected) are shown. A) superior view (left posterior, right anterior). B) right lateral view. SMA, supplementary motor area.

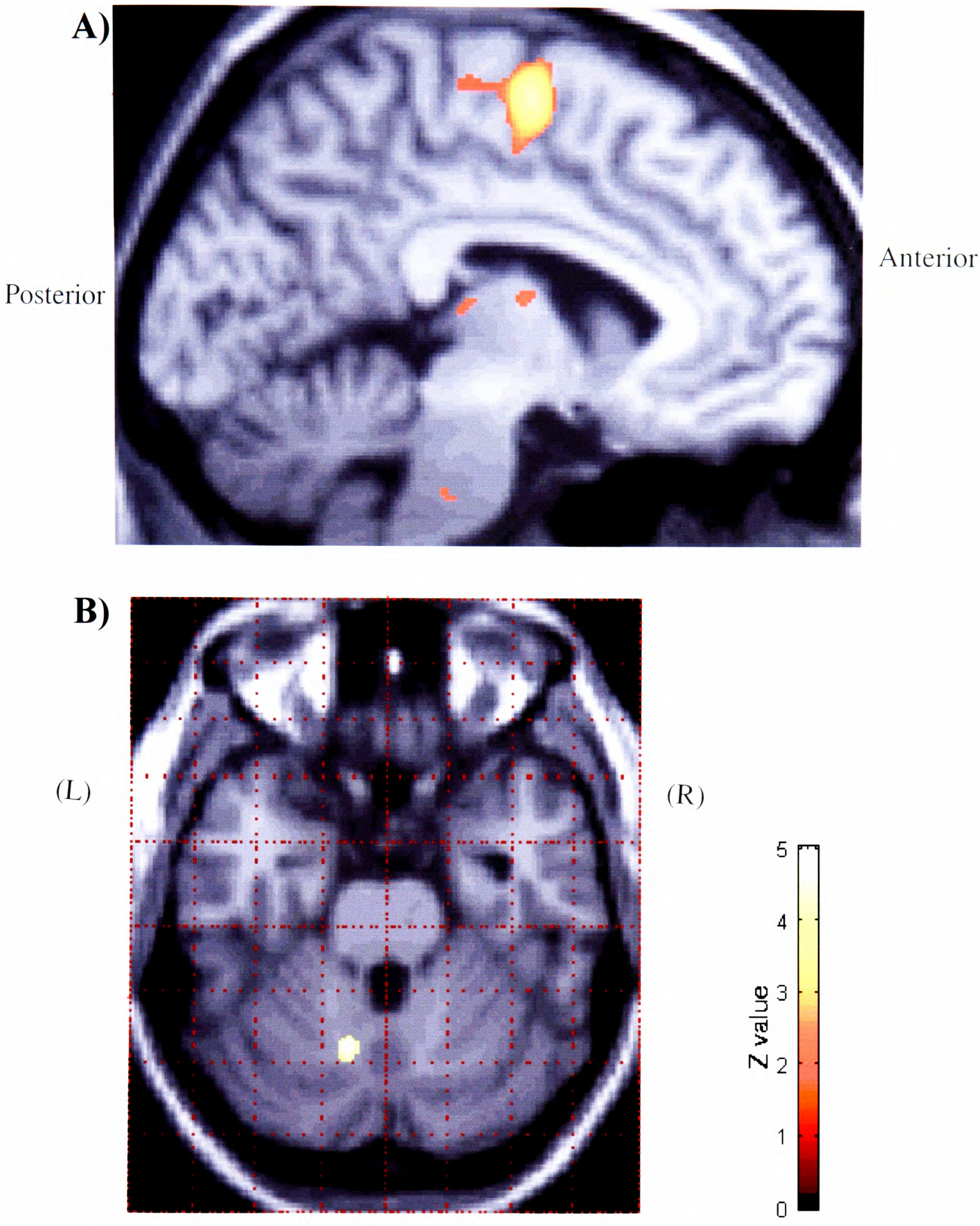
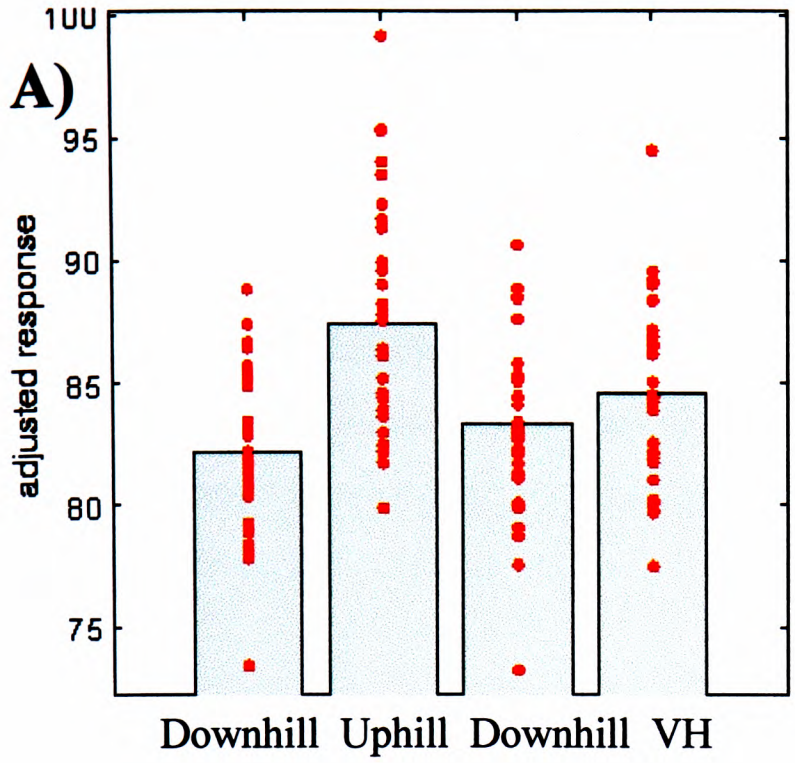
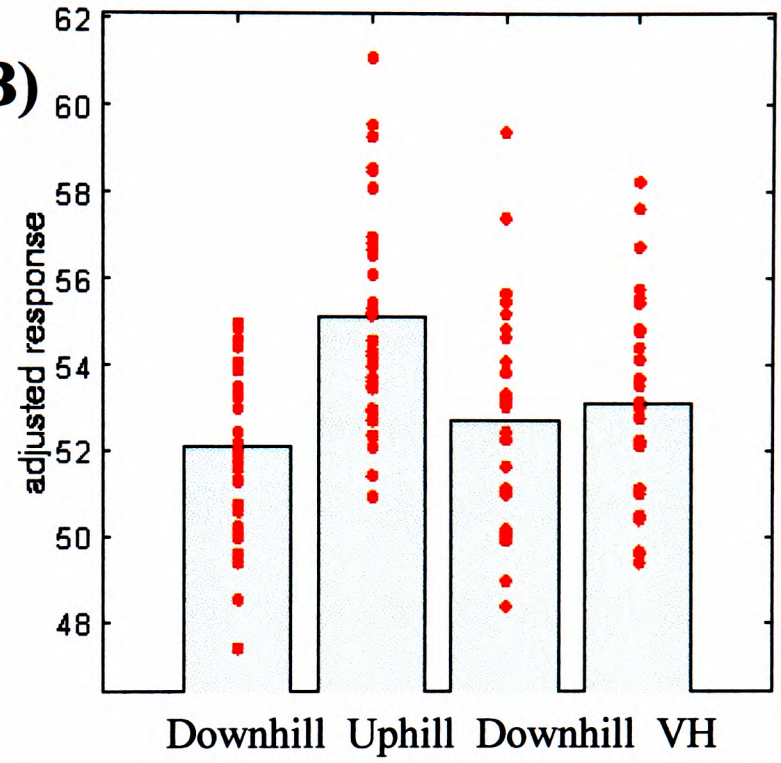
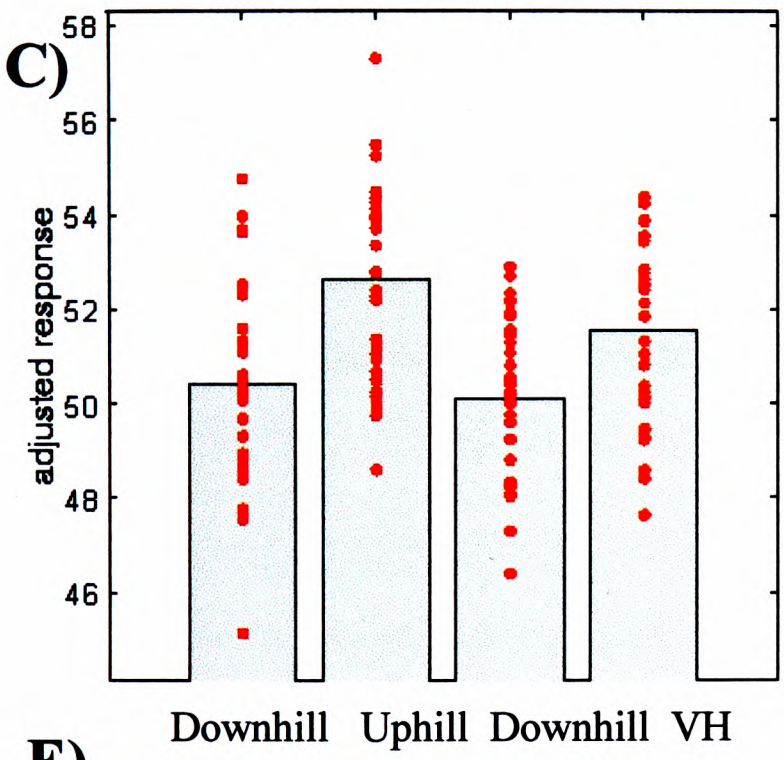
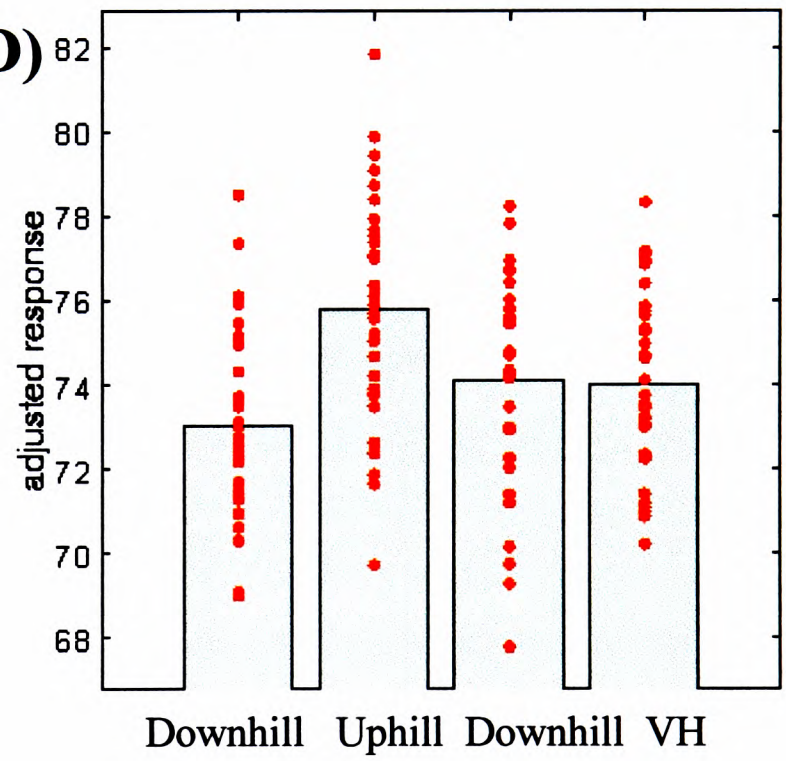


Figure 4.15. Areas of activation during volitional hyperventilation (compared to imagination of freewheeling downhill) overlayed on a 'typical' MRI. Areas with $p<0.01$ (uncorrected) are shown. A) saggital section (x=-8) showing activation of the SMA. B) transverse slice (z=-28) showing less activation of the cerebellum than during imagination of exercise (figure 4.9).









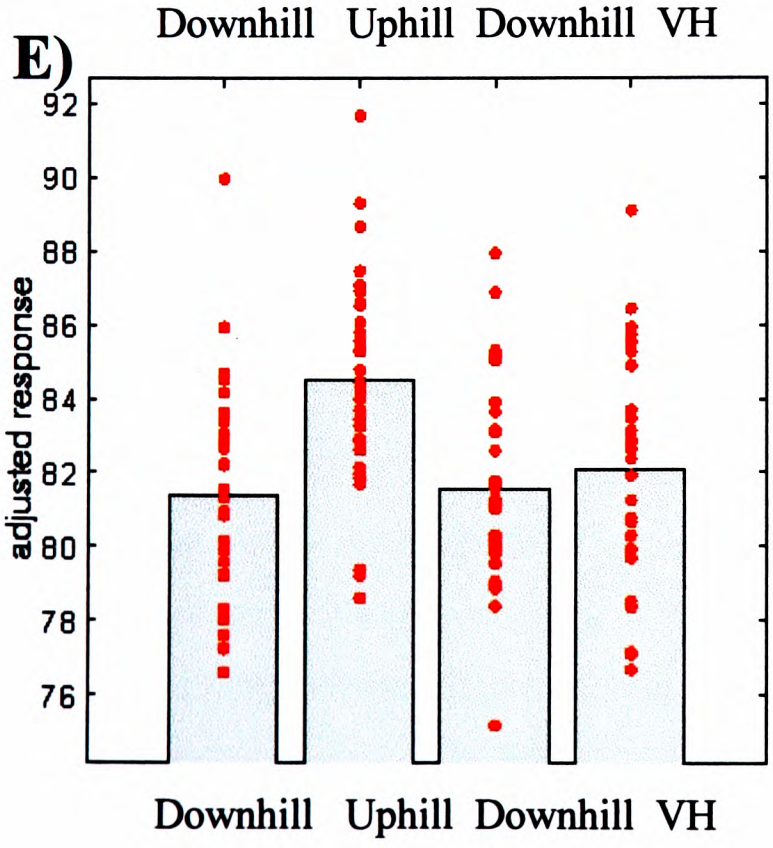


Figure 4.16. Response plots of the mean rCBF (arbitrarily adjusted to a mean global flow of 50ml/min/dl) for the location of maximal activation in the areas indicated. A) SMA (Z=5), B) right middle frontal gyrus (Z=3.4), C) right lateral sensorimotor cortex (Z=4.1), D) left cerebellum (Z=4.2), E) right cerebellum (Z=4.2). The Z scores refer to those during imagination of exercise. Each red data point refers to the voxel intensity for an individual repeat in a single subject. The grey bars represent the mean response.

Three activations remained significant at the *voxel* level after correction for multiple comparisons; left (two peaks, Z=7, Z=4.8) and right (one peak, Z=5.1) lateral sensorimotor cortices. Other activations associated with volitional hyperventilation were in the insular cortex, SMA, pons/medulla, cingulate gyrus, temporal-parietal junction, cerebellum, thalamus and basal ganglia. Individual responses are indicated in figure 4.16 (for the same voxels as activated during imagination of exercise).

4.4 Discussion

The main findings of this study are:

1) Imagination of exercise is associated with activations of a number of 'motor' areas; the SMA, PMA, superolateral motor cortex, thalamus and the cerebellum, all of which had been predicted on the basis of previous studies of increased breathing.
2) There were additional activations in the right middle frontal gyrus incorporating Brodmann's Areas 9 and 46, the left insular cortex and in the posterior parietal cortex.
3) These activations differed from those occurring during volitional hyperventilation, which resulted in highly significant activations of left and right lateral sensorimotor cortex and the SMA and lesser activations of the thalamus and brainstem. There was no activation of the middle frontal gyrus or posterior parietal cortex during volitional hyperventilation.

4.4.1 Cardiorespiratory data

The protocols elicited robust, repeatable cardiorespiratory responses similar to that seen in the study described in Chapter Three, despite the study being performed within the confines of a PET scanner. HR increased during imagination of exercise but not during volitional hyperventilation or imagining freewheeling downhill and whilst V_T decreased during imagination of exercise, the increase in $\dot{V}_I$ was comparable to that described in Chapter Three. The intention during the volitional hyperventilation task was to match the increases in *f* seen during imagination of exercise, no instructions were made regarding V_T (the assumption being that it would remain constant in both tasks as it had done in Chapter Three). Whilst the increase in *f* was similar, the total $\dot{V}_I$ was not well matched between imagination of exercise

and volitional hyperventilation ($p<0.01$ unpaired t-test). It is therefore possible that some of the differences in brain activations between the two groups discussed below can be attributed to this difference in total $\dot{V}_I$. Imagination of freewheeling downhill did not result in changes in cardiorespiratory variables, with the exception of a slight increase in $P_{ET}CO_2$ in group B subjects (figures 4.4, 4.6).

4.4.2 Areas of activation

4.4.2.1 Dorsolateral prefrontal cortex (DLPFC)

The most striking difference between the two groups was the strong activation of the right middle frontal gyrus that occurred during imagination of exercise but not during volitional hyperventilation (figure 4.7). This activation had not been predicted and has not been observed in previous PET studies of breathing, but since one of the peaks within the cluster remained significant after correction for multiple comparisons (Brodmann's Area 9, Z=4.8) it is reasonable to conclude activation. There were two other peaks in this region (Z=3.3 and 3.4). The extent of the activation was extremely similar to that observed in a previous study of 'willed' action (Frith *et al.* 1991) in which word generation and movement selection were studied and the generation of responses were not fully specified by the stimulus applied; there was a requirement for response selection and 'will'. The fact that activation of the dorsolateral prefrontal cortex (DLPFC) occurred during 'willed' action of both modalities suggests that it was a consequence of decision making rather than being related to a specific type of action. Consistent with this is both animal and human data using 'delayed response tasks[1]'. Monkeys with bilateral lesions of the DLPFC cannot perform a delayed response task (Goldman 1971) and similar defects have been observed in patients with DLPFC lesions (Ferreira *et al.* 1998). Transcranial magnetic stimulation of the DLPFC in humans results in increased errors in delayed response tasks (Pascual Leone & Hallett 1994) and DLPFC lesions result in a generalised failure of planning and a stereotyped, predictable behaviour (reviewed by (Fuster 1997).

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[1] For example; a monkey watches whilst food is placed in one of two holes. The holes are then covered and a screen lowered in front of the monkey so it can no longer see them. When the screen is raised the monkey chooses the hole it believes contains the food.

The DLPFC does not project directly to the motor cortex, but is extensively connected with the SMA, PMA and the posterior parietal cortex (Cavada & Goldman Rakic 1989; Lu *et al.* 1994), all of which were activated during imagination of exercise. An interesting feature of the DLPFC activation in the current study is that it was exclusively on the right side. A recent fMRI study (Henson *et al.* 1999) showed right DLPFC activation to be associated with memory retrieval; the degree of activation of DLPFC has been proposed to be related to the effort of retrieval (Schacter *et al.* 1996). This could be related to the process of imagining a previously performed exercise task. Right-sided activation is also consistent with the theory that the 'vigilance' system is predominantly in the right hemisphere as evidenced by PET studies (Pardo *et al.* 1991) and the fact that right-sided lesions result in more severe damage to attention than left-sided lesions (Weintraub & Mesulam 1987).

4.4.2.2 Supplementary motor area and premotor area

During imagination of exercise the most statistically significant activation (Z=5, survives correction for multiple comparisons) was in a superior precentral cortical area extending into the vertex and including the supplementary motor area (SMA), figure 4.7. This activation was bilateral and included 3 peaks (table 4.2). A similar area was also activated during volitional hyperventilation (figure 4.13), but did not quite survive correction for multiple comparisons (p=0.057 corrected). Activation of the SMA has consistently been observed during tasks involving increased respiratory muscle activity (Colebatch *et al.* 1991; Ramsay *et al.* 1993; Fink *et al.* 1996), including during exercise and the post-exercise state (Fink *et al.* 1995). The SMA has been subdivided into three functional areas; the supplementary eye field and the rostral and caudal SMA's (Tanji 1996). Imagination of movement has been shown to lead to increased activation in the rostral SMA compared to more caudal activation during actual movement (Stephan *et al.* 1995), so in the current study, one would predict both rostral and caudal SMA activation since the task involves both actual movement (breathing) and imagined movement (imagined exercise). Activation of the SMA during imagination of exercise occurred despite the fact that the control task of imagining freewheeling downhill included a 'non-exercise' motor task of imagining applying the brakes which might itself be expected to activate the SMA. This could be a consequence of the known somatopy of the

SMA and the fact that the type of motor task being imagined in the two tasks was very different.

The premotor area (PMA) on the right side was activated during imagination of exercise, but not during volitional hyperventilation. PMA activation has been identified in studies of imagined movement (Rao *et al.* 1993) and also in previous PET studies of breathing (Colebatch *et al.* 1991; Ramsay *et al.* 1993; Fink *et al.* 1996). A major source of input to the PMA is the posterior parietal association area, which was also activated during imagination of exercise and is discussed below.

4.4.2.3 Cerebellum

There were a number of cerebellar activations during imagination of exercise (figure 4.7, 4.9, table 4.2), consistent with previous studies involving increased breathing (Ramsay *et al.* 1993; Gozal *et al.* 1994; Corfield *et al.* 1995; Fink *et al.* 1995; Gozal *et al.* 1995; Fink *et al.* 1996). The cerebellar activations during volitional hyperventilation were minor compared to those occurring during imagination of exercise (table 4.3), but given that this was a different group of subjects and the increases in $\dot{V}_I$ between the two groups were different, quantitative comparisons of the differences in cerebellar activations between the two conditions is complicated (see Appendix). The greater increase in $\dot{V}_I$ may be responsible for the increased activation during imagination of exercise, alternatively given the role of the cerebellum in cardiovascular regulation (e.g. Paton *et al.* 1990) it is possible that the cerebellar activations during imagination of exercise are related to the increase in HR. Of note is the fact that cerebellar activations were observed during exercise (Fink *et al.* 1995) but not immediately following exercise. This may indicate that the cerebellar activation during exercise is related to leg movement, but when considered in conjunction with the current study it would also be consistent with the hypothesis that cerebellar activations are related to HR, which falls rapidly on cessation of exercise.

4.4.2.4 Precentral representation of breathing

The superolateral motor cortex was activated bilaterally during imagination of exercise (Z of 4 and 3.5) in areas previously shown to be activated during tasks involving increased respiratory muscle activity (Colebatch *et al.* 1991; Ramsay *et al.* 1993; Fink *et al.* 1996). There was a minor activation in the breathing motor cortex during volitional hyperventilation (Z=2.6); this may well be due to the fact that the $\dot{V}_I$ and the V_T were lower during this task than during imagination of exercise and thus too small for the sensitivity of the scanner. The original evidence for the motor cortical localisation of breathing came from Foerster (1936) who found that electrical stimulation of a cortical region close to the vertex elicited a hiccup. This localisation has been confirmed using transcranial magnetic stimulation in which a maximal effect on the contralateral diaphragm was found 3cm lateral to the vertex and 2-3cm anterior to the auricular plane (Maskill *et al.* 1991). This superolateral motor 'breathing' cortex was activated during imagination of exercise despite a number of differences with previous breathing studies. First, the increases in $\dot{V}_I$ in the current study occurred entirely via increases in *f*, whereas in previous studies breathing was increased via both *f* and V_T. Secondly, the nature of the control task differed; previous studies have used passive ventilation (Colebatch *et al.* 1991; Ramsay *et al.* 1993; Fink *et al.* 1996) during which respiratory related motor activity is absent or minimal. The current study used a control state in which normal resting breathing was occurring; this raises an interesting question regarding whether or not motor cortical drive to breathe occurs at rest. If some motor cortical drive to the diaphragm occurs at rest, then the magnitude of the activation between the control and experimental states would be smaller in the current study, thus decreasing the sensitivity of the contrast.

4.4.2.5 Insular cortex

The *left* insular cortex was activated during imagination of exercise, consistent with a SPECT study of mild exercise (Williamson *et al.* 1997). The *right* insular cortex was activated during volitional hyperventilation in the current study, of note is the fact that hyperventilation that includes a V_T component increases HR, yet activation of the insular cortex has not been observed in previous PET studies of breathing. The role of the insular cortex in cardiovascular control is unclear, electrical stimulation of the left insular evokes bradycardia, whereas

stimulation of the right insular evokes tachycardia in man (Oppenheimer *et al.* 1992), although this lateralisation is the opposite way around in the rat. The insular cortex projects to the lateral hypothalamus and the ventrolateral medulla (Yasui *et al.* 1991) and also to the nucleus of the solitary tract (Saper 1982), all of which are implicated in cardiovascular control. Given that both excitatory and inhibitory neural activity will be identified with PET scanning and the potential for non-specific effects of electrical stimulation in the studies of Oppenheimer *et al* (1992), it is difficult to explain the lateralisation of insular cortical activation in the current study, nevertheless it may indicate that the increase in HR during imagination of exercise is centrally generated.

4.4.2.6 Lateral sensorimotor activations

There was strong lateral sensorimotor activation during both the volitional hyperventilation task (bilateral, Z=7, 5.1, surviving corrections for multiple comparisons) and the imagination of exercise (right side, Z=4.1). A previous PET study of breathing found lateral sensorimotor areas activated during volitional expiration (Ramsay *et al.* 1993) and this region may well correspond to sensorimotor representation of the mouth and upper airways. In order to maintain experimental naivety no specific instructions were given to subjects as to whether they should employ mouth or nose breathing and no EMG recordings were made of facial muscle activity. These lateral sensorimotor activations may be a consequence of jaw activity (which could not easily be observed under the facemask). Two differences between the current study and previous PET studies of breathing might explain the absence of activations in these regions in previous PET studies of breathing; first the use of a nasal mask in previous studies and secondly, the use of passive hyperventilation as a control task.

4.4.2.7 Postcentral activations

Imagination of exercise resulted in widespread superolateral somatomotor activation (figure 4.7, 4.8) including precentral peaks (already discussed) and peaks in the parietal lobe (Z>3.1). There are two likely explanations for these postcentral activations; they could represent areas of sensation related to breathing, or alternatively be posterior parietal association areas.

Sensory representation of breathing

Only one previous PET study of breathing has observed parietal activations with similar coordinates to those seen in the current study (Fink *et al.* 1996). Interestingly this was during a study of *increasing inspiratory force* at a relatively low total $\dot{V}_I$ whereas other studies in which parietal activations did not occur (Colebatch *et al.* 1991; Ramsay *et al.* 1993) have been of *increases in total* $\dot{V}_I$. In addition, the control task in this study (which resulted in normal resting breathing) compared to that used in previous studies (passive hyperventilation, thus controlling for the sensory activations associated with breathing) might explain the lack of parietal activations in previous studies. Electrical stimulation of the phrenic nerve in cats elicits cortical potentials in 3b, 3a and 4γ (Davenport *et al.* 1985) in an intermediate position between the hindlimb and forelimb projections (*i.e.* in an area corresponding to the trunk). However a study using electrical stimulation of chest wall afferents in man (Gandevia & Macefield 1989) in conjunction with evoked-potential recording found a cortical focus closer to the vertex than that for cutaneous afferents of the chest wall. Since the areas of sensory representation of breathing in man have not been fully defined, it is not possible to positively identify the parietal activations in the current study as being breathing related, indeed the absence of similar activations during volitional hyperventilation may indicate that they are unrelated to $\dot{V}_I$.

Parietal activations and visuomotor integration

An alternative explanation for the parietal activations seen during imagination of exercise is that they are related to the imagination of exercise, rather than the consequence of an increase in $\dot{V}_I$. This is consistent with the fact that volitional hyperventilation did not result in parietal activations, either in the current study or in previous studies. It is also consistent with the fact that the maxima of these activations have coordinates that are too posterior to be primary somatosensory cortex, rather they may be in the precuneus. The posterior parietal cortex is classically implicated in integrating visual inputs in order to produce targeted movements. Stephan *et al* (1995) found that both imagining and executing movements activated the intermediate and caudal parts of the superior parietal lobe (Brodmann Area 7, including the

precuneus). Visual imagery evoked during memory retrieval also activates the precuneus (Fletcher *et al.* 1995). Posterior parietal activation has been associated with activation of the DLPFC (Fink *et al.* 1999) and appears to be involved in integrating sensory input and generating responses in conjunction with the SMA and PMA, hence the term 'posterior parietal association area'. Interestingly, Fink *et al* (1995) also identified parietal activity in the post-exercise state, albeit with a low level of significance (figure 4.16), consistent with the 'exercise' components of the current study.

4.4.2.8 Thalamus

The thalamus was activated during both volitional hyperventilation and imagination of exercise (Z of 3.5 and 4.1 respectively), consistent with previous PET studies of increased breathing (Ramsay *et al.* 1993; Fink *et al.* 1995; Fink *et al.* 1996). Activation of the thalamus is also consistent with the overall patterns of 'motor' activation seen in the current study; thalamus, SMA, PMA and cerebellum, although thalamic activation was also observed in a study of CO_2 stimulated breathing in which the motor cortex was not activated (Corfield *et al.* 1995). This latter observation is in keeping with the fact that the thalamus is a sensory relay; single unit recording studies in decerebrate paralysed cats have shown that a population of thalamic neurones increase their firing rate in response to hypercapnia, carotid sinus nerve stimulation and chest expansion (Chen *et al.* 1992). It is therefore not possible to determine whether the thalamic activations in the current study are related to sensory or motor activity.

4.4.2.9 Differences between imagination of exercise and volitional hyperventilation

Quantification of the differences between imagination of exercise and volitional hyperventilation is made difficult given that they were performed in a separate group of subjects and the absolute changes in $\dot{V}_I$ differed between the two groups. One way of quantifying the difference is discussed in the Appendix. At a qualitative level the main differences between imagination of exercise and volitional hyperventilation seem to be the activations of the DLPFC and posterior parietal cortex during imagination of exercise. Another interesting difference was the activation of the pons/medulla during volitional hyperventilation which was not seen during imagined exercise. The reason for this is unclear,

however it is further evidence that during imagination of exercise the increase in breathing is not driven in the same way as during volitional hyperventilation. There was no evidence for activation of the 'hypothalamic locomotor region' (Eldridge *et al.* 1981) or the mesencephalic locomotor region (Bedford *et al.* 1992).

4.4.2.10 Parametric analysis of activations associated with increases in $\dot{V}_I$

An alternative way of considering the data is to perform an analysis of group A subjects (performing imagination of exercise and imagination of freewheeling downhill) with $\dot{V}_I$ as a covariate, treating both protocols as equivalent. This approach is reasonable given the range of $\dot{V}_I$ in the current study (7.2 to 27.9 l/min in group A subjects), but has limitations, the most serious of which is it treats imagination of freewheeling downhill and imagination of cycling uphill to be part of the same continuum, with the only difference modelled being $\dot{V}_I$. However, such a comparison might shed light on which areas are activated 'all or nothing' during an imagery task, and which are activated in proportion to 'effort'. Areas not surviving correction for multiple comparisons were not considered, since there was no clear *a priori* hypothesis regarding this analysis (the table of statistics including these less significant activations is given in the Appendix). Areas whose voxel intensity were significantly correlated with $\dot{V}_I$ (and survived correction for multiple comparisons) included the left posterior cerebellum, the PMA, the DLPFC and the temporal pole/inferior frontal gyrus. That activation of the cerebellum and the PMA are correlated with $\dot{V}_I$ is not surprising; they have been shown to be related to increased breathing in a number of studies. However, the fact that the DLPFC and the temporal pole are both activated in proportion to $\dot{V}_I$ despite not being typical 'motor' areas involved in breathing is extremely interesting. As discussed earlier, the amount of DLPFC activation has previously been proposed to be related to the 'effort' of memory retrieval (Schacter *et al.* 1996), the current study may indicate its role in 'effort' *per se*; subjects did not find it more difficult to imagine exercise than freewheeling downhill, in fact the opposite was reported. Activation of regions that have not previously been associated with breathing with this analysis is possibly the best evidence so far that these regions represent neural correlates of 'central command'.

4.4.3 Study limitations

4.4.3.1 Determining the 'significance' of activations; the problem of multiple comparisons

A problem with all neuroimaging studies is how best to analyse them in order to calculate the significance of activations (Friston *et al.* 1991). The standard analyses within SPM involves carrying out statistical tests independently at each individual voxel in the brain, so the validity of the approach is dependent on the activation of voxels being truly independent, since when carrying out a number of statistical tests, some will show significance by chance alone. Brain voxels are not independent since the intrinsic resolution of the relationship between blood flow and neural activity means that activations are likely to occur over several voxels (so-called 'physiological non-independence'), so the activation of one voxel is not independent of the activation of an adjacent voxel. Also, using smoothing as a processing step increases the likelihood that adjacent voxels are not independent. Several approaches have been used to combat this problem:

1) The SPM program incorporates a Bonferroni-type correction for multiple comparisons. This correction includes strong control for type I errors (the likelihood of accepting a hypothesis which is in fact false), but only weak controls for type II errors (the likelihood of falsely rejecting a true hypothesis).
2) A 'region of interest' analysis can be performed (Worsley *et al.* 1995). This involves selecting regions of the brain for which *a priori* hypotheses exist. Correction for multiple comparisons is then based on the number of voxels in the selected regions rather than the whole brain, followed by a Bonferonni correction for the number of regions of interest.
3) Uncorrected p values are quoted and if $p<0.001$ ($Z>3.09$), significance is assumed. This is the least stringent of the three options described but is used extensively in neuroimaging research (Friston *et al.* 1996).

If an *a priori* hypothesis exists then there is no need to correct for multiple comparisons, however this hypothesis must be for an individual voxel, rather than a region as in the current study. As yet there is no consensus regarding the approach that should be taken when there are several fairly broad regions of interest and *a priori* hypotheses as existed for the SMA, PMA, superolateral motor cortex and cerebellum in the current study. A number of alternative

approaches are also possible, including the use of nonparametric statistical tests which incorporate fewer assumptions about the nature of the data, discussed by Frackowiak *et al.* (1997).

4.4.3.2 Task performance

A problem with PET scanning studies is whether or not the subject is producing a robust, repeatable response over a number of repetitions and judging the success of imagery tasks such as those in the present study is difficult. An indication is given by the repeatability of the cardiorespiratory responses (figure 4.3, 4.4). Subjects' own reports of their imagery indicated that they found it hardest to imagine freewheeling downhill and easiest to imagine exercise. However, all subjects claimed that there were no repeats in which they were not attempting to perform the task as instructed and no scans were excluded from the analysis on this basis. Hypnosis *per se* results in alterations in rCBF (Rainville *et al.* 1999), but given the balanced scan order it is unlikely that changes in hypnotic depth would have systematically affected one protocol more than another. An additional advantage of balanced scan order is that time (and therefore movement occurring over time) should not have resulted in spurious activations, although it will decrease statistical power by increasing variance.

4.4.3.3 CO_2 control

Despite adding CO_2 to the inspired air during protocols involving hyperventilation there was a statistically significant fall in $P_{ET}CO_2$ during imagination of exercise (figure 4.4). $P_{ET}CO_2$ affects global blood flow (Kety & Schmidt 1948), although this effect is more pronounced above isocapnia than below. Consistent with this is the fact that within the range of $P_{ET}CO_2$ in the current study the correlation with global CBF was poor (figure 4.10). However in a study of Ramsay *et al* (1993) similar activations were observed over a 33 Torr range of $P_{ET}CO_2$, so the fall in $P_{ET}CO_2$ in the current study (3.3±1.8 Torr) seems fairly insignificant. Nevertheless it is possible that systematic, task related falls in $P_{ET}CO_2$ in the current study could have influenced results.

4.4.4 Implications for 'central command' in exercise

Imagination of exercise resulted in neural activations with striking similarities to those previously observed during actual exercise and the post-exercise state by Fink *et al.* (1995). This is despite a number of differences in the physiology of the response. The ventilatory response to imagination of exercise was via *f* with no contribution of V_T, and the increases in HR and $\dot{V}O_2$ were modest compared to that occurring in actual exercise. Additionally the entire study was conducted under hypnosis, whose neural correlates are poorly characterised. However, the crucial difference between the current study and that of Fink *et al.* (1995) is that exercise was not and had not been occurring, so activations in the current study can not be attributed to some effect of peripheral feedback, rather they represent a genuine feedforward response.

The overall patterns of activation seen during imagination of exercise include the main components of motor circuitry that interact with the primary motor cortex; the SMA, PMA, thalamus and cerebellum. This is despite fairly minimal activation of the motor cortex (with the exception of the superolateral motor cortex, associated with ventilatory tasks). This absence of motor activity confirms the EMG evidence obtained in Chapter Three that the increases in $\dot{V}_I$ during imagination of exercise were not dependent upon increased muscle activity in the rest of the body. As discussed in Chapter One, 'central command' could potentially originate from any site involved in motor control, so the fact that accessory motor areas are activated during the current study is encouraging. Since the neural activations associated with CO_2 breathing do not result in activations in the motor cortex, SMA, PMA or cerebellum (Corfield *et al.* 1995) it is reasonable to suggest that activations of 'motor' areas in the current study are related to increases in breathing elicited by 'higher centres' rather than by increases in breathing *per se*. Since 'motor' areas are also activated by volitional increases in breathing, it could be argued that activations of the cerebellum, SMA, PMA and thalamus say little about 'central command'. However, since there were substantial differences between activations during volitional hyperventilation and imagined exercise, it is reasonable to conclude that imagination of exercise is a genuine 'exercise-like' response, and not merely a volitional increase in breathing by compliant subjects. It also indicates that during imagination

of exercise the increase in breathing is not driven in the same way as during volitional hyperventilation.

The most striking activations during imagination of exercise which have not previously been observed in breathing neuroimaging studies and not observed during volitional hyperventilation in the current study are the activations of the right middle frontal gyrus (dorsolateral prefrontal cortex) and the posterior parietal cortex. When activated together, these regions are strongly implicated in selection and generation of responses and are also important for working memory, so it could be hypothesised that this represents activation of a respiratory motor programme that is based on previous exercise performance and is continually updated in response to sensory information integrated in the parietal association areas. Activation of the DLPFC is also seen during imagination *per se*, so it is possible that activation in the current study is a consequence of the fact that I was studying imagination rather than actual exercise. However, since there was a significant correlation between $\dot{V}_I$ and voxel intensity in the DLPFC (figure 4.11), this seems unlikely. The DLPFC is involved in the learning of new tasks and its activation decreases when a task becomes familiar (Seitz *et al.* 1990; Jenkins *et al.* 1994); at first sight this does not hold well with the theory that exercise hyperpnoea is a learnt, familiar response. However, if a motor programme exists for exercise hyperpnoea, it would need to be continually updated and modified according to peripheral feedback, so such a programme could be considered as a continued learning and thus still require DLPFC activation. Indeed, integration of exercise drives and an updating of the respiratory motor programme would need to occur during every exercise task. This hypothesis could be examined by studying the neural correlates of manipulating the respiratory motor programme for exercise (e.g. by adding external dead space during a number of exercise trials). This type of task has previously been shown to modulate the ventilatory response to exercise in the short term in man (Helbling *et al.* 1997) and plasticity of the motor programme for exercise has also been demonstrated in goats using thoracic dorsal rhizotomy (Mitchell 1990).

Additional evidence that activation of the DLPFC is not merely a consequence of imagination is that the parametric analysis on the neural correlates of increases in $\dot{V}_I$ indicated that DLPFC activation was linearly related to the $\dot{V}_I$. Certainly the fact that activation of an area such as the DLPFC which is not considered to be a 'breathing' area is directly correlated with $\dot{V}_I$ is an extremely interesting finding. The current study suggests that activation of these areas is part of a feedforward mechanism rather than a consequence of peripheral feedback from exercising limbs; exercise was not occurring in the current study and there was no widespread motor cortical activation.

4.5 Summary

Imagination of exercise appears to be a good model of 'central command' in actual exercise since it has remarkably similar neural correlates compared to actual exercise, and these correlates differ substantially from those seen during volitional hyperventilation. The posterior parietal cortex and prefrontal areas such as the dorsolateral prefrontal cortex may play a role in generating exercise hyperpnoea via their connections with the premotor and supplementary motor areas. This hypothesis seems reasonable based on previous studies of actual exercise (Fink *et al.* 1995) and on the current study, in which similar activations were observed despite the absence of widespread motor activity and peripheral feedback. It is also consistent with studies indicating the role of the posterior parietal cortex in execution of learned movements and memory retrieval (Sadato *et al.* 1996), and with studies implicating both DLPFC and posterior parietal cortex in working memory (D'Esposito *et al.* 1995). In addition, activation of the dorsolateral prefrontal cortex during imagination was correlated with $\dot{V}_I$, which may indicate some relationship between prefrontal activity and 'effort'.

Chapter Five

Cardiorespiratory responses to alterations in actual and suggested work rates during exercise under hypnosis

Fere libenter homines id quod volunt credunt. Julius Ceasar.

Et feminae id quod matres eis narrant credunt. Judith Thornton.

5.1 Introduction

The studies described in Chapter Three showed that a proportion of the cardiorespiratory response to exercise is mimicked by *imagination* of exercise whilst at rest. However, the relationship between this response and 'central command' during exercise is unclear. Owing to the redundancy of control mechanisms (Waldrop *et al.* 1986), the magnitude of any response to 'central command' differs according to the work rate and on the level of peripheral feedback. In addition, whilst the hypnotic state allows a degree of isolation from the environment, the subject is still aware of their movement (or absence of movement), so the responses to hypnotic suggestions may be improved if the subject performs actual exercise rather than just imagining exercise. I therefore studied the effect of hypnotic suggestions on the cardiorespiratory responses to actual exercise. Two types of study were performed, both under hypnosis. First, *constant load* exercise tasks with *altered suggestion* (both increase and decrease), secondly *alterations in load, without informing the subject*. In the former the intention was to keep peripheral feedback constant and increase or decrease 'central command' independently. In the latter, the aim was to maintain 'central command' constant and manipulate peripheral feedback.

5.2 Methods

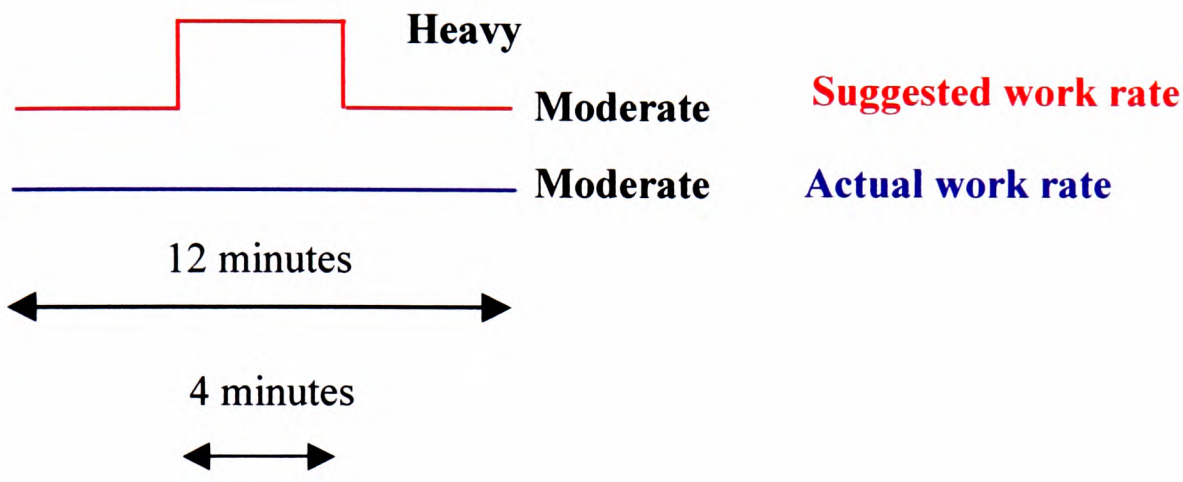
Subjects were instrumented for cardiorespiratory recordings as described in Chapter Two. Subjects did not perform more than two protocols (i.e. 24 minutes exercise) during any single visit to the laboratory.

5.2.1 Protocols

5.2.1.1 Protocol 1. Constant load exercise with suggested increase in work rate under hypnosis

In an attempt to upregulate 'central command' whilst maintaining peripheral feedback at a constant level, fourteen hypnotised subjects exercised at a *constant* moderate work rate for 12 minutes. After 4 minutes of this it was *suggested* that the work rate had *increased* to the 'heavy'

work rate for a further 4 minutes, after which the subject was told that the work rate had returned to 'moderate' for a further 4 minutes:



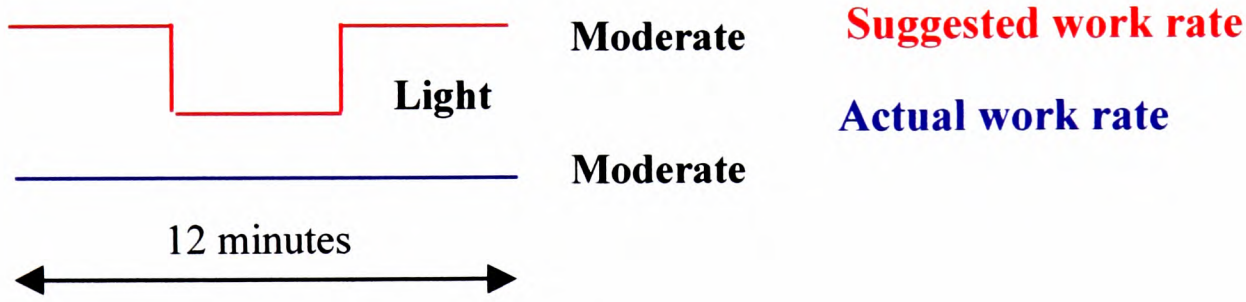
The precise instructions given by the hypnotist were as follows:

> *'We're just going to help you put your feet into the pedals...that's good...just make yourself comfortable...Now when I tell you to I want you to start pedalling and it's going to be at the moderate rate...you're going to pedal for 12 minutes ...during this time you'll hear various noises as people walk around and noises from the bits of equipment that are around you but these won't worry you at all, you'll find you can easily concentrate on maintaining a good exercise rhythm...So I'll count down from 5 to 1 and then I'd like you to start pedalling at the moderate work rate...5, 4, 3, 2, 1 go...left, right, left, right, left, right...that's very good, keep up that rate...remember this is the moderate work rate...that's the 1st minute gone, 3 minutes to go at this rate...very good indeed...now the second minute is coming up... very good, keep going at this rate...feeling good, feeling fine, no change in the level...that's 3 minutes...Now at the next minute you're going to feel the load increase to the heavy load, as if you were cycling up a steep hill...so you are going to feel the load's getting much harder... So when I count down from 5 to 1 I'd like you to see in your own mind's eye yourself cycling up a hill...5, 4, 3, 2, 1 now the load's feeling harder and you find yourself having to push harder against the pedals ...that's very good...still feeling relaxed, not worrying about a thing...that's been 1 minute at this harder work rate ...that's very good...you're having to work hard at this level...that's been 2 minutes at this level...that's very good, keep going...still feeling fine, just having to push very hard against the pedals at this heavy rate...we're coming up to 3 minutes now ... that's very good...As we come up to the next minute once again in your mind you'll find that the effort decreases as if you're cycling along a flat road...5, 4, 3, 2, 1 so now the load is back to the moderate load ...we'll do this for 4 minutes and then stop and rest...that's very good...feeling you're on a flat road and it's fairly easy to pedal...that's 1 minute gone at this level...just 3 minutes to go at this level... on the flat as it were...just 2 minutes to go now...that's very good, still pedalling at this rate... not worrying about a thing...we're coming up to the last minute now...very good, keep pedalling...this is the last minute now and*

then you can rest...Now at the end of the minute, when I count down from 5 to 1 I'd like you to stop pedalling and we'll help you to take your feet out of the pedals...5, 4, 3, 2, 1 and stop pedalling...that's very good, now relax totally... that was very good indeed...deeply, deeply relaxed...'

5.2.1.2 *Protocol 2. Constant load exercise with suggested decrease in work rate under hypnosis*

In an attempt to downregulate 'central command' whilst maintaining peripheral feedback at a constant level, five hypnotised subjects exercised at a *constant* moderate work rate for 12 minutes. After 4 minutes of this it was *suggested* that the work rate had *decreased* to the light' work rate for 4 minutes, after which the subject was told that the work rate had returned to 'moderate' for a further 4 minutes:



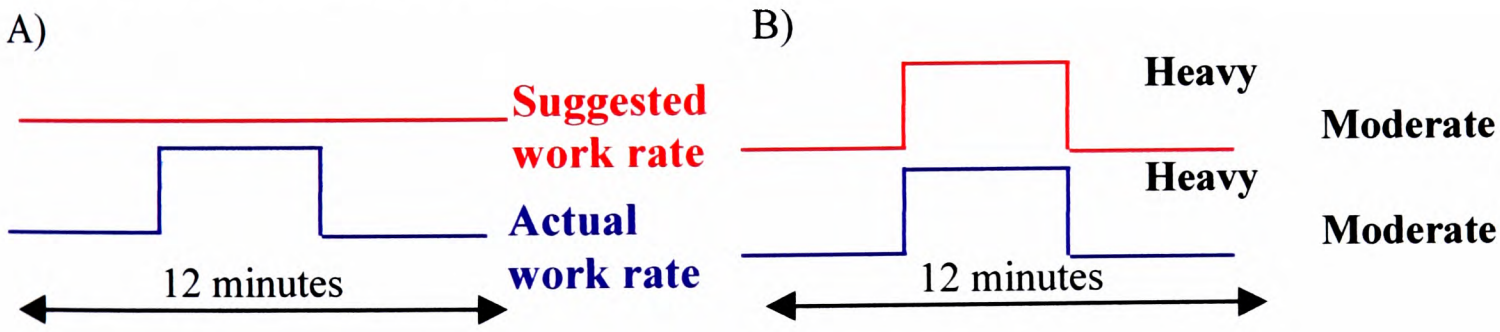
The precise instructions given by the hypnotist were:

'We're just going to help you put your feet into the pedals...that's good...just make yourself comfortable...Now when I tell you to I want you to start pedalling and it's going to be at the moderate rate...you're going to pedal for 12 minutes ...during this time you'll hear various noises as people walk around and noises from the bits of equipment that are around you but these won't worry you at all, you'll find you can easily concentrate on maintaining a good exercise rhythm...So I'll count down from 5 to 1 and then I'd like you to start pedalling at the moderate rate...5, 4, 3, 2, 1 go...left, right, left, right, left, right...that's very good, keep up that rate...remember this is the moderate work rate...that's the 1st minute gone, 3 minutes to go at this rate...very good indeed...now the second minute is coming up... very good, keep going at this rate...feeling good, feeling fine, no change in the level...that's 3 minutes... Now at the next minute you're going to feel the load decrease to the light work load, as if you were coasting down a hill...so you are going to feel the load's getting much easier... So when I count down from 5 to 1 I'd like you to see in your own mind's eye yourself cycling down a hill...5, 4, 3, 2, 1 now the load's feeling easier and you find that it's really no effort at all to turn the pedals ...that's very good...still feeling relaxed, not worrying about a thing...that's been 1 minute at this easy work rate...that's very good...just coasting down a hill...that's been 2 minutes at this level...that's very good, keep going...still feeling fine, just bowling

along...we're coming up to 3 minutes now...that's very good...As we come up to the next minute once again in your mind you'll find that the effort increases back to the moderate work rate as if you're cycling along a flat road...5, 4, 3, 2, 1 so now the load is back to the moderate load ...we'll do this for 4 minutes and then stop and rest...that's very good...feeling you're on a flat road and it's fairly easy to pedal ...that's 1 minute gone at this level...just 3 minutes to go at this level... on the flat as it were...just 2 minutes to go now...that's very good, still pedalling at this rate... not worrying about a thing...we're coming up to the last minute now...very good, keep pedalling...this is the last minute now and then you can rest...Now at the end of the minute, when I count down from 5 to 1 I'd like you to stop pedalling and we'll help you to take your feet out of the pedals...5, 4, 3, 2, 1 and stop pedalling ...that's very good, now relax totally... that was very good indeed...deeply, deeply relaxed...'

5.2.1.3 Protocol 3. Increased work rate with constant suggested work rate under hypnosis

In an attempt to keep 'central command' constant whilst modulating peripheral feedback three hypnotised subjects performed two repeats of a 12 minute exercise task. Between 4 minutes and 8 minutes the work rate *increased*. In the first repeat (A) the exercise rate increased but the subject wasn't told this. In the second repeat (as a control, B), the exercise rate increased and the subject is made aware of this:



The precise instructions given by the hypnotist were as follows (repeat A):

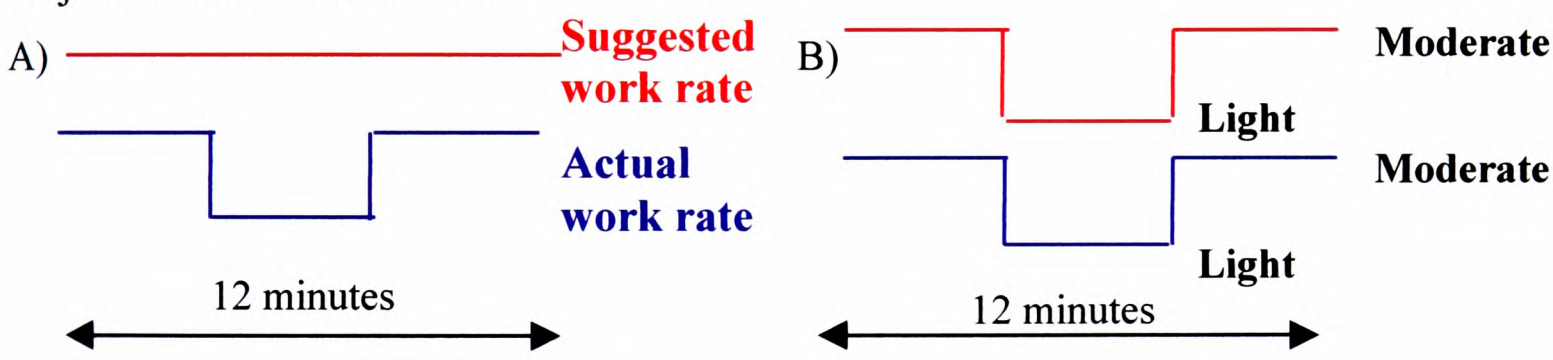
We're just going to help you put your feet into the pedals...that's good...just make yourself comfortable... Now when I tell you to I want you to start pedalling and it's going to be at the moderate rate...you're going to pedal for 12 minutes ...during this time you'll hear various noises as people walk around and noises from the bits of equipment that are around you but these won't worry you at all, you'll find you can easily concentrate on maintaining a good exercise rhythm ... So I'll count down from 5 to 1 and then I'd like you to start pedalling at the moderate rate ... 5, 4, 3, 2, 1 go...left, right, left, right, left, right...that's very good, keep up that rate...remember this is the moderate work rate...that's the 1st minute gone, 11 minutes to go...very good indeed...now the second minute is coming up... very good, keep going at this

rate...feeling good, feeling fine, no change in the level...that's 3 minutes.....this is the last minute now and then you can rest...Now at the end of the minute, when I count down from 5 to 1 I'd like you to stop pedalling and we'll help you to take your feet out of the pedals...5, 4, 3, 2, 1 and stop pedalling...that's very good, now relax totally... that was very good indeed...deeply, deeply relaxed...'

Suggestions for repeat (B) were the same as for protocol 1 (described above).

5.2.1.4 Protocol 4. Decreased work rate with constant suggested work rate under hypnosis

Hypnotised subjects performed two repeats of 12 minutes exercise, between 4 minutes and 8 minutes the work rate *decreased*. In the first repeat (A), the exercise rate decreases but the subject isn't told this. In the second repeat (as a control, B), the exercise rate decreases, and the subject is made aware of this:



The suggestions given by the hypnotist during repeat (A) were the same as during protocol 3 and during repeat (B) were as given during protocol 2.

5.2.2 Analysis

5.2.2.1 Steady state analysis

Breath-by breath data from each subject was divided into 30 second epochs (protocol 1, 3 and 4) or 15 second epochs (protocol 2) and averages were calculated for each epoch. Where deltas are indicated (figures 5.2, 5.5, 5.6, 5.8), the average value from 3 minutes to 3.5 minutes after the onset of exercise (protocols 1, 3 and 4), or 3.5 minutes to 3.75 minutes after the onset of exercise (protocol 2) was taken as the control value and subtracted from each average. Deltas were then averaged across the group and all data were expressed as mean ± S.E.M. Paired t-tests were performed on all values quoted in tables 5.2 and 5.3.

5.2.2.2 *Breath-by-breath analysis of transients*

In order to analyse any transient changes in cardiorespiratory variables that were not maintained during the steady state, a breath-by-breath analysis was performed on the data from protocols 3 and 4. In this approach, individual breaths before and following the altered load or suggestion (i.e. after 4 minutes of exercise) were given consecutive numbers. Data were then averaged between subjects for each breath number. Use of a repeated measures ANOVA on ranks and *post hoc* test (Student-Newman-Keuls) allowed determination of *when* a change in cardiorespiratory variables becomes significant (e.g. a change may occur 3 breaths after a change in work rate). After determining *when* a change becomes significant, breath-by-breath data from the two repeats of protocols 3 and 4 were compared using an unpaired t-test in order to test whether the two repeats differed from each other (e.g. figure 5.7).

The approach of allocating a number to each breath is a reasonable method for analysing transients, but its usefulness depends upon the amount of variation between subjects in the time when the breath was taken (and therefore on breathing frequency) and decreases over time. As an example, this time variation was quantified for the 14 subjects who performed protocol 1 (table 5.1). Breath number 0 was taken to be the last breath before the suggested change in load (i.e. occurring at 4 minutes).

Breath number	*Mean time at which breath was taken ± SD (seconds)*
0	0±0
5	11±4
10	25±7
15	40±12
20	56±16
25	69±19

Table 5.1. Variability in the time at which specific breaths were taken following a suggestion of an increase in work rate (protocol 1).

In the studies presented in this chapter, breath-by-breath analysis is presented for 10 breaths following a change or suggested change in work rate.

5.3 Results

5.3.1 Constant work rates with altered suggestion

5.3.1.1 Constant actual work rate with suggested increase in work rate

After 4 minutes of exercise at a constant moderate work rate, a suggestion that the work rate *increased* resulted in sustained hyperventilation (figure 5.1) and hypocapnia and an increase in HR (table 5.2, figures 5.2, 5.3, n=14). The magnitude of the increased $\dot{V}_I$ was similar to that seen when the work rate *actually* increased to the heavy rate but unlike the latter it occurred via changes in *f*, with no change in V_T (table 5.2). $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ increased significantly (table 5.2) during the suggested increase in work rate compared to that during the moderate exercise. When after 8 minutes the subjects were told that the work rate had returned to the moderate level $\dot{V}_I$ remained elevated, albeit decreasing, for *ca.* 30 seconds (figure 5.3). $\dot{V}_I$ in the 30 seconds following this instruction was 7.2±1.9 l/min above that preceding suggestion. Subjects were unsure about whether or not the exercise load had in fact increased or remained the same:

"...I could feel that the load had changed, it felt different, my chest was tighter and I was breathing more, but I don't think the load actually did change..."

5.3.1.2 Constant actual work rate with suggested decrease in work rate

Suggestions of a *decrease* in work rate did not result in any significant change in cardiorespiratory variables (repeated measures ANOVA on 15 second epochs, n=5 figure 5.5), however two subjects did show a clear decrease in breathing and HR (e.g. figure 5.4). When comparing epochs using a paired t test there was a small but significant *transient* decrease in $\dot{V}_I$ and *f* between 6.75 and 7 minutes (compared to 3.5-3.75 minutes), table 5.2. Three of the five subjects thought the load hadn't changed, the other two believed that the work rate actually did decrease

"It was really easy, I was absolutely flying along"

There was no significant difference in $\dot{V}_I$ or HR during moderate exercise prior to the suggested decrease in work rate (protocol 2) compared to that prior to the suggested increase in work rate (protocol 1), (table 5.2).

5.3.2 Altered actual work rates with <u>constant suggested</u> work rates

5.3.2.1 Increased actual work rate with constant suggestion

All three subjects said that they were aware of the increase in work rate during the first repeat, even though they weren't informed of the change. The increase in work rate (from 49 to 98 Watts) with a suggestion that the work rate had remained constant resulted in an increase in $\dot{V}_I$, HR, V_T and f (table 5.3). There was no steady state difference between the repeats of protocol 3 (suggestion that the work rate had remained *constant* in repeat A, suggestion that the work rate had *increased* in repeat B), as shown by comparison of 30 second epochs between the two repeats, figure 5.6. Analysis of breath-by-breath data for the transition at 4 minutes showed no maintained significant difference in $\dot{V}_I$, $P_{ET}CO_2$, f, or V_T (repeated measures ANOVA on ranks) for the 10 breaths following the transition compared to those preceding the transition. However, there was a transient difference in the HR responses between the two repeats. The increase in HR was significantly different (from the last breath before the change in work rate) from the 3rd breath onwards when the subject was informed of the increase in work rate (B), but not until the 5th breath when the subject was not informed of the increase in work rate (A), figure 5.7. Having shown the breaths to be significantly different from baseline, an unpaired t-test was performed on each breath in order to compare the constant suggestion (A) with the increased suggestion (B). There was a significant difference on breaths 2 and 3 ($p=0.041$ and 0.0018 respectively), but not on breath 4 ($p=0.052$) and not on breath 5 onwards.

5.3.2.2 Decreased actual work rate with constant suggestion

The decrease in work rate (from 54±14 to 8 Watts) resulted in a decrease in HR (table 5.3), but no significant decrease in $\dot{V}_I$. There was no steady state difference between the two repeats of protocol 4 (comparison of 30 second epochs between the two repeats), figure 5.8. Breath-by-

	$\dot{V}_I$ (litres/min)	f (Breaths/min)	V_T (litres)	$P_{ET}CO_2$ (Torr)	HR (bpm)	$\dot{V}_{O_2}$ (ml min^{-1})	$\dot{V}_{CO_2}$ (ml min^{-1})
Actual moderate exercise	21±1.7	17.1±1.4	1.3±0.1	45.6±1.3	99±4	1105±79	882±84
Actual moderate, suggested heavy (n=14) (protocol 1)	33.4±2.1 *	26.2±1.5 *	1.3±0.1	40.5±1.1 *	109±2 *	1268±105 * (n=11)	1165±122 * (n=11)
Actual heavy exercise	34.8±1.9 *	23.7±0.9 *	1.5±0.1 *	45.8±0.4 *	128±4 *		
Actual moderate	26.7±5.3	19.6±0.8	1.4±0.2	42.8±1.9	114±7	1145±147	933±167
Actual moderate, suggested light (n=5) (protocol 2)	24±4.8 *	15.4±0.9 *	1.4±0.2	43.8±1.6	112±9	1133±176 (n=6)	1002±175 (n=6)
Actual light exercise	18.3±2.9 *	17.5±2.1	1.1±0.3	40.3±1.1	106±3	431±39	372±32

Table 5.2. Cardiorespiratory data from protocols 1 and 2. * indicates $p<0.05$ compared to moderate exercise, paired t-test. 'Heavy' and 'moderate' refer to predetermined work rates that the subjects had previously been familiarised to, designed to result in 50% and 30% of HR_{max} respectively. 'Light' refers to unloaded pedalling. Control values are taken as the mean ± S.E.M between 3 minutes and 3.5 minutes after exercise onset. Values during the protocols are mean ± S.E.M for 7.5 to 8 minutes of the procedure. $P_{ET}CO_2$ values were not corrected for f, V_T or $\dot{V}_{CO_2}$; consequently whilst $P_{ET}CO_2$ appeared to increase during exercise it is likely that arterial PCO_2 did not increase (Jones *et al.* 1979).

	$\dot{V}_I$ *(litres/min)*	f *(Breaths/min)*	V_T *(litres)*	$P_{ET}CO_2$ *(Torr)*	HR *(bpm)*
Moderate exercise	23.3±3	16.9±1.9	1.4±0.1	45.3±1.4	98±3
Heavy exercise, suggested moderate (protocol 3A, n=3)	39.4±4.4 *	18.8±1.6*	2.1±0.2 *	46.8±1.8	121±4 *
Moderate exercise	21.9±1.1	18.6±1.9	1.2±0.1	43.9±2.2	102±2
Heavy exercise, suggested heavy (3B)	37.5±5.5 *	24.2±3.7	1.6±0.1	42.2±3.3	125±5 *
Moderate exercise	23.3±5.3	16.2±1.4	1.5±0.5	42.5±1.6	118±2
Light exercise suggested moderate (protocol 4A, n=3)	18.3±2.9	17.5±2.1	1.1±0.3	40.3±1.1	106±3
Moderate exercise	21.9±9.7	17.6±0.2	1.3±0.6	42.6±2.2	115±4
Light exercise suggested light (4B)	15.3±6.6	17.1±2	1±0.5	39.5±0.9	103±3 *

Table 5.3. Cardiorespiratory data for protocols 3 and 4. * $p<0.05$, paired t test versus moderate exercise.
Control values are taken from 3 to 3.5 minutes after exercise onset and values for each protocol from 7.5 to 8 minutes.

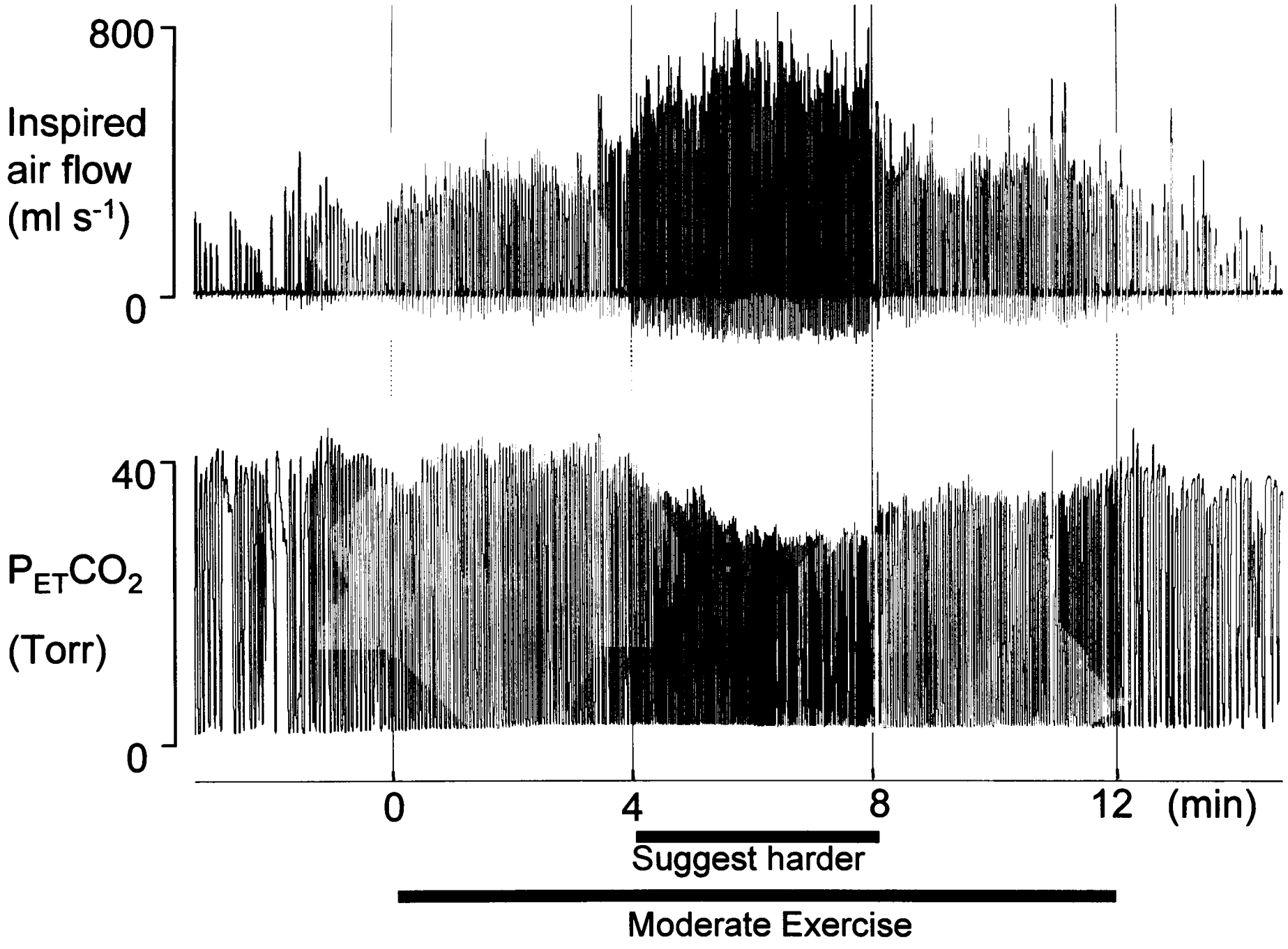
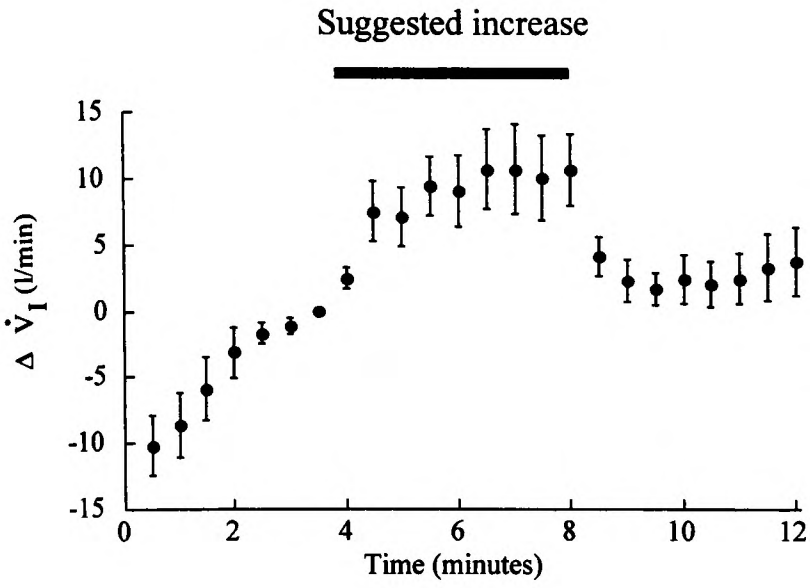
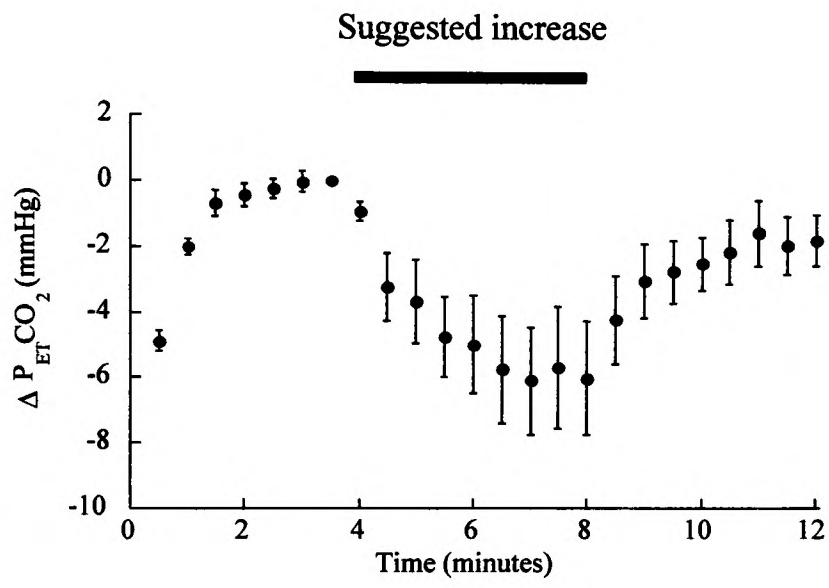
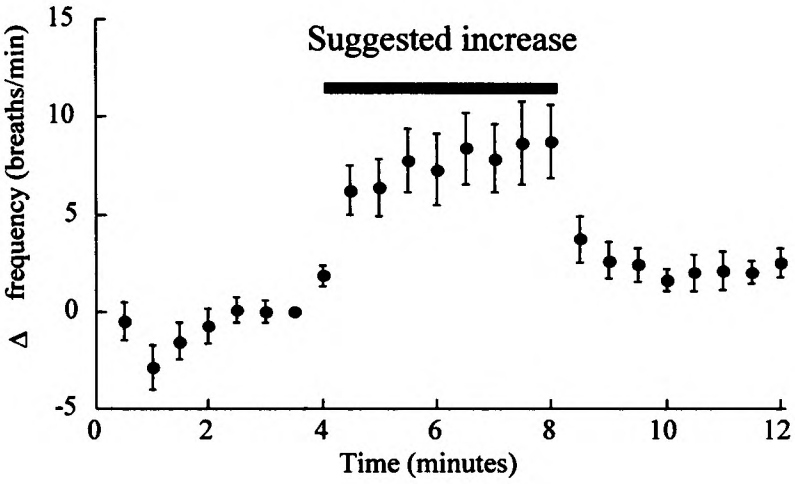
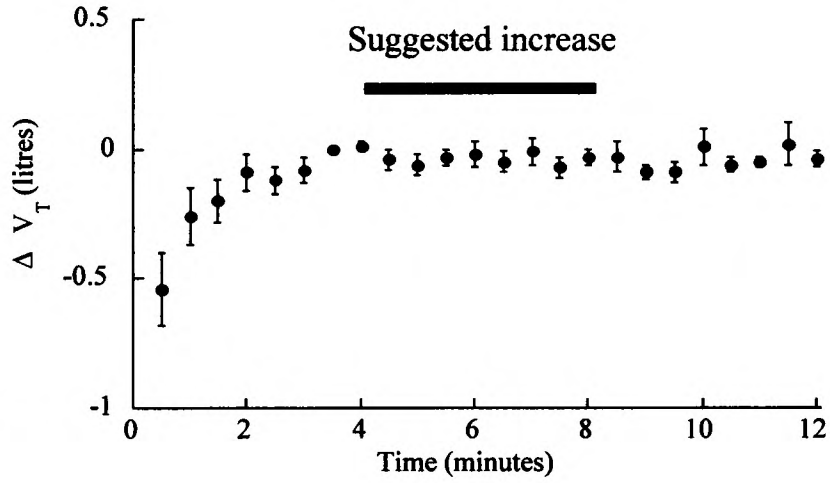


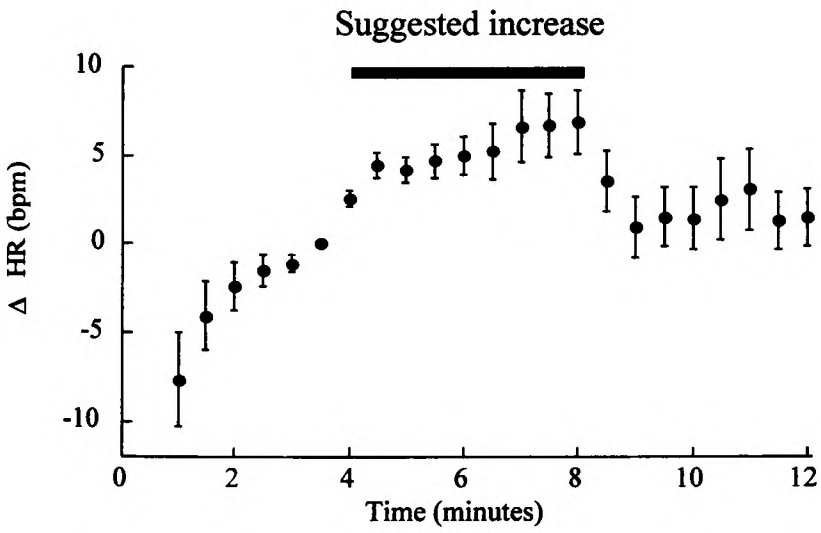
Figure 5.1. Raw data from one subject showing the response to a suggested increase in exercise load during a constant moderate exercise task (protocol 1). After 4 minutes of exercise it is suggested to the subject that the work rate has increased. This results in hyperventilation and hypocapnia which is reversed when after 8 minutes the subject is told that the work rate has returned to moderate. The slight negative flow signal is due to valve imperfections in the facemask and was of negligible volume.











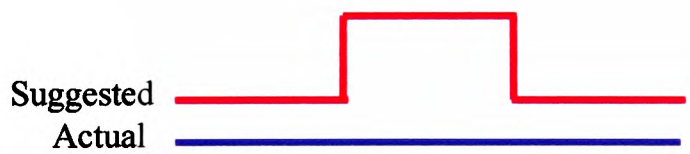


Figure 5.2. Group data for protocol 1 (constant moderate work with a suggestion of increased load). Each data point is the mean ± S.E.M of a 30 second epoch in 14 subjects, expressed as the change from the steady-state moderate exercise values (taken as the mean of the variable obtained between 3 and 3.5 minutes of constant moderate exercise). A) $\Delta \dot{V}_I$, B) $\Delta P_{ET}CO_2$, C) Δf, D) ΔV_T, E) Δ HR.

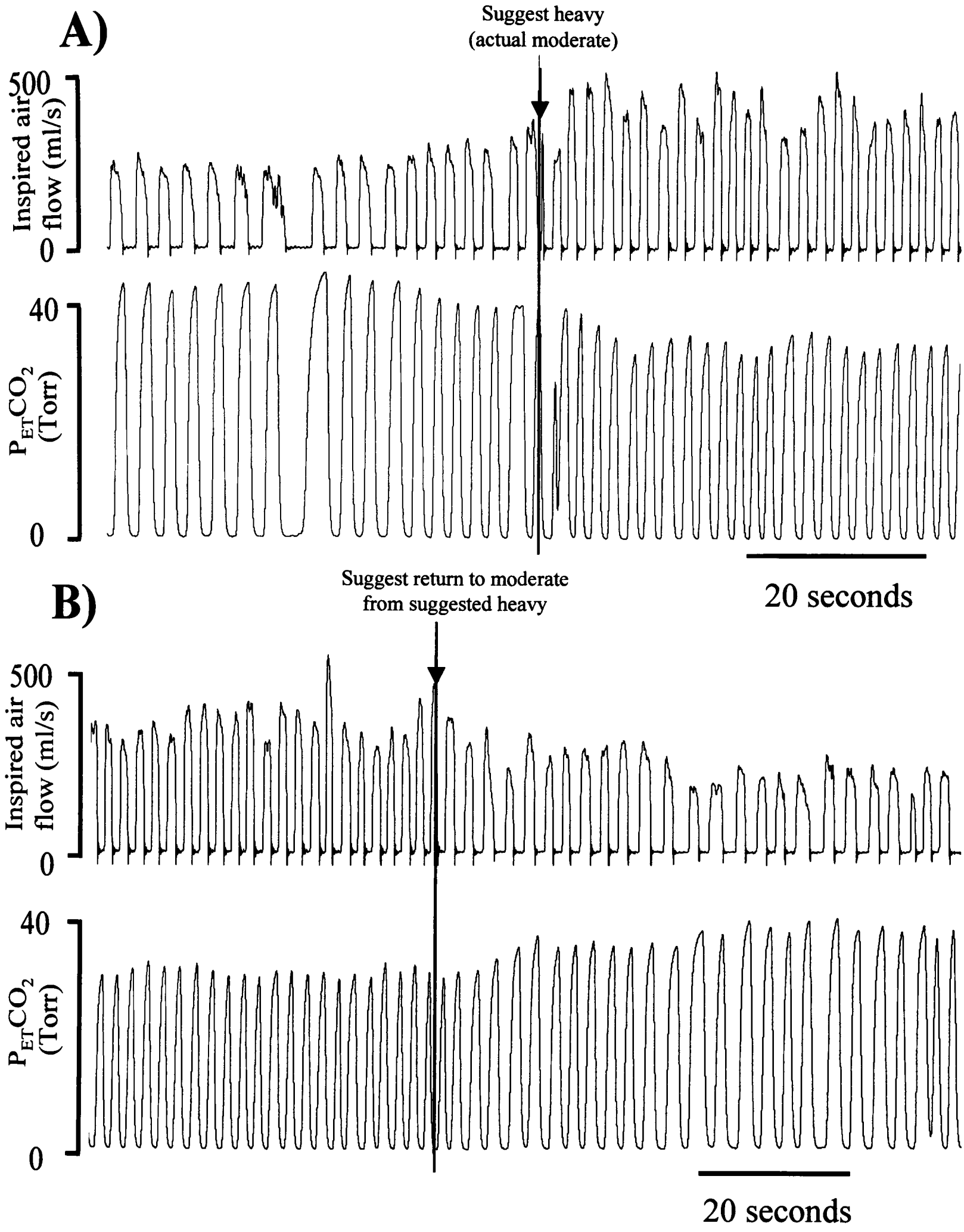


Figure 5.3. Raw data showing A) the immediate hyperventilation and hypocapnia occurring when it is suggested to the subject that the work rate has increased during a constant moderate exercise task. B) When the suggestion of an increased work rate is removed inspired air flow remains elevated for *c.a.* 30 seconds before it returns to that occurring during steady-state moderate exercise.

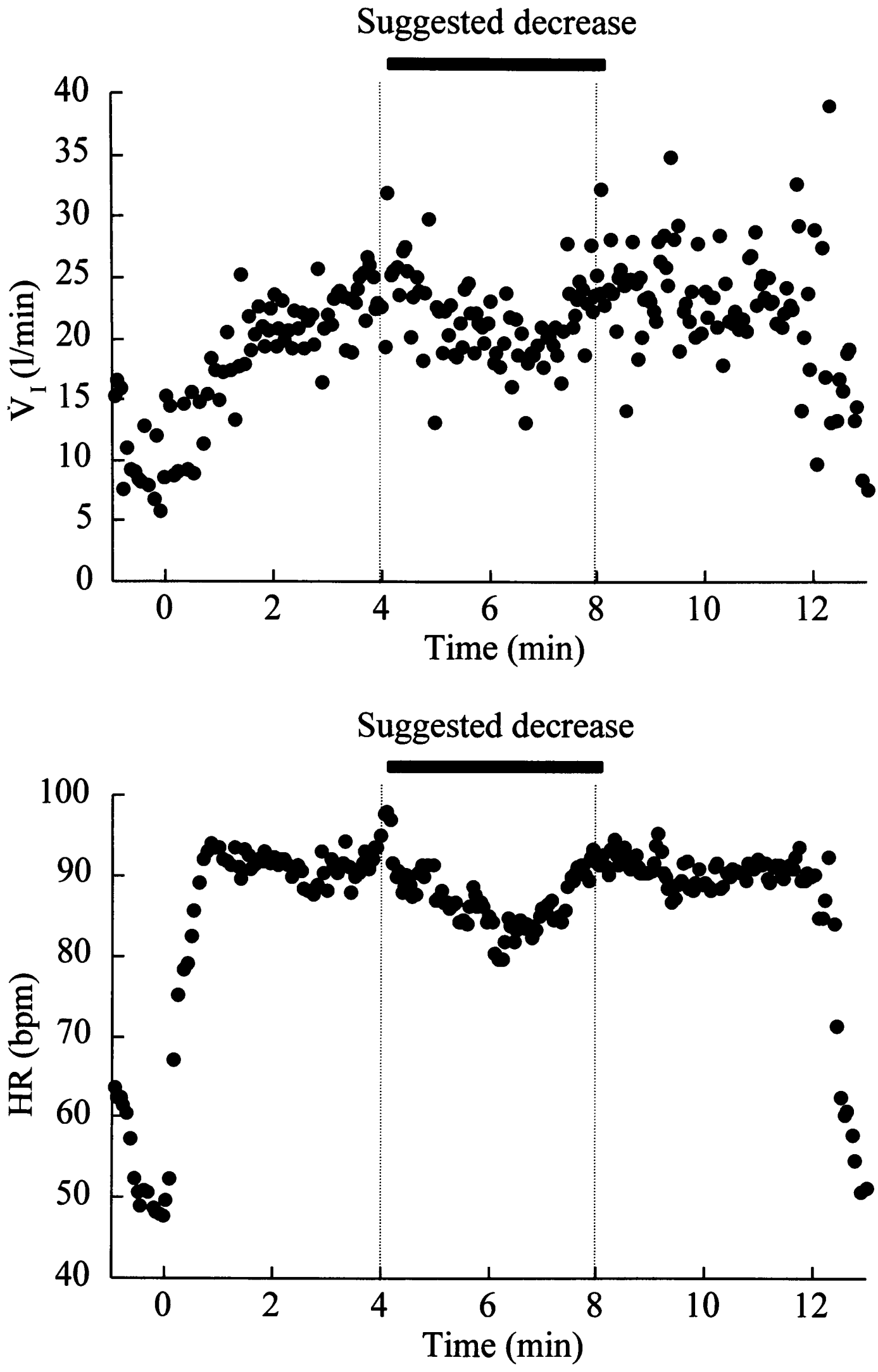


Figure 5.4. Data for one subject performing protocol 2 (constant moderate work with a suggestion of decreased load). Each data point represents a single breath. A) $\dot{V}_I$, B) HR.

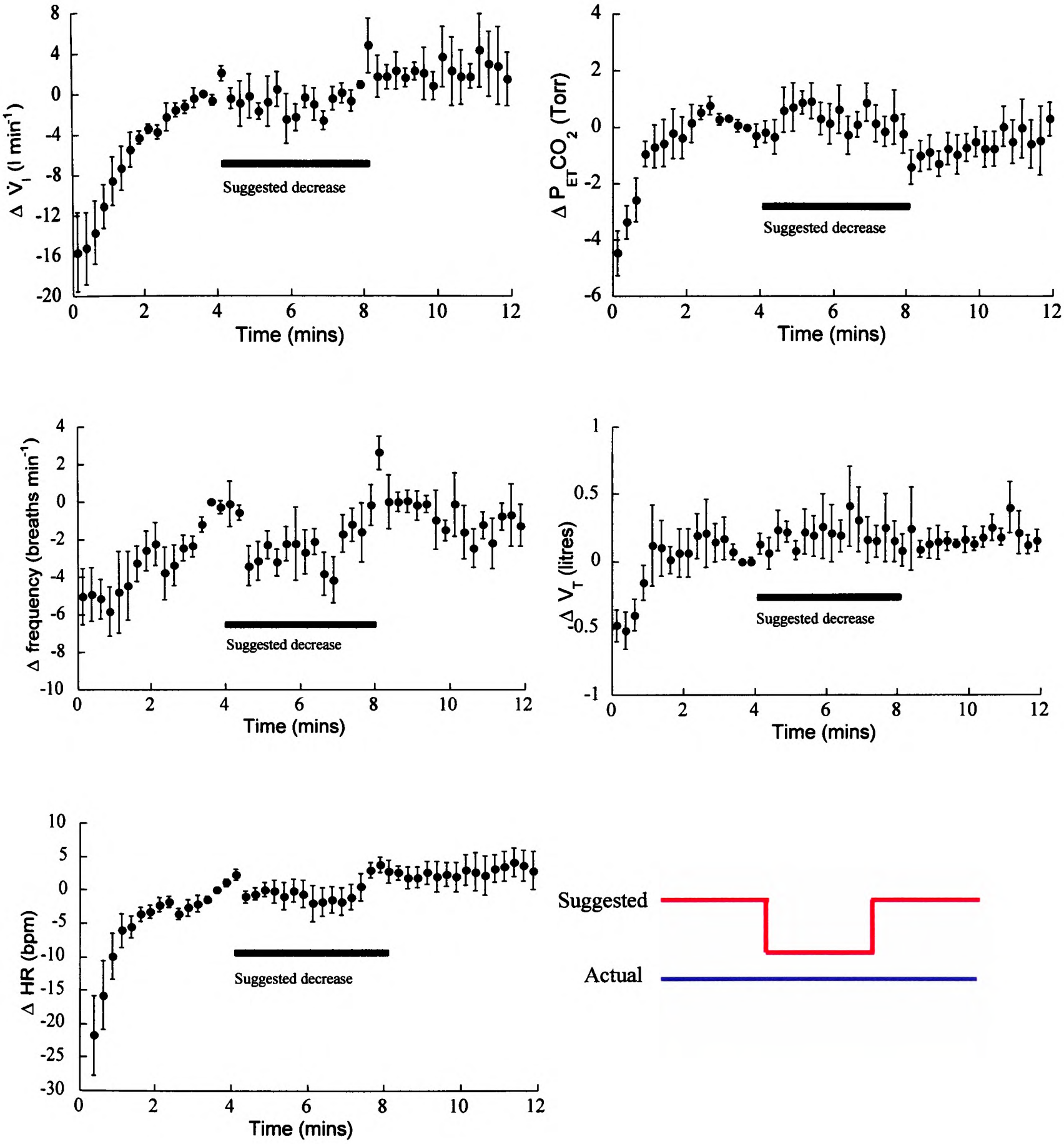


Figure 5.5. Group data for protocol 2 (constant moderate work with a suggestion of decreased load). Each data point is the mean ± S.E.M of a 15 second epoch in 5 subjects, expressed as the change from the steady-state moderate exercise values (taken as the mean of the variable obtained between 3.5 and 3.75 minutes of constant moderate exercise). A) Δ $\dot{V}_I$, B) Δ $P_{ET}CO_2$, C) Δ *f*, D) Δ V_T, E) Δ HR.

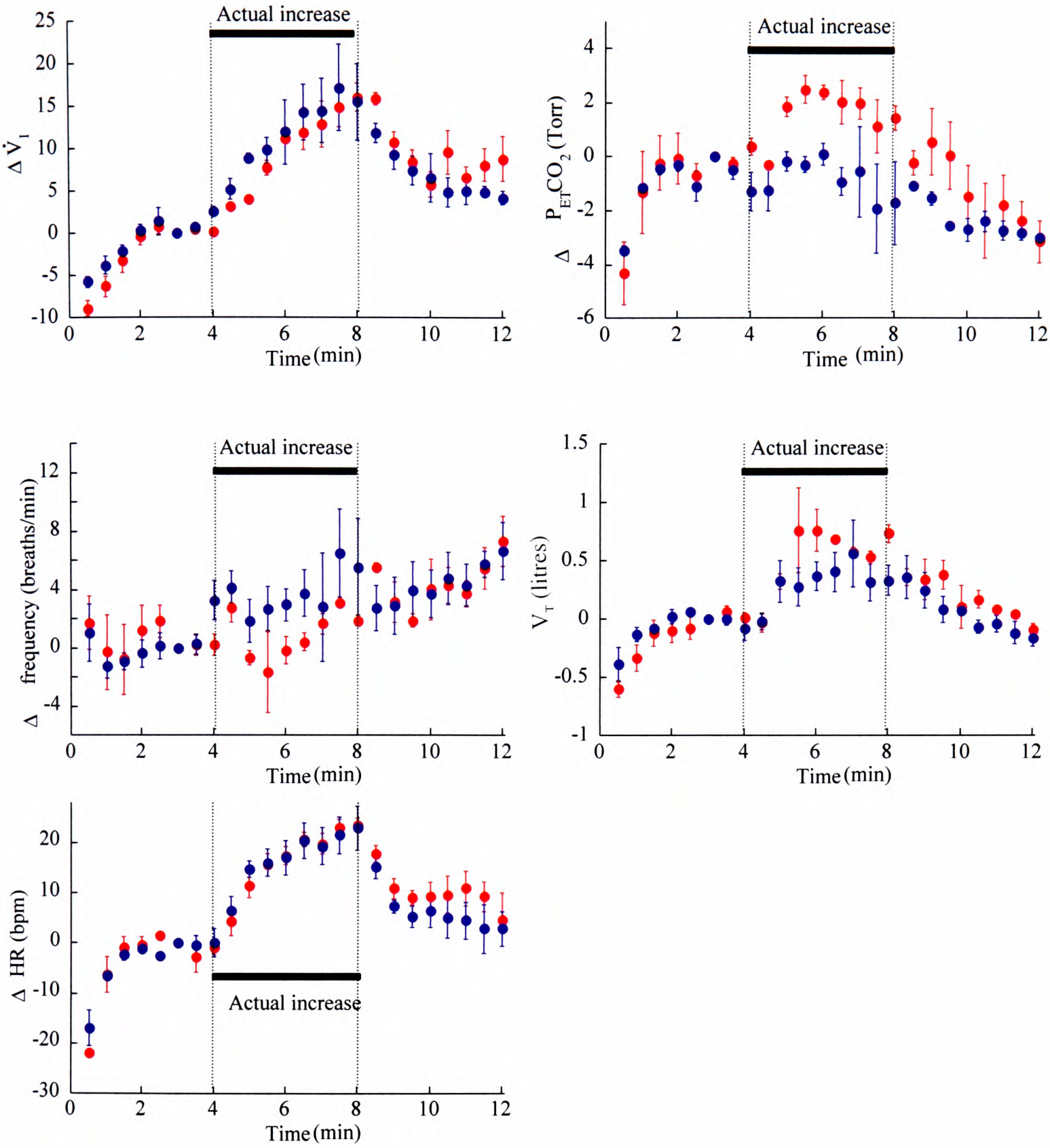


Figure 5.6. Response to a step *increase* in work rate during exercise under hypnosis with (●) and without (●) informing the subject of the change (protocol 3). Subjects exercise for 12 minutes, during the middle 4 minutes the work rate increases. Each data point is the mean ± S.E.M of a 30 second epoch in 3 subjects, expressed as the change from the steady-state moderate exercise values (taken as the mean of the variable obtained between 3 and 3.5 minutes of constant moderate exercise). A) Δ $\dot{V}_I$, B) Δ $P_{ET}CO_2$, C) Δ *f*, D) Δ V_T, E) Δ HR.

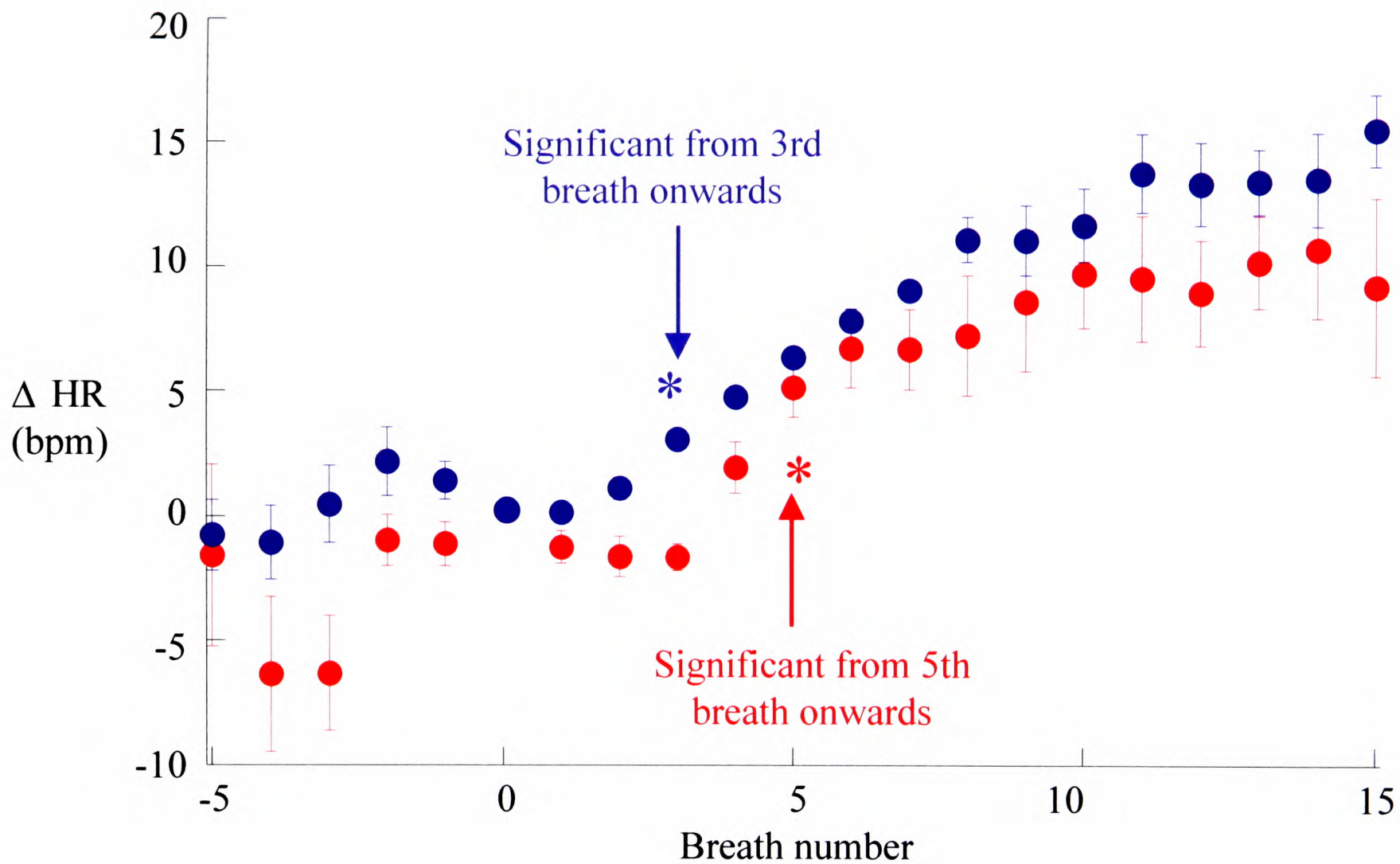
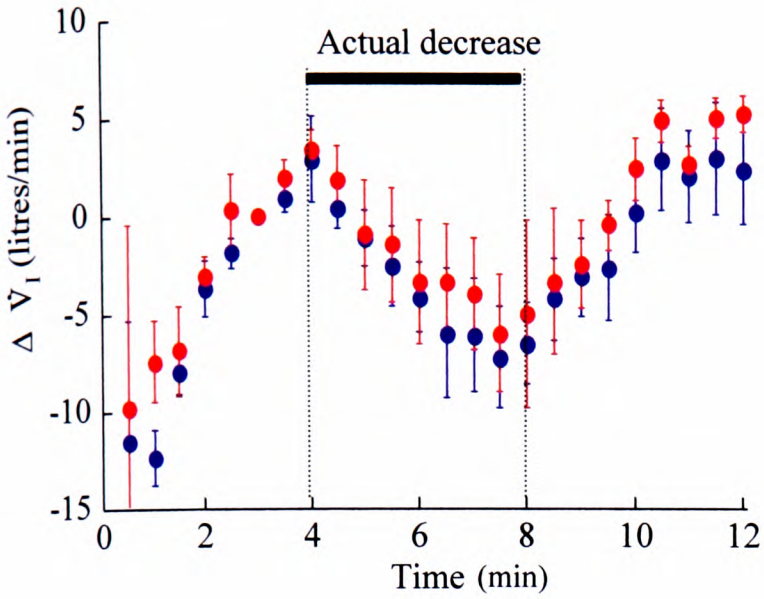
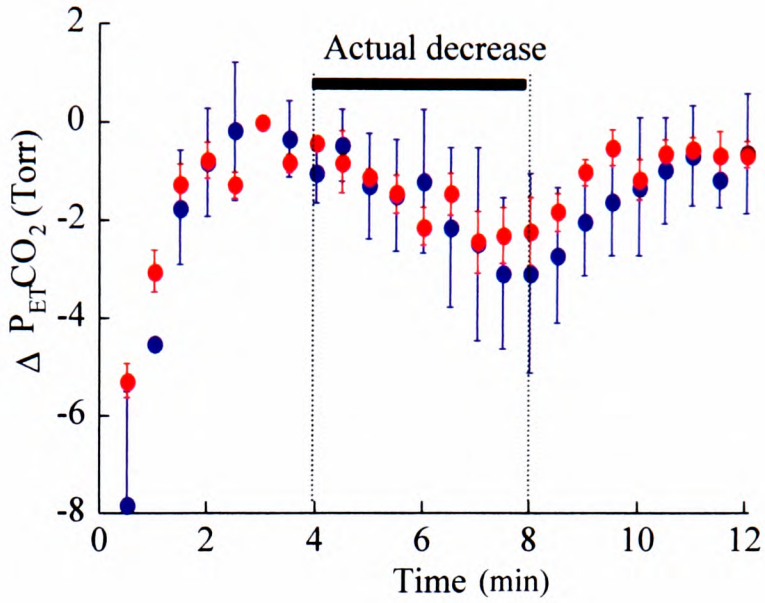
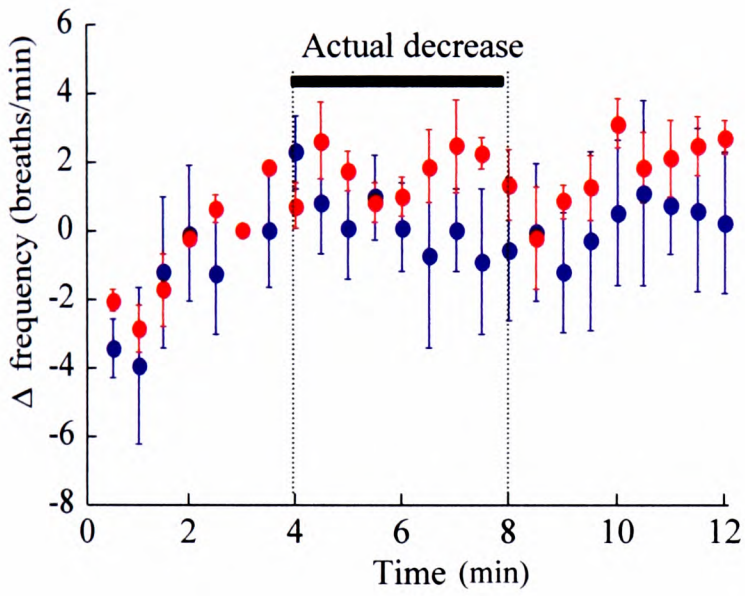
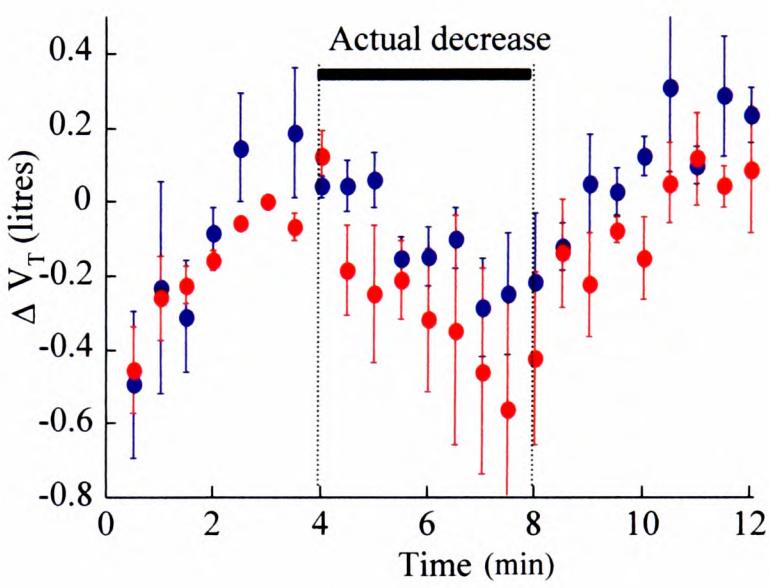


Figure 5.7. Transient HR responses to *increases* in work rate under hypnosis with (●) and without (●) informing the subject of the increase in work rate (protocol 3). Each data point represents the mean and SEM of 3 subjects. * * indicates significance tested using a repeated measures ANOVA on ranks and a *post hoc* SNK test.









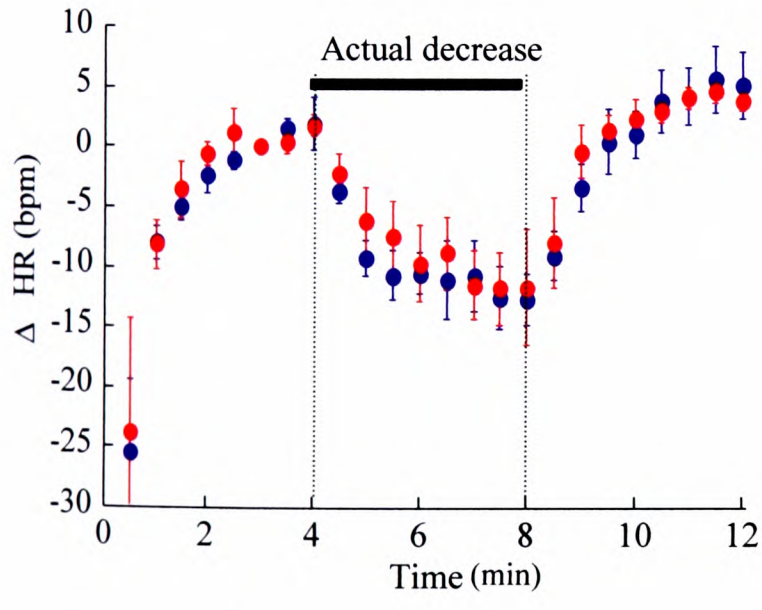


Figure 5.8. Response to a step decrease in work rate during exercise under hypnosis with (●) and without (●) informing the subject of the change (protocol 4). Each data point is the mean ± S.E.M of a 30 second epoch in 3 subjects, expressed as the change from the steady-state moderate exercise values (taken as the mean of the variable obtained between 3 and 3.5 minutes of constant moderate exercise). A) Δ $\dot{V}_I$, B) Δ $P_{ET}CO_2$, C) Δ *f*, D) Δ V_T, E) Δ HR.

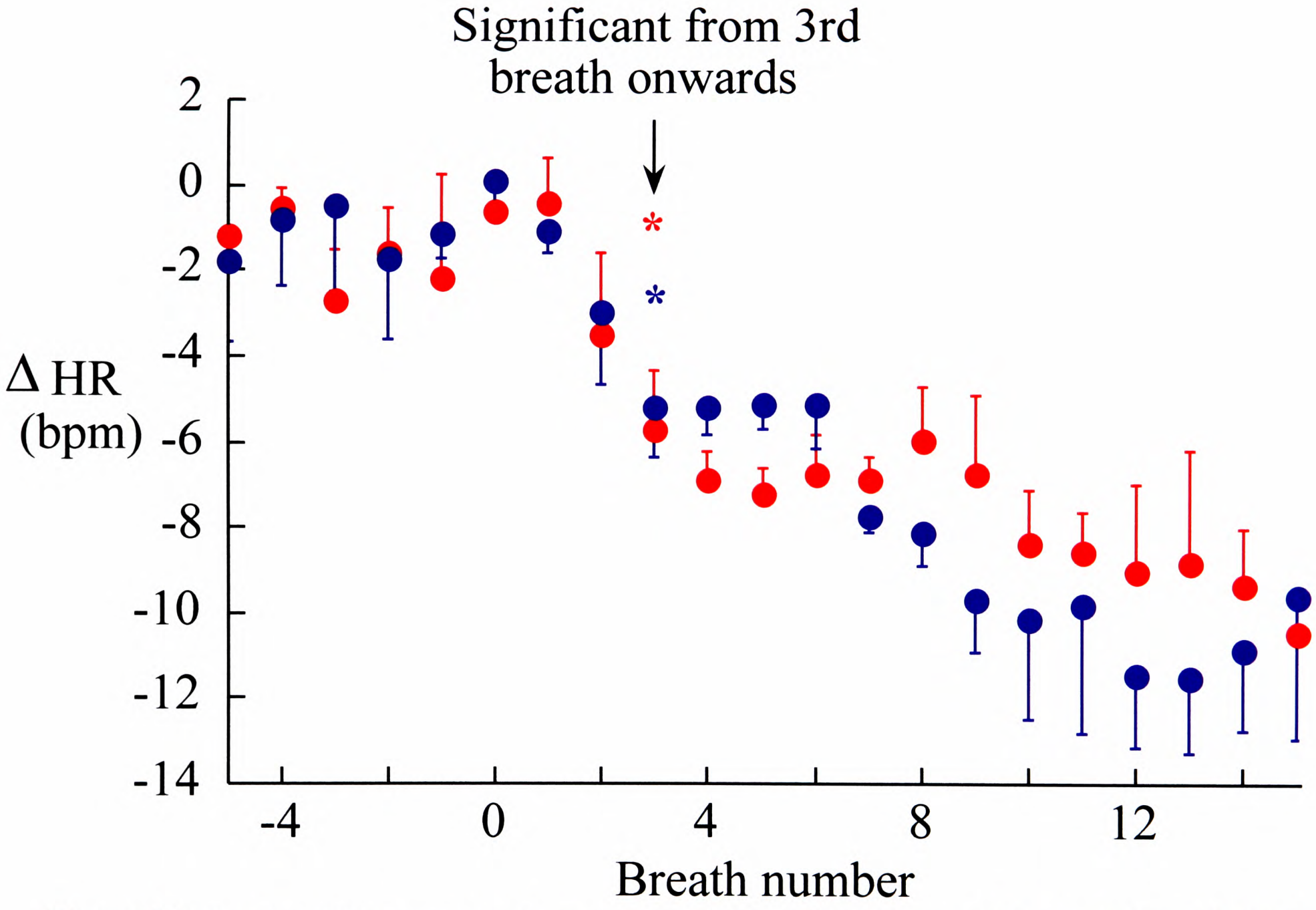


Figure 5.9. Transient HR responses to *decreases* in work rate under hypnosis with (●) and without (●) informing the subject of the decrease in work rate (protocol 4). Each data point represents the mean ±SEM in 3 subjects. * indicates significance as tested using a repeated measures ANOVA on ranks followed by a *post hoc* SNK test.

breath analysis was performed on the first 10 breaths after the change and showed no significant differences in the transients between the two repeats. A repeated measures ANOVA on ranks did not yield any significant differences in $\dot{V}_I$, $P_{ET}CO_2$, f or V_T. HR had significantly decreased by the 3rd breath during both the actual decrease without informing the subject (A) and the control (B), figure 5.9. All three subjects reported that they were aware of the load decreasing during both repeats:

"I know the load decreased, I felt it. You can't fool me that easily..."

5.4 Discussion

The main findings of this chapter are: 1) Subjects performing constant moderate exercise under hypnosis hyperventilate and become hypocapnic if told that the work rate has *increased.* 2) When after 8 minutes the subjects are told that the work rate had returned to the moderate level ventilation remains elevated for *ca.* 30 seconds. This is in contrast to the post-hyperventilation pauses seen in Chapter Three following imagination of exercise. 3) During constant moderate exercise a suggestion that the work rate has *decreased* does not alter steady-state cardiorespiratory variables. 4) When the work rate increases, steady-state cardiorespiratory variables increase whether or not the subject is informed of the change. However, there is a transient difference, with HR taking longer to rise if the subject is not informed of the change in work rate.

5.4.1 Constant moderate work rate with increased and decreased suggestion

5.4.1.1 Suggested increase

Subjects performing constant moderate exercise hyperventilate and become hypocapnic if told that the work rate has increased. Astonishingly, the magnitude of the ventilatory response was the same as if there was an actual increase in work rate suggesting that this protocol was more successful in highlighting the role of 'central command' than imagination of exercise at rest. This might reflect a requirement for peripheral feedback (e.g. from exercising muscles, or an increased $\dot{V}_{CO_2}$) to enable the full cardiorespiratory response to exercise to be expressed. This

hyperventilation was rapid in onset (figure 5.3) and then increased further and persisted for the four minutes of suggestion, so it seems that the hyperventilation was not merely an alerting response, rather it represents both the rapid phase I response and also 'slow central drive'. Although the increase in HR was more modest, it was *ca.* 35% of that occurring during an actual increase in work rate, too much to be accounted for solely by the increased work of breathing (10% total $\dot{V}_{O_2}$ during exercise, Aaron *et al.* 1992) indicating that the hypnotic suggestion resulted in some uncoupling of central versus peripheral mechanisms in control of HR during exercise.

Similar to the studies performed at rest described in chapter three, the hyperventilation during protocol 1 was entirely via an increase in *f* with no change in V_T. As discussed in chapter three, this may reflect either a unique feature of imagination under hypnosis, alternatively it may indicate that 'central command' is a frequency based drive to breathe and that changes in V_T are a consequence of the magnitude of peripheral feedback (e.g. muscle afferents or locomotor-respiratory coupling).

Krogh & Lindhard (1913) were the first to describe the effect of altered perception of work rate on ventilatory responses to exercise (in the awake state):

> *"...In one experiment the subject J.L. was told to work at a rapid rate and with a heavy load (3kg) but at the signal to begin the current was not turned on to the magnets of the ergometer. The resulting respiration during the first few seconds was practically the same as if there had been a heavy load, though the actual work performed was insignificant..."*

The use of hypnosis as a tool to modify perception of exercise load was first reported by Nemtzova (Nemtzova & Shatenstein 1936) who found that suggestions of an increase in work rate led to a 50% increase in $\dot{V}_I$. Similar results were reported more recently (Morgan *et al.* 1973) but interpretation of this study is difficult given the use of explicit cues for breathing, e.g.

> *"...your breathing will be very irregular and you will be very tense ...you will lack the ability to 'catch your wind' as you exercise..."*

This group of workers (Morgan *et al.* 1976) did repeat their earlier study without explicit cues regarding cardiorespiratory variables and observed an increase in $\dot{V}_I$ during a suggested increase in work rate, albeit without any increases in HR, $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$. Use of a metronome to signal pedalling frequency during the period of suggestion in these studies may also inadvertently have driven breathing frequency. Interestingly the study of Morgan *et al* (1976) was repeated in the awake state as well as under hypnosis, and this resulted in similar (but smaller) increases in breathing in response to suggested increases in work rate. Other authors (e.g. (Krogh & Lindhard 1913), have found that suggestions of altered work rate result in only transient changes in cardiorespiratory variables in the awake state.

5.4.1.2 Suggested decrease

Suggestion of a *decrease* in work rate did not result in a convincing steady-state decrease in $\dot{V}_I$ or HR although this might reflect the small number of subjects performing the protocol (n=5), since two of the subjects showed a maintained decrease in HR and $\dot{V}_I$ and the group data for $\dot{V}_I$ and f showed a significant decrease for part of the 4 minutes of suggestion (table 5.2). It is possible that metabolism sets a minimum level of $\dot{V}_I$ that can be increased but not decreased by 'central command' but this would not explain how two of the subjects were able to decrease their breathing and HR below that occurring during the preceding exercise period. There was no change in pedalling frequency so it is difficult to see how cycling efficiency could have been increased. It is possible that a relative hyperventilation was occurring in the first 4 minutes of exercise; this would allow a decrease in the 4 minutes of suggestion. However, a paired t-test comparing the moderate exercise prior to the suggested *decrease* (protocol 2) with that occurring prior to a suggested *increase* (protocol 1) showed them not to be significantly different, so this seems unlikely. An alternative explanation would be that 'after-discharge' contributes to exercise hyperpnoea in the steady-state. If this was the case it could explain the absence of an immediate decrease in breathing when it is suggested that the work rate has decreased, yet allow a decrease in the steady-state when 'after-discharge' has declined.

Daly & Overley (1966) made suggestions of rest during exercise under hypnosis and found that this resulted in a decrease in $\dot{V}_I$ and $P_{ET}CO_2$ although this was compared with exercise *in the awake state, not the hypnotic state*; since the authors also reported that $\dot{V}_I$ was lower and $P_{ET}CO_2$ higher under hypnosis compared to awake, this comparison of the two exercise protocols is inappropriate. Nemtzova *et al.* (1936) and Morgan *et al.* (1973) have performed protocols similar to that used in the current study and found a maintained decrease in $\dot{V}_I$ of *ca.* 25% during a suggested decrease in work rate. Unfortunately interpretation of the latter study is complicated by the use of explicit cardiorespiratory cues.

5.4.1.3 The post-hyperventilation state

In contrast to the post-hyperventilation expiratory pauses described following imagination of exercise in Chapter Three, no such phenomenon was observed during the off-transition from a suggested increase in work rate (protocol 1), indeed there was a tendency towards post-hyperventilation hyperpnoea (figure 5.2 and 5.3) since $\dot{V}_I$ was still 7.2±1.9 l/min above the pre-hyperventilation value, despite a $P_{ET}CO_2$ of 3.2±1.1 Torr below that at the onset of the hyperventilation. The post-hyperventilation hyperpnoea may be what is commonly described as 'short-term potentiation' (STP), 'after-discharge' or the 'flywheel effect'. This phenomenon of a response persisting after the stimulus has been removed has been demonstrated to occur following a variety of respiratory stimuli in man (e.g. hypoxia; Georgopoulos *et al.* 1990, exercise; Jeyaranjan *et al.* 1989 and volitional hyperventilation; Mills 1946, Swanson *et al.* 1976). It has also been demonstrated in animal studies following peripheral muscle stimulation, vagal stimulation and electrical stimulation of the brainstem (reviewed by Waldrop *et al.* 1996). This raises the question as to why in the current study post-hyperventilation expiratory *pauses* occur at rest when the instruction to imagine exercise is removed, but post-hyperventilation *hyperpnoea* occurs following cessation of an imagined increased work rate during constant exercise (figure 5.3). I have no definitive explanation for this, but it is consistent with the hypothesis that 'central command' *inhibits* the respiratory centres of the pons and medulla whilst stimulating breathing via an independent pathway. When framed within this hypothesis the current results could be explained: At rest imagination of exercise elicits hyperventilation

via a direct pathway not including the respiratory oscillator, when this central drive ceases expiratory pauses ensue (owing to the central stimulus having inhibited the respiratory oscillator). In contrast, during exercise (protocol 1) afferent feedback (e.g. from exercising limbs and arterial chemoreceptors) maintains stimulation of the respiratory oscillator, thus post-hyperventilation hyperpnoea occurs. This theory was originally proposed by Krogh & Lindhard (1913)

> *"The assumed irradiation of impulses might as far as we can see influence the respiration in three different ways... through the cortex centres of the respiratory muscles producing respiratory movements independently of and superseding the normal action of the respiratory centre"*

Evidence of a direct pathway from higher centres to the respiratory muscles comes from the phenomenon of neurosurgically induced 'Ondine's Curse' where high cervical cord damage abolishes automatic breathing whilst voluntary control is maintained (Severinghaus & Mitchell 1962). In addition trans-cranial electrical stimulation can result in diaphragm activation with a latency similar to that seen for other muscles (Gandevia & Rothwell 1987); suggesting a rapidly conducting oligo-synaptic pathway exists for diaphragmatic activation. Furthermore, electrical stimulation of the motor cortex in cats (Bassal & Bianchi 1981) inhibits the activity of some inspiratory propriobulbar and bulbospinal neurones, indicating that higher centres activating breathing may indeed inhibit parts of the ponto-medullary rhythm generator. Studies in anaesthetised cats also support the idea that higher centre drive does not necessarily act via the medullary cardiorespiratory centres. Bauer *et al* (1989) showed that the increases in HR, ABP and ventilation caused by muscular contraction were attenuated by injection of an excitatory amino acid inhibitor into the ventrolateral medulla, but that the cardiorespiratory increases caused by electrical stimulation of the hypothalamus were not affected.

5.4.2 Altered work rate with constant suggestion

In protocol 3 (*increases* in *actual* work rate, with and without informing the subject) there was no difference in steady-state cardiorespiratory variables according to whether or not the subject was informed of an increase in work rate. There are two possible explanations for this. First it may be that 'central command' cannot over-ride the effects of stimuli resulting from the

changes in metabolic rate accompanying the increase in work rate. Alternatively, this result may be a 'false negative'; since subjects were aware of the alteration in work rate even when not informed of it, the aim of the protocol (to keep 'central command' constant whilst varying peripheral factors) may not have been achieved. Subjects were far more willing to believe that a work rate had changed when in fact it hadn't (protocols 1 and 2), than to believe that it hadn't changed when in fact it had (protocols 3 and 4). If perception is an indicator of central drive then 'central command' was not maintained constant in protocols 3B and 4B. However, the fact that the HR transient during the transition from a 'moderate' to a 'heavy' work rate was slower if the subject wasn't informed of the work rate change (B) suggests that a degree of uncoupling of central and peripheral drives was achieved during this protocol. These findings may indicate that 'central command' is an essential drive during only the first seconds of an increase in work rate and subsequently peripheral factors can account for the entire cardiorespiratory response to exercise. This hypothesis is supported by the studies in which the ventilatory response to electrically induced leg exercise had a slower on-transient than the response to voluntary exercise, but that the steady state response was identical (Adams *et al.* 1984). Moreover, it seems that whilst 'central command' may be able to *increase* cardiorespiratory variables above that indicated by peripheral feedback, it is not possible for 'central command' to *decrease* HR and $\dot{V}_I$ below that required. In other words, if 'central command' and peripheral feedback generate conflicting signals the net cardiorespiratory drive can be more than that required by metabolism, but not less. Clearly if either perfusion or $\dot{V}_I$ are lower than that required, CO_2 will start to rise and this will generate a peripheral feedback signal that will increase cardiorespiratory variables.

There was no difference in transient or steady-state responses to an *actual* decrease in work rate between the two repeats of protocol 4. This is perhaps surprising; the transition to a lower work rate should mean that peripheral drives are decreasing and when viewed from a metabolic perspective, central drive could maintain cardiorespiratory variables above that required (as seen in protocol 1). Protocol 4 (decrease in work rate) might therefore be more likely to yield positive results than protocol 3 (increase in work rate). However this was not the case and it is possible that such an effect might be masked by the slowness of the cardiorespiratory kinetics

for decreases in work rate compared to increases, i.e. by the phenomenon of 'after discharge'. Also, examination of the cardiorespiratory variables in table 5.3 indicates that there may not have been a sufficient difference between the 'moderate' and 'light' work rates to detect any transient differences; steady-state cardiorespiratory variables were not significantly lower at the 'light' work rate than at the 'moderate'.

5.4.3 Study limitations: quantifying 'central command'

An obvious problem with studying 'central command' during exercise is finding an objective way of measuring its magnitude. In the absence of such a measure it is necessary to maintain a constant level of peripheral feedback and then to assume that any changes in cardiorespiratory variables are due to 'central command', i.e. to use the cardiorespiratory response as the indicator variable. An alternative approach is to equate 'central command' with *perception* of work rate and to quantify perception (e.g. using a Borg scale (Borg 1982). However, perception is clearly influenced by peripheral feedback, so is not a good measure of 'central command'. A Borg scale was not used in this study as it was clear from the outset that hypnotic suggestions modify the relationship between perception and an actual event, so any quantification of perception is likely to be misleading.

However, there is some evidence to suggest that perception or 'effort' is genuinely related to 'central command'. Hobbs (1982) trained baboons to lift weights and performed a tendon vibration study similar to the human study of Goodwin *et al* (1972) and found strikingly similar results to those seen in humans. Clearly, experimental naivity is not an issue with baboons so there would appear to be a direct correlation between motor outflow, 'central command' and the cardiorespiratory response. In addition, Gandevia & McCloskey (1977) showed that in the presence of partial unilateral weakness induced by neuromuscular blockade, normal subjects overestimated the weight of objects, suggesting that they were rating weight according to the efferent motor drive necessary to perform the task and not the afferent feedback from the limb. An interesting study to determine whether cardiorespiratory changes occur due to perceived or actual work rate could make use of the so-called 'size-weight illusion' (Burgess & Jones 1997). If asked to lift two objects of equal mass but different volume the larger object feels lighter.

Consequently whilst the actual work is constant, the perceived work is less when lifting the larger object and this might be reflected in cardiorespiratory changes.

Two types of responses were observed in this study when a subject was aware that the experimental condition was not the same as the condition suggested by the hypnotist; they either played along with it, e.g.

> *"I knew the load had changed, it decreased, but I tried to imagine it hadn't"*

or they continued in a state of quiet disbelief without any concern for the suggestion:

> *"I knew the load had changed, you can't fool me that easily"*

It is possible that this difference in strategy may produce different effects on cardiorespiratory variables, although this would be difficult to quantify. Given the fact that subject's did not always believe the suggestions that were being made to them it was important to have randomised protocols, since the nature of a subjects response may be altered by the protocols they had previously performed.

5.5 Summary

The main findings of the studies described in this chapter are:

1) If hypnotised subjects were told that the work rate had *increased* whilst they were in fact performing *constant* moderate exercise they hyperventilated and became hypocapnic and HR increased.
2) The breathing pattern in the post-hyperventilation state in this study compared to that in the study described in Chapter Three indicates that 'central command' may actually *inhibit* the respiratory rhythm generator whilst increasing cardiorespiratory variables via an independent pathway.
3) Suggestions that the work rate had *decreased* whilst performing *constant* moderate exercise did not result in any maintained steady-state alterations in cardiorespiratory variables.
4) If the work rate was increased the steady-state cardiorespiratory variables were identical whether or not the subject was informed of the increase. However, there was a transient

difference, with HR taking longer to rise if the subject was not informed of the increased work rate.

5) The results confirm and extend those of Chapter Three. A powerful drive to breathe originating from higher centres arises when exercise or increased work rate is imagined under hypnosis. Hypnosis may be a useful tool with which to investigate 'central command' in humans since it may allow partial uncoupling of central versus peripheral drives to breathe.

Chapter Six

Electrical stimulation of the midbrain in awake man

The mode of application of the stimulus must be 'physiologically meaningful', that is, it should not be grossly different from that which the mechanism is designed to handle.

SM Tenney.

6.1 Introduction

Animal studies (Smith *et al.* 1960; Eldridge *et al.* 1981; Bedford *et al.* 1992; Angyan 1994; Angyan 1996) have shown that electrical stimulation of the hypothalamus, thalamus, basal ganglia and mesencephalic locomotor region can cause locomotion together with a cardiorespiratory response even when peripheral feedback is abolished. Together these findings suggest that subcortical areas may integrate descending locomotor and cardiorespiratory drives and thus may contribute to 'central command' during exercise. However, there is little human data supporting this; thalamic and midbrain activity has been demonstrated during and immediately following exercise (Fink *et al.* 1995) using PET scanning and also in the studies of imagination of exercise in Chapter Three. However, PET does not have sufficient resolution to reliably identify *specific* midbrain nuclei (e.g. the subthalamic nucleus which is *ca.* 5x2mm in man; Mai *et al.* 1997) and fMRI studies are complicated by the fact that the brainstem moves *ca.* 1mm in time with cardiac and respiratory cycles (Poncelet *et al.* 1992). This is sufficient to cause considerable inaccuracies when recording data in which the task involves alterations in cardiorespiratory variables (and therefore in brainstem movement). Consequently other ways of assessing the role of these brain regions in 'central command' are required.

Between 1950 and 1970 ablation of specific thalamic and midbrain nuclei played a major role in the treatment of Parkinson's Disease. With the introduction of levodopa in 1968 surgical treatment declined substantially. However, recent improvements in stereotaxic surgery and a growing appreciation of the limitations of levodopa therapy have renewed interest in the use of surgery for patients with Parkinson's. Surgery has also become an option in patients with other movement disorders, including benign essential tremor and the dystonias. The two main surgical options[1] are electrolytic lesioning (Gill & Heywood 1997) or implantation of electrodes for chronic high frequency electrical stimulation of the basal ganglia (Limousin *et al.* 1995). Both of these treatments decrease the output of the basal ganglia, which are

[1] Transplantation of foetal dopaminergic neurones has been attempted with variable clinical benefit and is not yet a common treatment (Kordower *et al.* 1997).

pathologically overactive in these disorders, thereby facilitating movement and relieving symptoms of akinesia and bradykinesia. During surgery electrical stimulation of the ventralis intermedius thalamus (VIM), ventralis oralis posterior thalamus (VOP), subthalamic nucleus (STN), substantia nigra (SN) and internal pallidum (GPi) is performed in awake patients. This surgical procedure therefore provides the ideal opportunity to study the cardiovascular effects of electrical stimulation of subcortical structures in awake man and so perform a similar study to that which has been performed many times in animals. Since many Parkinson's patients have impaired autonomic function it is of interest to study the effects of chronic electrical stimulation on cardiovascular autonomic reactivity in patients with implanted electrodes.

6.2 Methods

Subjects were patients undergoing stereotaxic neurosurgery for either the placement of electrical stimulating electrodes (group A) or electrolytic lesioning (group B) of the subthalamic nucleus, pallidum, VIM thalamus or VOP thalamus. Fourteen patients had Parkinson's Disease, one had benign essential tremor, one myoclonic dystonia and one had spasmodic torticollis. All patients gave written informed consent after receiving an account of the nature of the study. Experiments were carried out with local ethics committee approval (COREC 99.083).

6.2.1 **Protocols**

Intraoperative experiments (groups A and B)

Surgery was performed as described in Chapter Two. In patients undergoing surgery on the STN, the electrode trajectory passes through the ventral lateral thalamic nuclei, into the STN and then the substantia nigra (figure 6.1). In patients undergoing surgery on the pallidum, a more lateral trajectory is used, the electrode passing through the external then internal pallidum (figure 6.1). At each location, electrical stimulation was performed with pulse widths, frequencies and voltages chosen by the surgeon. Patients were instrumented with a 3 lead ECG and an arterial cannula and data were recorded on an AS/3 Anaesthetic monitor

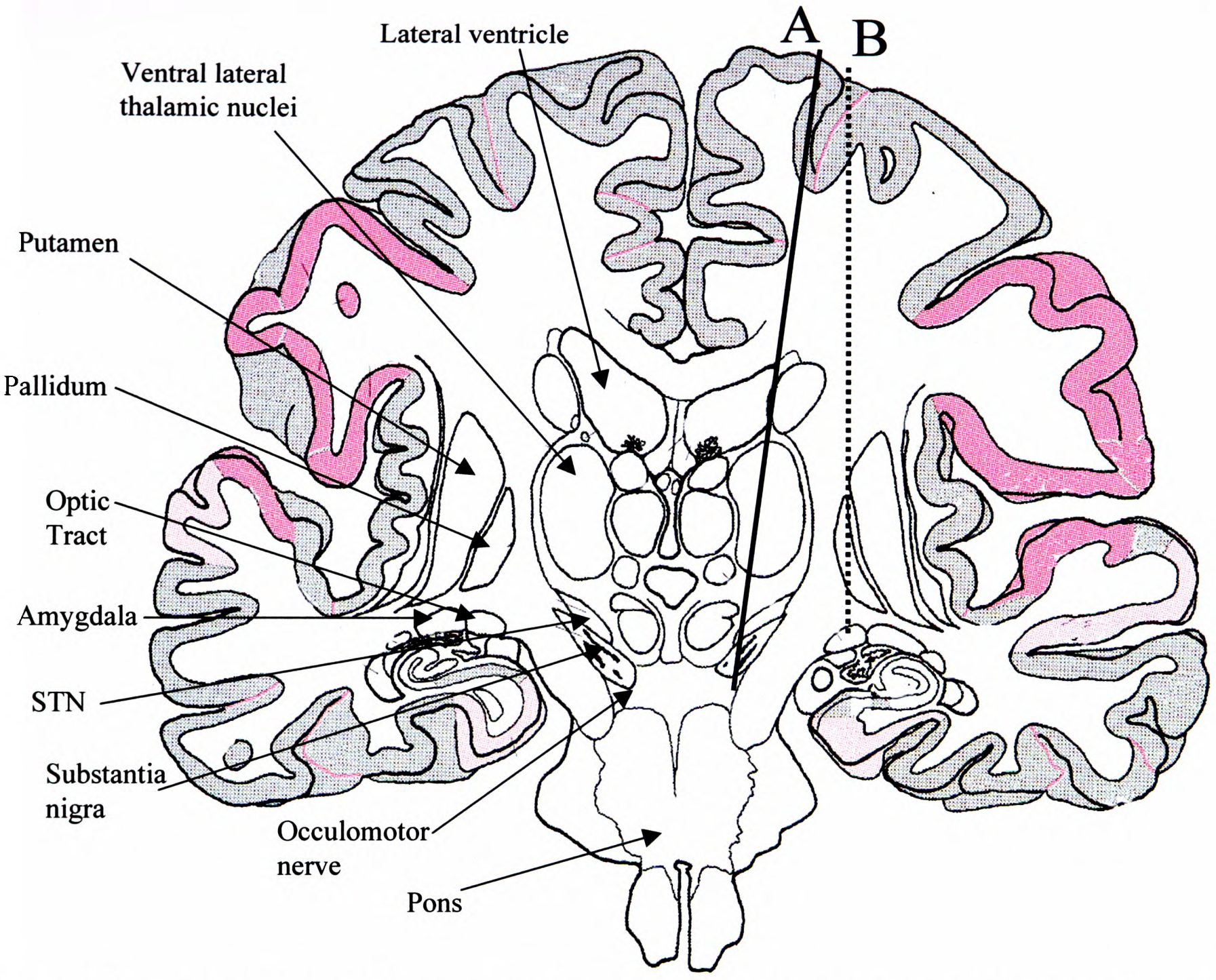


Figure 6.1. Anatomy and electrode trajectories for stereotaxic neurosurgery. This coronal section shows the major structures that are stimulated/lesioned and the surrounding structures. The solid line (A) shows the trajectory for surgery on the STN, the dotted line (B) shows the trajectory used for surgery on the pallidum. During surgery on the STN electrical stimulation of the ventral lateral thalamic nuclei and the substantia nigra is also performed. From Mai *et al.* (1997) with permission.

(Datex-Engstrom). Analogue outputs from the anaesthetic monitor were sampled at 500 Hz, digitised (BIOPAC MP100WS) and recorded on a Power Macintosh computer using Acqknowledge software for subsequent analysis.

Postoperative experiments (group A only)

Patients with implanted stimulating electrodes were studied an average of 3±1 months postoperatively. Two types of experiments were performed on these patients:

a) Assessment of autonomic function. Patients were asked to lie supine for 15 minutes with stimulators on and a further 15 minutes after stimulators had been turned off. The order of these two procedures was randomised.
b) Assessment of exercise pressor response. Patients were asked to perform a constant static handgrip at 30% of their maximum voluntary contraction (MVC) for 2 minutes with their dominant arm using a handgrip dynamometer. This protocol was repeated with and without stimulation, separated by a period of at least 20 minutes. The order of the assessments was randomised.

Patients were instrumented with a 3 lead ECG (HME LifeTrak 20). Beat-by-beat finger arterial blood pressure was obtained using a Finapress at heart level (2300, Ohmeda) and HR was triggered from either the ECG or the Finapress. Analogue outputs were sampled at 500Hz, digitised and recorded on a Power Macintosh computer for subsequent analysis.

6.2.2 Data analysis

Intraoperative studies: Control values for HR and MAP were taken as the mean value for the 5 seconds preceding electrical stimulation. Stimulation values are the mean of a 5 second period during stimulation. Data for electrical stimulation were pooled for an individual subject according to the nucleus in which the electrode was situated (determined according to the number of mm above or below the target point chosen from the fused MRI and CT scans), and the frequency of stimulation (>90Hz was considered high frequency, <90Hz low frequency). The mean response was calculated for each patient calculated for each nucleus at both high

and low frequency stimulation and then averaged across all patients. Control and electrical stimulation data were compared using a paired t-test.

Postoperative studies: 15 minutes of resting data with and without electrical stimulation was analysed for baroreflex sensitivity (BRS) and heart rate and blood pressure variability (HR/BPV) as described in Chapter Two. Data from the study of handgrip exercise are presented as case studies.

6.3 Results

6.3.1 Intraoperative studies

Fifteen patients (7 male) aged 57±2 years with a disease duration of 13±1 years were studied intraoperatively (surgical target; n=1 thalamus, n=8 STN, n=6 pallidum). Patient characteristics are given in table 6.1.

6.3.1.1 Electrical stimulation of the thalamus, STN and substantia nigra

HR increased during high frequency electrical stimulation of the thalamus (5±3 bpm, $p<0.05$), STN (5±3 bpm, $p<0.01$) or substantia nigra (6±3 bpm, $p<0.01$), (figures 6.2, 6.4). MAP also increased significantly (figures 6.2, 6.4). Stimulation parameters were 0.5±0.2ms, 100Hz, 1.3±0.2 Volts (thalamus), 0.5±0.1ms, 100±3Hz, 1.5±0.2 Volts (STN), 0.4±0.2ms, 101±1Hz, 1.9±0.4 Volts (substantia nigra). Electrical stimulation resulted in improved motor signs including decreased tremor, improved rigidity and a lessening of bradykinesia. The duration of the electrical stimulation averaged 80±14 seconds. Low frequency (15±8Hz) electrical stimulation of the thalamus, STN or substantia nigra did not affect either HR or MAP (figure 6.5) and did not improve motor signs.

6.3.1.2 Electrical stimulation of the pallidum

Neither high nor low frequency electrical stimulation of the pallidum had any effect on HR or ABP (figure 6.3-6.5). Stimulation parameters were 0.4±0.2ms, 100Hz, 1.9±0.2 Volts (high

frequency stimulation) and 0.4±0.2ms, 2.4±0.4Hz, 2.2±0.4 Volts (low frequency stimulation). Electrical stimulation of the pallidum was accompanied by improvements in rigidity and akinesia. The duration of electrical stimulation was 94±21 seconds.

6.3.2 Post-operative studies

Seven patients (3 male) aged 56±5 years and with a disease duration of 10±2 years, were studied postoperatively. Patient characteristics are given in table 6.2.

6.3.2.1 Resting autonomic function with and without electrical stimulation

It was not possible to record blood pressure using the finapress in three patients owing to tremor and rigidity in all limbs. There was no change in baroreflex sensitivity (BRS) with and without electrical stimulation in the four patients in whom it could be measured, as shown in table 6.3. However, the number of slopes from which it was measured was fewer than generally recommended (e.g. between 0 and 4 slopes in 15 minutes in Parkinson's patients, compared to 46 in the same time in a spasmodic torticollis patient). Chronic electrical stimulation of the STN (n=5) or pallidum (n=1) did not affect blood pressure variability (BPV, n=5) or heart rate variability (HRV, n=6).

6.3.2.2 Handgrip exercise

This was performed with and without electrical stimulation by five patients, but there were a number of difficulties. These included a) patients having insufficient strength to be able to perform handgrip exercise with a force great enough to increase blood pressure or HR, b) handgrip inducing tremor which resulted in a variable force of contraction, c) tremor preventing blood pressure recordings from the finapress. Initial study showed that 30% of maximum voluntary contraction did not generate any increase in cardiovascular variables, consequently patients were asked to perform a maximum contraction and maintain as much of this force as they good over the course of 2 minutes. The aim of this was to achieve a maximal

Patient	*Age*	*Sex*	*Disease duration (years)*	*Surgery* [1]	*Comments* [2]
01	58	F	9	L STN stim.	Previous R VIM les. Sinemet, selegiline.
02	55	M	12	Bilat STN stim.	Previous L STN les. Madopar, clonazepam.
03	60	F	19	L STN stim.	Previous R STN les. Pergolide, sinemet.
04	54	F	12	Bilat STN stim.	Pergolide, madopar.
05	40	M	13	L pall les.	Sinemet, selegiline.
06	54	M	9	L pall les.	Sinemet, pergolide, selegiline.
07	78	F	11	R pall les.	Madopar, sinemet.
08	47	F	15	R STN les.	Sinemet, selegiline, orphenadrine, bromocriptine.
09	67	M	6	L STN les.	Madopar, Clonazepam, Propanolol.
10	63	M	8	L pall les.	Previous R pall les.
11	67	F	7	Bilat pall stim.	Spasmodic torticollis[3], not PD. Diazepam, fluoxcetine.
12	50	M	24	R STN les.	Previous L pall les. Apomorphine, sinemet.
13	64	M	17	L pall les.	Sinemet, pergolide.
14	64	F	9	Bilat STN stim.	Sinemet, baclofen, pergolide, procyclidine, domperidone, apomorphine
15	32	F	20	L VOP les.	Benign essential tremor[4] not PD. Target VOP thalamus

Table 6.1. Characteristics of patients studied intraoperatively. Abbreviations: STN subthalamic nucleus, pall. internal globus pallidum, VOP ventralis oralis posterior thalamus, VIM ventralis intermedius thalamus, les. lesion, stim. stimulator implantation, VIM ventral interomedial thalamus.

[1] Lesion or implant site is chosen according to the symptoms of the patient. The pallidum is targeted in patients with severe drug induced dyskinesias (Krack *et al.* 1998) and also improves akinesia and rigidity. The STN is preferable for tremor removal [Gill, 1997 #10] and in patients with widespread tremor and gait problems.
[2] Only drugs related to Parkinsonian or cardiovascular symptoms are listed (other drugs included anti-depressants, anti-emetics, analgesics, anxiolytics and sedatives). Levodopa (a dopamine precursor) is the most common pharmacological strategy for managing PD, usually in conjunction with a peripheral decarboxylase inhibitor such as carbidopa or selegiline. These combinations are marketed as Madopar and Sinemet. The usefulness of levodopa is limited by its tendency to cause involuntary movements (dyskinesias) and it's rapid 'on-off' effects. Other drug strategies include ACh antagonists (benztropine, orphenadrine, procyclidine), drugs mimicking the action of dopamine (pergolide, apomorphine, bromocriptine) and drugs causing release of dopamaine (amantadine). For a review of the drug treatment of Parkinson's Disease, see Rang (1999).
[3] Spasmodic torticollis is a focal dystonia of the neck, accompanied by head tremor, reviewed by Berardelli *et al* (1998).
[4] Benign essential tremor; a tremor of unknown cause but believed to involve thalamic and basal ganglia circuitry, reviewed by Findley & Koller (1987).

Patient	*Age*	*Sex*	*Disease duration (years)*	*Surgery*	*Stimulation parameters (Volts, Hz, μsec)*			*Comments*
02	55	M	12	L STN stim	4.2	170	90	Previous L STN les. Madopar, clonazepam. R STN stim implanted but not working.
03	60	F	19	L STN stim	3.6	185	60	Previous R STN les. Pergolide, sinemet.
04	54	F	12	Bilat STN stim	4.9	130	90	Pergolide, madopar.
11	67	F	7	Bilat pall stim	3.5	140	100	Spasmodic torticollis, not PD. Diazepam, fluoxcetine.
14	64	F	9	Bilat STN stim	3.0	130	90	Sinemet, baclofen, pergolide, procyclidine, domperidone, apomorphine
16	62	M	5	R STN stim	2.8	180	90	Madopar. Previous L VIM les.
17	27	M	10	Bilat pall stim	6.5	130	210	Myoclonic dystonia not PD.

Table 6.2. Characteristics of patients studied postoperatively. Details as for table 6.1.

	Stimulated	*Unstimulated*
Total (ms $mmHg^{-1}$)	2.4±0.4	2.9±0.3
Down-slope	2.1±0.4	2.9±0.6
Up-slope	2.7±0.4	2.9±0.3

Table 6.3. Baroreflex sensitivity (lag 0) assessed by the spontaneous sequence method with and without chronic high frequency electrical stimulation. All values are mean ± SEM, n=4. Up-slope, slope of the correlation lines between R-R interval lengthening in response to spontaneous *increases* in SBP; Down-slope, slope of the correlation lines between R-R interval shortening in response to spontaneous *decreases* in SBP. There was no significant difference in baroreflex sensitivity with and without electrical stimulation (paired t-test).

	Stimulated	*Unstimulated*
HR variability		
RR mean	840±60	810±34
RR variance (SD)	26.1±3.3	28.7±8.1
ln LF power	4.9±0.3	4.6±0.2
ln HFpower	3.8±0.4	3.8±0.3
BP variability		
Variance		
ln Systolic BP	2.1±0.2	2.1±0.2
ln Diastolic BP	1.5±0.2	1.4±0
LF power		
ln Systolic	2.2±0.3	1.9±0.4
ln Diastolic	1.1±0.4	0.4±0.4
HF power		
ln Systolic	0.9±0.4	0.8±0.5
ln Diastolic	-0.1±0.7	-0.7±0.6

Table 6.4. HR and blood pressure variability with and without chronic high frequency electrical stimulation. All values are mean ± SEM, n=5 (blood pressure) or 6 (HR). HF, high frequency (0.15-0.40 Hz); LF, low frequency (0.04-0.15 Hz). N.B. Spectral data were not normally distributed and so a natural log transformation was performed prior to statistical analysis (indicated by ln). There was no significant difference in any variable with or without electrical stimulation (paired t-test).

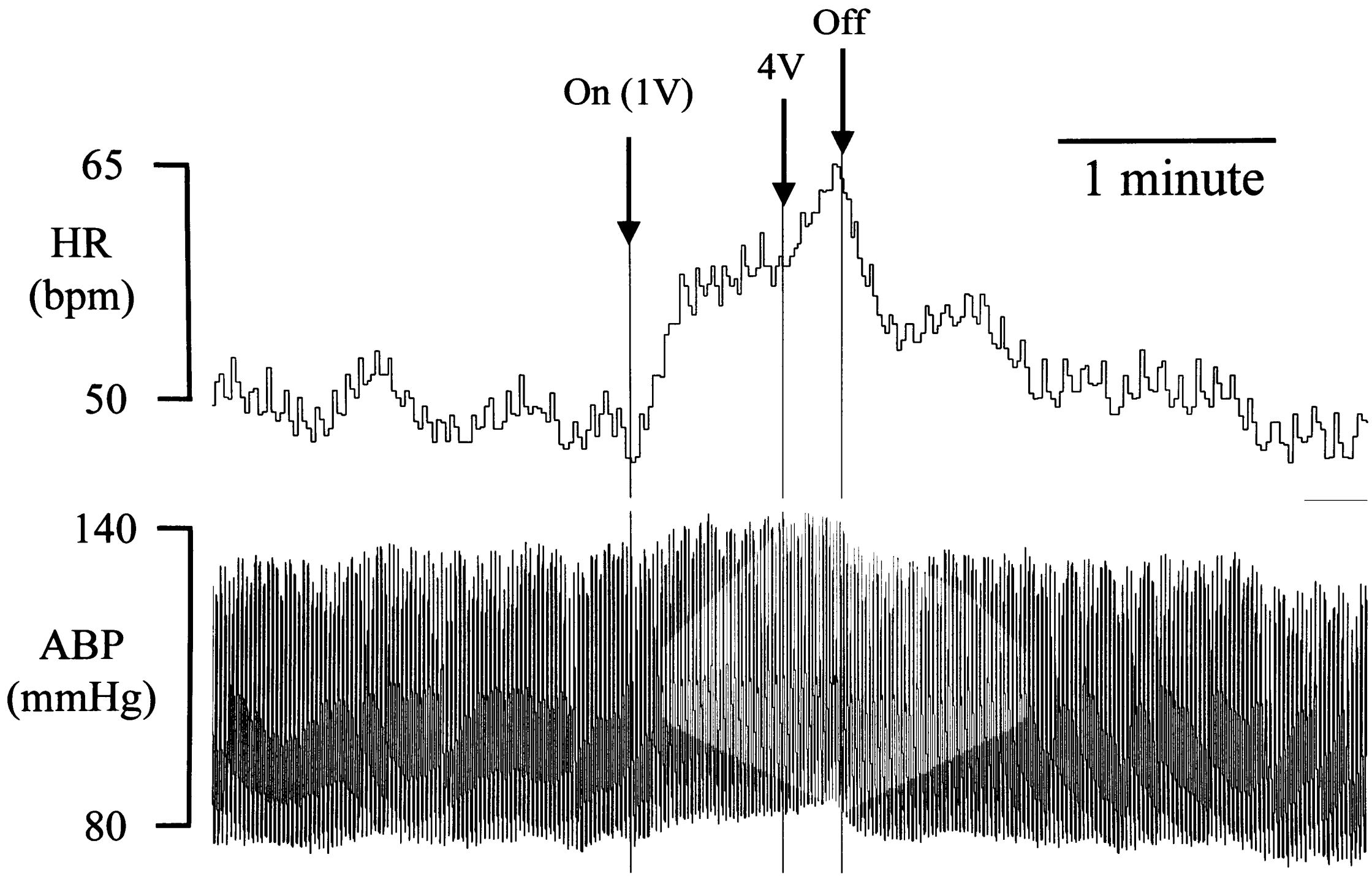


Figure 6.2. Raw data trace showing the effect of electrical stimulation of the subthalamic nucleus (frequency 100Hz, pulse width 1ms) on HR and ABP.

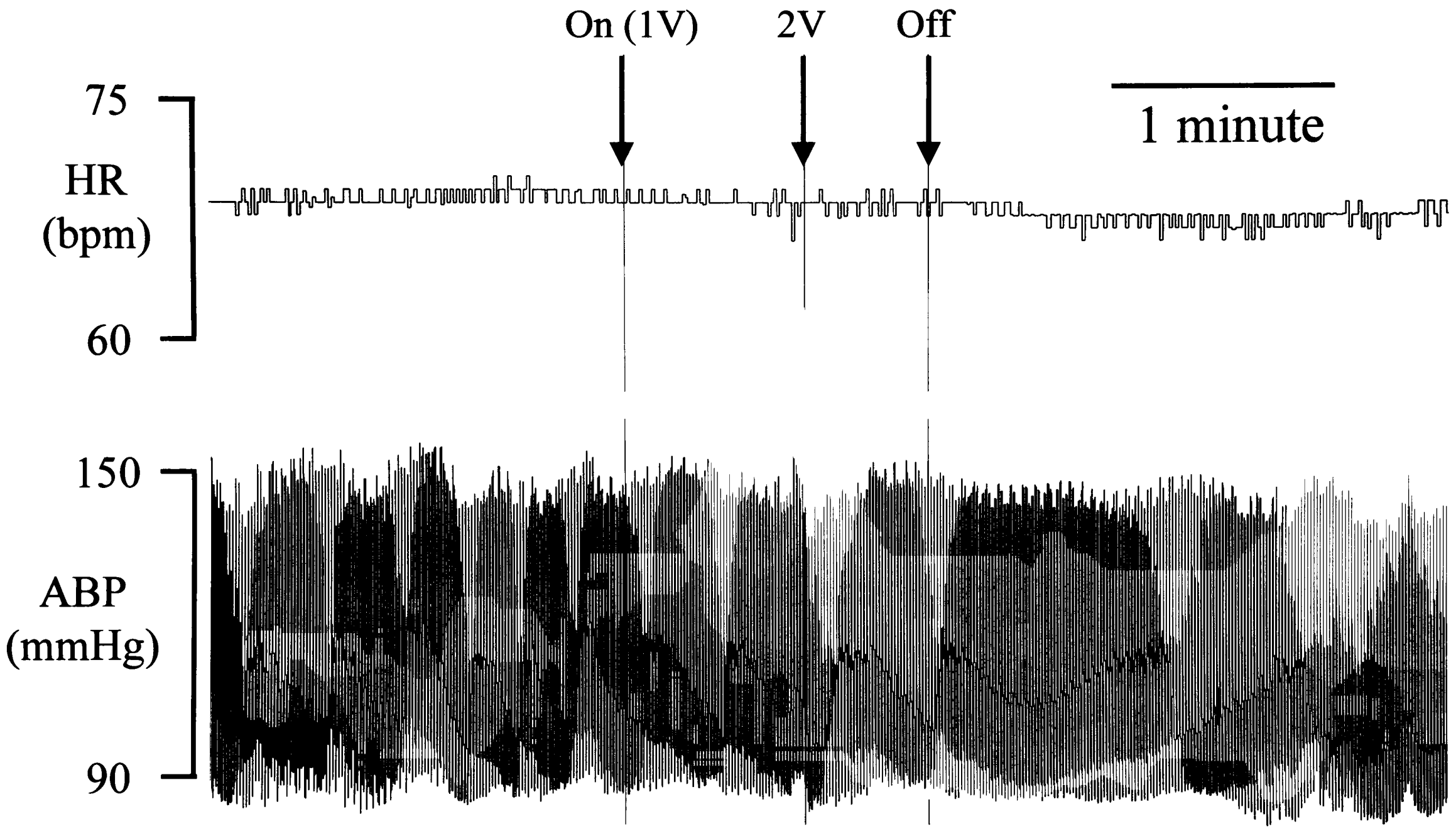
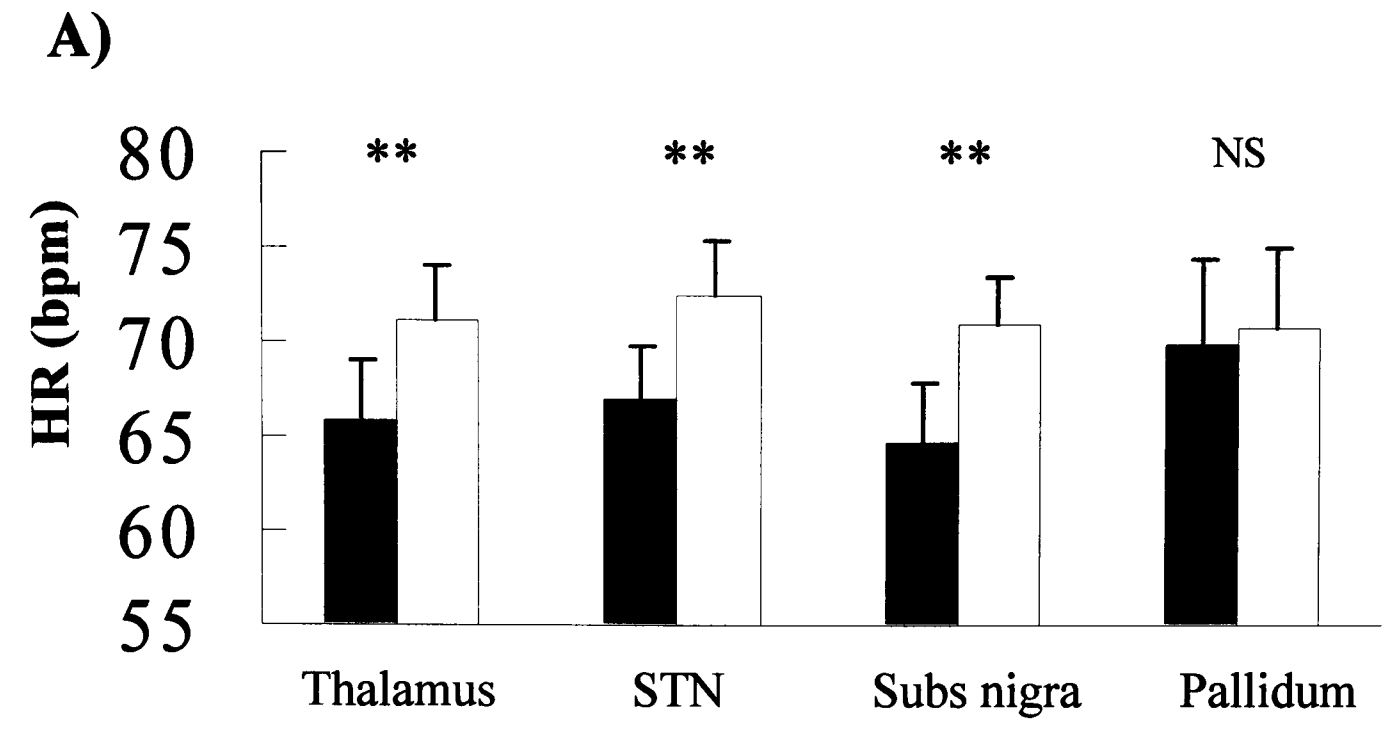


Figure 6.3. Raw data trace showing the effect of electrical stimulation of the internal pallidum (frequency 100Hz, pulse width 1ms) on HR and ABP.



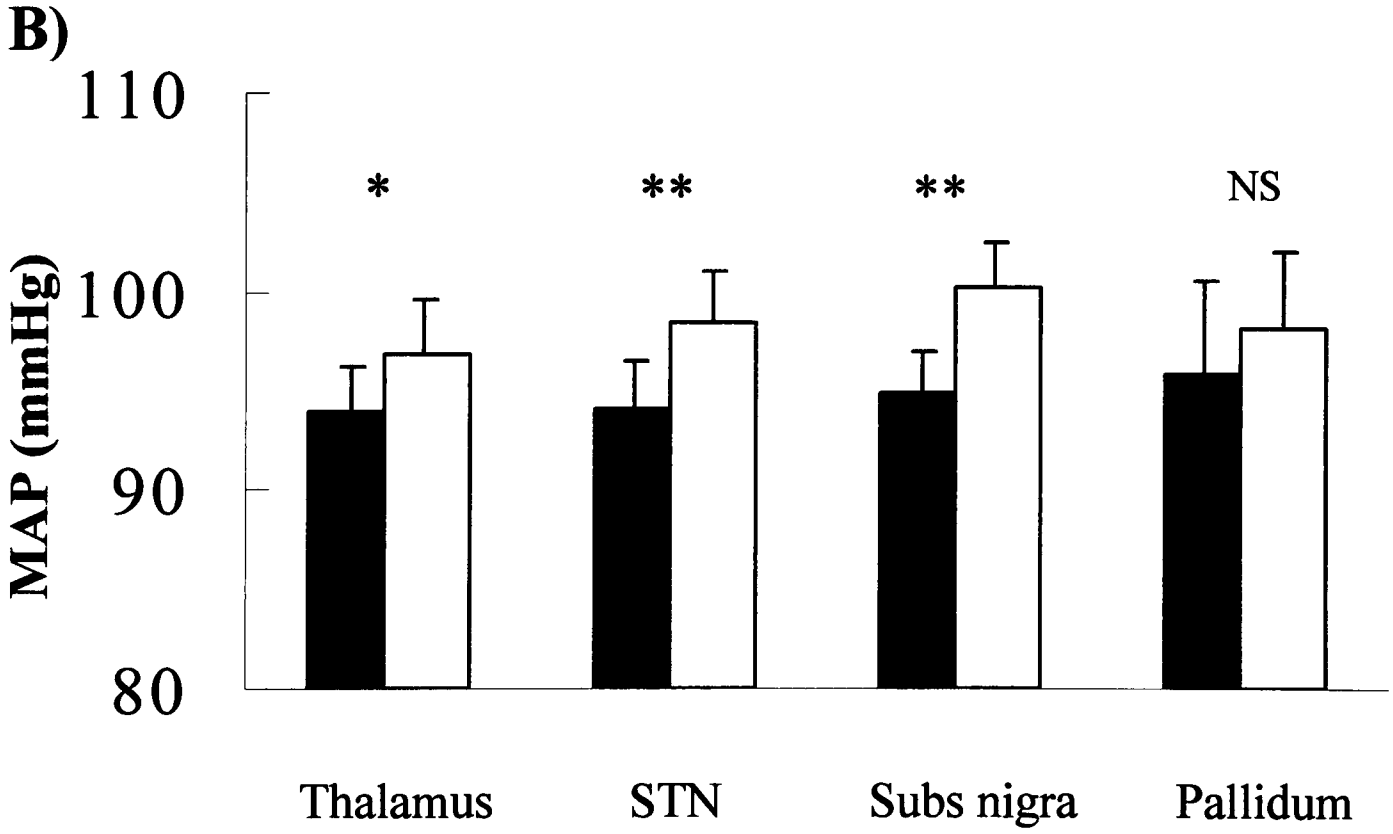


Figure 6.4. Group data (n=9 thalamus, n=8 STN and substantia nigra, n=5 pallidum) for the effects of **high frequency** electrical stimulation on A) HR, B) MAP. Black bars are control, white are during stimulation. Thalamus refers to either the ventralis intermedius or ventralis oralis posterior nuclei. STN; subthalamic nucleus. * indicates $p<0.05$, ** $p<0.01$, NS not signficant, paired t-test.

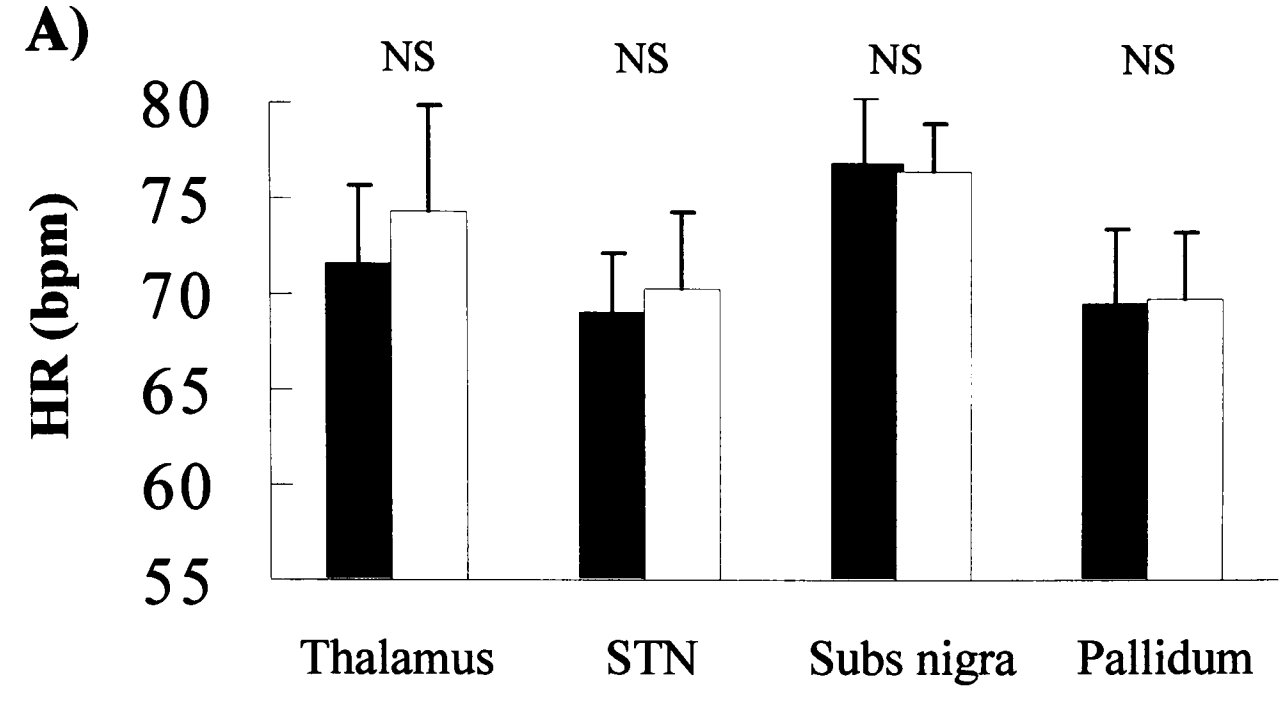


Figure 6.5. Group data (n=3 thalamus, n=5 STN, n=4 substantia nigra, n=7 pallidum) for the effects of **low frequency** electrical stimulation on A) HR, B) MAP. Black bars are control, white are during stimulation. Thalamus refers to either the ventralis intermedius or ventralis oralis posterior nuclei. STN; subthalamic nucleus. There was no significant change in HR or MAP during low frequency stimulation of any nucleus.

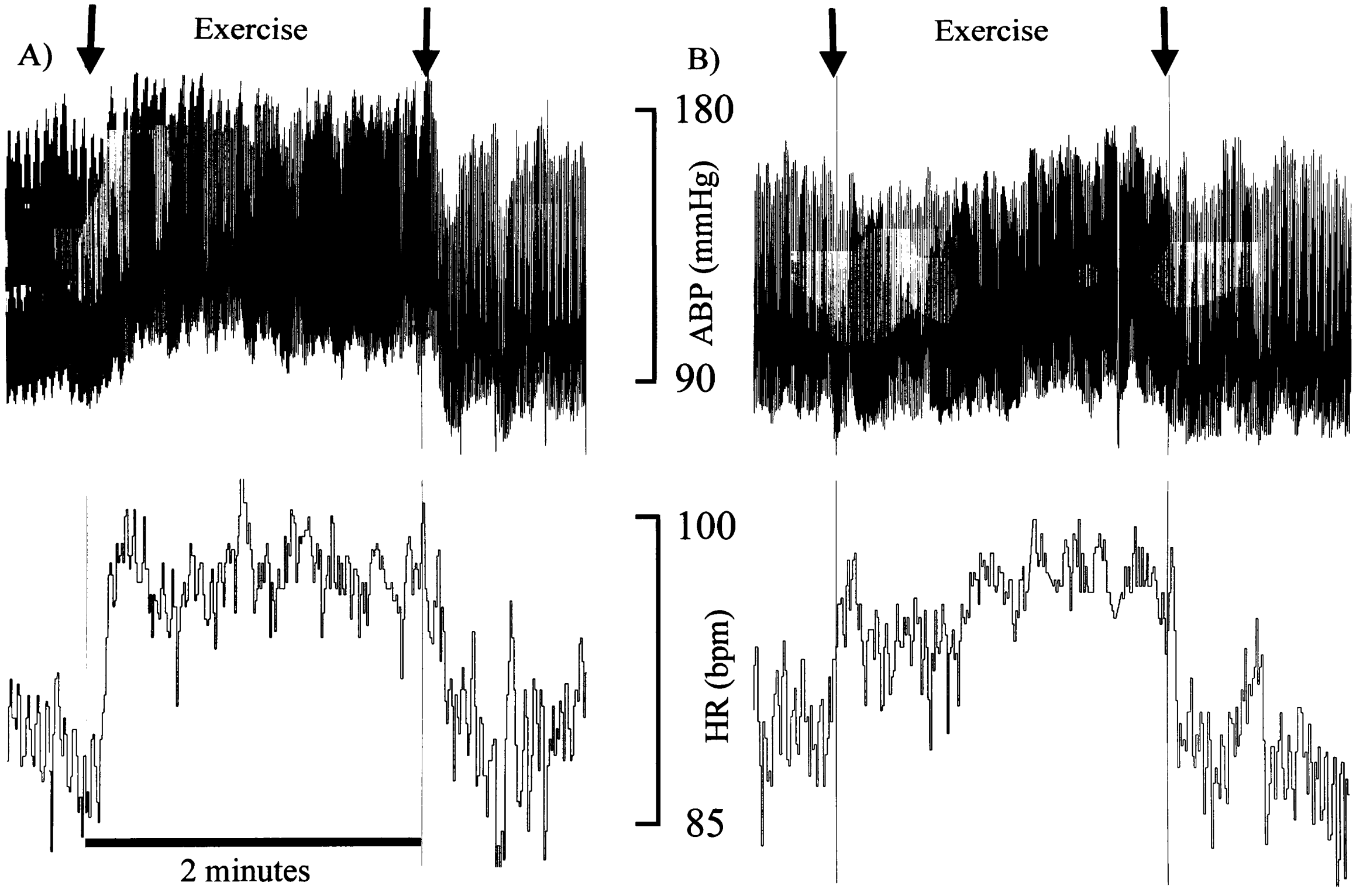


Figure 6.6. Raw data of the response to static handgrip. A) normal response. B) response during bilateral electrical stimulation of the STN. The HR response is maintained but the pressor response is markedly reduced during electrical stimulation.

sympathetic activation within the shortest possible time. Given the differences in the precise protocol performed this data is presented as case studies:

Patient 02: There was no ABP response to handgrip exercise during electrical stimulation of the STN (right side). Power started at 27kgW and declined to 18kgW. No ABP recordings possible when performing exercise without electrical stimulation owing to tremor and rigidity in all four limbs. HR increased by *ca.* 7bpm both with and without electrical stimulation of the STN.

Patient 04: This patient could only perform 1 minute of exercise at 5kgW with and without electrical stimulation of the STN (bilaterally). This did not increase ABP and the increase in HR was *ca.* 3bpm both with and without electrical stimulation of the STN.

Patient 11: This patient performed handgrip exercise with a power of *ca.* 10kgW and this was similar when repeated with and without bilateral electrical stimulation of the pallidum. The ABP response to exercise in this patient was smaller with electrical stimulation of the pallidum than without (figure 6.6).

Patient 14: Contraction with a power of *ca.* 10kgW was performed both with and without electrical stimulation of the STN. This did not result in any change in ABP: the increase in HR was *ca.* 4bpm in both repeats.

Patient 17: This patient was unable to generate a constant power of contraction, but performed intermittent maximal contraction (28 kgW) on a baseline of moderate force (15 kgW). ABP and HR therefore fluctuated during exercise and there was no clear difference between the exercise performed with and without bilateral electrical stimulation of the pallidum.

6.4 Discussion

The major findings of this study are:

1) High frequency electrical stimulation of the thalamus, subthalamic nucleus and substantia nigra resulted in increases in HR and MAP in awake man, but stimulation of the internal pallidum had no effect. High frequency electrical stimulation was accompanied by a facilitation of movement, including a lessening of akinesia and bradykinesia, a decrease in rigidity and a decrease in tremor.

2) Low frequency electrical stimulation of any of thalamic or midbrain nuclei did not affect cardiovascular variables.
3) These findings are consistent with animal studies in which electrical stimulation of the midbrain results in parallel activation of the cardiorespiratory and locomotor systems and indicates a potential role for these regions in 'central command' during exercise.
4) Chronic electrical stimulation of either the STN (n=5) or the pallidum (n=1) does not alter cardiovascular autonomic reactivity, indicating that the acute cardiovascular effects of electrical stimulation are not maintained in the long term.

6.4.1 Electrical stimulation of the midbrain in awake man

Electrical stimulation of the thalamus, substantia nigra or subthalamic nucleus results in a 6% increase in HR and a 5% increase in MAP. This is consistent with data from anaesthetised rats in which electrical or chemical stimulation of the substantia nigra increases HR by 15% and ABP by 50% (Lin & Yang 1994). Vagotomy abolished the tachycardia but not the pressor responses, and spinal transection abolished the pressor response but not the tachycardia in the rat. Therefore it seems that the tachycardia induced by substantia nigra stimulation is mediated entirely via vagal withdrawal and that the pressor response is mediated by sympathetic efferents. In the current study the magnitudes of the increases in cardiovascular variables were less impressive than in the study of Lin & Yang (1994). This could be a consequence of the depressed autonomic function seen in many of the patients in the current study (table 6.3, 6.4) and in previous studies of cardiovascular autonomic reactivity in basal ganglia disorders (Mesec *et al.* 1993). Additional explanations for the modest responses seen in this study compared to animal studies include the influence of 'descending inhibition' from the cortex due to patients being studied awake rather than anaesthetised or decorticated, the different stimulation parameters, a species differences and the presence of basal ganglia pathology. An interesting future study in these patients would be to study the cardiovascular effects of electrical stimulation in the presence of autonomic blockers in order to delineate the relative roles of vagal withdrawal and sympathetic activation.

There are a number of reasons to believe that the increases in HR and ABP were not merely an alerting response to the stressful situation of functional neurosurgery. First, the patient was not aware of whether or not the electrode was actually turned on or what stimulation parameters were being used. Consequently, even if baseline cardiovascular variables differed owing to stress there was no additional stress related to electrical stimulation. In addition, electrical stimulation of the pallidum (albeit in a separate group of patients) had no effect on cardiovascular variables, and there is no reason to expect that stimulation of the pallidum is any less stressful than stimulation of the thalamus, subthalamic nucleus or substantia nigra. Also there was no cardiovascular response to low frequency electrical stimulation of any site on cardiovascular responses. Responses were voltage dependent, maintained for the duration of stimulation and fell rapidly on cessation (e.g. figure 6.2). Consequently it seems likely that the cardiovascular responses seen in the current study are a direct consequence of the electrical stimulation on the neurones within the region of the electrode.

An interesting finding was the fact that low frequency stimulation of any structure did not affect HR or ABP. There were two broad ranges of frequencies of stimulation in the current study; 2-20Hz and >100Hz, a single cut-off point was therefore made at 90Hz, above which stimulation was considered 'high frequency', below which it was considered 'low frequency'. The stimulation ranges used during neurosurgery are based on the reported firing rate of the neurones in the basal ganglia (40Hz for the STN; DeLong *et al.* 1985). High frequency electrical stimulation has similar effects to an electrolytic lesion and is proposed to be due to overdrive suppression[1] and hence inhibition of the 'stimulated' region. It can therefore be used during neurosurgery to see what effect a lesion would have prior to actually performing one. In contrast, low frequency electrical stimulation increases basal ganglia output and are used during neurosurgery to test for localisation of the electrode. The fact that low frequency electrical stimulation of the basal ganglia had no effect on cardiovascular variables may indicate a threshold effect; a decrease in basal ganglia output (caused by high frequency stimulation) increases cardiovascular variables, whereas an increase in basal ganglia output

[1] Overdrive suppression results from a high frequency stimulation that keeps Na^+ channels in their refractory state and therefore renders the neurones unable to fire action potentials.

(caused by low frequency stimulation) has no effect. If the basal ganglia were to play a role in integrating cardiorespiratory drives during exercise it would seem logical for a decrease in basal ganglia output (that facilitates movement) to lead to an increase in cardiorespiratory variables.

6.4.1.1 Potential mechanisms

The basal ganglia are extensively interconnected with other midbrain nuclei, many of which have been shown to affect cardiovascular and autonomic function. These include the hypothalamus, parabrachial nucleus, raphe nucleus, red nucleus, ventral tegmentum, locus ceruleus and the periaqueductal gray. There are therefore a multitude of ways in which the electrical stimulation of the current study could be affecting cardiovascular variables. However, in contrast to the effects of stimulating the thalamus, STN or substantia nigra, high frequency electrical stimulation of the pallidum did not affect either HR or ABP. The lack of effect of pallidal stimulation implicates a specific mechanism by which the basal ganglia may be altering cardiac autonomic function. The STN and the substantia nigra have excitatory projections to the pedunculopontine nucleus (Lee *et al.* 1988; Lavoie & Parent 1994). This is part of the mesencephalic locomotor region (MLR), stimulation of which increases HR and ABP and causes a concomitant locomotion in decerebrate rats (Bedford *et al.* 1992; Chong & Bedford 1997). Electrical stimulation of the STN or substantia nigra could therefore evoke this pathway. The pallidum on the other hand has an inhibitory effect on the pedunculopontine nucleus via a GABAergic pathway (Kodsi & Swerdlow 1997), so would not increase cardiovascular variables. However, the relationship between the frequency of electrical stimulation and the output of basal ganglia nuclei is poorly understood; in the current study the effects of high frequency electrical stimulation are believed to be *inhibitory* on the stimulated nucleus, this does not fit well with the above hypothesis regarding the pedunculopontine nucleus.

The basal ganglia and cardiovascular control

Electrical and/or chemical stimulation of a number of midbrain nuclei has cardiovascular effects (Angyan 1978; Tan *et al.* 1983; Dampney *et al.* 1984; Ward 1988; Angyan 1991;

Angyan 1994; Lin & Yang 1994; Angyan 1996) In addition to the studies in which midbrain nuclei have been stimulated electrically or chemically and cardiovascular responses monitored, studies have also indicated that the central arc of the baroreflex contains at least one dopaminergic synapse within the basal ganglia. Sino-aortic denervation or carotid occlusion results in a decrease in tyrosine hydroxylase[1] activity in the striatum and a decrease in striatal dopamine (Alexander *et al.* 1984). Rats with nigrostriatal lesions show increased blood pressure variability (Alexander *et al.* 1986). Systemic administration of phenylephrine to activate the baroreflex increases striatal dopamine release (Yang & Lin 1993), an effect that is attenuated in rats with nigrostriatal lesions. In addition, the fact that Parkinson's is associated with abnormal autonomic function (Netten *et al.* 1995) also implies a role for the basal ganglia in cardiac autonomic control.

The basal ganglia and motor control

Whilst there is a large literature on the role of the basal ganglia in cardiovascular control, their main function is in control of the motor system, including the planning, initiation and execution of movements and motor learning (reviewed by Jueptner & Weiller 1998). These roles are exemplified by the movement disorders of Parkinson's, Huntington's, dystonia and essential tremor, all of which are believed to be basal ganglia disorders and result in a variety of motor side effects including tremor, rigidity, bradykinesia, chorea and akinesia. Inputs to the basal ganglia (figure 6.7, 6.8) arise from the cortex and the intralaminar nuclei of the thalamus, entering the basal ganglia circuit via the putamen and the caudate (together known as the striatum). Within the basal ganglia there are both direct and indirect pathways by which processing occurs, there is also a degree of somatotopy within these circuits; parallel pathways controlling different body parts pass through the basal ganglia. The major output of the basal ganglia is via the internal pallidum (GPi) and the substantia nigra par reticulata (SNr) which project to the ventral lateral, ventral anterior and mediodorsal nuclei of the thalamus. Electrical stimulation of these thalamic nuclei can evoke movement (Miall *et al.* 1998), as can

[1] The enzyme that catalyses the conversion of tyrosine to dopa, which is the rate limiting step in dopamine and NA synthesis.

electrical stimulation of the basal ganglia themselves (Arushanian & Dutov 1977; Angyan 1978; Angyan 1991; Angyan 1994; Angyan 1996).

6.4.1.2 Relationship of the current study to parallel activation and 'central command'

The evidence already discussed shows that the basal ganglia can influence both the cardiovascular system and the locomotor system. However, the relationship between the current study and animal studies of 'parallel activation' of the cardiovascular and locomotor system is unclear. Even in studies of the hypothalamic locomotor region (Eldridge *et al.* 1981), where electrical or chemical stimulation evokes convincing changes in cardiorespiratory variables even after paralysis, there remains a question regarding the relevance of this neural circuitry to exercise. The fact that lesions in the hypothalamic locomotor region do not affect the cardiorespiratory response to exercise (Ordway *et al.* 1989) is testament to this. In the current study electrical stimulation of the thalamus, STN and substantia nigra resulted in increases in HR and ABP and a facilitation of movement. As discussed above, I do not believe that this is an alerting response, however it may not necessarily be related to 'central command' *per se*. A difference between the current study and those performed in animals is that electrical stimulation in man did not actually drive movement; I did not observe the classical 'parallel activation' of locomotor and cardiorespiratory systems seen in the studies of Eldridge *et al* (1981, 1985). However, the most disabling symptom of Parkinson's Disease, that of akinesia was relieved by electrical stimulation (as judged by improved performance of motor tasks such as finger tapping and fist clenching), indeed this is the main purpose of surgery. Consequently, whilst movement *per se* was not occurring, *facilitation* of movement clearly was. A similar phenomenon is observed in animal studies at low stimulation currents. Eldridge *et al* (1981, 1985) found that electrical stimulation evoked cardiorespiratory responses at lower currents than that required to elicit locomotion. The likely explanation for this is that the neural networks controlling cardiorespiratory systems are well above threshold at rest, while locomotor networks are below threshold. Therefore a small activation of a common driving mechanism would have a demonstrable effect on the systems above threshold without any noticeable effect on the systems below threshold. It is possible that increasing the stimulation voltage in the current

study may have caused movement and a larger cardiovascular response to occur, but this was not attempted as it is unpleasant for the patient and so would probably have led to an alerting response. High voltages could be attempted under anaesthesia, but interpretation of this would be complicated since anaesthesia would alter the amount of 'descending inhibition' from the cortex. Of course, the fact that movement was not driven by electrical stimulation in the current study removes the need for paralysis controls; the cardiovascular response in the current study is clearly not dependent upon peripheral feedback from exercising muscles. A tentative circuit diagram explaining the concomitant effects of basal ganglia stimulation on cardiovascular variables and locomotion is given in figure 6.9.

6.4.1.3 Basal ganglia stimulation; excitation or inhibition?

Of crucial importance to interpretation of this study is the relationship between stimulation parameters (frequency, pulse width, voltage) and the consequences (excitation or inhibition) for local neurones. The mechanism by which high frequency electrical stimulation is believed to relieve motor symptoms in these patients is believed to be 'overdrive suppression' and hence *inhibition* of the region surrounding the electrode. This is consistent with the reported firing frequency of the STN (*ca.* 40Hz, DeLong *et al.* 1985). However, whilst optimal effects are achieved at frequencies >100Hz, in chronically stimulated patients the threshold to produce an anti-Parkinsonian effect is about 30Hz (Limousin *et al.* 1998), indicating that another mechanism might also be contributing. Given the circuitry of the basal ganglia (figure 6.7) it can be seen that stimulating the STN may result in *activation* of the *external* pallidum (which will in turn *inhibit* the *internal* pallidum, thus decreasing basal ganglia outflow). However, defining the precise relationship between the frequency of electrical stimulation and the effect on basal ganglia output would require combining electrical stimulation during surgery with electrophysiological recordings of the basal ganglia.

In addition to the varying effects of electrical stimulation according to frequency, it could not be determined in the current study whether the effects were due to stimulation of neurones with cell bodies in the stimulated region or fibres of passage. In animal studies this question is addressed by performing chemical stimulation with excitatory amino acids or GABA

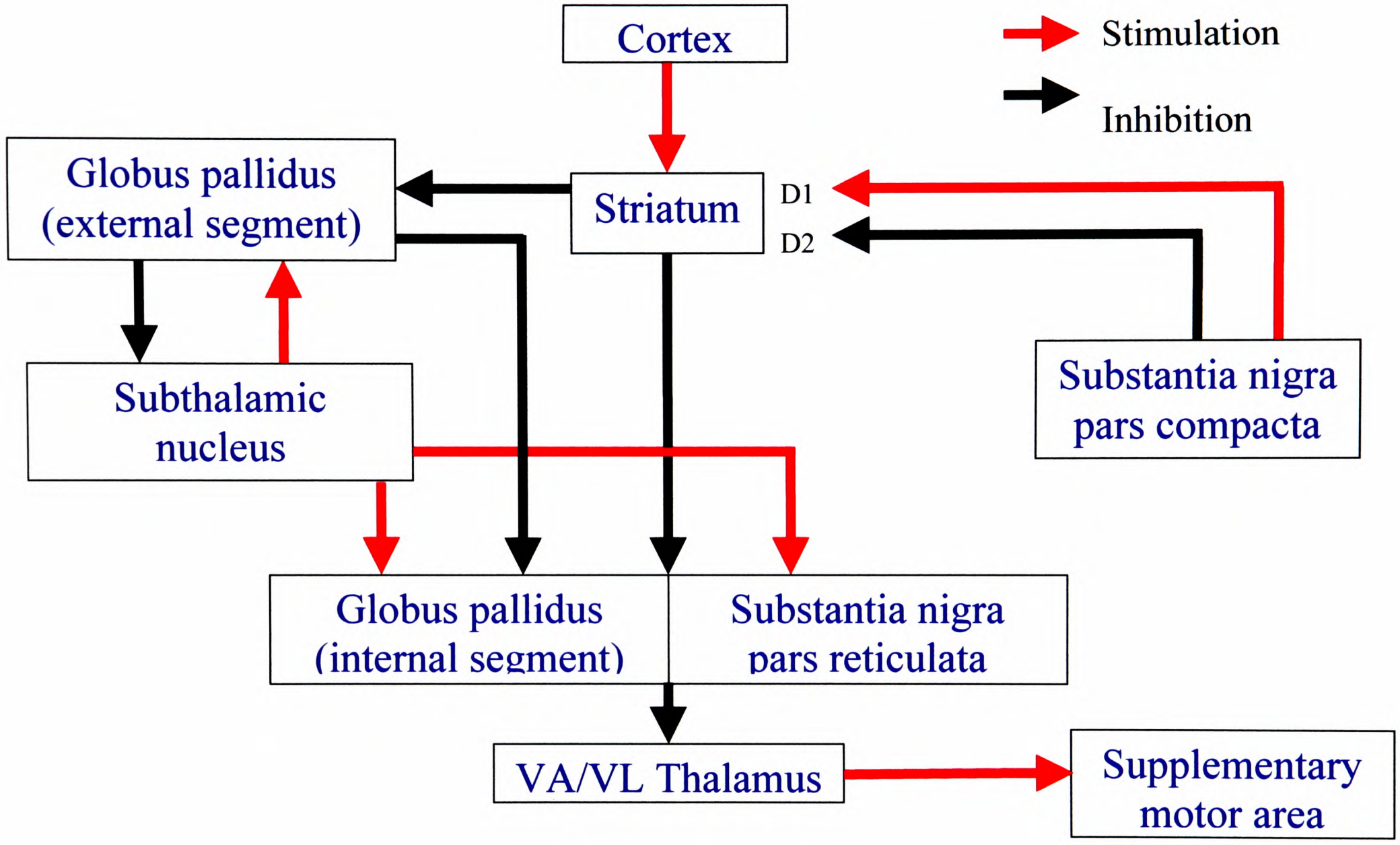


Figure 6.7. Circuit diagram of the basal ganglia and their inputs and outputs. The striatum consists of the caudate nucleus and the putamen. Two main pathways exist within the basal ganglia; the direct pathway from the striatum to the internal pallidum and substantia nigra and the indirect pathway via the external pallidum and the subthalamic nucleus. The direct pathway facilitates movement and the indirect pathway inhibits movement. The projection from the substantia nigra to the striatum is mediated by dopamine (which acts on D1 and D2 receptors in the striatum and has the effect of stimulating the direct pathway and inhibiting the indirect pathway, thereby facilitating movement). VA ventral anterior nucleus, VL ventrolateral nucleus.

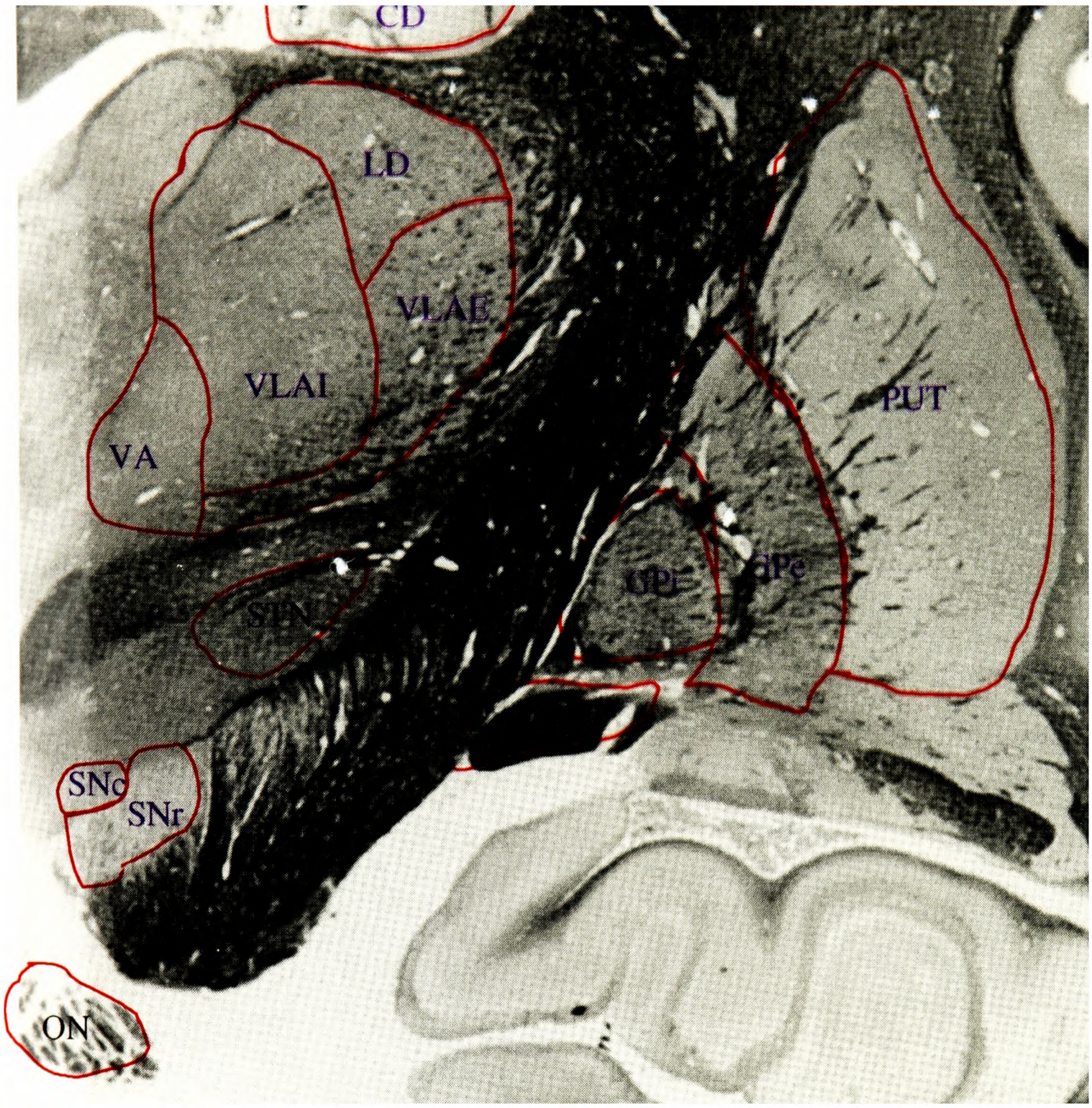


Figure 6.8. Coronal section (formalin fixed), of the region stimulated and lesioned in the studies described in Chapter Six. Abbreviations: CD caudate, ON occulomotor nerve, PUT putamen, GPE external globus pallidus, GPI internal globus pallidus, SNc substantia nigra par compacta, SNr substantia nigra par reticulata, OPT optic tract, STN subthalamic nucleus, VA ventroanterior thalamic nucleus, VLA ventrolateral anterior thalamic nucleus, LD lateral dorsal thalamic nucleus. From Mai *et al.* (1997) with permission.

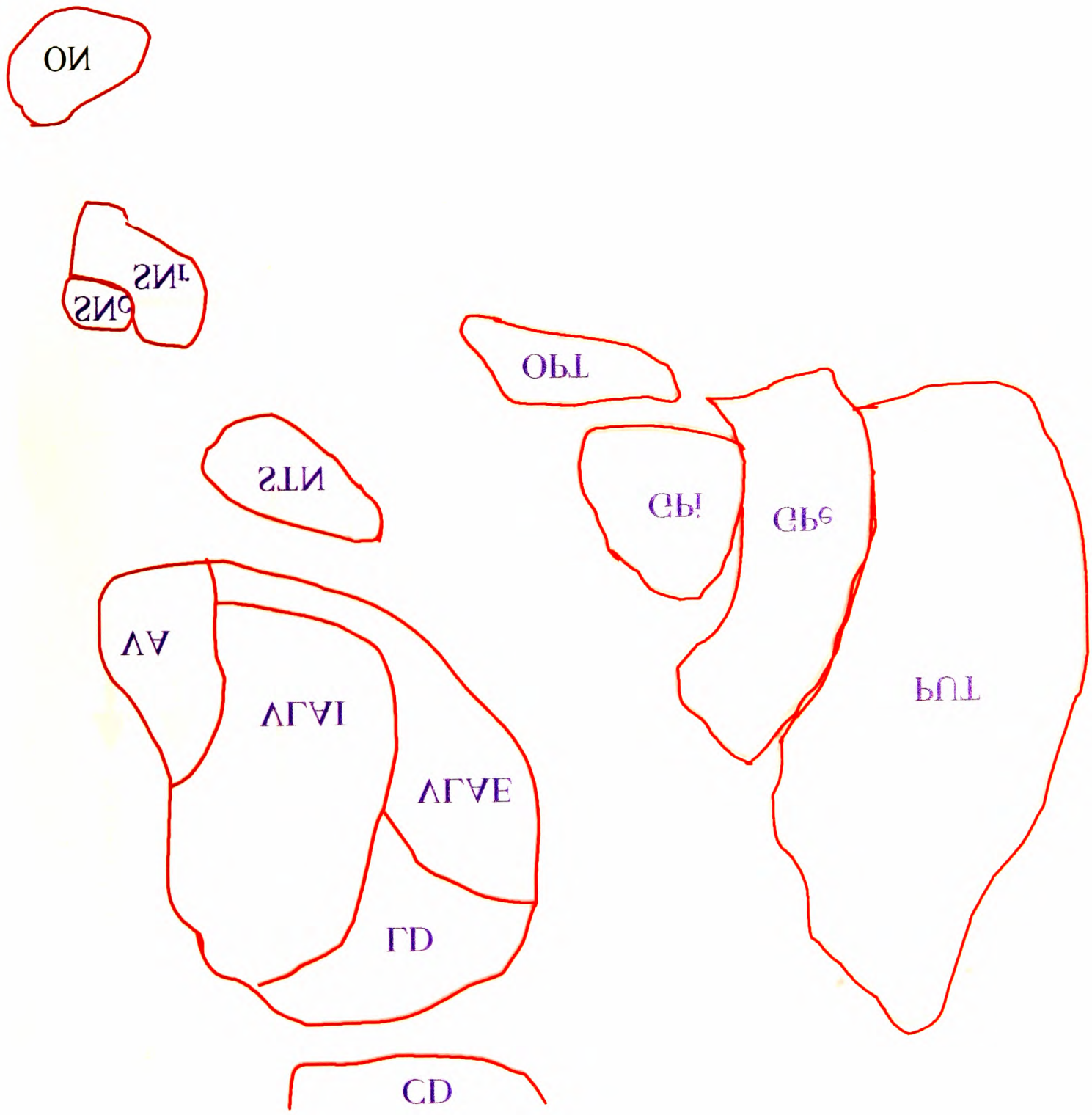

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Figure 6.8. Coronal section (formalin fixed), of the region stimulated and lesioned in the studies described in Chapter Six. Abbreviations: CD caudate, ON occulomotor nerve, PUT putamen, GPE external globus pallidus, GPI internal globus pallidus, SNc substantia nigra par compacta, SNr substantia nigra par reticulata, OPT optic tract, STN subthalamic nucleus, VA ventroanterior thalamic nucleus, VLA ventrolateral anterior thalamic nucleus, LD lateral dorsal thalamic nucleus. From Mai *et al.* (1997) with permission.

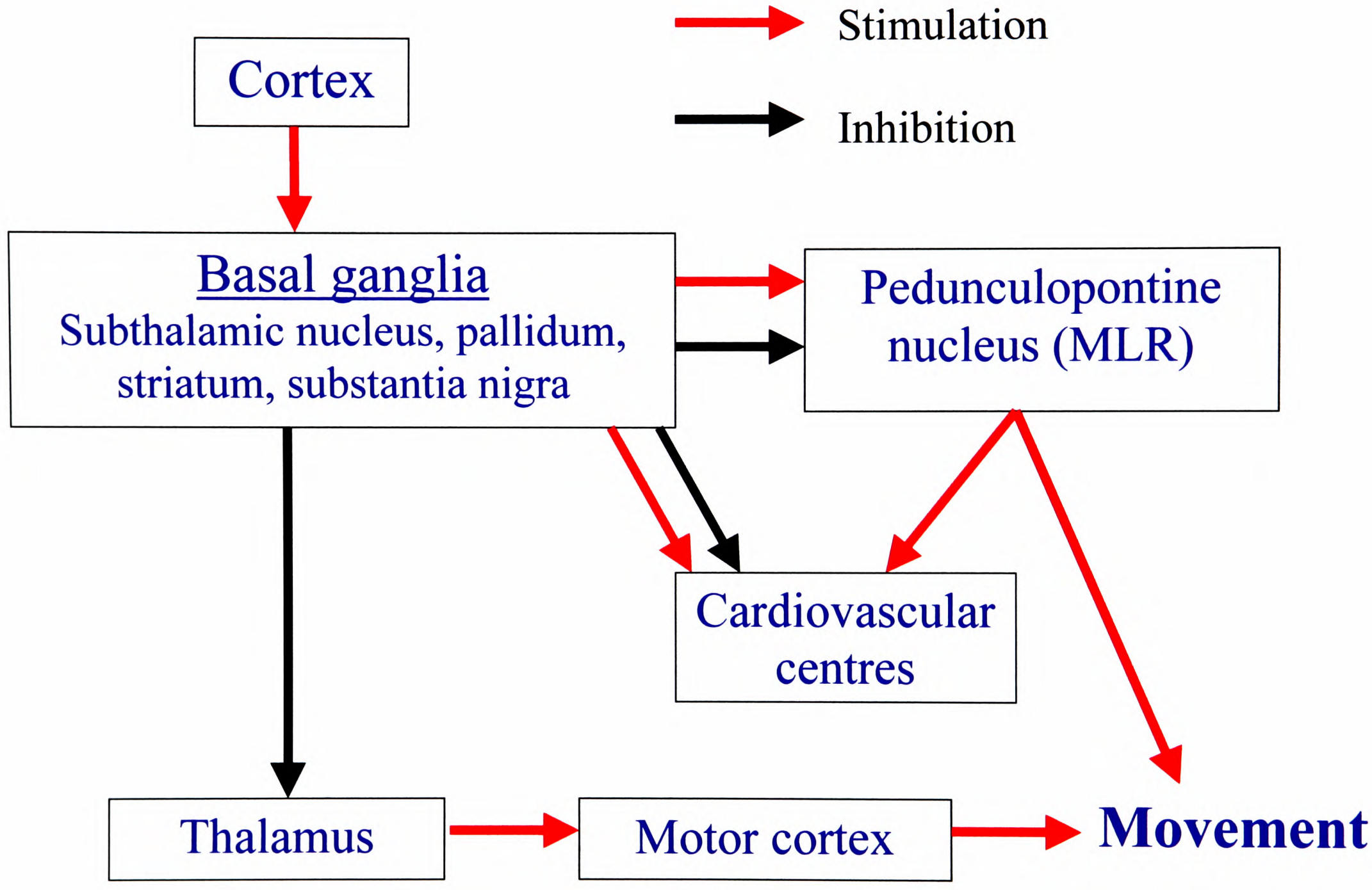


Figure 6.9. Simplified diagram of the ways in which the basal ganglia might act to affect cardiovascular variables in parallel with movement. MLR; mesencephalic locomotor region.

inhibitors. This elicits similar effects to electrical stimulation indicating both that effects were due to stimulation of neurones with cell bodies in the region of the electrode, and also that the frequencies of stimulation were *excitatory* and therefore differ to the *inhibitory* effects which are presumed to be the basis of much of the current study.

Direct comparison of the *current* passed in this study compared to those performed in animals is not possible given the fact that some authors have quoted parameters in terms of current whilst others quote voltage (and none mention impedance). The currents used during this study were in the order of 1-5mA, with an impedance of *ca.* 800Ω (although this varies according to the frequency of stimulation, type and location of electrode and its orientation in relation to nerve fibres). This is higher than that used in animal studies of the basal ganglia (Angyan 1994, used 0.1-0.5mA) and of the hypothalamus (<12μA by Eldridge *et al.* 1981). However, amongst studies quoting stimulation parameters in terms of voltage, the range 1-10V is common, considerably greater than that used in the current study (mean across all stimulations, 1.8±0.1V). One might expect differences in the stimulation required in this human study compared to in animal studies owing to the different nature of the areas being stimulated, the nature of the effects being studied (increasing cardiovascular variables was not the specific aim of current study), species differences, or to the varying amounts of descending cortical inhibition in the experimental preparations. Whilst direct electrical stimulation of the midbrain could never be considered a physiological manoeuvre, the use of an awake patient instead of decorticated or anaesthetised animals decreases the likelihood of studying non-specific effects or alerting responses. The patients in the current study would have had a near-maximal descending inhibition compared to animal studies, additionally they could not generally tolerate >5 volts and yet cardiovascular responses were still observed. This suggests that stimulation voltages in many animal studies have been unphysiologically high. Clearly to resolve these issues a comparable study needs to be performed in primates with chronic electrical stimulators implanted.

6.4.1.4 The influence of descending inhibition on responses to electrical stimulation

> *"It has been remarked that Life's aim is an act, not a thought. Today the dictum must be modified to admit that, often, to refrain from an act is no less an act than to commit one, because inhibition is co-equally with excitation a nervous activity."*
>
> Charles Sherrington, 1933

The studies in this chapter were performed on awake, unsedated patients whereas the majority of animal studies used decorticated or anaesthetised animals, the exception being the studies of Smith *et al.* (1960) in chronically instrumented dogs and Angyan (1994) who studied chronically instrumented awake cats. The use of an awake preparation is obviously more applicable to normal physiology, since descending inhibition is intact. This descending inhibition may come from almost any level in the neurological hierarchy above the areas stimulated in the current study. The lack of descending inhibition in animal studies is illustrated by the fact that in many decorticate preparations spontaneous locomotion is reported (Eldridge *et al.* 1981; Millhorn *et al.* 1987) along with a high resting breathing frequency (DiMarco *et al.* 1983). The degree of descending inhibition probably influences the stimulus parameters required to elicit a response and might explain the modest responses seen in the current study.

6.4.1.5 Postoperative studies

The patients studied postoperatively were severely disabled and all but two (who didn't have Parkinson's Disease) had extremely poor cardiovascular reactivity (as indicated by the poor HR and blood pressure variability and the low baroreflex sensitivity). For example in a study population aged 50±1 years (compared to 56±5 years in the current study) baroreflex sensitivity was 10.7±1.2 ms/mmHg (compared to 2.4±0.4 ms/mmHg during electrical stimulation in the patients in the current study), (Laitinen *et al.* 1998). However there was no difference in cardiovascular autonomic reactivity with and without chronic high frequency electrical stimulation. This is despite evidence from animal studies that the basal ganglia appear to be involved in the baroreflex (discussed above) and the fact that rats with nigrostriatal lesions show increased blood pressure variability (Alexander *et al.* 1986).

The exercise pressor response was depressed in all of the Parkinson's patients, this has been observed in a number of previous studies (Mesec *et al.* 1993; Netten *et al.* 1995; Linden *et al.* 1997). There was a decrease in the blood pressure response to handgrip exercise during electrical stimulation in the only patient who successfully maintained a constant force of contraction for 2 minutes that was sufficient to increase cardiovascular variables. Of note is the fact that this patient had bilateral stimulation of the pallidum, which had no acute effect on cardiovascular variables in the intraoperative studies. I have no good explanation for the fact that stimulation of the pallidum did not affect cardiovascular variables acutely but seemed to have effects when chronically stimulated. The relationship between acute and chronic electrical stimulation and the consequences for neural remodelling have not yet been determined (discussed by Samii *et al.* 1999). The decreased exercise pressor response is consistent with results from a study of 5 exercise trained baboons which showed a decrease in the MAP response to exercise following a 'subthalamic' lesion (Hobbs 1982) whilst having a normal pressor response to startle. Unfortunately the stereotactic coordinates of the lesion in the baboon study were not given, so its relationship to the site of electrical stimulation in the current study is unknown. However it does indicate that the basal ganglia may play an obligatory role in integrating central and peripheral exercise drives. The decreased pressor response during electrical stimulation seen in the current study may indicate that a portion of the blood pressure response is centrally generated or is dependent on a drive that passes through the basal ganglia. A study of beagles showed that a lesion in the hypothalamus did not alter exercise responses (Ordway *et al.* 1989), in addition a study by Bauer et al. (1989) showed that microinjection of an excitatory amino acid antagonist into the ventrolateral medulla attenuated the pressor response to muscular contraction but did not affect the pressor response to posterior hypothalamic stimulation. Consequently there is a good deal of doubt regarding the redundancy or lack of redundancy amongst brainstem nuclei. Clearly, the role of the midbrain in integrating exercise drives would be best answered by studying animals with electrodes implanted for chronic stimulation. Since the implanted leads used in patients have four electrodes accurate positioning would allow assessment of a number of midbrain regions in the same animal. Such animals would also allow assessment of exercise responses with and without electrical stimulation on a number of occasions, rather than following an all-or-nothing lesion. It would also be possible to perform studies on these animals with varying

degrees of autonomic blockade, paralysis, or tendon vibration to alter the central and peripheral drives.

6.4.2 Study limitations

6.4.2.1 Limitations of pathology

This study was performed in patients with severe pathology of the basal ganglia; for example Parkinsonian symptoms do not develop until *ca.* 90% of the dopaminergic cells of the nigrostriatal pathway are lost. I was therefore studying disease states rather than 'control' humans. The basal ganglia pathology in these patients could alter the neural circuitry of 'central command' itself, making it difficult to draw inferences regarding normal exercising humans. The nigrostriatal pathway of the basal ganglia is not the only dopaminergic projection in the brain that is impaired in Parkinson's. For example, the mesolimbic pathway arising in the midbrain (mainly in the A10 cell group) and projecting to the limbic system, in particular the nucleus accumbens is also affected. Autonomic control regions such as the raphe nuclei, sympathetic and parasympathetic nuclei, hypothalamus, amygdala and the dorsal vagal nucleus stain for Lewy bodies (Greenfield 1997) in Parkinson's patients. The basal ganglia themselves are also implicated in autonomic control (Lin *et al.* 1982; Lin & Yang 1994) as already discussed. It is therefore not surprising that Parkinson's is associated with a high incidence of autonomic failure (between 15 and 40% depending on the autonomic criteria used (Netten *et al.* 1995). Whilst the patients in the current study did not show classic autonomic symptoms, their cardiovascular reactivity was poor and this degeneration of autonomic control will affect the relevance of the current study to intact exercising man. Clearly this is an invasive study that cannot be performed in normal humans with intact basal ganglia so it is impossible to exclude the possibility that abnormal basal ganglia are a prerequisite for these responses. However, the responses do not appear to be a unique feature of Parkinson's; patients with spasmodic torticollis and benign essential tremor yielded comparable results in the current study.

6.4.2.2 Limitations of medication and previous surgery

A number of patients in this study had previously had surgery for their disorder. Owing to the relatively small number of patients in the study it was not possible to exclude or pool patients on the basis of previous lesional surgery, which included thalamotomy, pallidotomy and subthalamic nucleotomy (table 6.1). However, qualitatively similar results were observed in patients who had not undergone previous surgery. Most patients were also taking medication of some sort. In all but one Parkinson's patient[1] anti-Parkinsonian medication was withdrawn *ca.* 14 hours prior to surgery so they were in the 'off' state when the intraoperative studies were performed. Patients had recently come round from a general anaesthetic, were receiving oxygen from a nasal catheter and 6 patients had anti-hypertensives during surgery, all of which might be expected to affect cardiovascular function. The post-operative studies were all performed with patients on their normal medication and this will have affected cardiovascular autonomic function. For example, dopamine causes hypotension (Pollak *et al.* 1987) which can be prevented by domperidone (a peripheral dopaminergic blocker). However, since autonomic function was assessed with and without stimulation within a period of *ca.* 30 minutes and the order of assessments (stimulated versus unstimulated) was randomised, this is unlikely to have systematically altered the results obtained in this study.

6.4.2.3 Limitations of techniques

The aim of the electrical stimulation during neurosurgery is not to increase cardiovascular variables, so responses might well have been bigger if stimulus parameters had been optimised for this purpose as would occur in animal studies. In addition, stimulation is fairly unsystematic and is not performed for set periods at set voltages, rather it is optimised according to the patients immediate symptoms. Nevertheless, the environment of functional neurosurgery provides a unique opportunity to study well-established doctrines based on animal studies.

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[1] The clinical problem in this patient was drug-induced dyskinesias, so she was studied in the "on" state.

Given the sparsity of spontaneous fluctuations in blood pressure and HR it was difficult to calculate baroreflex sensitivity in Parkinson's patients. Consequently phenylephrine infusion or neck suction might have been a more appropriate way of measuring baroreflex sensitivity and cardiovascular autonomic reactivity. Owing to their severely disabling symptoms, it was difficult to assess exercise responses in the Parkinsonian patients; they clearly had impaired sympathetic responses and this would affect the relevance of any exercise study in such patients. However, future exercise studies could usefully be performed in patients with stimulators implanted for other movement disorders such as those in the current study with spasmodic torticollis and myoclonic dystonia; these patients have good cardiovascular reactivity.

6.4.3 Implications for 'central command' in exercise

Much has been made in the literature on 'central command' of studies involving electrical stimulation of the midbrain and the potential role of this region in generating cardiovascular and respiratory responses during exercise. The basal ganglia and other midbrain nuclei are certainly well placed to play a role in generating and integrating cardiorespiratory drives during exercise given their role in control of movement. They possess the neural connections to influence both motor and autonomic centres and the current study indicates that decreasing basal ganglia output may increase both HR and ABP in parallel with a facilitation of movement in man. The increases in cardiovascular variables in this study were modest and are probably not of functional significance for the patients in whom the stimulation is performed, nevertheless they demonstrate an important principle based on animal studies that until now had not been studied in man. The increases in cardiovascular variables are not due to a non-specific effect of stimulation or an alerting response, since electrical stimulation of the pallidum was without effect and there was also no effect of low frequency electrical stimulation. This is consistent with results seen in animal studies, indeed it is considerably more convincing than the use of electrical stimulation in a decorticate animal. The fact that exercise was not occurring in the current study also indicates that the cardiovascular effects of electrical stimulation of the midbrain are not dependent upon peripheral feedback rather they represent a genuine feedforward response.

However, it should be reiterated that simply because electrical stimulation evokes an 'exercise-like' response does not necessarily mean that the stimulated region plays any role in exercise. Such a conclusion would require a study of electrical stimulation combined with exercise. The decrease in the pressor response to exercise in a patient during bilateral electrical stimulation of the pallidum may indicate a role for the basal ganglia in integration of exercise drives. Whilst midbrain activity has been identified during exercise using PET scanning (Fink *et al.* 1995), the relationship between an increased regional cerebral blood flow and neural activity is unclear. An immunocytochemistry study of rats post-exercise showed extensive staining for a transcription factor (c-*fos*) in a number of midbrain nuclei including the hypothalamic locomotor region, the parabrachial nuclei and the periaqueductal grey (Iwamoto *et al.* 1996), but there was no marked increase in basal ganglia staining. Evidence for the role of the midbrain in the cardiorespiratory responses to exercise is therefore lacking, but given the connections of midbrain nuclei any role is likely to be complex. Studies of awake exercising animals with chronically implanted electrodes (both stimulating and recording) should enable a more systematic evaluation of this question.

6.5 Summary

This study provides the first evidence that in man, electrical stimulation of the midbrain can increase HR and blood pressure as it has been shown to do in animal studies. This increase in cardiovascular variables is accompanied by facilitation of movement and so could be related to the concept of 'parallel activation' of the locomotor and cardiorespiratory systems that has been proposed to form part of the 'central command' accompanying exercise. In addition, since exercise is not occurring in the current study, the response in man may be a genuine feedforward response and not one that is dependent on peripheral feedback. This does not preclude a role for the basal ganglia in integration of central and peripheral drives; indeed the observation (albeit in one patient) that the blood pressure response to handgrip exercise is diminished when basal ganglia outflow is inhibited suggests that the basal ganglia may well play a role in integration of exercise drives.

Chapter Seven

The sympathetic nervous system and arrhythmia

The art of reading is to skip judiciously.

P.G. Hamerton.

7.1 Introduction

Alterations in autonomic balance occur in a number of pathologies, for example during Parkinson's Disease (Netten *et al.* 1995) as discussed in Chapter Six. Whilst the autonomic failure associated with Parkinson's is rarely associated with arrhythmias, other alterations in autonomic balance, such as the increase in sympathetic activity occurring in ischaemia (Cinca *et al.* 1980; Lombardi *et al.* 1983) can be profoundly arrhythmogenic. Previous studies (Podzuweit 1980; Podzuweit *et al.* 1989) have shown that in the *in-vivo* non-ischemic pig heart a localised subepicardial[1] infusion of noradrenaline (NA) is effective in causing a sustained ventricular arrhythmia and a profound depression of haemodynamics. I hypothesised that NA resulted in the formation of a new pacemaker at the infusion site which would in turn result in an altered activation sequence that could be studied using epicardial electropotential mapping.

The electrophysiological mechanisms underlying the arrhythmic effects of sympathetic stimulation and NA are not fully understood. Abnormal automaticity and triggered activity are potential mechanisms, since they can both lead to ventricular tachycardia and be provoked by catecholamines (Lerman 1993) or exercise (Sung *et al.* 1983). The resulting ventricular tachycardia can be terminated by β-adrenergic blockade (Sung *et al.* 1983; Lerman 1993), Ca^{2+} channel antagonists and acetylcholine (Wit & Cranefield 1977). At the level of the single cell, the final common mechanism of these arrhythmias is believed to be Ca^{2+} overload (Lubbe *et al.* 1992). It is generally accepted that transmembrane entry of a small amount of 'trigger' Ca^{2+} is required to elicit a much larger Ca^{2+} release from the sarcoplasmic reticulum (SR) (reviewed by Fabiato 1983); Ca^{2+}-induced Ca^{2+} release (CICR). However, recently (Hobai *et al.* 1997) it was reported that when Ca^{2+} entry is blocked by 5mM nickel (Ni^{2+}), membrane depolarisation *alone* triggered *ca.* 80% of the Ca^{2+} transient in the guinea-pig ventricular myocyte at 37°C. This mechanism was not seen unless cells were pre-stimulated with cAMP. It is therefore of interest to see if 'voltage-activated Ca^{2+} release' (VACR) is functionally important in eliciting

[1] 'Subepicardial' is taken to include the entire ventricular wall including Purkinje fibres, endocardium, M cells and epicardium.

contraction in a multicellular cardiac preparation and this is the second main focus of this chapter. Two preparations were used; the isolated guinea-pig papillary muscle and the isolated perfused frog heart. Contraction was measured in the presence of 5mM (papillary) or 8mM (frog heart) Ni^{2+} to block CICR. If cAMP stimulates voltage-activated Ca^{2+} release as it appears to do in single cells (Hobai *et al.* 1997) then the increase in cAMP with sympathetic activity might cause a larger Ca^{2+} transient and so accelerate Ca^{2+} overload. This would add to the number of potential mechanisms for the arrhythmic effects of NA.

7.2 Anaesthetised pigs

7.2.1 Methods

Experiments were carried out in accordance with a British Home Office Project Licence (PPL 30/00608) and a Personal licence (30/3550).

Anaesthesia and surgery

Male domestic pigs (weight 24±5 kg) were premedicated with azaperone (5 mg/kg i.m., Stresnil, Janssen) and a surgical plane of anaesthesia was induced with 2-3% halothane (Fluothane, ICI) in 100% O_2 via an Ayre's T-piece with a mask and bag. After surgery halothane was replaced with a bolus infusion of α-chloralose (Sigma) (100 mg/kg i.v.) and repeated every two hours as required. Stainless steel needle electrodes were inserted into each limb and into the chest wall to monitor the ECG.

A tracheostomy was performed and a cuffed endotracheal tube (9 mm, Vygon) was introduced *ca.* 8 cm into the trachea. Fluid filled catheters (Portex) were inserted into two femoral arteries and one femoral vein for sampling of arterial blood, monitoring of arterial blood pressure, and infusion of anaesthetic and fluids, respectively. Artificial ventilation (Mark II ventilator, Penlon) commenced and was varied to maintain $PaCO_2$ between 35 and 45 mmHg. A midline thoracotomy was performed and the chest wall retracted laterally. Following incision of the pericardium the heart was suspended in a pericardial cradle. Cranial projections of the vagi were cut. A Millar catheter (Mikro-Tip, TC-510 control unit, Millar Instruments Inc., Houston) pressure sensor was inserted into the left ventricle via the apical dimple in order to

measure LVP. An elasticated sock (figure 7.1B, 7.2A) containing 127 unipolar stainless steel electrodes (Biomedical Instruments Designers, Montreal) was slipped over the ventricles. In addition, one custom-built silver electrode was attached to the right atrium using continuous suction. See figure 7.1 and 7.3 for details of the experimental set-up. After completion of surgery, at least one hour elapsed before experiments were begun. The thoracic cavity was covered by plastic film to reduce cooling. Core body temperature was measured by a rectal thermistor and maintained at 38±1^{o}C by heating lamps beneath the operating table. Fluid was replaced by an i.v. saline drip (*ca.* 100 ml/hr). Arterial blood samples were analysed for pH, blood gases and electrolytes (Na^{+}, K^{+}, Ca^{2+}) (Radiometer ABL505, Copenhagen). ABP was measured using a saline-filled pressure transducer (SensoNor 840, Norway), calibrated in the mid-axillary line. Analogue inputs were sampled at 200Hz (MP100WS) employing Acqknowledge software for the Macintosh. HR and the rate of change of LVP (LVdP/dt) were computed on line. During three experiments, two-dimensional echocardiography (HP SONOS 5500) was used to estimate LV ejection fraction.

Epicardial electropotential mapping

63 of the sock electrodes (inter-electrode spacing *ca.* 15 mm) spanning the whole of the ventricular epicardium and the right atrial electrode were connected to a 64 channel Unemap cardiac mapping system (Uniservices, Auckland). The animal and all cable shields were earthed to the system (mains) ground. Unipolar epicardial electropotentials (referred to the system ground) were continuously sampled at 900 Hz with a rolling buffer of 8 s and were digitally stored for subsequent analysis.

Electropotential signal analysis

After epicardial signal acquisition using the electrode sock the electropotentials for a single heart cycle were identified from the recorded data, as shown in figure 7.2B. The most negative electropotential slope was computed for each signal and this was chosen to mark the instant at which the associated sock electrode detected excitation of the adjacent portion of epicardium. An *event marker* was used to identify the time of excitation for each signal. For example, the solid black lines in figure 7.2B schematically illustrate event markers for electrode signals 97 and 121. The set of event markers were then used to order the signals,

such that the signal that detected earliest activation was listed first, as illustrated by electrode signal 90 in figure 7.2B. For the earliest signal, the marker time was referred to as the *datum*, as shown by the dashed line. *Activation times* were then computed for all other signals by taking the difference between their marker times and the datum. Figure 7.2B schematically depicts the activation times for signals 97 and 121. By definition, the activation time was zero for the datum signal.

2-dimensional activation maps

From the known topographical organisation of the epicardial electrodes, a scalar finite element field was fitted to the activation times using a smoothly continuous two-dimensional interpolation of nodal parameters. Estimated 3-dimensional locations of the sock electrodes were plotted using the Hammer projection (Raisz 1962). This 2-dimensional topography represents the ventricular epicardial surface, where the left ventricle (LV) makes up the central portion of the projection, the right ventricle (RV) comprises the border regions, but the apex is retained as a single point (figure 7.8). Left anterior and posterior descending coronary arteries are superimposed for anatomical clarity.

Nodal activation times were computed from the set of spatially arranged electrode activation times using a 2-dimensional finite element fitting technique, which incorporated linear least squares (Hunter *et al.* 1997). Red portions of this 2-dimensional *activation map* highlight regions of earliest epicardial activation, whereas blue denotes regions of latest epicardial activation. In the data shown in figure 7.8, epicardial activation was first detected on the RV free wall (indicated by o), and this was taken to be the site of earliest activation breakthrough. The wave of epicardial excitation proceeded primarily circumferentially from this region and ceased at the posterobasal epicardium (*), adjacent to the interventricular septum.

3-dimensional activation maps

To produce epicardial activation maps with anatomical realism the 3-dimensional epicardial activation sequence was reconstructed by fitting a scalar field of activation times onto the epicardial surface of an anatomically accurate mathematical model of the canine ventricles (Nielsen *et al.* 1991), as shown in figure 7.9. The estimated 3-dimensional locations of the

electrodes were projected onto the epicardial surface of the anatomical model and the epicardial activation time field was approximated using linear interpolation of a set of parameters defined at spatial locations, which coincided with the finite element nodes used to define the geometry. The nodal epicardial activation times were computed using a standard finite element fitting technique (as for the 2-dimensional activation maps). Figure 7.9 shows the 3-dimensional epicardial activation sequence from anterior and posterior viewpoints.

Protocols

Fourteen pigs were prepared as described. At least one hour elapsed between completion of surgery and onset of experiments.

Protocol 1: Subepicardial NA infusion

Ventricular arrhythmias were induced by subepicardial infusions of NA bitartrate (10 μM in 0.9% saline, 150 μ l/min) using a microinjection pump and a 25 gauge hypodermic needle (figure 7.4). The concentration and infusion rate were chosen because they elicited reproducible arrhythmias within two to five minutes after the onset of infusion. The needle was inserted at randomly selected locations throughout the ventricular subepicardium. In cases where subepicardial infusion of NA did not elicit a ventricular arrhythmia within five minutes (approximately 20% of infusions), the needle was removed and infusion was begun in another part of the ventricle.

Protocol 2: Ventricular electrical pacing

A custom built roving bipolar electrode was used to pace hearts (0.5-1.5 V, 2 ms width, n=3) at the same site in which NA had previously been infused. The pacing rate was matched to the rate observed during the preceding arrhythmia. The aim of this protocol was to see the extent to which electrical pacing mimicked the electrical and haemodynamic changes occurring during a NA induced arrhythmia.

Protocol 3: Subepicardial saline infusion

In order to investigate whether fluid infusion *per se* could elicit arrhythmias, the saline vehicle was infused (150 µl/min) subepicardially for *ca.* 10-15 minutes in six animals.

Measurements and data analysis

Ventricular electropotential mapping using the elasticated sock was performed for control, arrhythmia and recovery states. In ten pigs an additional suction electrode was used to detect right atrial activation. Trans-oesophageal or epicardial surface echocardiography was used to estimate LV ejection fraction during control, arrhythmia and recovery states in three pigs. Total ventricular epicardial activation times were calculated as described in Chapter Two. All data are expressed as mean ± SEM. MAP, LVP, HR, LVdP/dt and total ventricular activation times for control, arrhythmic and recovery states were compared using a repeated measures ANOVA and a *post-hoc* Tukey test. Significance was accepted at $p<0.05$.

7.2.2 Results

Values for pHa, $PaCO_2$ and PaO_2 were 7.4±0.02, 43±2 mmHg and 298±22 mmHg, respectively (n=14).

7.2.2.1 Basic properties of the arrhythmia

Infusion of NA into the ventricular subepicardium elicited a reproducible ventricular arrhythmia on 29 separate occasions in 14 animals. The time to onset of this arrhythmia was highly variable (range 48 to 140s, and *ca.* 20% of infusions in which the arrhythmia could not be evoked). Figure 7.5 shows the ECG and haemodynamic changes occurring during this arrhythmia (raw data from one animal). At the onset of the arrhythmia there was an instantaneous change in the amplitude and direction of the QRS complex, which masked the P wave (figure 7.6). A tachycardia and haemodynamic depression (MAP, LVP and LVdp/dt max) occurred. On cessation of the NA infusion, normal sinus rhythm and haemodynamic performances were restored within five minutes. Group data are shown in figure 7.7.

After an interim recovery (*ca.* 15 minutes) without removing the needle, NA could elicit arrhythmias in the same site that had previously elicited an arrhythmia. These had comparable depression of haemodynamics when compared to the previous arrhythmia. Arrhythmias could not be provoked by infusion of the saline vehicle.

7.2.2.2 Epicardial maps of NA induced arrhythmias

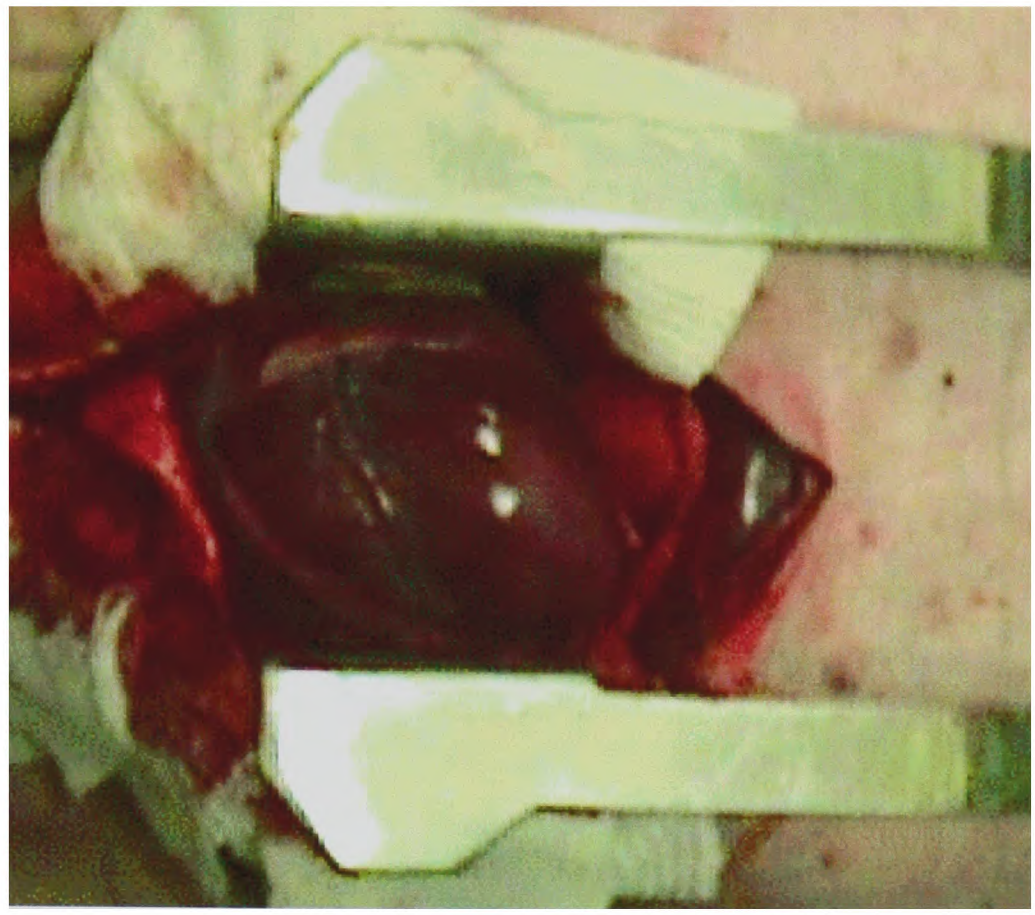
Under control conditions the site of earliest ventricular epicardial breakthrough was on the RV free wall and spread radially across the RV and apex over the septa and across the LV (figure 7.8 and 7.9). In most pigs 2-4 sites of epicardial breakthrough were observed, with the RV free wall always activated first. The total ventricular epicardial activation time under control conditions was 20±4ms. NA infusion resulted in an arrhythmia and a typical activation map is shown in figure 7.8. The total ventricular epicardial activation time increased from 18 ms under control conditions (A) to 34 ms during the arrhythmia (B). Within two minutes of stopping the NA infusion the cardiac activation sequence and the total ventricular activation time were restored to their control states (C).

In all pigs the site of earliest epicardial activation consistently matched the NA infusion site and the total ventricular epicardial activation time increased from 20±4 ms under control conditions to 50±9 ms during the NA-induced ventricular arrhythmia (figure 7.7). Right atrial activation followed the earliest ventricular epicardial activation (20 observations in 10 pigs) during the NA induced arrhythmia as shown in figure 7.10. The first two cycles show control conditions where right atrial excitation preceded ventricular activation. Following the ventricular repolarization of the second cycle, a spontaneous ventricular depolarisation was observed. Subsequently, earliest ventricular epicardial activation continued to precede right atrial activation on a one-to-one basis.

7.2.2.3 Epicardial electrical pacing

NA infusion resulted in an arrhythmia and an increase of the total ventricular epicardial activation time from 19 ms to 43 ms. Following recovery, the epicardium was electrically stimulated at the same location and this resulted in an increase in total ventricular epicardial

A)



B)

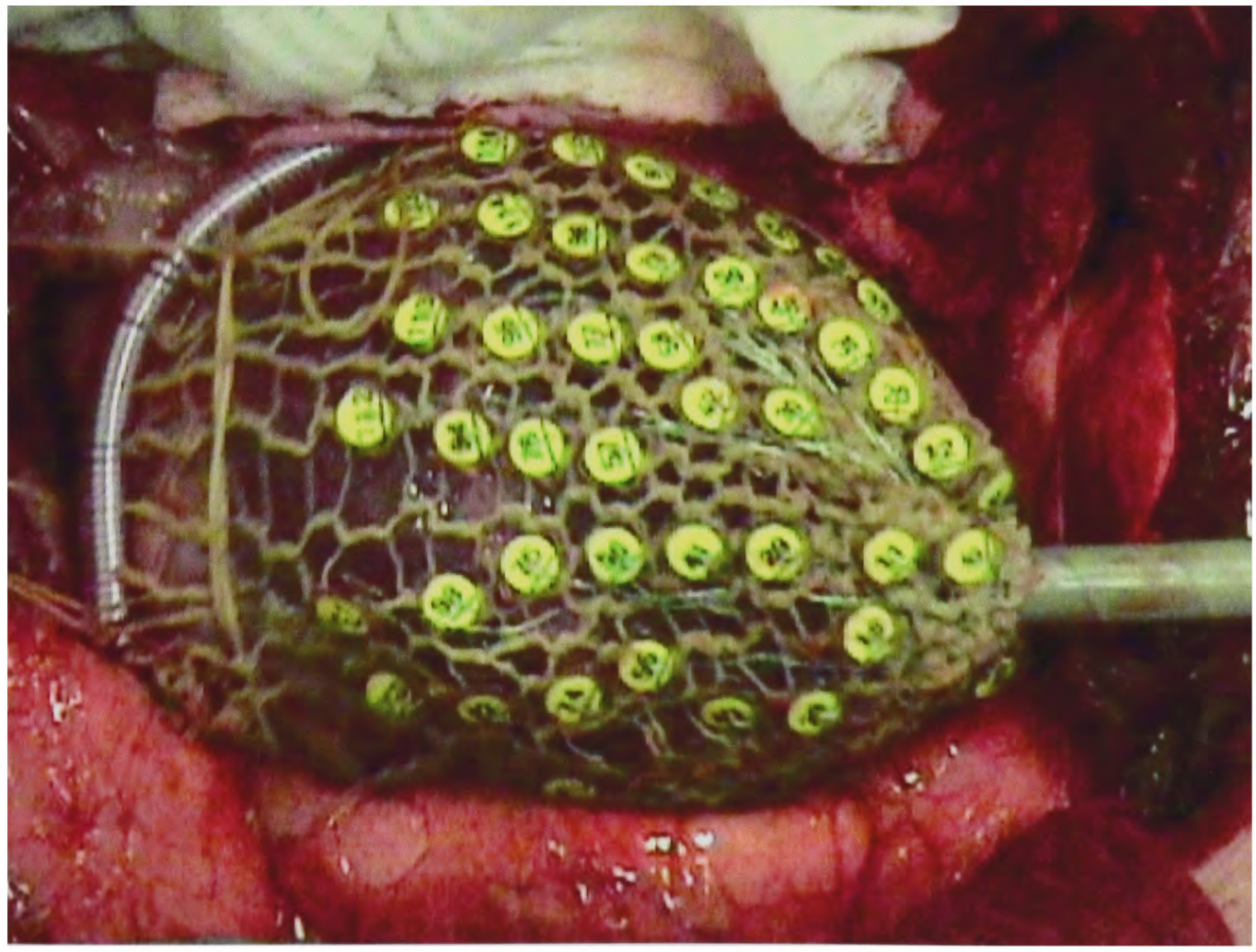
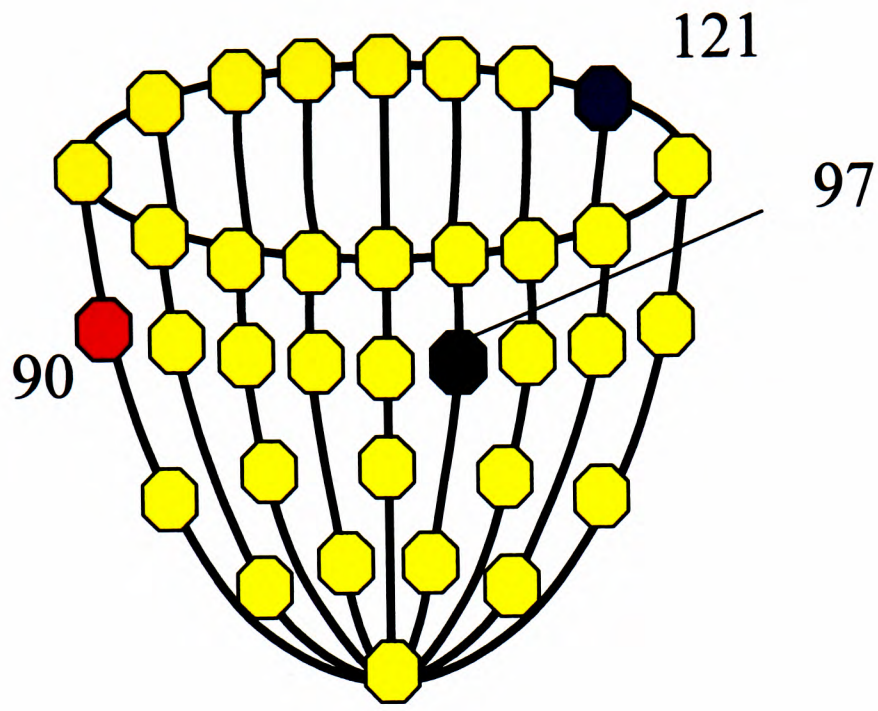


Figure 7.1. A) A midline thoracotomy is performed and the pericardium removed to expose the heart. B) The elasticated sock is placed over the ventricles. Each electrode consists of a wire implanted into a yellow plastic button (numbered), with all the wires leaving at the apical end of the sock (right).

A)



B)

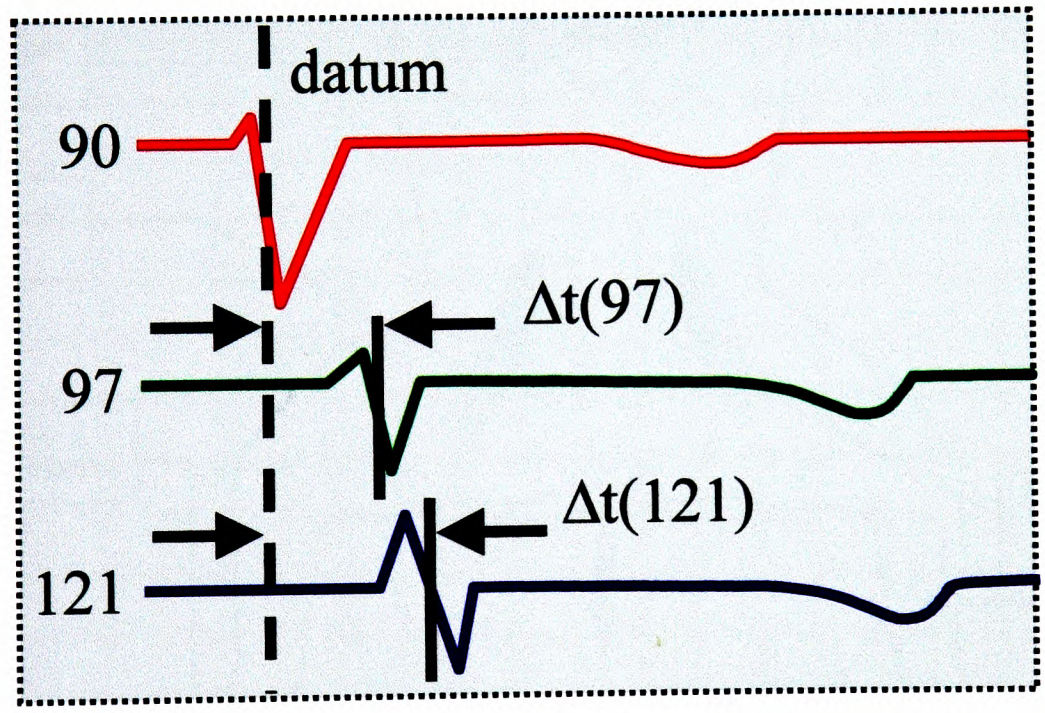


Figure 7.2. A) Schematic diagram of epicardial electrode sock. B) Schematic diagram of the way in which local activation times were calculated. See text for explanation.

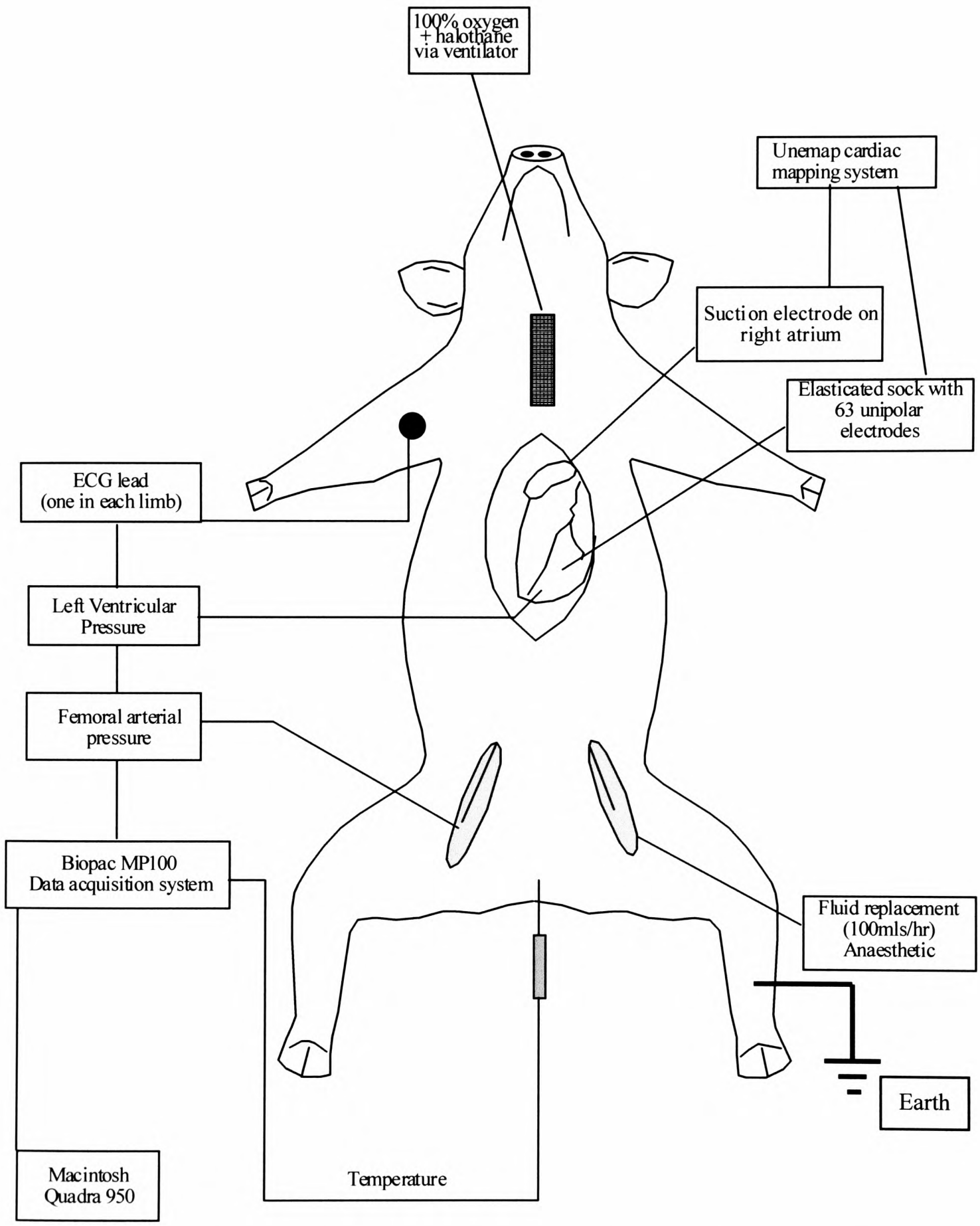


Figure 7.3. Anaesthetised pig setup. The pig is thoractomised and ventilated and ECG leads are placed in each limb. Cannulas are placed in a femoral artery and vein. A pressure transducer is introduced into the left ventricle. An elasticated sock with 63 unipolar electrodes is placed over the venticles and an additional bipolar electrode is attached to the right atrium.

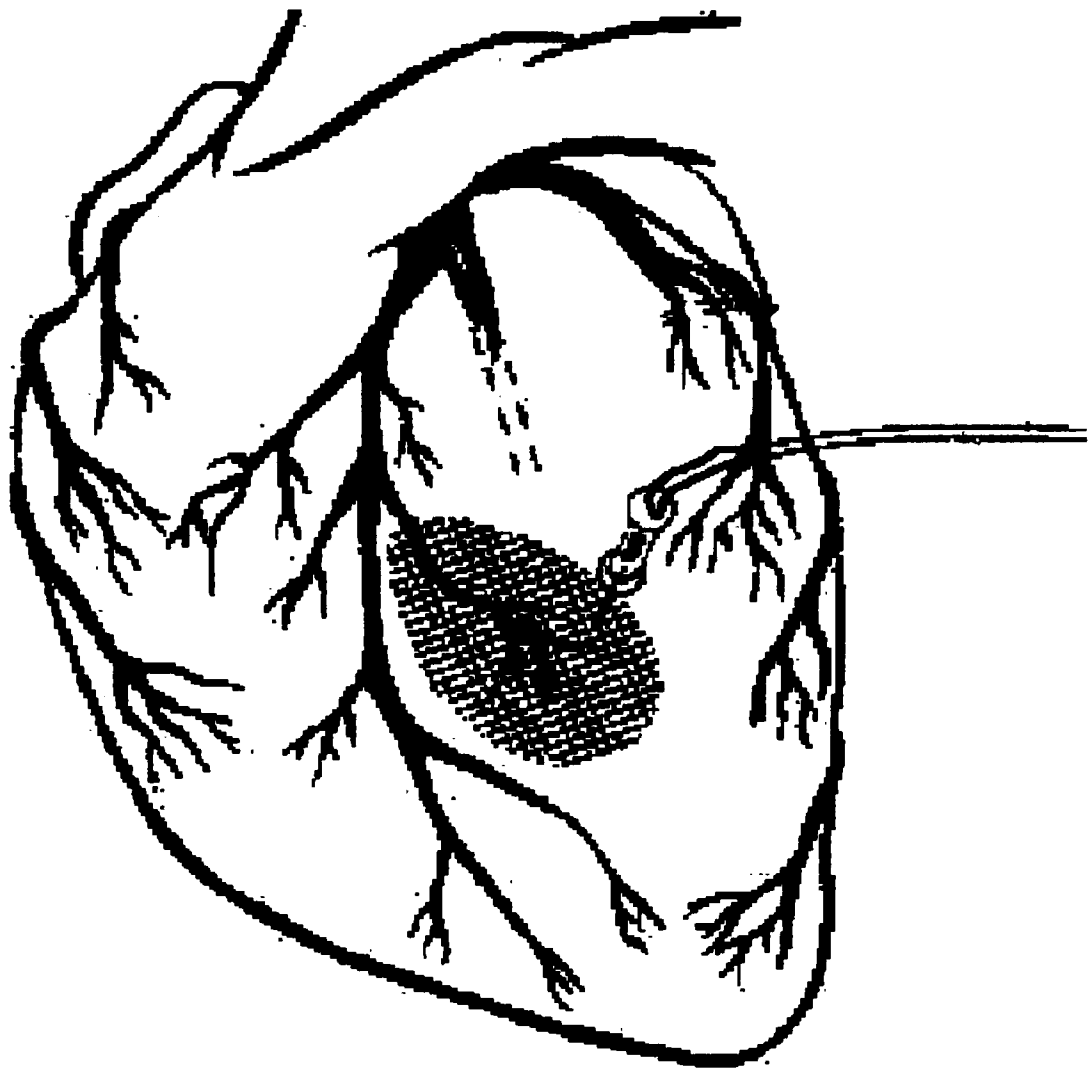


Figure 7.4. Schematic representation of the subepicardial infusion of NA. A needle was inserted into the ventricular wall and NA was infused at 150μl/min. The region of influence of NA is indicated by the hatched area.

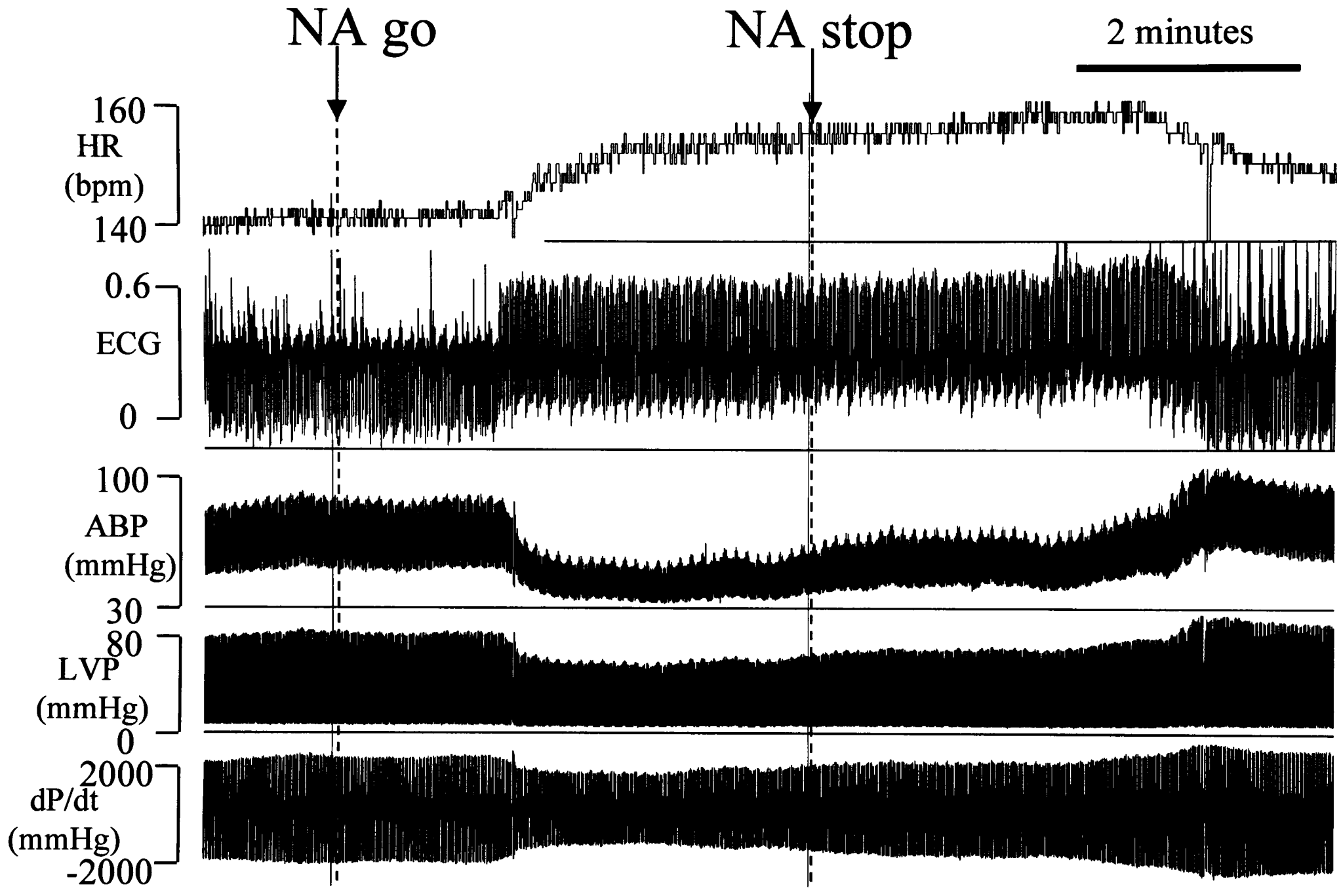


Figure 7.5. Raw data showing the effect of a subepicardial infusion of NA on cardiovascular function. After *ca.* 60 seconds the ECG complex inverts and HR increases. Arterial blood pressure (ABP), left ventricular pressure (LVP) decrease. These changes are reversed when NA infusion is stopped.

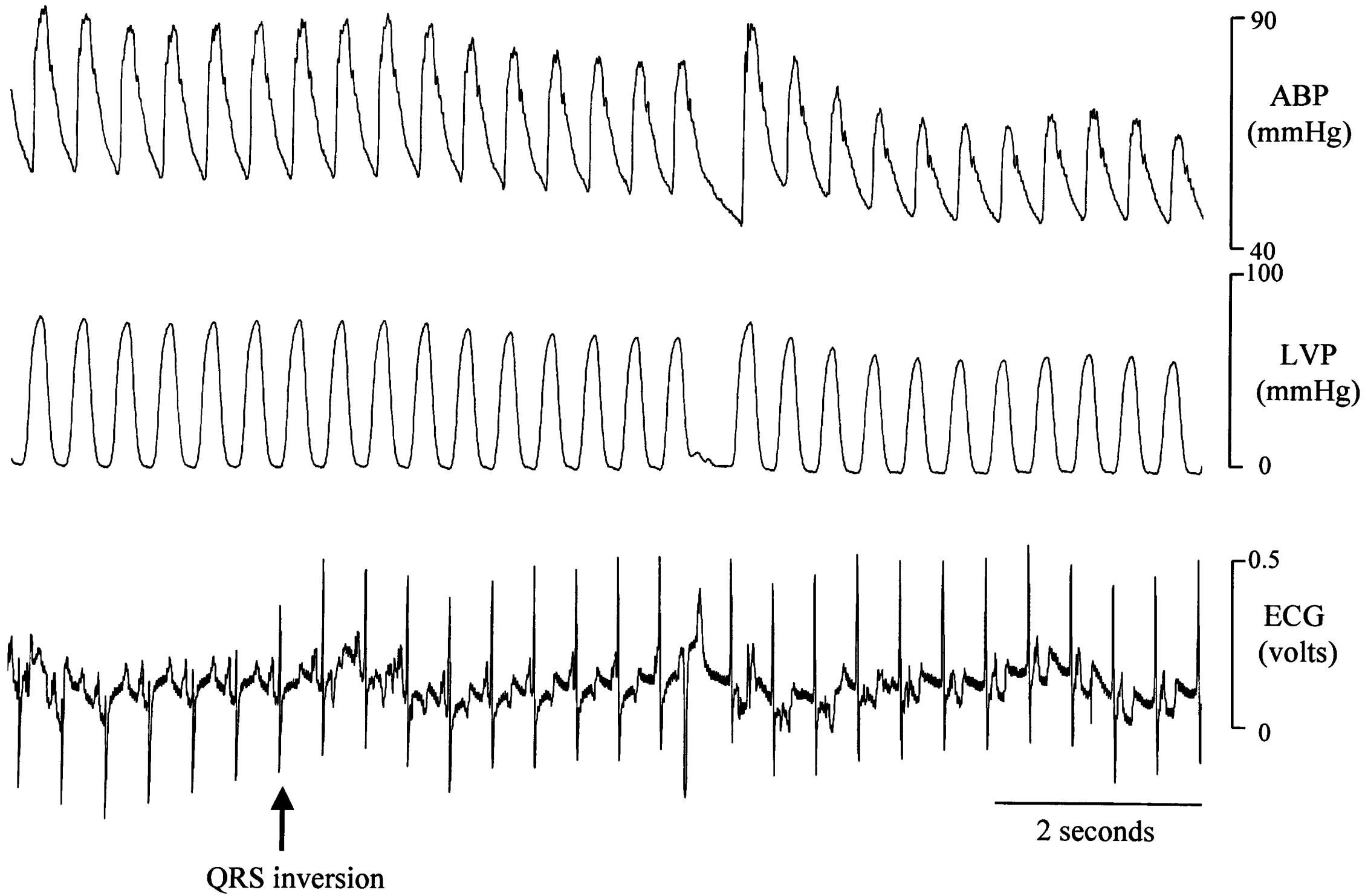


Figure 7.6. Raw data trace showing the changes occurring at the onset of a NA induced arrhythmia. QRS inversion of the ECG occurs prior to any change in haemodynamics.

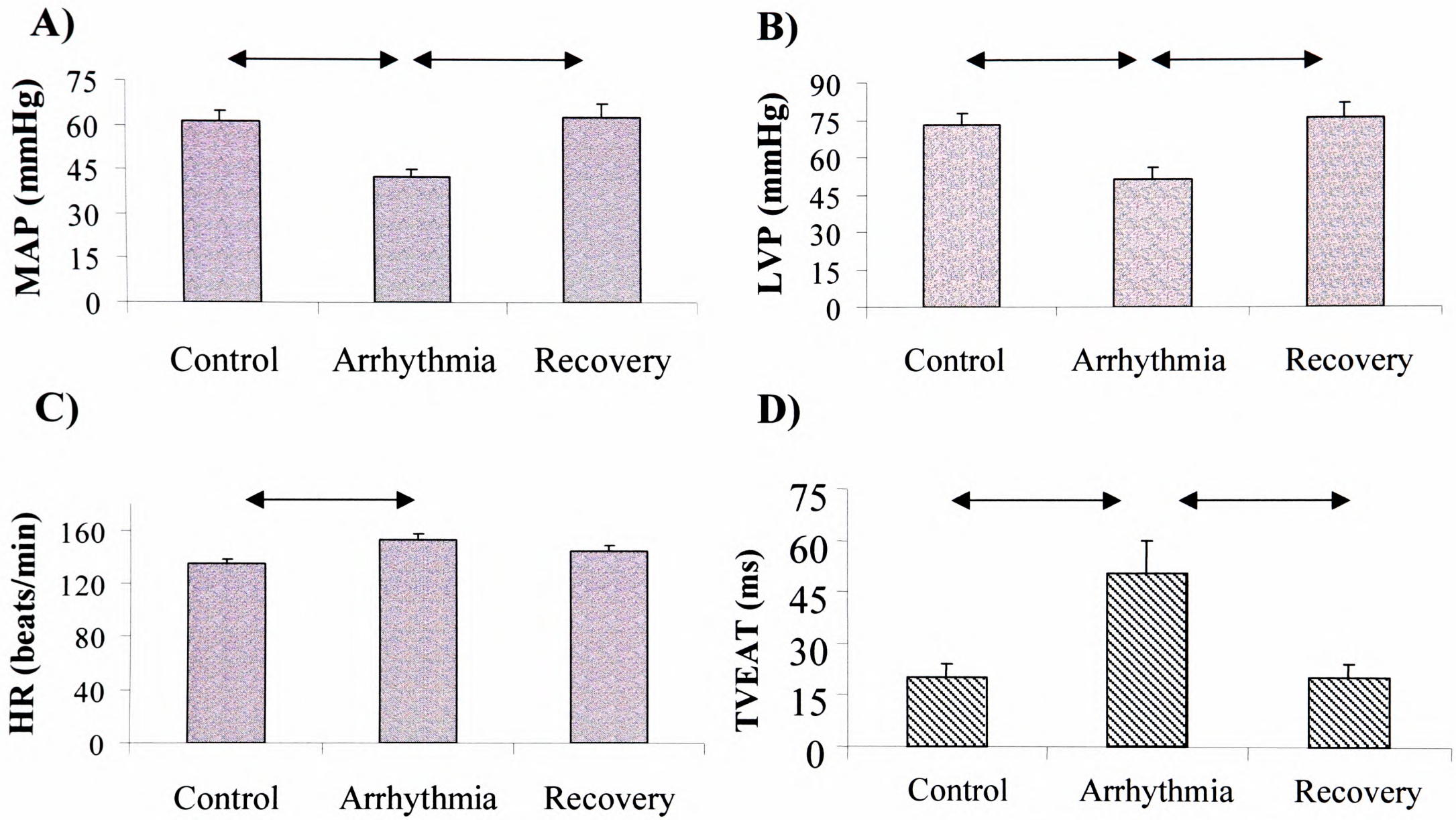


Figure 7.7. Effect of subepicardial infusion (150 μL/min) of 10 μmol/L NA on hemodynamics and total ventricular epicardial activation time (TVEAT). Values correspond to (Control) those before infusion, (Arrhythmia) the maximum hemodynamic depression during NE infusion and (Recovery) the values reached after termination of the arrhythmia. (A) Mean arterial blood pressure (MAP); (B) Maximum systolic left ventricular pressure (LVP); (C) Heart rate (HR); (D) Total ventricular epicardial activation time. Arrows indicate $p<0.05$ (repeated measures ANOVA and *post-hoc* Tukey test).

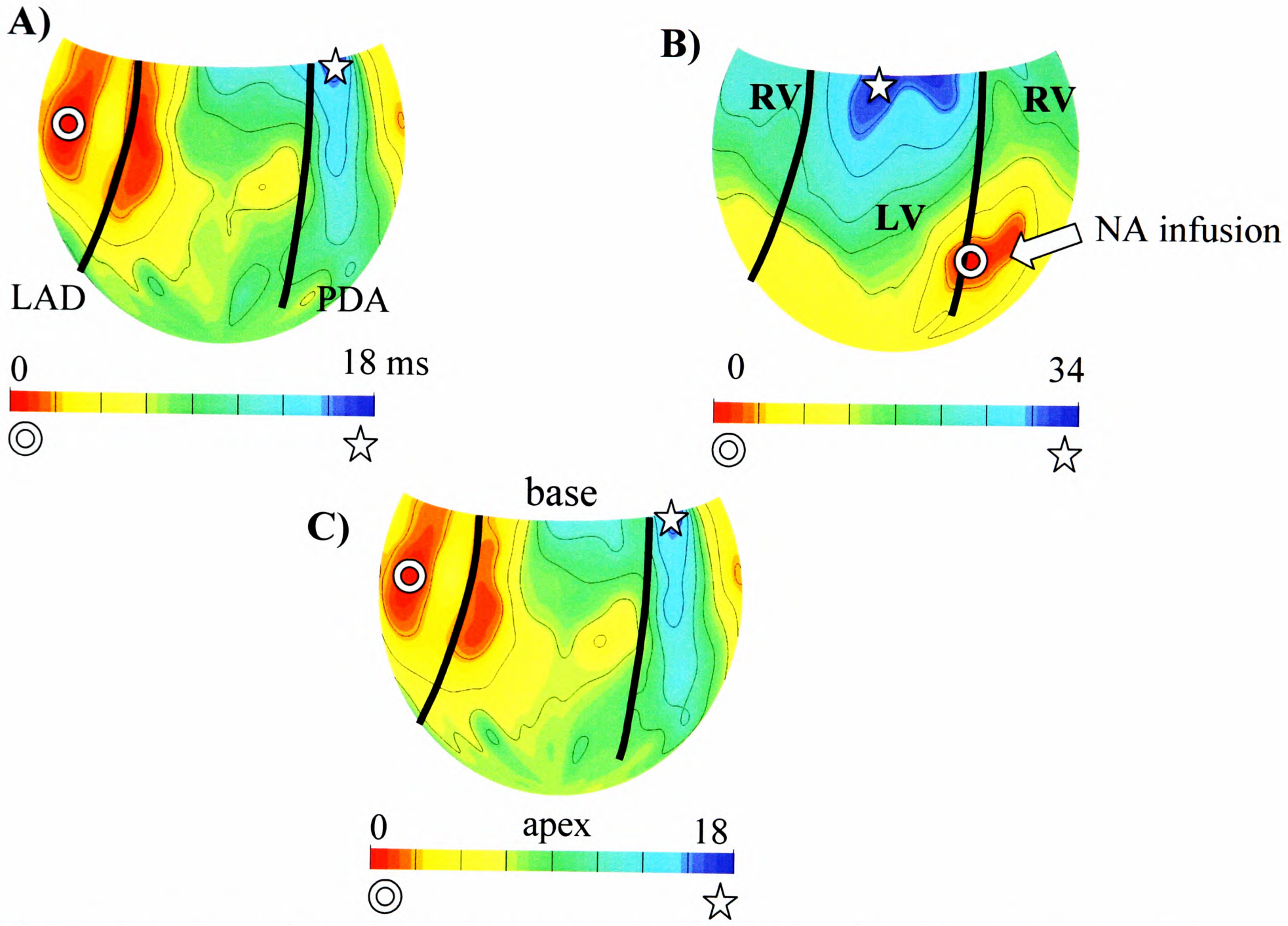


Figure 7.8. 2-dimensional epicardial activation sequences during A) normal sinus rhythm, B) arrhythmia induced by subepicardial NA infusion, C) recovery. O and * indicate the earliest and latest sites of epicardial activation respectively. The LAD and PDA are included for anatomical clarity. During the arrhythmia, the point of earliest epicardial activation shifted to the NA infusion site and the total ventricular epicardial activation time increased.

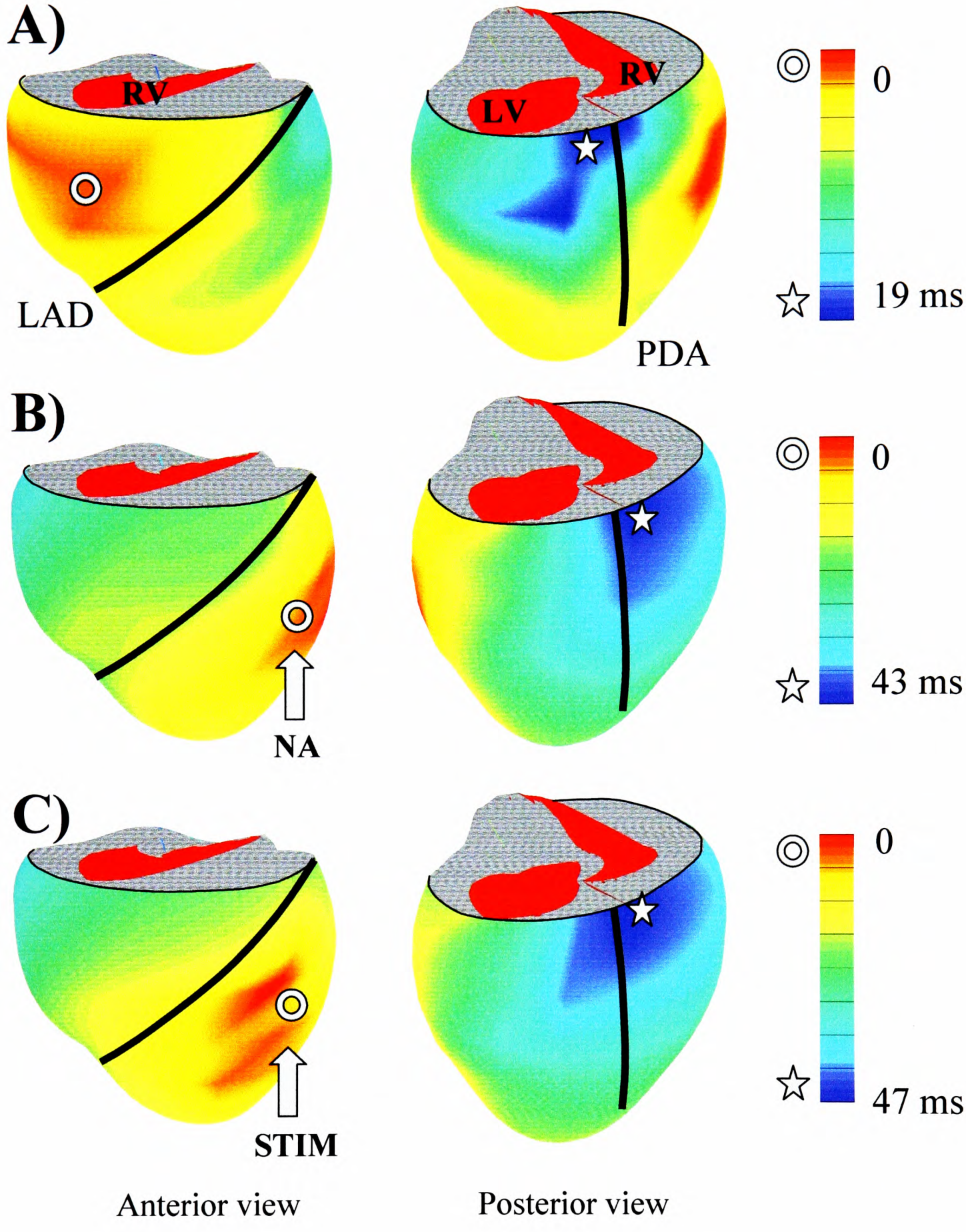


Figure 7.9. Epicardial activation sequences during A) normal sinus rhythm, B) an arrhythmia evoked by subepicardial NA infusion, and C) epicardial electrical stimulation. Regions of earliest (o) and latest (*) epicardial activation are highlighted, the left anterior descending (LAD) and posterior descending (PDA) coronary arteries are also indicated. During the ventricular arrhythmias, the location of earliest epicardial activation shifted to the infusion/stimulation site (arrows). LV, left ventricle; RV, right ventricle.

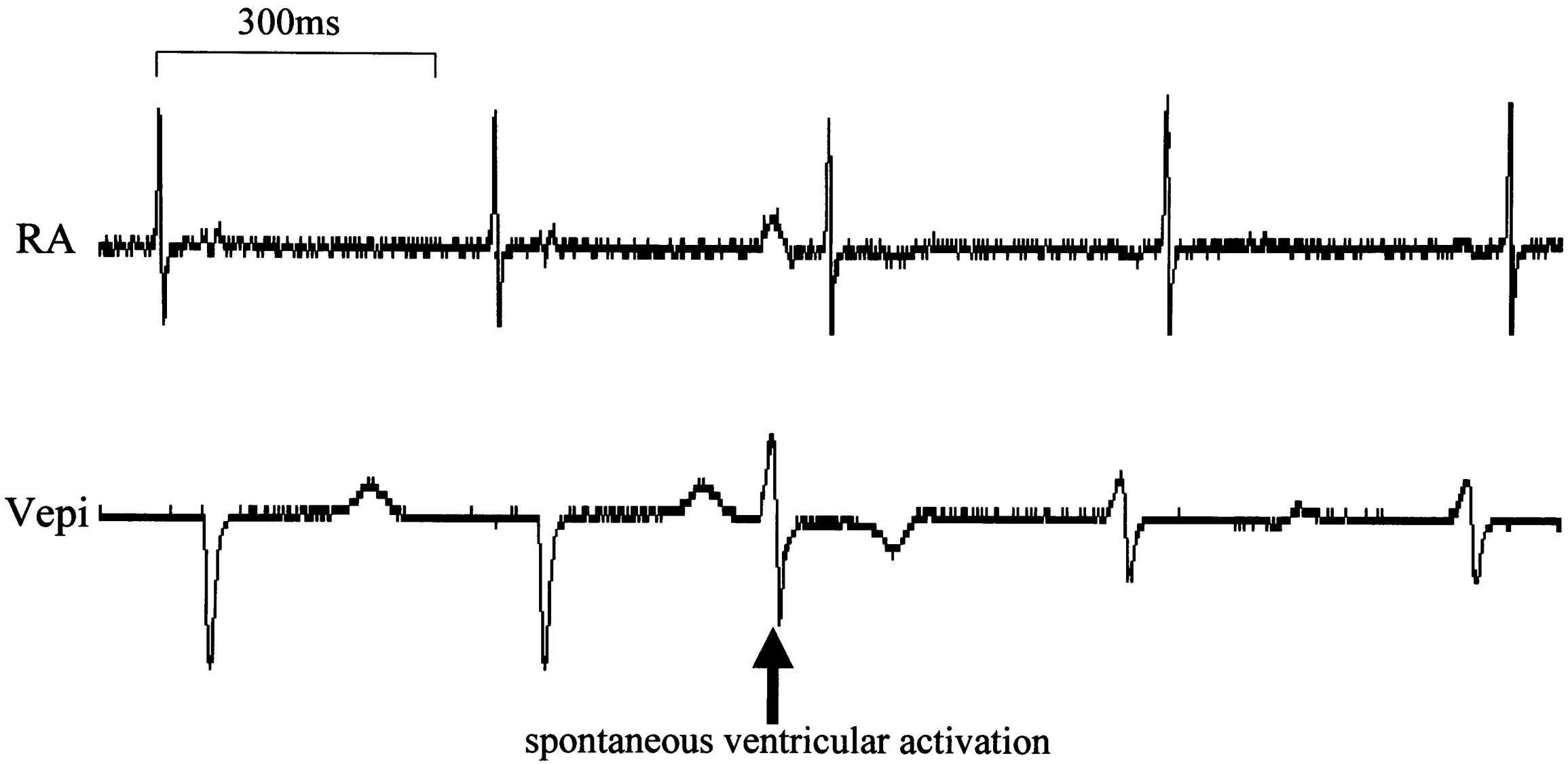


Figure 7.10. Right atrial (RA) and earliest ventricular (Vepi) electropotential recordings at the onset of a NA induced ventricular arrhythmia. The first two cycles show RA activation preceding ventricular activation, subsequently a spontaneous ventricular activation occurs, after which RA activation follows Vepi activation.

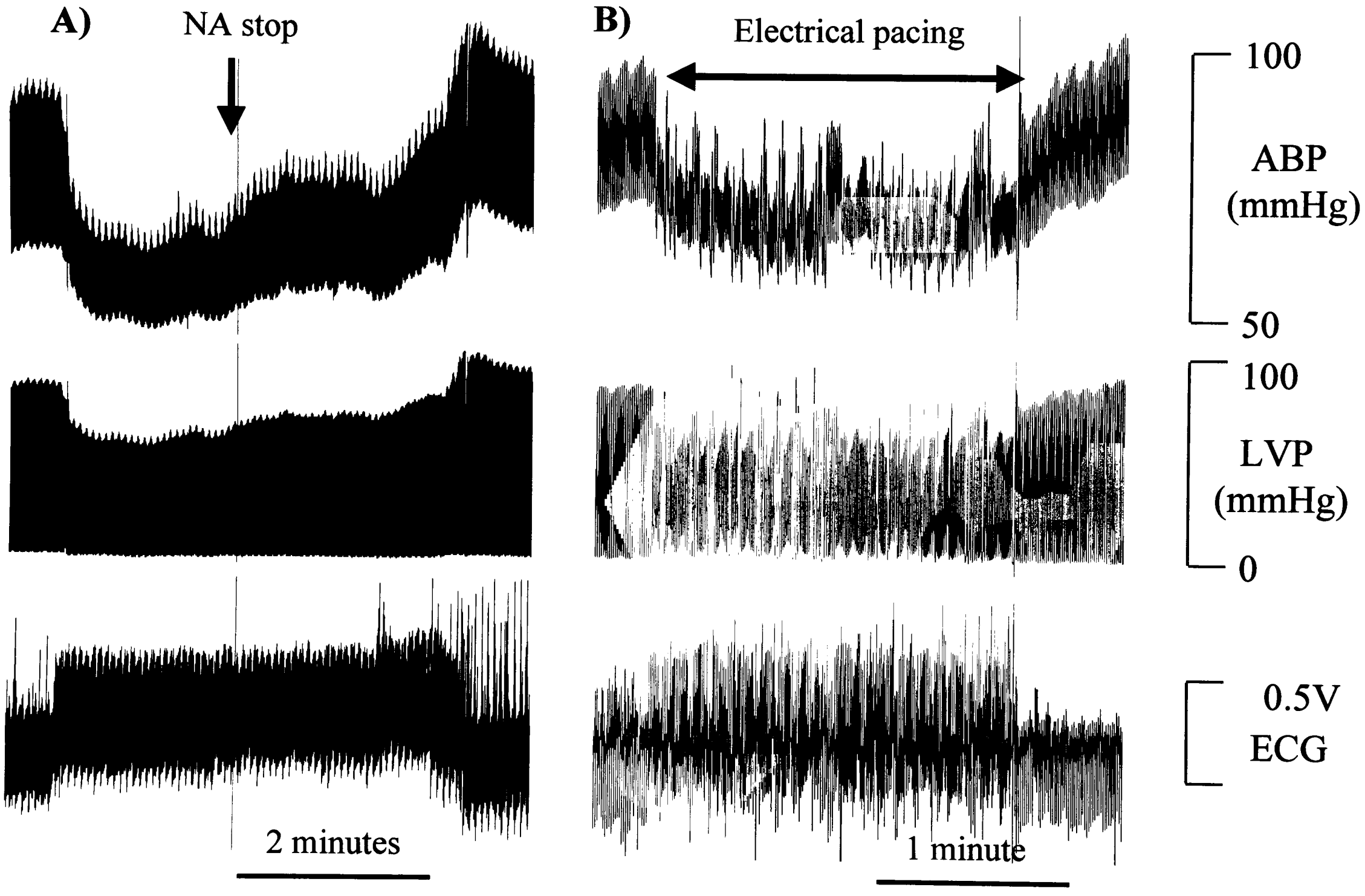


Figure 7.11. Comparison of the haemodynamic effects of a NA induced arrhythmia (A) and an electrical pacing induced arrhythmia (B).

activation time from 19 ms (control) to 47 ms. The activation maps for the electrically and chemically induced arrhythmias were qualitatively similar (figure 7.9). MAP decreased similarly during both arrhythmias (figure 7.11) as did the LV ejection fraction which decreased from approximately 55% to approximately 25%.

7.3 Isolated guinea pig papillary muscle

7.3.1 Methods

Apparatus

The organ bath is illustrated diagrammatically in figure 7.12. It consisted of a perspex bath (HSE, Germany) with a volume of *ca.* 5mls. A force transducer (HSE F30) was mounted on one end of the bath to a manipulator assembly, allowing the tension to be varied. The superfusing solution was bubbled with 95% O_2 and 5% CO_2 and flowed into the tissue bath under the force of gravity, the flow rate being controlled by means of a Hoffman clip (normal flow rate 5mls/min, range 0-30mls/min). The height of the outlet tube could be adjusted to alter the fluid level in the bath and was connected to a peristaltic pump. The temperature of the solution in the bath was adjusted according to two temperature probes (one in the bath and one in the heater) allowing regulation within ±0.1°C. The whole assembly was mounted over an air table. The composition of the guinea pig Tyrode solution was (all in mM): NaCl 120, KCl 4, $MgCl_2$ 2, NaH_2PO_4 0.1, glucose 11, $NaHCO_3$ 23, $CaCl_2$ 2. The solution was bubbled with 95% O_2, 5% CO_2 and was adjusted to pH 7.4. When low Ca^{2+} guinea pig Tyrode solution was used, [Ca^{2+}] was 0.5mM.

Surgery

Male albino Dunkin-Hartley guinea pigs weighing 160-250g were killed by concussion followed by exsanguination. The thorax was opened and the apex of the ventricle removed. 5mls of Tyrode solution (containing 1000 units of heparin) were perfused in to each ventricle before the heart was removed and then pinned with it's anterior side uppermost in a dissection dish with a Sylgard base. The right ventricle was opened to expose the papillary muscles by cutting along the junction of the right ventricular anterior free wall and the septum. A suitable papillary muscle (approximately 4-5mm long and less than 1mm wide) was identified and a

6/0 silk suture passed underneath. The needle end of the suture was bent into a hook and the papillary muscle was securely tied around the tendons. The muscle was then carefully dissected from the ventricle and mounted in the tissue bath. The valvular end of the muscle was secured to the force transducer by means of the hook and the wall end clipped to the base of the bath. Bath temperature was maintained at 37±1°C.

Measurements

A bipolar platinum stimulating electrode was placed at the end of the muscle that was clipped to the base of the bath. A stimulus with a fixed pulse duration of 200μsec and 1.5 times threshold was administered (Digitimer DS2A Isolated Stimulator) in all experiments. Papillary muscles were stretched to the length at which maximum tension was developed by moving the force transducer assembly and allowed to equilibrate for 30 minutes. Force measurements were amplified and filtered (45-60Hz notch) before being digitized and recorded at 200Hz on a Power Macintosh using Acqknowledge software. Contractions were normalised to initial control force and then pooled and expressed as a percentage of the initial force ±SEM. Comparisons were performed using a paired t-test.

Protocols

Thirteen guinea pigs were sacrificed and right ventricular papillary muscles prepared. Muscles were stimulated with a voltage 1.5 times threshold and superfused (5ml/min) with HCO_3 buffered Tyrode solution at 37°C.

Protocol 1: Testing diffusion limitation. Given the thickness of a papillary muscle (*ca.* 1mm) it was necessary to determine how long it would take a divalent cation such as Ni^{2+} to diffuse to the centre of the muscle and hence block Ca^{2+} entry throughout the muscle. In order to do this, muscles were stimulated at 1 Hz and normal Tyrode (Ca^{2+} 2mM) was changed to a rapid superfusion (40 ml/min) of low Ca^{2+} Tyrode (500μM Ca^{2+}).

Protocol 2: The effect of 5mM Ni^{2+}. To test for contraction in the absence of Ca^{2+} entry, the preparation was rapidly superfused (40ml/min) in low Ca^{2+} (500μM) Tyrode containing 5mM

Ni^{2+} (to block Ca^{2+} entry, (Wright 1996) and 5mM Cs^{+} (to stabilise the preparation). Muscles were stimulated every 60 seconds following the switch to the Ni^{2+} Tyrode.

7.3.2 Results

In pilot experiments, rapid superfusion of the papillary muscle with 5mM Ni^{2+} in normal Tyrode solution resulted in spontaneous contractions at a rate of about 120/min. Subsequent experiments were carried out using low Ca^{2+} Tyrode (0.5mM Ca^{2+}) containing 5mM Cs^{2+} as this was found to prevent spontaneous contractions.

Protocol 1: Testing for diffusion limitation

Rapid superfusion with low Ca^{2+} Tyrode caused contraction to decrease to 39±3% of control force with a decay of $t_{0.5}$ of 49±7 seconds (figure 7.13).

Protocol 2: The effect of 5mM Ni^{2+}

After switching to 5mM Ni^{2+} and 5mM Cs^{2+} in low Ca^{2+} Tyrode, contraction with a magnitude of 39±14% compared to control could still be elicited after 13 minutes in eleven preparations of the thirteen studied (figures 7.13B and 7.14).

7.4 Isolated frog heart

7.4.1 Methods

Surgery

Frogs (Rana Temporaria) were concussed, pithed and pinned to a cork dissecting board. A longitudinal incision was made from the sternum to the xiphoid process and the front portion of the ribcage removed to expose the heart. The pericardium was removed and the heart was manoeuvred cranially to display its posterior aspect. The sinus venosus was dissected away from the surrounding tissue and a silk ligature loosely tied around it. A transverse cut was made in the sinus venosus and a Syme's cannula filled with Ringer's solution was advanced into the sinus venosus a few mm and secured with the silk ligature. The heart was removed from the thoracic cavity and mounted on a clamp stand. The level of fluid in the cannula was

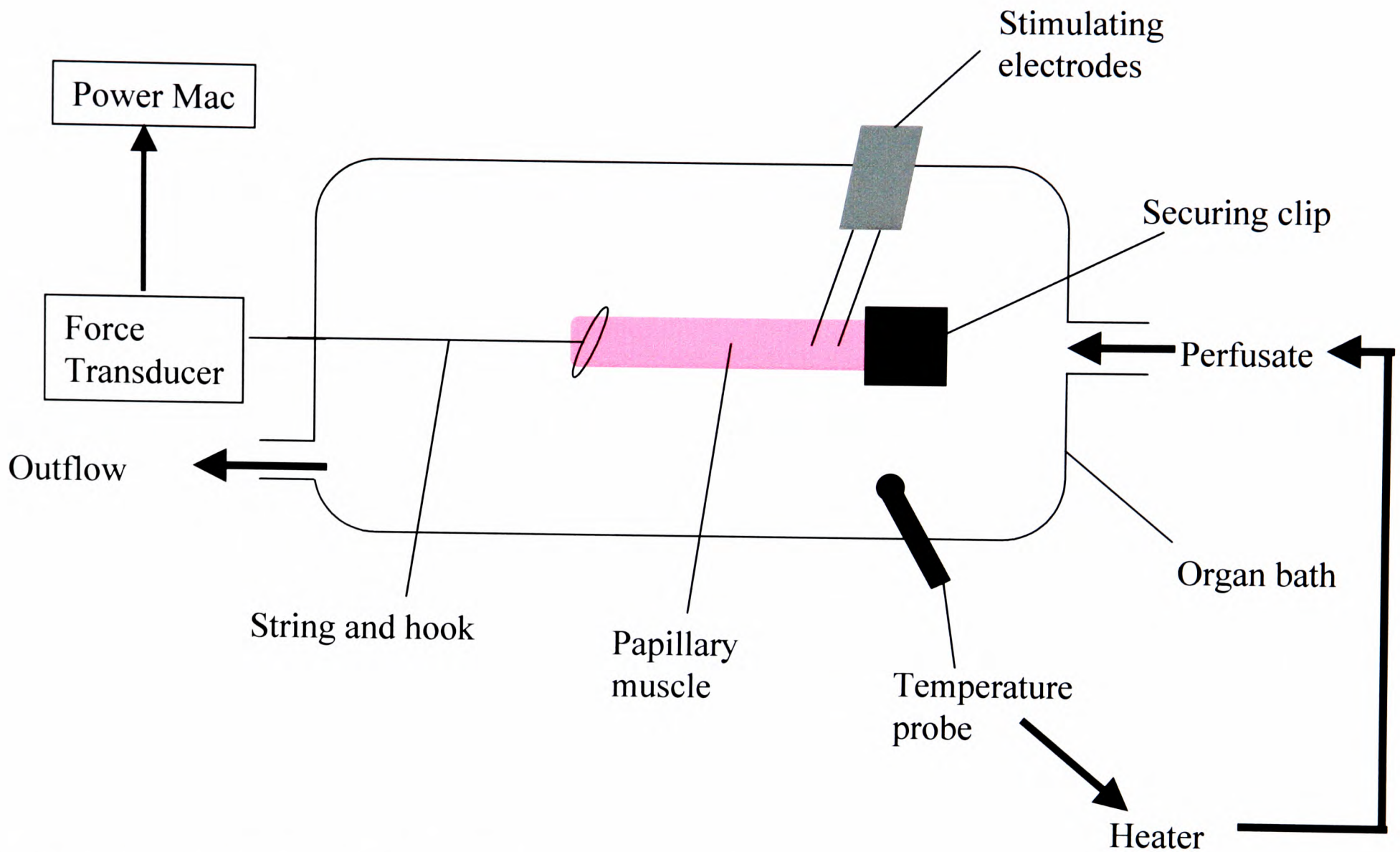


Figure 7.12. Isolated guinea pig papillary muscle setup. The papillary muscle is placed in an organ bath (volume 5mls). It's wall end is secured to the floor of the bath with a plastic clip, a string and hook is attached to the valvular end, the hook being attached to a force transducer. The muscle is electrically stimulated at it's wall end. Temperature and flow rate of perfusate can be controlled.

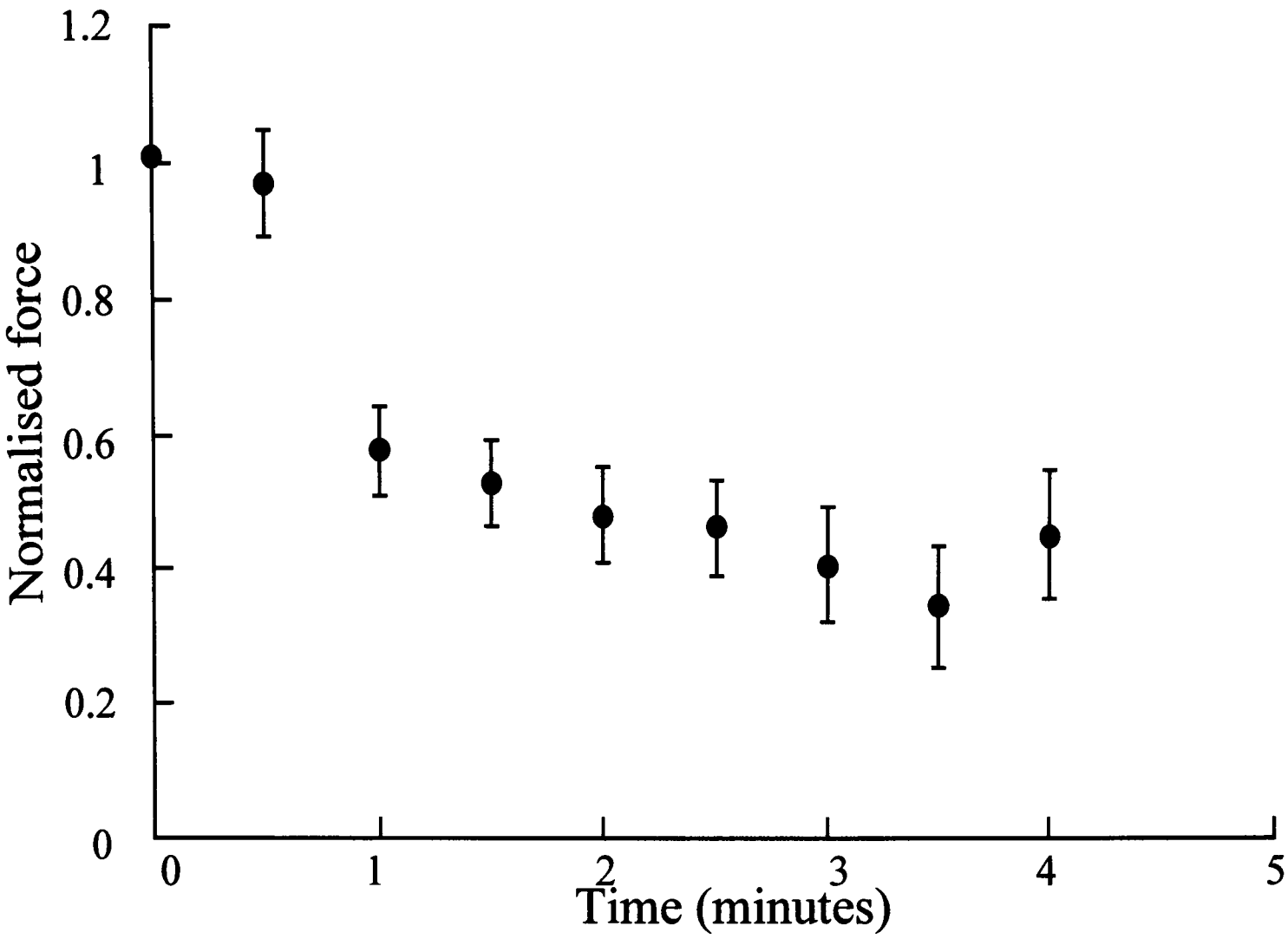


Figure 7.13A. Contractile response to rapid superfusion with 500μM Ca^{2+} Tyrode. Contractions were normalised to initial control force. Data are presented as mean ±S.E.M. n=13.

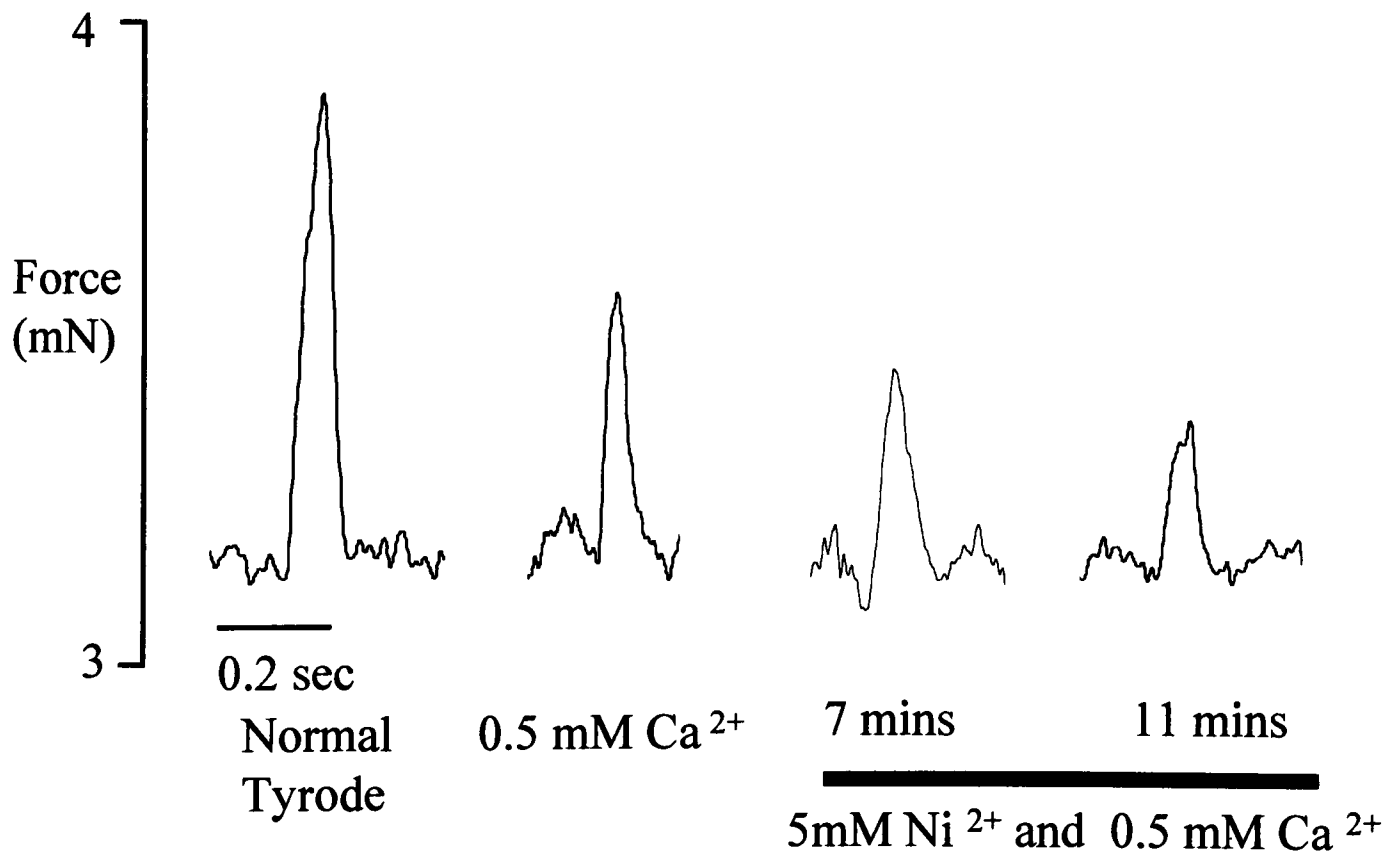


Figure 7.13B. Contractile response of a single preparation to superfusion with 500μM Ca^{2+} Tyrode followed by 500μM Ca^{2+} and 5mM Ni^{2+} Tyrode. Note that contraction could be elicited after 11 minutes of superfusion with Ni^{2+}

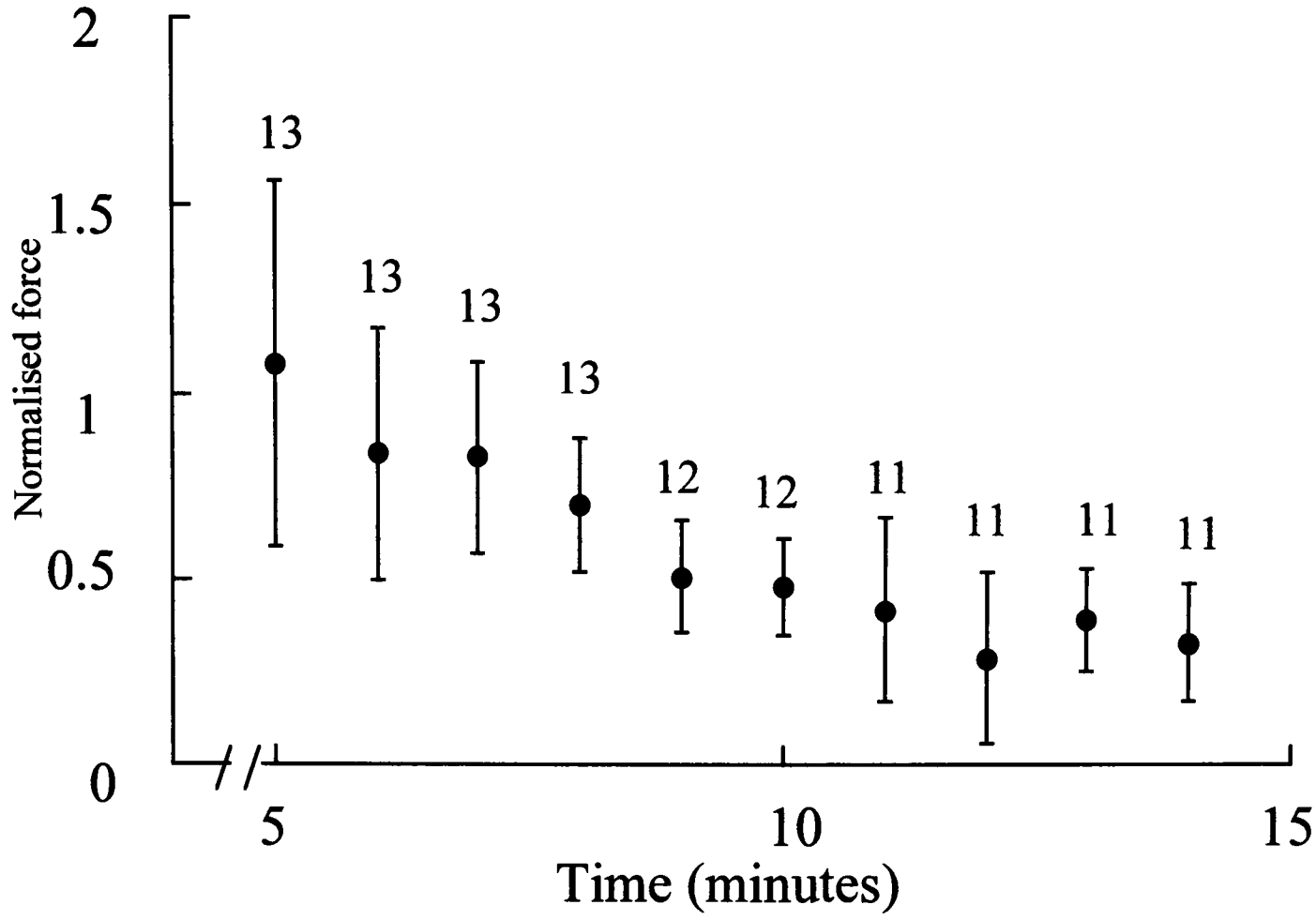


Figure 7.14. Contractile response to electrical stimulation every 60 s in papillary muscle (n=13) superfused with Tyrode containing 5mM Ni^{2+} to block Ca^{2+} entry. Contractions in normal Tyrode were 0.56 ± 0.09 mN. Contractions were normalised to initial control force and are presented as mean ± S.E.M. The number of muscles in which contraction could be elicited is indicated above each data point. Contraction with a magnitude of 52 ± 16 % compared to control was still present after 13 minutes of superfusion with Ni^{2+}.

maintained at approximately 1cm to ensure a constant perfusion pressure, using an adjustable tap around the inflow tube. Once fixed to the clamp stand any remaining extraneous tissue was removed. The surface of the heart was kept moist throughout the experiment by application of Ringer's solution.

Measurements

A small hook attached to a silk thread was placed in the apex of the ventricle and the thread attached to a force transducer (figure 7.15). The transducer was calibrated prior to beginning the experiment with 1, 2 and 5g weights. Hearts were paced from the ventricle using two flexible wires placed on the ventricle attached to a pulse generator. Signals were amplified and recorded (Biopac MP100WS on a Power Macintosh at 200Hz using Acknowledge software. The composition of the frog Ringer was (mM): NaCl 111, KCl 2, $CaCl_2$ 1.1, HEPES 5, glucose 10. The pH was adjusted to 7.4 with NaOH as required. Low Ca^{2+} frog Ringer had a $[Ca^{2+}]$ of 0.3mM.

Protocols

Eight frog hearts were prepared as described. Hearts were allowed to stabilise for 15 minutes to determine their resting beating rate, then paced from the ventricle at a rate 5 bpm above this. A further 10 minute stabilisation period followed. Five protocols were then performed, the order of which was randomised (with the exception of protocol 3 which was always performed last). Each protocol lasted 5 minutes, with 10 minutes recovery between each protocol, or until the preparation had returned to control values of contraction. Contractions were normalised to initial control force and then pooled and expressed as a percentage of the initial force ±SEM. Comparisons were performed using a paired t-test.

Protocol 1: Testing diffusion limitation. To assess the diffusion limitation to divalent cations, hearts were perfused with low Ca^{2+} (0.3mM) Ringer.

Protocol 2: Testing for functional sarcoplasmic reticulum. Voltage-activated Ca^{2+} release is clearly going to be dependent upon the amount of functionally releasable Ca^{2+} in the intracellular stores of myocytes. Given the controversy surrounding the amount of

sarcoplasmic reticulum present in the frog heart (Page & Niedergerke 1972), hearts were perfused with 2mM caffeine (which releases Ca^{2+} from intracellular stores via its action on the ryanodine receptor) in either normal Ringer (protocol 2A) or low Ca^{2+} Ringer (protocol 2B).

Protocol 3: The effect of 8mM Ni^{2+}. To test for the existence of voltage-activated Ca^{2+} release hearts were perfused with normal Ringer containing 8mM Ni^{2+} (protocol 3A) and following recovery, with low Ca^{2+} Ringer containing 8mM Ni^{2+} (protocol 3B).

7.4.2 Results

In two pilot experiments performed with atrial pacing instead of ventricular pacing, 8mM Ni^{2+} caused atrioventricular block and dissociation of atrial and ventricular contraction. Given the need to maintain a constant beating rate of the ventricle and that the aim of the study was to look for VACR in ventricular tissue, all protocols were performed using ventricular pacing.

Protocol 1: the effect of low Ca^{2+} Ringer

Perfusion with low Ca^{2+} Ringer resulted in a rapid decrease of force to 28±4% of control with a $t_{0.5}$ of 8±2 seconds (figure 7.16).

Protocol 2: the effect of caffeine

2mM caffeine in normal Ca^{2+} Ringer resulted in an increase in the force of contraction (144±11% control, figure 7.17) which was maintained for the duration of exposure (n=7). 2mM caffeine in low Ca^{2+} Ringer resulted in a transient increase in force followed by a decrease to that seen in low Ca^{2+} solution alone (figure 7.18).

Protocol 3: the effect of 8mM Ni^{2+}

8mM Ni^{2+} in normal Ringer arrested frog hearts after 11± 2 seconds (figure 7.19) and after 9±1 seconds in low Ca^{2+} Ringer (both n=8, table 7.1). There was no significant difference between these times. When perfusion with normal Ringer was resumed, hearts recovered to control force within 5 minutes.

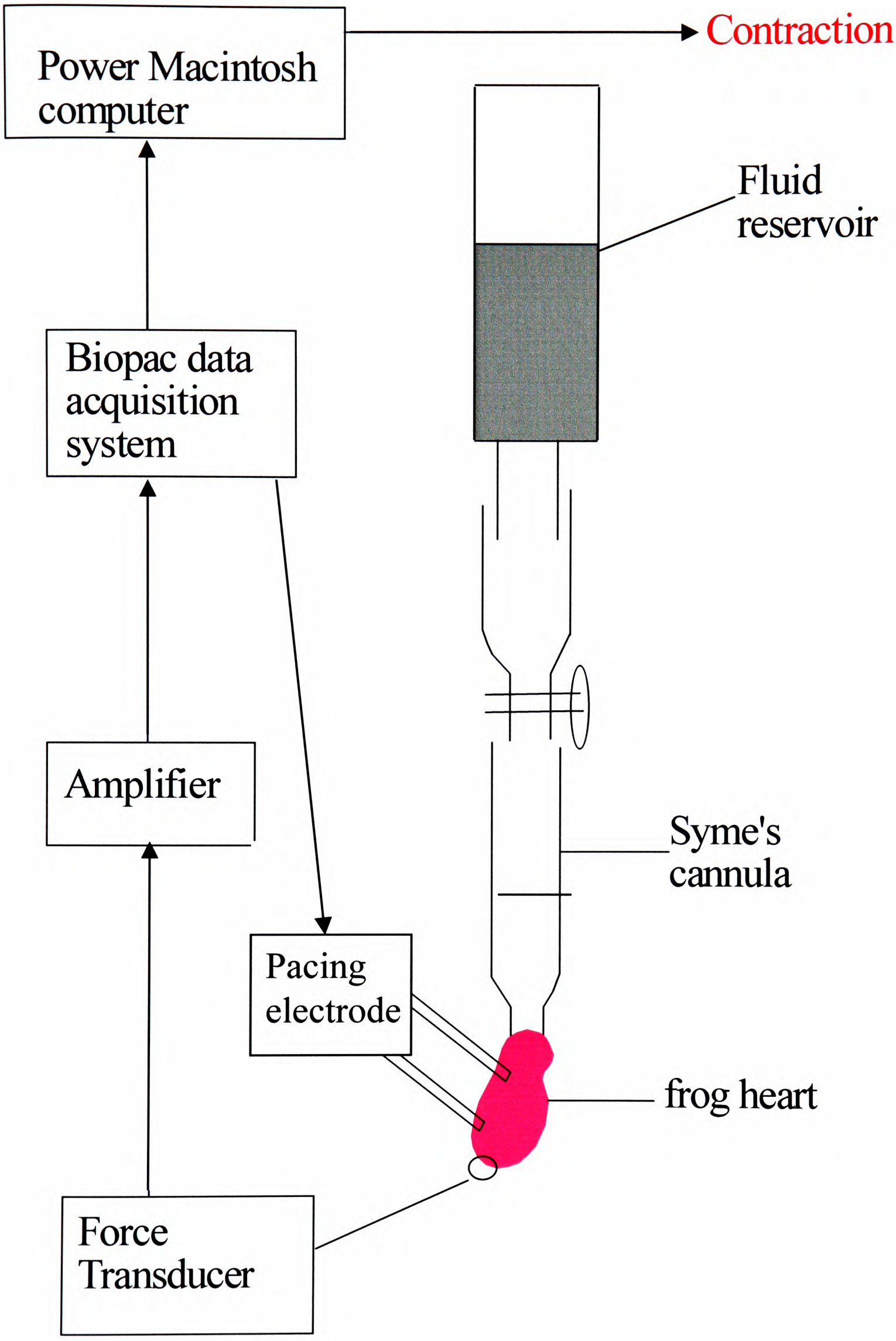


Figure 7.15. Apparatus for perfusion of the isolated frog heart. A Syme's cannula is inserted into the sinus venosus and securely tied. A small hook is placed through the apex of the ventricle to enable contraction to be measured.

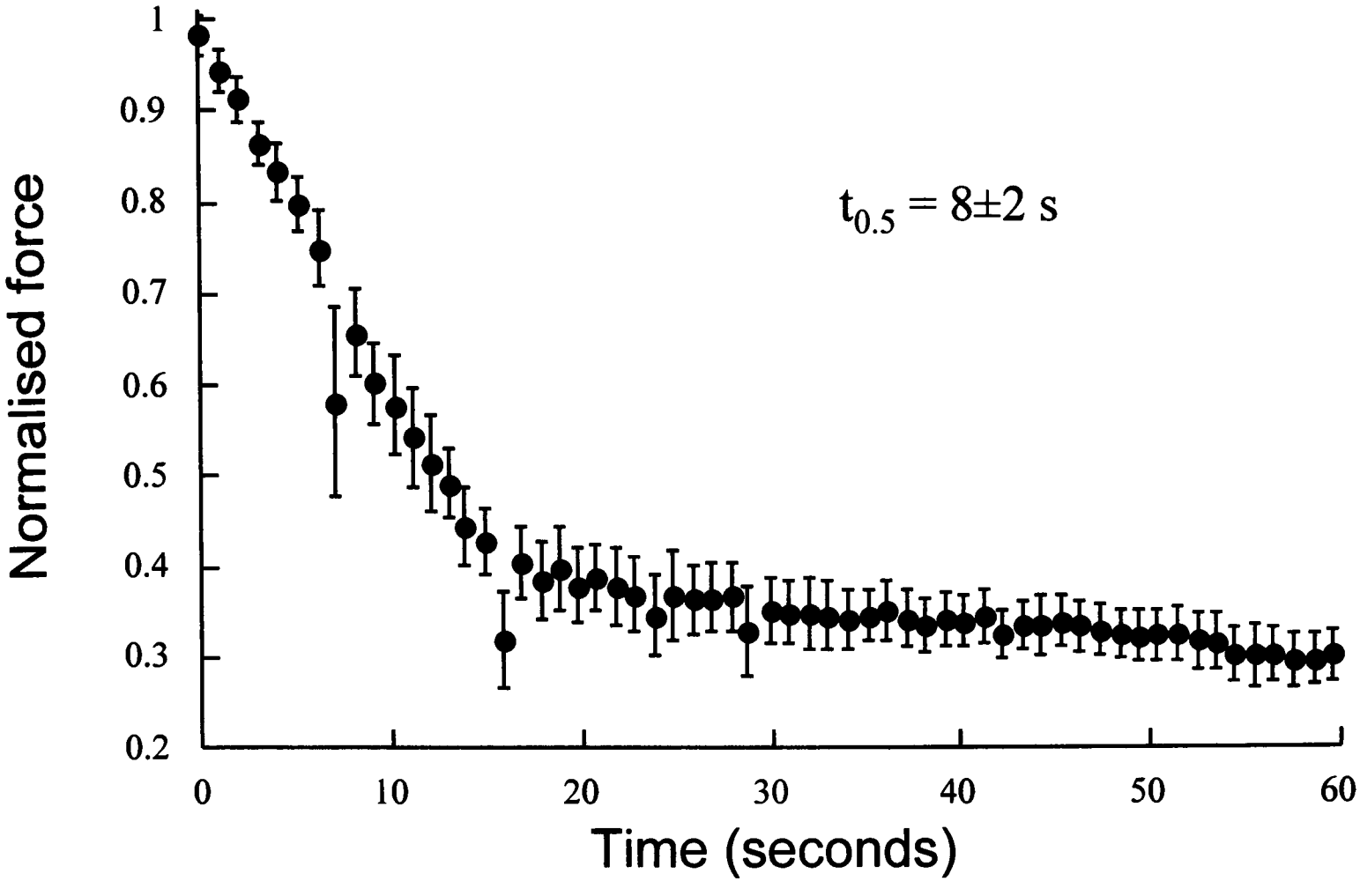


Figure 7.16. The effect of perfusion with low Ca^{2+} Ringer on contraction in the isolated perfused frog heart. Contraction decreased to 28±4% of control force with a $t_{0.5}$ of 8±2 seconds.

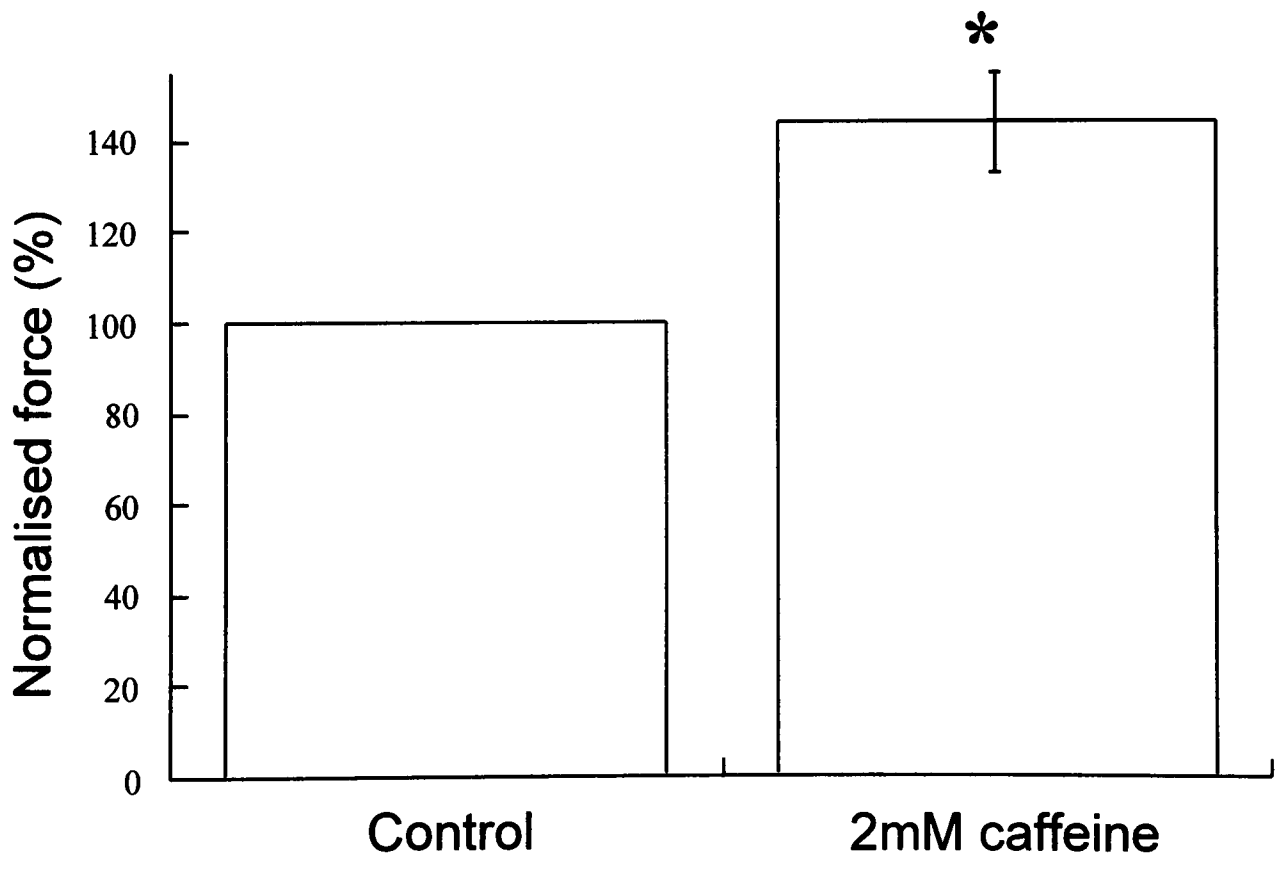


Figure 7.17. 2mM caffeine in normal Ringer solution resulted in a sustained increase in force of contraction (144±11% of control) in the isolated perfused frog heart ($p<0.05$, paired t test).

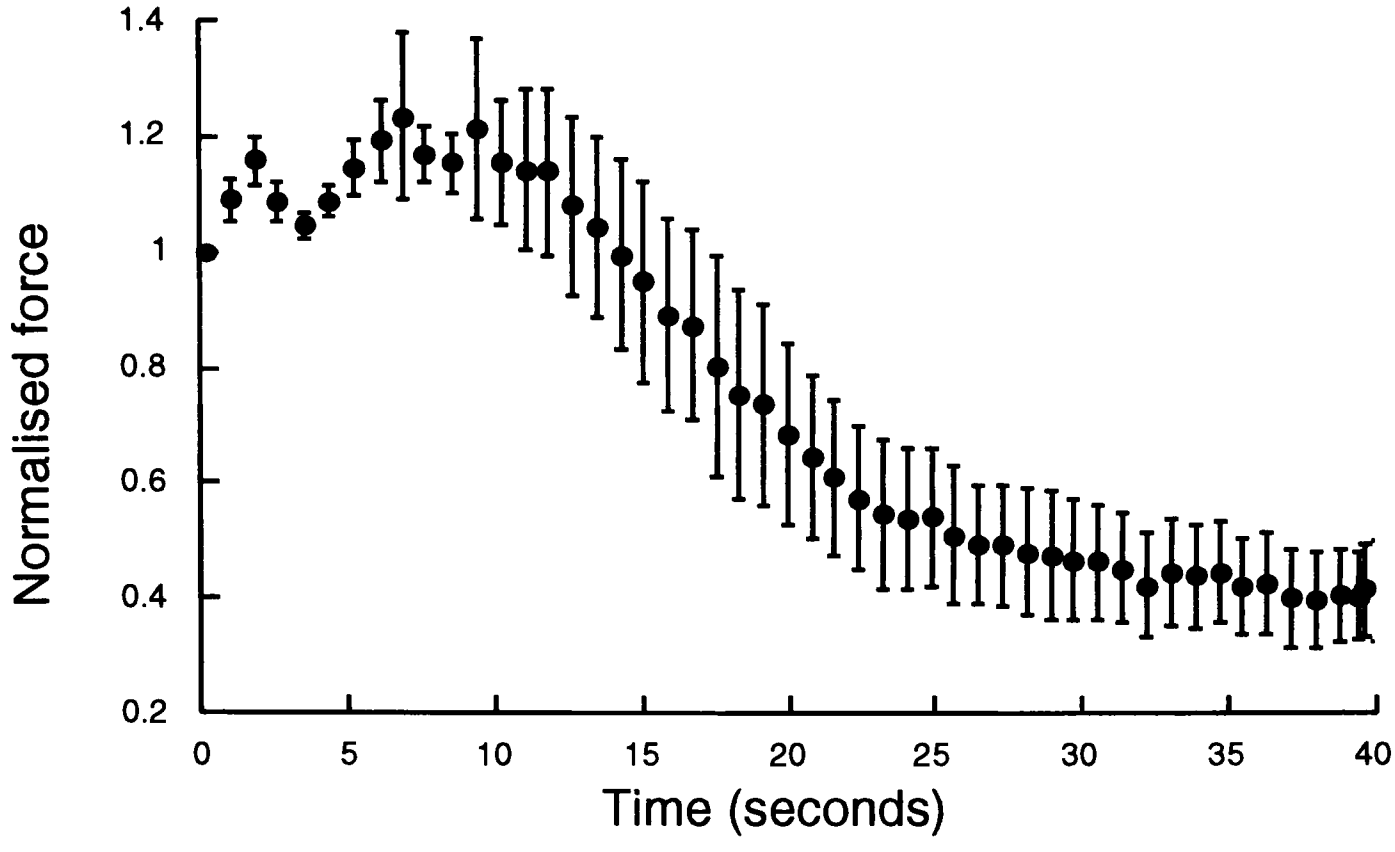


Figure 7.18. The effect of perfusion with 2mM caffeine in low Ca^{2+} Ringer. A transient increase in force occurred, followed by a decrease to that seen in low Ca^{2+} Ringer alone.

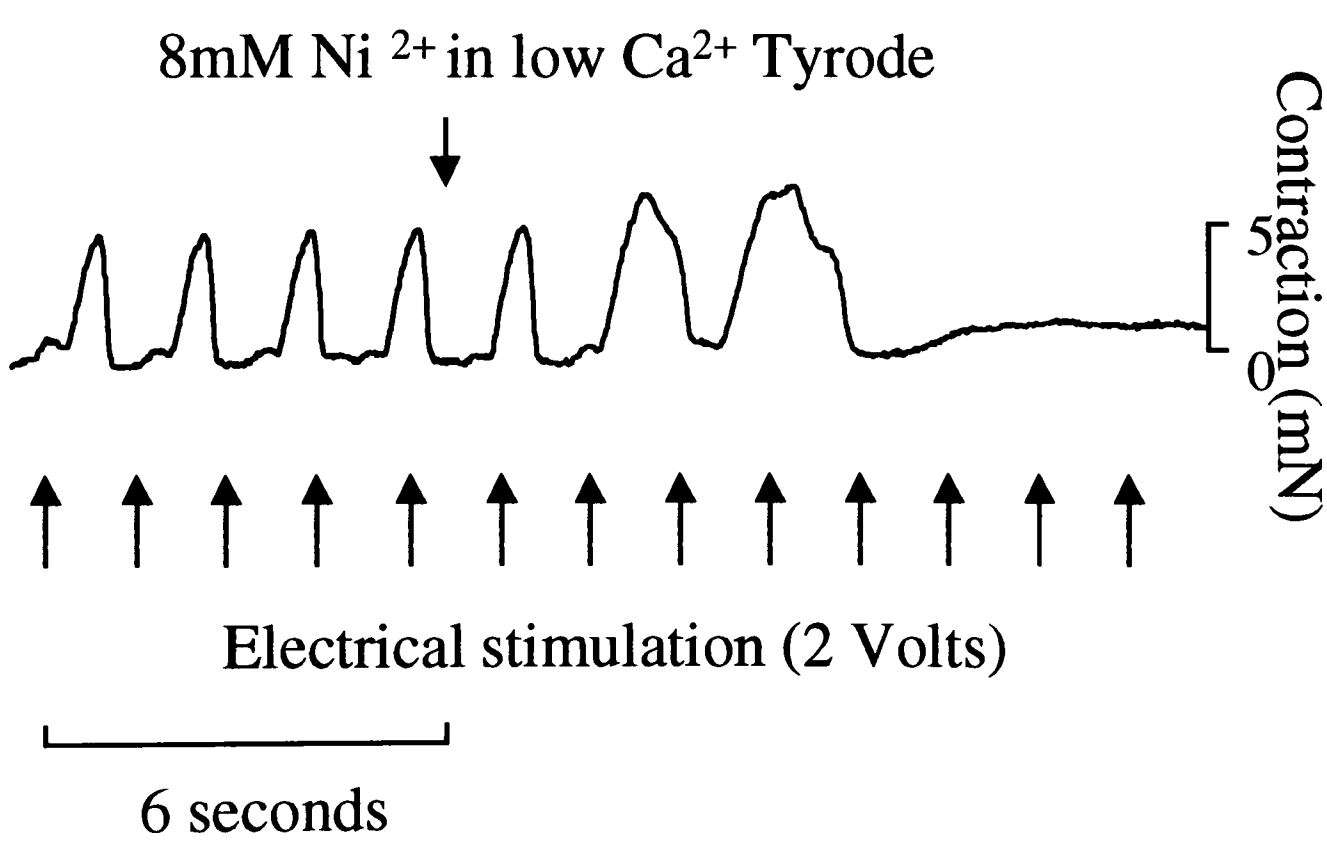


Figure 7.19. Raw data trace showing the effect of 8mM Ni^{2+} on ventricular contraction in the isolated perfused frog heart. Electrical pacing of the venticle was performed to maintain a constant beating rate. 8mM Ni^{2+} abolished contraction in *ca.* 5 seconds.

Solution	*Time to no observable force (seconds)*
8mM Ni^{2+} in normal Ringer	11±2
8mM Ni^{2+} in low Ca^{2+} Ringer	9±1

Table 7.1. Time taken for 8mM Ni^{2+} to arrest frog hearts (n=7). The time was not significantly different between normal and low Ca^{2+} ringer (paired t-test).

7.5 Discussion

The main findings of this chapter are: 1) Infusion of NA subepicardially into the *in-vivo* pig heart elicits a reproducible alteration in the sequence of electrical activation. The site of earliest breakthrough of electrical activation moves from the right ventricular free wall to the site of NA infusion. This alteration in activation sequence explains the altered QRS morphology of the 3 lead ECG and the decreases in MAP, LVP and LVdP/dt max. 2) A similar pattern of activation and depression of haemodynamics is elicited when the epicardium is electrically stimulated at the NA infusion site. 3) In the guinea-pig papillary muscle 5mM Ni^{2+} reduces but does not block stimulated contraction, consistent with the existence of a voltage-activated Ca^{2+} release mechanism. 4) In the isolated perfused frog heart 8mM Ni^{2+} rapidly abolishes ventricular contraction; there is no evidence for the existence of voltage-activated Ca^{2+} release in the frog heart.

7.5.1 Electrical activation pattern of the ventricle during an arrhythmia elicited by subepicardial NA infusion

7.5.1.1 Mechanism of the arrhythmia induced by subepicardial NA infusion

A stable ventricular arrhythmia could be elicited by subepicardial infusion of NA. This arrhythmia is likely to be due to an effect of NA directly on the ventricular myocytes and not a reflex, since it could be elicited in the absence of autonomic innervation (O'Neill 1995). The fact that the ECG changes occurred prior to the haemodynamic changes (figure 7.6) and that electrical pacing of the epicardium resulted in similar alterations in activation times and haemodynamics compared to the NA induced arrhythmia also indicate that a reflex is unlikely to be responsible. Previous studies indicate that the arrhythmia is mediated via an effect of NA

on β receptors (since it can be terminated by β blockade, Podzuweit 1980), which in turn increases intracellular cAMP (the arrhythmia can be elicited by 8-Br-cAMP, Podzuweit 1980). Catecholamines can enhance automaticity in Purkinje fibers (Pappano & Carmeliet 1979), induce after-depolarizations in canine ventricular myocytes (Marchi *et al.* 1987) and cause re-entry in the ventricle (Hisatome & Arita 1995).

7.5.1.2 Spread of the activation wave

Total ventricular epicardial activation time under control conditions in this study was 20±4ms. This corresponds well to previous epicardial mapping studies in the pig (20.1±2.3ms in the study of Cinca *et al* (1997) but is considerably shorter than that measured in humans (range in eight previous studies 64-125 ms; (Klein *et al.* 1982; Wiener *et al.* 1982; Wiener *et al.* 1984; Untereker *et al.* 1985; Vassallo *et al.* 1986; Lacroix *et al.* 1990; Hood *et al.* 1992; Hatala *et al.* 1995). Several of these authors also found increases in total activation times during ventricular tachycardia (induced by programmed electrical stimulation), consistent with the current pig study. The reason for this difference in total ventricular activation under control conditions between the species is unclear, it is unlikely to be due to the open-chest preparation used; whilst absolute potentials are dramatically altered by exposure to air, isochrones are not markedly affected (Green *et al.* 1991). Most of these human studies were performed in hearts which had varying degrees of myocardial infarction which could have affected total ventricular activation time via a generalised slowing of conduction, whilst leaving activation sequences the same as that seen in the healthy heart. However, studies on patients with Wolff-Parkinson-White syndrome also recorded total ventricular epicardial activation times of *ca.* 60ms (Pieper & Pacifico 1992). The differences between the current study and human studies may therefore reflect species-dependent differences in conduction velocity or in the extent of the Purkinje network.

The site of earliest epicardial breakthrough consistently moved to the site of NA infusion during the arrhythmia; this occurred regardless of where the infusion needle was placed in the ventricular wall. Another consistent finding was that during the arrhythmia there was only ever a single site of epicardial breakthrough of the activation wave, whereas during control

conditions there were always several, consistent with human studies. Almost all previous studies (pig, dog and human) have tended to show a qualitatively similar pattern of epicardial activation, but the number of breakthrough points has differed and is a function of the spatial resolution of the electrodes. Using 1124 electrodes in the dog heart 20-35 epicardial breakthrough sites were observed (Arisi *et al.* 1983), using 117 electrodes in human hearts 12 were observed (Pieper & Pacifico 1992). In both these studies neighbouring breakthroughs merged into a single major breakthrough within 1-2ms, thus they would be missed in studies such as the current one in which spatial resolution was not high. In a study of 76 patients with prior myocardial infarction (Hatala *et al.* 1995) there were normally 2±0.5 epicardial breakthrough sites and the site of earliest breakthrough was on the anterior right ventricular epicardium, consistent with the current studies in the pig under control conditions (figure 7.8A). After the emergence of the RV epicardial breakthrough, the activation wave in these patients spread radially over the RV and progressed right-to-left over the apex and anterior and posterior septa to activate the LV. In 64% of patients there was a further distinct breakthrough site in the LV, in a patient without structural heart disease breakthrough sites were observed on the inferior RV and at the apex. These activation sequences are broadly similar to those seen in the pig heart in the current study, although as already mentioned the total ventricular epicardial activation time was markedly shorter in the pig than in man.

Right atrial activation followed ventricular activation in every arrhythmia in which it was recorded. This was also true of the arrhythmia evoked by electrical stimulation of the ventricle. This is consistent with a retrograde conduction of the activation wave through the AV node during the arrhythmia. In a previous study O'Neil (1995) showed this arrhythmia to be terminated by vagal stimulation. This might also be consistent with retrograde conduction; vagal innervation of the ventricle is sparse, so termination of the arrhythmia by vagal stimulation might be occurring at the AV node which is more densely innervated. This is of considerable importance and could be tested directly using His Purkinje recordings as is performed in clinical electrophysiological studies. A role for the AV node in the current study would also be consistent with clinical evidence that idiopathic ventricular tachycardia is usually not terminated by vagal manoeuvres (Slama *et al.* 1985), but arrhythmias involving the AV node generally respond well to vagal stimulation (Waxman *et al.* 1980).

7.5.1.3 Cellular origin

In the current study it was not possible to determine the exact location of the needle within the ventricular wall. The tissue type (purkinje, M cell, epicardial or endocardial) in which the arrhythmia originated is therefore left to speculation but is of considerable importance since it influences the likely arrhythmic mechanism and what type of manoeuvres are likely to be effective in terminating the arrhythmia (Antzelevitch *et al.* 1995). It also affects the clinical relevance of the current study; in a human study in which endocardial and epicardial maps were recorded simultaneously during ventricular tachycardia endocardial activation preceded epicardial activation in >90% of tachycardias (35 patients), (Harris *et al.* 1987). Moreover the epicardial activation pattern was not integral to the ventricular tachycardia mechanism; epicardial activation began and ended within the period of endocardial activation and arrhythmias were also observed on the endocardium that did not alter the epicardial activation sequence.

There are several reasons to suspect that the arrhythmia did not originate in the Purkinje network, but arose in the ventricular wall. First, electrical pacing of the *epicardium* was successful in eliciting arrhythmias. The slight difference in the activation times produced by electrical stimulation as opposed to the NA infusion may be due to the fact that the electrical pacing was performed on the epicardial surface, whereas the NA infusion was subepicardial. Secondly as discussed above, under control conditions there were multiple sites of epicardial breakthrough, whilst during the arrhythmia only one breakthrough site was ever observed. This is consistent with the activation wave spreading across the epicardial surface from an initial ectopic focus in the ventricular wall; if the arrhythmia originated in the Purkinje system, multiple breakthrough sites might still occur (as occurs in humans with ventricular tachycardia whose origin is endocardial).

The use of an endocardial basket catheter (e.g. (Eldar *et al.* 1996) in combination with plunge electrodes (e.g. (Chung *et al.* 1997) and an epicardial sock would allow a 3-dimensional representation of the ventricular wall and therefore a more precise localisation of the origin of the arrhythmia. An alternative would have been to perform endocardial ventricular pacing and to determine the total ventricular activation time during this manoeuvre in comparison with

that during the arrhythmia. Since conduction parallel to the fibre direction is *ca.* ten times faster than perpendicular to fibre direction (Clerc 1976), a NA induced arrhythmia originating in the Purkinje fibres might be expected to have a similar total ventricular activation time compared to endocardial electrical pacing. If on the other hand endocardial ventricular pacing resulted in a shorter total ventricular activation time than that occurring during the arrhythmia it would be consistent with a subepicardial origin for the NA induced arrhythmia. Another way of answering this question would be to infuse NA at the site of normal epicardial breakthrough. If this resulted in an increase of total ventricular epicardial activation time, similar to that seen during other arrhythmias, it would indicate that the arrhythmia is unlikely to originate in the Purkinje system.

7.5.1.4 Effect of altered activation sequence on haemodynamics

Ventricular tachycardia in the normal heart is generally asymptomatic and relatively benign (Trappe *et al.* 1988). Therefore a particularly interesting feature of the arrhythmia induced by sub-epicardial NA infusion is the extent to which the haemodynamics are compromised. In the present study, epicardial mapping showed that the order of activation of the ventricle was substantially altered during the arrhythmia, consistent with studies showing a dependence of stroke volume on activation sequence (Nakano 1964; Grover & Glantz 1983; Burkhoff *et al.* 1986; Schwaab *et al.* 1999). Electrophysiological mapping studies of activation sequences (Lister *et al.* 1964) also support this concept, originally proposed by Wiggers (1925):

> *"the greater the distance the excitation wave has to travel from the pacing site to the Purkinje system, the greater is the asynchrony of contraction and therefore the weaker the contractile strength"*

The fact that haemodynamic depression was similar between electrical pacing and a NA induced arrhythmia at the same site (figure 7.9) and when two arrhythmias were induced consecutively at the same site may also indicate a direct link between the precise site of epicardial breakthrough of the activation wave and the degree to which haemodynamics are depressed.

7.5.1.5 Clinical relevance

Many reports documenting ventricular tachycardia (VT) in the apparently 'normal' heart have suffered from inadequate clinical assessment; detailed investigations including endomyocardial biopsy and cardiac catheterisation show that many such patients do in fact have structural heart disease (e.g. 70% in the study by Deal *et al* (1986). Ventricular tachycardia can therefore be an early indicator of cardiomyopathy rather than a primary electrical disease (Slama *et al.* 1985) and could therefore be used diagnostically. If structural heart disease is truly absent, the incidence of lethal arrhythmias in these patients is low (Trappe *et al.* 1988), reviewed by Wichter *et al.* (1995). When VT is present in the 'normal' heart, it is often provocable by exercise testing (Mont *et al.* 1991) and is sensitive to β-blockers, verapamil and adenosine (all drugs which may have little effect on VT following myocardial infarction). MIBG scintigraphy[1] often reveals regional abnormalities in cardiac sympathetic innervation in patients with VT and apparently 'normal' hearts (Gill *et al.* 1993; Mitrani *et al.* 1993; Wichter *et al.* 1994). In addition, heart rate variability studies have demonstrated increased sympathetic tone immediately prior to VT (Fei *et al.* 1993). Taken together, these findings imply a role of the sympathetic nervous system in the genesis of VT in the normal heart. Infusion of NA subepicardially may therefore be a useful experimental tool with which to investigate VT.

7.5.1.6 Limitations of epicardial mapping studies

Limitations imposed by the electrical parameters analysed

Analysis of epicardial electrical data was limited to an investigation of activation times (taken to be the point of maximum speed of change in voltage). Whilst this represents the gross order of breakthrough of the electrical wave to the epicardium, further analyses on other electrical parameters may be illuminating. The type of electrical parameter yielding the most information will vary according to the nature of the investigation. For example, maps of repolarization times or local refractory periods (e.g. Freigang *et al.* 1997) in the current study may have predictive value as to whether the subsequent beat was going to be normal or

arrhythmic. Since a major determinant of arrhythmia diagnosis and treatment is based on the width of the QRS complex, a study of the regional differences in QRS duration over the epicardial surface would be of considerable value. Studies of ischaemia and infarction could appropriately include mapping the regional differences of ST segment elevation or depression (e.g. as performed by Li *et al.* 1998) and regional changes in T-wave alternans might also be of diagnostic value (Nearing *et al.* 1991).

Limitations imposed by the number and location of electrodes

Given a relatively sparse electrode coverage (*ca.* 15mm spacing) and the fact that only epicardial electrodes were used it is not possible to draw conclusions regarding conduction velocity of the activation wave and the anisotropy of the epicardium. Pilot studies showed that the spatial resolution of electrodes appeared to be sufficient when considering *qualitative* changes in the order of breakthrough of the activation wave (maps were identical when inter-electrode spacing was halved). As already discussed, the number of epicardial breakthrough sites depends upon the spatial distribution of electrodes and therefore will alter the apparent spread of the activation wave. The current resolution is unlikely to be sufficient when considering electrical changes other than activation times or when diagnosing an arrhythmia type. For example, re-entry has been shown to be dependent upon cores of tissue with 3-5mm diameter (Kamjoo *et al.* 1997), so it is possible that in the current study re-entry was occurring but was beyond the resolution of the mapping system. An additional advantage of greater spatial resolution would be that when performing quantitative comparisons of activation maps more electrodes would enhance the potential to demonstrate statistically significant differences in electrical activity that did not result in such gross changes in activation sequence as seen in the current study.

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[1] A technique for non-invasively imaging regional sympathetic innervation using iodine-123 metaiodobenxylguanidine (MIBG).

7.5.2 Functional importance of voltage-activated Ca^{2+} release

7.5.2.1 Origins of the intracellular Ca^{2+} transient

Cardiac contraction is believed to depend upon the entry of extracellular Ca^{2+} to trigger Ca^{2+} release from the sarcoplasmic reticulum; Ca^{2+}-induced Ca^{2+} release (CICR). This transmembrane movement of trigger Ca^{2+} is predominantly via L type Ca^{2+} channels (Bers 1991), but 'reverse mode' Na^{+}/Ca^{2+} exchange also occurs during the plateau of the action potential (Leblanc & Hume 1990). Recently however, Hobai *et al* (1997) proposed that voltage-activated Ca^{2+} release can also occur in cardiac muscle as it does in skeletal muscle. This conclusion was based on the finding that intracellular Ca^{2+} transients occurred when transmembrane entry of Ca^{2+} was blocked with 5mM Ni^{2+}. Additional evidence for the existence of voltage-activated Ca^{2+} release comes from the use of voltage clamp in conjunction with cell shortening as a measure of contraction (Ferrier *et al.* 1998). Contraction-voltage relations for contractions elicited by CICR (determined from a post-conditioning potential of –40mV to inactivate VACR) were proportional to the amount of inward Ca^{2+} current. However, at more negative post-conditioning potentials and in the presence of cAMP the amplitude of contraction became independent of the magnitude of inward Ca^{2+} current.

The current studies were designed to test whether Ca^{2+} transients in the presence of Ca^{2+} entry blockers were functionally important in eliciting contraction. If contraction were triggered solely by Ca^{2+} entry inducing Ca^{2+} release from the SR, then 5mM Ni^{2+} would be expected to block stimulated contraction completely in the guinea pig papillary muscle. However, after 13 minutes there was still a stimulated contraction in the presence of 5mM Ni^{2+} (figure 7.13, 7.14). This is consistent with the idea that there may be another trigger for SR release in addition to Ca^{2+} entry, i.e. voltage activated Ca^{2+} release. This conclusion is dependent upon 5mM Ni^{2+} blocking all Ca^{2+} entry into the myocytes and thus on how fast Ni^{2+} diffuses to the core of the papillary muscle. Whilst the $t_{0.5}$ was short (49 seconds) compared to the time over which contraction could be elicited in the presence of Ni^{2+} (13 minutes), time constants are only a crude measure of how rapidly diffusion occurs. Owing to the difficulties posed by diffusion limitation, another preparation was also studied that was expected to have a much more rapid diffusion time; the isolated perfused frog heart. Amphibian hearts have no coronary vascular system; the ventricular wall has a large surface area owing to its spongy form and as long as

cardiac output is above 3-4mls/min this is sufficient to meet the metabolic needs of the heart. As expected, the diffusion time for Ca^{2+} was considerably faster than in the guinea pig papillary muscle ($t_{0.5}$ of 8±2 seconds, figure 7.16). 8mM Ni^{2+} abolished contraction in the frog heart after 10±2 seconds; this is not significantly different from the diffusion limitation measured in the preparation. There was therefore no evidence for the existence of voltage-activated Ca^{2+} release in the frog heart. This could either indicate a species difference between the frog and the guinea-pig with the latter possessing a voltage-activated Ca^{2+} release mechanism, alternatively it may merely demonstrate the difficulty in blocking Ca^{2+} entry in the papillary muscle preparation.

Hobai *et al* (1997) reported that the voltage-activated Ca^{2+} release mechanism was not evident in cells that had not been pre-stimulated with 8-Br-cAMP. However, this may be a feature of the experimental technique (whole-cell patch clamping) which dialyses the cell interior. Normal intracellular cAMP concentration is in the range of 2-20μM (Bogoyevitch *et al.* 1993; Bohm *et al.* 1994; Wynne *et al.* 1996) and in the guinea-pig papillary muscle this was sufficient to allow demonstration of contraction in the presence of 5mM Ni^{2+}. It is possible that pre-stimulation with NA might have allowed demonstration of VACR in the frog heart, however in pilot studies NA led to arrhythmias that could not be overdriven by ventricular pacing so perfusion with Ni^{2+} in the presence of NA was not attempted.

7.5.2.2 Functionality of frog sarcoplasmic reticulum

A number of histological studies suggest that frog sarcoplasmic reticulum (SR) is sparse and poorly developed (Page & Niedergerke 1972) and this is a potential problem when using this preparation to study SR dependent mechanisms. However functional SR release of Ca^{2+} has been shown in the frog heart (Chapman & Miller 1974) using caffeine (which releases Ca^{2+} from intracellular stores via its action on the ryanodine receptor, Rousseau *et al.* 1988; Rousseau & Meissner 1989). The functionality of SR in the isolated frog hearts used in the current study was therefore tested using perfusion with 2mM caffeine. In *mammalian* cardiac muscle caffeine results in a transient increase in force (while caffeine is releasing Ca^{2+} from the SR), followed by a decrease below baseline force (O'Neill & Eisner 1990). This is not

what was seen in the *amphibian* preparation used in these studies. When frog hearts were perfused with caffeine a maintained increase in force was observed. This could be due to additional non-specific effects of caffeine (e.g. antagonism of adenosine receptors, direct effect on myofibrils, effect on phosphatases), alternatively it suggests that functional Ca^{2+} stores are present in the frog heart, but even when empty contraction continues with external Ca^{2+} as the trigger. Consistent with this was the observation that perfusion with caffeine in low Ca^{2+} Ringer resulted in a transient increase in force which then decayed to that seen in low Ca^{2+} Ringer alone. If external Ca^{2+} alone is sufficient to allow contraction then the frog heart may not be an appropriate preparation in which to investigate voltage-activated Ca^{2+} release. Therefore even if contraction had occurred in the presence of 8mM Ni^{2+} this would still not be definitive evidence for the existence of voltage-activated Ca^{2+} release unless it is possible to substitute external Ca^{2+} in the Ringers solution.

7.5.2.3 Implications for arrhythmogenesis

The actions of NA on cardiac cells occur via activation of the β-1 receptor, which in turn stimulates adenylate cyclase and so an increase in cAMP and activation of protein kinase A (Kameyama *et al.* 1985). This has a number of actions in myocytes including:

- Stimulation of L-type Ca^{2+} channels (Reuter & Scholz 1977).
- Phosphorylation of phospholamban leading to stimulation of the Ca^{2+} ATPase on the SR membrane, thereby increasing store loading (Rapundalo *et al.* 1989).
- Activation of a Cl^- current (Bahinski *et al.* 1989; Harvey & Hume 1989).
- Phosphorylation of Troponin 1 (Solaro *et al.* 1976).
- Increase in I_K (Tsien *et al.* 1972), thereby speeding repolarization.
- Activation of the background Na^+ conductance (Egan *et al.* 1987).
- Stimulation of the Na^+/K^+ ATPase (Wanless *et al.* 1985; Dobretsov *et al.* 1998).

A number of these actions of NA are thought to be arrhythmogenic, most notably the stimulation of L-type Ca^{2+} channels and the SR Ca^{2+} ATPase, both of which may lead to cellular Ca^{2+} overload. If the voltage-activated Ca^{2+} release mechanism exists and is stimulated by cAMP, it would provide an important target for adrenergic modulation of cardiac contractility and an additional mechanism by which cAMP could be arrhythmic.

7.5.2.4 Limitations of studies on voltage activated Ca^{2+} release

The primary limitation of the studies in which I investigated voltage activated Ca^{2+} release is whether or not under the circumstances of the experiment, all entry of Ca^{2+} entry into the cytoplasm from the extracellular space has been blocked. The ideal way of ensuring this would be to substitute Ca^{2+} with Ba^{2+} in the perfusate and to use a Ca^{2+} chelator such as EGTA. However, Hobai *et al.* (1997) reported that the VACR mechanism is dependent upon low levels of extracellular Ca^{2+} *per se* and not simply on the presence of a divalent cation, since they could not demonstrate the mechanism when Ca^{2+} was replaced with Ba^{2+}. They hypothesised that the VACR protein had an obligatory Ca^{2+} binding site, although an alternative explanation would be that some Ca^{2+} entry is still occurring. In pilot studies on the guinea-pig papillary muscle, Ba^{2+} replacement led to spontaneous contractions (consistent with previous studies, e.g. Valenzuela & Vassalle 1991) so I was unable to test for the presence of VACR in the absence of external Ca^{2+}.

Whilst the studies of Hobai *et al.* (1997) showed no demonstrable Ca^{2+} current in the presence of 5mM Ni^{2+}, this is a little surprising given previous studies specifically designed to measure Na^{+}/Ca^{2+} exchange current suggest that block at this concentration is only *ca.* 85% and that inhibition is near-maximal at these concentrations (Wright 1996). Despite the cloning of the Na^{+}/Ca^{2+} exchanger there are still no membrane permeant high affinity inhibitors that could be used in studies of voltage-activated Ca release. Mn^{2+}, Cd^{2+} and several peptides (XIP, No 7943, FRCRCF) inhibit Na^{+}/Ca^{2+} exchange, but all are relatively non-specific and also inhibit K^{+} channels (Kimura *et al.* 1987). Proving the presence or absence of VACR is extremely difficult given the apparent requirement for external Ca^{2+} and the absence of good blockers for Na^{+}/Ca^{2+} exchange. An alternative approach for proving total block of Ca^{2+} entry would require studies of $^{(45)}Ca^{2+}$ entry into isolated myocytes, a technique considerably more sensitive than those used currently (recording Ca^{2+} currents with patch clamping, or Ca^{2+} fluorescence with confocal microscopy).

In several pilot experiments on the isolated guinea-pig papillary muscle, superfusion with 5mM Ni^{2+} in normal Tyrode resulted in spontaneous contractions at a rate of about 120/min. These were prevented by substituting low Ca^{2+} Tyrode containing 5mM Cs^{2+}. The reason for

the stabilising effect was not investigated but there are several possible reasons. As well as blocking Ca^{2+} *entry*, Ni^{2+} blocks Ca^{2+} *extrusion* from myocytes, thus making them susceptible to Ca^{2+} overload and use of low Ca^{2+} Tyrode may help prevent this. 5mM Cs^{2+} could be anti-arrhythmic by blocking I_f in any Purkinje tissue that may be present in the preparation, or by blocking I_{K1} (Denyer & Brown 1990). However, the fact that such a manoeuvre was necessary illustrates how profoundly sensitive cardiac tissue is to Ca^{2+} overload; consequently even if VACR exists its role in arrhythmogenesis is likely to be minor.

7.6 Summary

A number of studies have documented regional abnormalities in cardiac sympathetic innervation in patients with VT and apparently 'normal' hearts. Subepicardial infusion of NA may therefore be a useful experimental tool with which to investigate VT in the normal heart. This study demonstrates the electrical and haemodynamic changes that may result from such an abnormality.

The cellular mechanism for the arrhythmic effect of NA is thought to include Ca^{2+} overload as the final mechanism. If contraction were triggered solely by Ca^{2+} entry inducing Ca^{2+} release from the SR, then 5mM Ni^{2+} would be expected to block stimulated contraction completely in the guinea pig papillary muscle. However, there was still a stimulated contraction in the presence of 5mM Ni^{2+}; consistent with the idea that there may be another trigger for SR release in addition to Ca^{2+} entry, i.e. voltage activated Ca^{2+} release. There was no evidence for the existence of this mechanism in the frog heart. This could either indicate a species difference between the frog and the guinea-pig with the latter possessing a voltage-activated Ca^{2+} release mechanism, alternatively it may demonstrate the difficulty in blocking Ca^{2+} entry in the papillary muscle preparation. VACR may be an additional mechanism by which NA is arrhythmogenic but its precise role in normal physiology remains to be determined.

Chapter Eight

Concluding Discussion

There are more things in heaven and earth, Horatio,
Than are dreamt of in your philosophy
Shakespeare.

This chapter discusses the main results of the thesis, together with suggestions for further work.

Dynamic exercise is associated with a rapid increase in $\dot{V}_I$, followed by a rise to a steady state. There is also a rapid increase in cardiac output with the onset of exercise and a redistribution of blood flow towards exercising muscles. The way in which these cardiorespiratory changes are brought about and coordinated with muscular activity is unclear, but there are two general schools of thought, 'peripheral feedback' and 'central command'. The two theories are not mutually exclusive and a number of studies have suggested that there is a high degree of redundancy in the steady-state response.

The origins of 'central command'

Electrical stimulation of subcortical sites in animal studies including the thalamus, hypothalamus, basal ganglia and pedunculopontine nucleus causes locomotion and a cardiorespiratory response even when peripheral feedback is abolished. These areas could potentially be involved in 'central command' in exercise, but there is little evidence to support this in humans. Neuroimaging studies of exercise and the post-exercise state in man have identified thalamic, cerebellar and cortical sites that might be involved in exercise hyperpnoea (Fink *et al.* 1995; Williamson *et al.* 1997). However head movement can be a major confound when exercise is combined with neuroimaging and the presence of peripheral feedback from exercising muscles makes it difficult to attribute neural activations to 'central command' unless the post-exercise state is also studied (Fink *et al.* 1995). Therefore an appropriate alternative model of a situation resembling exercise is required. One such model could be imagination of exercise under hypnosis which results in increases in breathing (Levin & Egolinsky 1936; Nemtzova & Shatenstein 1936; Agosti & Camerota 1965; Daly & Overley 1966; Arvidsson *et al.* 1970; Wang & Morgan 1992). Unfortunately, interpretation of several of these studies is complicated by the use of explicit cues regarding cardiorespiratory variables. Consequently it was necessary to characterise the cardiorespiratory responses to imagination of exercise in experimentally naïve subjects without any suggestions regarding cardiorespiratory variables (**Chapter Three**). Imagination

of exercise under hypnosis resulted in a robust and repeatable hyperpnoea, hypocapnia and an increase in HR. In contrast, volitional hyperventilation at a similar breathing frequency did not increase HR and imagination of freewheeling downhill (performed as a control for cognitive effort) did not affect cardiorespiratory variables.

Imagination of exercise therefore seems to be a reasonable model with which to investigate the neural correlates of 'central command' and this was performed using PET scanning in the studies described in **Chapter Four**. These studies identified clear differences between imagination of exercise and volitional hyperventilation and indicated that 'central command' might be related to activations of the dorsolateral prefrontal cortex and the posterior parietal cortex acting in conjunction with areas typically associated with motor tasks such as the SMA, PMA, cerebellum and thalamus. These are remarkably similar to those seen during actual exercise and the post-exercise state (Fink *et al.* 1995), despite an absence of movement and hence peripheral feedback in the current study.

The contribution of 'central command' during exercise?

A major problem when investigating the roles of 'central command' and peripheral feedback in exercise is finding an objective measure of the magnitude of each drive. Whilst individual aspects of peripheral feedback can be measured (e.g. by recordings from muscle afferents or from the carotid sinus nerve), the total response cannot. Likewise, measuring the magnitude of 'central command' in man is near impossible, especially when its origin is unknown. Imagination of exercise under hypnosis may be a good model with which to investigate the neural correlates of 'central command' but it does not provide any insight into the relative contributions of 'central command' and peripheral feedback during actual exercise. I therefore investigated the effects of hypnotic suggestions on the cardiorespiratory responses to actual exercise (**Chapter Five**) in order to uncouple 'central command' from peripheral feedback. The ventilatory response to a *suggested increase* in work rate during a constant moderate exercise task was equivalent to that occurring when the exercise rate actually increased. In contrast, a *suggested decrease* in work rate did not alter steady-state cardiorespiratory variables. It therefore seems that 'central command' can mimic the entire ventilatory response to an increase in work rate and a portion of the HR response. In contrast,

'central command' cannot decrease cardiorespiratory variables below that indicated by peripheral feedback.

Clearly a powerful drive to breathe originating from higher centres arises when exercise or increased work rate is imagined under hypnosis. Of interest is the fact that this drive increases breathing frequency with no increase in tidal volume. This is similar to data obtained both awake (Decety *et al.* 1991; Wuyam *et al.* 1995) and under hypnosis (Agosti & Camerota 1965) and might indicate that the increase in tidal volume during exercise is related to some element of peripheral feedback that is not occurring in the current study. The relationship between frequency and tidal volume can be altered by the vagus nerve (Winning *et al.* 1985), by locomotor-respiratory coupling (Bramble & Carrier 1983) and can be separated experimentally by chemical stimulation of the ventrolateral medulla (Tolentino Silva *et al.* 1993).

Is there evidence for the existence of a 'motor programme' for breathing in exercise?

It has been hypothesised that the ventilatory response to exercise is a result of activation of a 'motor programme' for breathing. This theory arises from the fact that metabolism, perfusion and $\dot{V}_I$ are so tightly coupled during exercise that blood gases do not change during moderate exercise, hence the 'missing error signal' (Somjen 1992). The neural correlates of imagination of exercise under hypnosis in the current study were similar to those for actual exercise (Fink *et al.* 1995) and this may be evidence in favour of the existence of a respiratory motor programme since imagination of exercise under hypnosis evokes a genuine 'exercise-like' response that is clearly not related to peripheral feedback. The repeatability of the response to imagination of exercise might also be taken as evidence in favour of a motor programme for breathing in exercise; a specific exercise intensity is being imagined and evokes a robust, repeatable response.

Imagination of movement has been shown to improve muscle strength (Yue & Cole 1992). This is proposed to be due to improvements in a central programme for muscle activity, since a comparable beneficial effect is seen in the same muscle on the 'untrained' side during both imagined (Yue & Cole 1992) and actual exercise training (Houston *et al.* 1983). Such a

motor programme would presumably originate in areas activated during imagination of movement and could therefore include the SMA, dorsolateral prefrontal cortex and posterior parietal cortex. The study of Yue & Cole (1992) indicates that motor programmes exhibit plasticity, and there is no reason to believe that the central drive to breathe should be any different (reviewed by (Turner *et al.* 1997)). Indeed, it could be hypothesised that if a centrally driven cardiorespiratory response to exercise is inadequate then peripheral feedback provides an error signal which has two functions; first to instantly correct the mismatch between $\dot{V}_{O_2}$ and $\dot{V}_I$, secondly to enable learning and incorporation of experience into subsequent exercise performance. The dorsolateral prefrontal cortex and the posterior parietal cortex may be involved in such a motor programme, since they were activated both during imagination of exercise under hypnosis (**Chapter Four**) and in the post-exercise state (Fink *et al.* 1995). Plasticity of the respiratory motor programme has been demonstrated in goats using thoracic dorsal rhizotomy; this decreases peripheral feedback and leads to a severe hypercapnia during exercise, which is followed by a progressive recovery over subsequent exercise trials (Mitchell 1990). Plasticity of the ventilatory response to exercise has also been demonstrated using exercise trials with added external dead space in goats (Martin & Mitchell 1993) and man (Helbling *et al.* 1997). This type of approach to studying plasticity of the exercise respiratory motor programme could be improved using dynamic end-tidal forcing to manipulate $P_{ET}CO_2$ during exercise, followed by investigation of subsequent exercise trials. If this type of adaptive respiratory learning could be studied during neuroimaging the neural correlates of respiratory motor programmes might finally be revealed.

Evidence for 'parallel activation' of cardiorespiratory and locomotor systems from the midbrain?

The 'subthalamic locomotor region' and a number of other midbrain nuclei are believed to be involved in 'central command' given results from animal experiments (Eldridge *et al.* 1981) in which electrical stimulation can cause locomotion together with a cardiorespiratory response. I saw little evidence for activation of these areas in the PET study described in **Chapter Four**. This might be due to insufficient sensitivity of the experimental technique, or

alternatively that imagination of exercise does not evoke midbrain activity. Unfortunately, it has not yet been possible to combine neuroimaging with exercise and reliably identify activation of midbrain nuclei in man. Consequently an alternative study was performed (**Chapter Six**) which took advantage of an increasingly common neurosurgical treatment for intractable movement disorders. Electrical stimulation of the thalamus and midbrain is performed in these patients whilst they are awake, thus providing the opportunity to study the phenomenon of parallel activation of locomotion and cardiorespiratory systems. This study demonstrated that increases in heart rate and blood pressure could be evoked by electrical stimulation of midbrain nuclei. Whilst electrical stimulation did not result in movement *per se*, it was accompanied by facilitation of movement (a reduction in akinesia and bradykinesia), so it may represent a parallel activation of the locomotor and cardiorespiratory systems, with the former activation being below threshold. This type of response is seen at low stimulation currents in animal studies (Eldridge *et al.* 1981), with a more exercise-like situation of co-ordinated locomotion and increases in cardiorespiratory variables occurring with increasing electrical stimulation. However, there is little if any evidence to suggest that the locomotion and cardiorespiratory changes evoked by electrical stimulation of the midbrain has any relevance to exercise.

Whilst studying these patients is clearly important in order to investigate the role of these brain regions in 'central command' in humans, it is likely that further insight will come from implanting stimulators in monkeys. Electrical stimulation could then be performed under a variety of circumstances including autonomic blockade, anaesthesia, exercise, and in both normal monkeys and those with MPTP-induced Parkinsonism. It could also be combined with deep brain recording in order to determine the precise effects of electrical stimulation on neural firing.

The autonomic nervous system and arrhythmia

Despite their abnormal autonomic function, arrhythmias are uncommon in Parkinson's Disease, yet many other autonomic disorders do result in arrhythmias. This is exemplified by the study described in **Chapter Seven** in which a localised subepicardial infusion of a sympathetic neurotransmitter (noradrenaline) in an anaesthetised pig results in an arrhythmia

that is accompanied by a profound depression of haemodynamics. Whilst most ventricular tachycardias in the 'normal heart' are benign in themselves (Wichter *et al.* 1995), they may be a sensitive predictor of future heart disease, since detailed electrophysiological investigations of patients with ventricular tachycardia and 'normal' hearts reveal subtle structural abnormalities in almost all patients (Deal *et al.* 1986). If electrocardiac mapping can be performed relatively non-invasively, for example by the use of endocardial basket catheters or body surface mapping, more subtle alterations in electrical activity in the heart could be used diagnostically.

There are numerous ways in which sympathetic nervous system activity can be arrhythmogenic, but a recent suggestion is that extracellular Ca^{2+} may not in fact be necessary for cardiac contraction and that voltage-activated-Ca^{2+}-release may occur in the heart as it does in skeletal muscle (Hobai *et al.* 1997; Ferrier *et al.* 1998). This hypothesis was based on the fact that an intracellular Ca^{2+} transient can occur in the absence of Ca^{2+} entry from the extracellular space. The studies on guinea pig papillary muscle in **Chapter Seven** indicated that it might be of functional significance in eliciting contraction, although there was no evidence for its existence in the frog heart. If such a mechanism exists it might provide an additional way in which sympathetic stimulation is arrhythmic, but the role of voltage-activated-Ca^{2+}-release in normal physiology remains to be determined.

Appendix

Supplementary PET data

Ye thought? Ye are not paid to think.

Kipling.

Corrections for global cerebral blood flow

Global CBF is not normally a parameter of interest in PET studies; one is actually interested in regional changes in blood flow occurring above the background of global flow (which may occur according to perfusion pressure, chemical and metabolic factors). Scans are therefore scaled to an arbitrary value of global CBF of 50 ml/min/dl in order to reduce variance between scans and therefore potentially increase the sensitivity of the regional analysis. There are two ways in which this scaling can be performed, analysis of covariance (ANCOVA) or proportional scaling. These two alternatives use different assumptions and models about brain blood flow.

Analysis of covariance uses the global CBF as a covariate in the analysis and assumes independence between the magnitude of a regional activation and the absolute overall magnitude of global flow. For example, a subject is scanned in two conditions and has a global flow of 50ml/min/dl and an activation is found in an area with a magnitude of 5 ml/min/dl. If the subject is scanned again in the same two conditions but this time has a global flow of 70ml/min/dl, the ANCOVA model predicts that the activation is still 5ml/min/dl, whereas proportional scaling predicts an activation of 7ml/min/dl. Since proportional scaling scales regional flows according to the overall global flow, it assumes that the relationship between regional and global flows is linear and passes through zero. This does not appear to be the case; in a study of visual cortex activation, the increase in rCBF accompanying opening the eyes showed no systematic dependence upon the level of gCBF over the range 24-67 ml/min/dl (Ramsay *et al*, 1993). This would indicate that the ANCOVA model might be more appropriate. However, if there is a systematic difference in global CBF between conditions then proportional scaling may be appropriate since the conditions of equal variance required in order to perform ANCOVA are violated. An additional reason to use proportional scaling is if qualitative 'count' measurements are being used to measure blood flow (with no absolute quantification possible unless an arterial cannula is used to sample blood). This was the case in the study described in Chapter Four; the addition of CO_2 to the inspired air in protocols 1 and 2 was not completely successful in maintaining isocapnia. $P_{ET}CO_2$ and hence global CBF were changing so the proportional scaling model was used.

Activations in 'MNI space'

The coordinates given for the activations described in Chapter Four are normalised to the template brain of Talairach & Tournoux (1988). This atlas was based on preserved brains and so is somewhat shrunken and warped compared to a living brain. This template has been superceeded by the 'MNI brain' which is an average of the MRI scans of 305 brains from the Montreal Neurological Institute (ICBM, NIH P-20 project). To facilitate comparisons with previous studies, the coordinates in Chapter Four are given according to the template of Talairach & Tournoux (1988) using a correction formula described by Meyer-Lindenberg (1998). Tables AI and AII are for the same activations as those in Chapter Four, represented in 'MNI space'.

Statistical comparison of the two experimental groups studied in Chapter Four

The PET data in Chapter Four consists of subjects performing 3 different protocols:

Protocol 1: Imagination of exercise

Protocol 2: Volitional hyperventilation

Protocol 3: Imagination of freewheeling downhill

As it was important not to alert subjects to the interest in breathing, subjects were divided into two groups, with group A performing imagination of exercise and imagination of freewheeling downhill, and group B performing volitional hyperventilation and imagination of freewheeling downhill. In order to say definitively that there is a difference in the neural correlates of imagination of exercise compared to volitional hyperventilation (and therefore to say that the cardiorespiratory increases associated with imagination of exercise were not merely volitional increases produced by compliant subjects) it is necessary to statistically compare the two groups. This was performed using a contrast specifying what activations in group A are significantly greater than in group B. This analysis requires the assumption that the two groups of subjects behave in the same way when imagining freewheeling downhill. In addition, this type of analysis will identify areas that 'deactivate' (i.e. show a decrease in rCBF) during imagination of freewheeling downhill in group B. It is therefore necessary to use an 'inclusive mask' with the contrast that identifies activations in Group A (contrast in table AIII). The results from this analysis are illustrated in figure AI and the coordinates with $Z>3.09$, $p<0.001$ (uncorrected) are in table AIV. No activations survived correction for multiple comparisons

Table AI. Coordinates of the activations as detailed in Chapter Four in 'MNI space' (as opposed to Talairach space). **Imagination of exercise** under hypnosis (compared to imagination of freewheeling downhill). Coordinates indicate the highest Z value within an area of activation. x, distance (mm) to right (+) or left (-) of the mid-saggital line; y, distance anterior (+) or posterior (-) to a vertical plane drawn through the anterior commissure; z, distance above (+) or below (-) the intercommissural (AC-PC) line. Numbers in brackets refer to Brodmann Areas where activations could be clearly attributed to a specific region. P values both corrected for multiple comparisons (C) and uncorrected (NC) are indicated. To aid statistical interpretation the 'traffic light' convention has been used; **green** = survives correction for multiple comparisons, **amber** = $p<0.001$ uncorrected ($Z>3.1$), **red** = $p<0.01$ uncorrected ($3.1>Z>2.3$).

Region	*Left*						*Right*					
	x	y	z	Z	P (C)	P (NC)	x	y	z	Z	P (C)	P (NC)
Supplementary motor area (6)	-10	-18	80	3.4	0.991	0.000	**4**	**-6**	**74**	**5**	0.011	0.000
							8	16	70	3.6	0.933	0.000
Ventral premotor area (6)							66	6	22	4.4	0.192	0.000
Middle frontal gyrus (9/46)							**48**	**28**	**40**	**4.8**	0.039	0.000
Middle frontal gyrus (9/46)							48	40	26	3.4	0.989	0.000
							42	26	50	3.3	0.999	0.001
Inferior frontal gyrus/temporal pole							30	30	-26	3.2	1	0.001
Lateral sensorimotor							68	-8	24	4.1	0.368	0.000
Sensorimotor	-50	-12	46	3.4	0.992	0.000	48	-6	60	3.3	0.999	0.001
	-48	-10	62	3.4	0.992	0.000						
Cerebellum: Anterior/lateral	-28	-58	-24	3.5	0.976	0.000	24	-58	-26	4.2	0.301	0.000
Anterior/medial	-14	-48	-28	4.2	0.311	0.000						
Posterior/medial	**-18**	**-60**	**-28**	**3**	1	0.001	**16**	**-44**	**-38**	**2.5**	1	0.007
	-16	**-36**	**-50**	**2.9**	1	0.002						
Posterior/lateral	-34	-36	-48	3.2	0.98	0.001						
	-30	**-46**	**-36**	**2.6**	1	0.005						
Superolateral sensorimotor	-20	-32	66	3.5	0.985	0.000	20	-26	74	4	0.517	0.000
Parietal lobe (precuneus) (7)	-12	-34	54	3.4	0.991	0.000						
Left parietal cortex	-16	-44	32	3.6	0.961	0.000						
Temporal/parietal junction							70	-38	28	4.6	0.068	0.000
Parahippocampal gyrus							36	-36	-6	3.7	0.84	0.000
							30	-46	6	3.7	0.889	0.000
Insular cortex	-54	-8	20	3.4	1	0.001						
	-44	-12	20	3.2	1	0.000						
Thalamus/fornix							0	-6	18	4.1	0.44	0.000
Tail of caudate/fornix	**-24**	**-38**	**12**	**3**	1	0.001						
Putamen							26	4	6	3.2	1	0.001
Body of caudate							22	2	22	3.2	1	0.001
							20	**-6**	**28**	**2.9**	1	0.002

Region	*Left*						*Right*					
	x	y	z	Z	P (C)	P (NC)	x	y	z	Z	P (C)	P (NC)
Supplementary motor area (6)	-6	-8	62	4.7	0.057	0.000						
Superolateral sensorimotor	**-6**	**-22**	**66**	**2.6**	1	0.005						
Posterior temporal/parietal	-52	-32	16	4.7	0.06	0.000						
Lateral sensorimotor	**-56**	**-2**	**22**	**7**	0.001	0.000	48	-16	42	3.9	0.658	0.000
	-64	**-12**	**18**	**4.8**	0.028	0.000	**62**	**-2**	**24**	**5.1**	0.008	0.000
Cerebellum	-44	-62	-42	3.9	0.71	0.000	12	-68	-50	3.2	1	0.001
	-34	**-72**	**-50**	**2.4**	1	0.008						
	-10	-62	-28	3.4	0.989	0.000						
Paracentral gyrus	-12	-10	52	3.5	0.98	0.000						
Insular cortex/operculum							52	-4	8	3.6	0.955	0.000
							30	-20	26	3.2	1	0.001
Occipital cortex (lingual gyrus)	**-22**	**-76**	**8**	**3**	1	0.001						
Thalamus	-10	-6	12	3.5	0.987	0.000						
Putamen							24	6	10	3.8	0.765	0.000
							28	-2	10	3.5	0.986	0.000
							38	-12	10	3.2	1	0.001
Pallidum	-26	-6	-4	3.2	1	0.001						
Pons/medulla	-10	-30	-36	3.9	0.693	0.000						

Table AII. Coordinates for the same activations detailed in Chapter Four, this time in 'MNI space'. Coordinates refer to maximally activated foci during **volitional hyperventilation** (tachypnea) under hypnosis (compared to imagination of freewheeling downhill), indicated by the highest Z value within an area of activation. Details as for table AI.

using this analysis, but four were significant at $p<0.001$ uncorrected. These were the cingulate gyrus (mid cingulate, Z=3.8 and posterior cingulate, Z=3.2), the occipital lobe (precuneus, Z=3.2) and the left cerebellum (Z=3.1). The dorsolateral prefrontal cortex narrowly avoided being significant at this level (Z=3). An equivalent analysis was performed to look at the increased activation occurring during volitional hyperventilation that were not activated during imagination of exercise (contrast in table AIII), figure AII, coordinates in table AV.

Contrast	Mask	Description
-1 1 1 -1	-1 1 0 0	Activations during imagination of exercise (compared to imagined freewheeling downhill) that do not either activate or 'deactivate' during volitional hyperventilation (also compared to imagination of freewheeling downhill).
1 –1 –1 1	0 0 –1 1	Activations during volitional hyperventilation (compared to imagined freewheeling downhill) that do not either activate or 'deactivate' during imagined exercise (also compared to imagination of freewheeling downhill).

Table AIII. Contrasts and masks for statistical comparison of the two groups of subjects. The order of scan entry was Group A (imagined freewheeling downhill), Group A (imagined exercise), Group B (imagined freewheeling downhill), Group B (volitional hyperventilation). The first contrast and mask therefore identifies the activations associated with imagined exercise that do not occur during volitional hyperventilation, the second contrast and mask identify the activations during volitional hyperventilation not associated with imagined exercise.

Covariates analysis on Group A subjects

A covariates analysis with $\dot{V}_I$ as the covariate of interest was performed on the data from Group A subjects. Only the activations which survived correction for multiple comparisons are given in Chapter Four since there was no real *a priori* hypothesis regarding this analysis; lesser activations are given in figure AIII for interest.

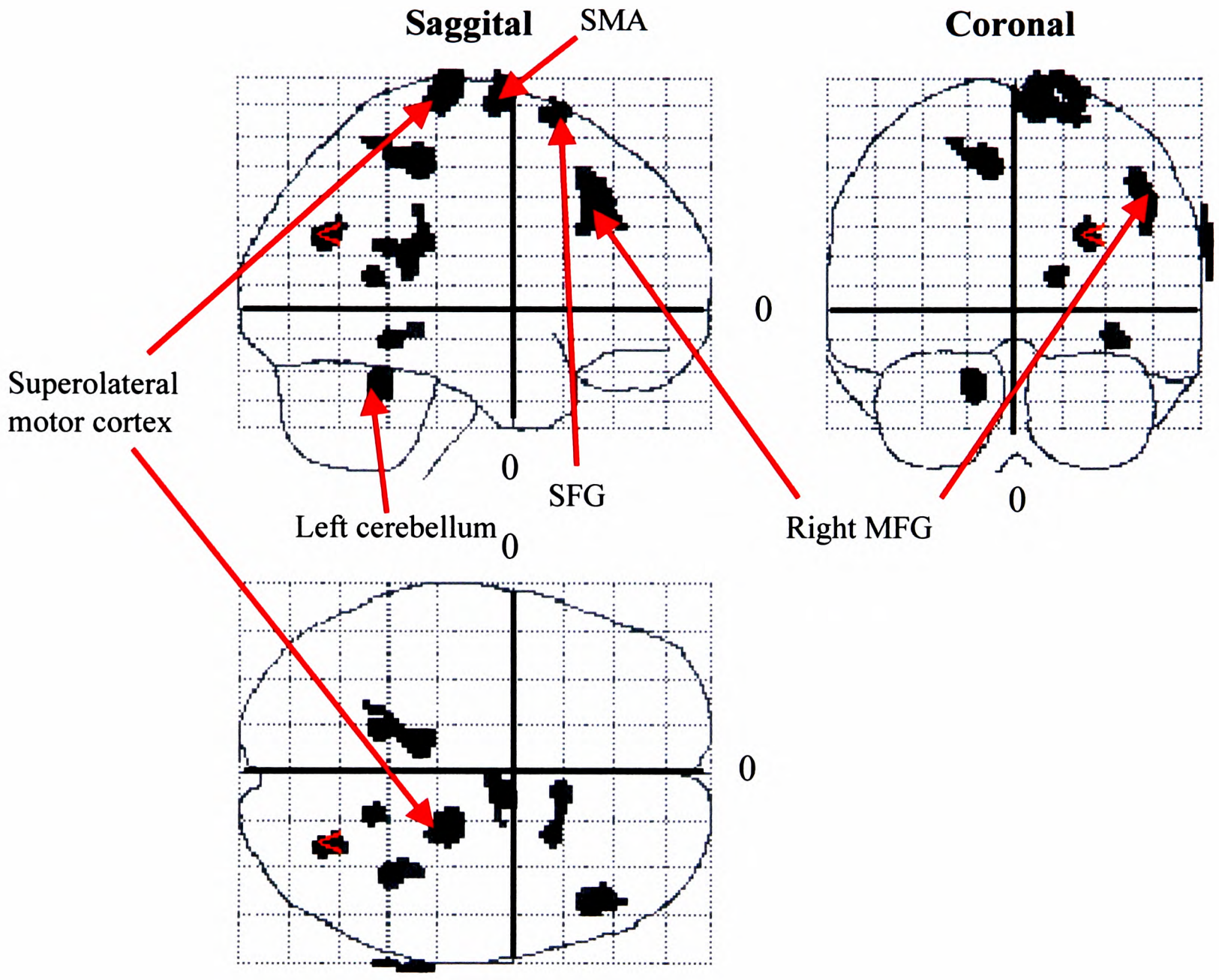


Figure AI. Areas showing increased activation during imagination of exercise compared to volitional hyperventilation (both compared to imagination of freewheeling downhill, see text for details). Activations with $Z>2.33$, $p<0.01$ uncorrected are shown as projections onto representations of stereotactic space. The solid lines indicate the origin for coordinates (horizontal and vertical planes through the anterior commisure). See text for details. Abbreviations; SMA supplementary motor area; MFG middle frontal gyrus; SFG superior frontal gyrus.

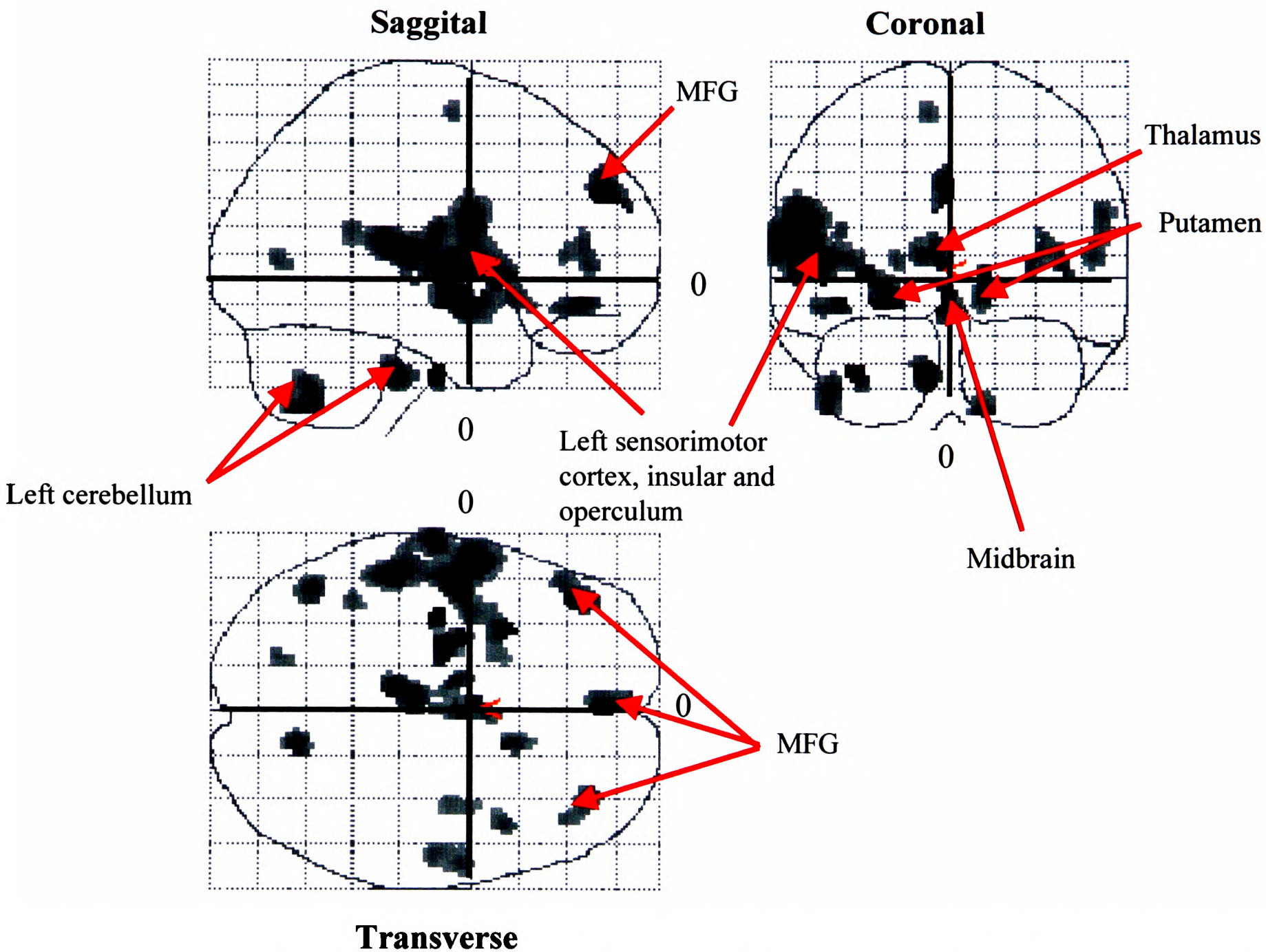


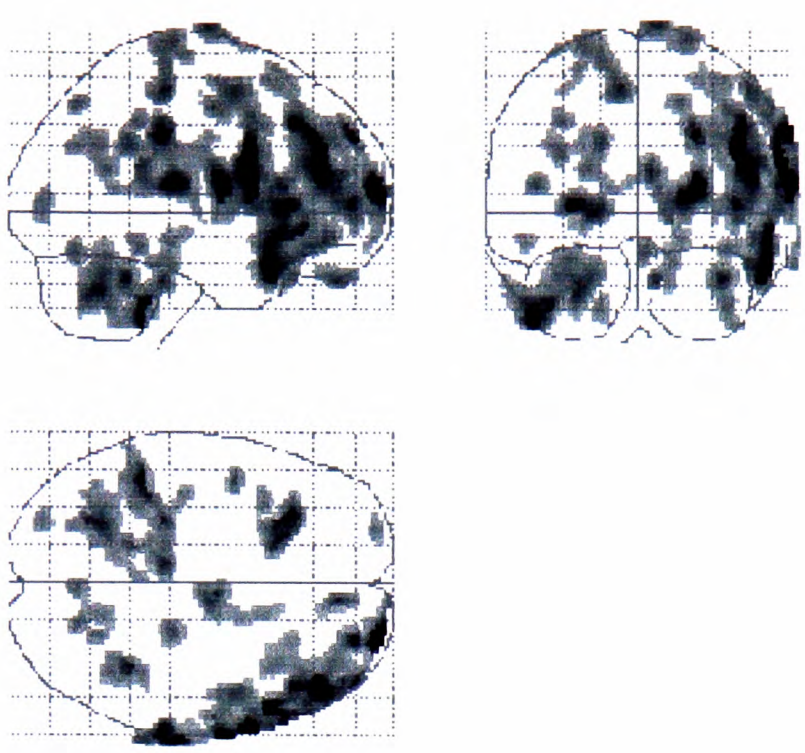
Figure AII. Areas showing increased activation during volitional hyperventilation compared to imagination of exercise (both compared to imagination of freewheeling downhill, see text for details). Activations with $Z>2.33$, $p<0.01$ uncorrected are shown as projections onto representations of stereotactic space. The solid lines indicate the origin for coordinates (horizontal and vertical planes through the anterior commisure). See text for details. Abbreviations; SMA supplementary motor area; MFG middle frontal gyrus

Region	*Left*						*Right*					
	x	y	z	Z	P (C)	P (NC)	x	y	z	Z	P (C)	P (NC)
Cingulate	**-8**	**-34**	**50**	**3.8**	0.798	0.000						
Posterior cingulate							**16**	**-52**	**12**	**3.2**	1	0.001
Occipital gyrus (precuneus)							**26**	**-70**	**26**	**3.2**	1	0.001
Cerebellum	**-16**	**-50**	**-28**	**3.1**	1	0.000						

Table AIV. Coordinates ('MNI space') refer to maximally activated foci during imagined exercise that were not activated during volitional hyperventilation (see table AIII for contrast). The dorsolateral prefrontal cortex narrowly avoided being significant (Z=3).

Region	*Left*						*Right*					
	x	y	z	Z	P (C)	P (NC)	x	y	z	Z	P (C)	P (NC)
Left sensorimotor	**-64**	**-14**	**18**	**4**	0.485	0.000						
	-56	**4**	**4**	**4**	0.59	0.000						
	-58	**-2**	**10**	**3.4**	0.995	0.000						
Inferior frontal gyrus	**-56**	**0**	**24**	**4.9**	0.02	0.000						
	-42	**42**	**-8**	**3.6**	0.933	0.000						
Superior frontal gyrus	**0**	**50**	**32**	**4.2**	0.295	0.000						
Insular/operculum	**-48**	**-8**	**8**	**4.4**	0.148	0.000	**42**	**12**	**4**	**3.1**	1	0.001
Superior temporal	**-52**	**-32**	**14**	**4.4**	0.178	0.000						
Thalamus	**-8**	**-24**	**10**	**3.8**	0.728	0.000						
	-10	**-6**	**10**	**3.4**	0.989	0.000						
Midbrain	**-2**	**-4**	**-8**	**4.3**	0.349	0.000						
Putamen	**-32**	**10**	**0**	**4**	0.501	0.000	**14**	**12**	**2**	**3.6**	0.962	0.000
Amygdala(?)	**-26**	**-8**	**-8**	**4.6**	0.069	0.000						

Table AV. Coordinates ('MNI space') refer to maximally activated foci during volitional hyperventilation that were not activated during imagined exercise (see table AIII for contrast).



P values & statistics: /usr/paterson/joode/PET

set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z	x,y,z {mm}
0.120 (26)	0.000 (3590, 5.82)	0.000 (5.82)	0.000 0.000	66 6 22
		0.004 (5.31)	0.000	48 40 20
		0.007 (5.18)	0.000	56 18 -26
	0.123 (546, 4.84)	0.034 (4.84)	0.000 0.000	-46 -40 -46
		0.119 (4.54)	0.000	-40 -38 -40
		0.736 (3.92)	0.000	-26 -30 -30
	0.226 (396, 4.61)	0.092 (4.61)	0.002 0.000	-30 28 4
		0.305 (4.28)	0.000	-20 22 0
	0.067 (690, 4.18)	0.402 (4.18)	0.000 0.000	-26 -60 -34
		0.999 (3.39)	0.000	-30 -56 -20
		1.000 (2.83)	0.002	-42 -60 -32
	0.299 (383, 4.16)	0.431 (4.16)	0.002 0.000	38 -46 -28
		0.999 (3.40)	0.000	40 -54 -40
		1.000 (3.20)	0.001	40 -42 -14
	0.735 (125, 4.02)	0.604 (4.02)	0.057 0.000	6 -6 18
		1.000 (2.71)	0.003	14 -8 10
	0.229 (429, 3.99)	0.638 (3.99)	0.001 0.000	-8 -28 56
		0.999 (3.38)	0.000	-26 -32 76
		1.000 (3.31)	0.000	-16 -30 68

Height threshold (u) = 2.33, p = 0.010
Volume (S) = 230201 voxels or 1589.5 Resels
Extent threshold (k) = 34 voxels, p = 0.300
Degrees of freedom due to error = 58.0
Expected voxels per cluster, E(n) = 34.3
Smoothness FWHM (mm) = 9.3 10.6 11.7
Expected number of clusters, E(m) = 20.2
(voxels) = 4.6 5.3 5.9

Figure AIII. Statistical output for the covariates analysis on Group A subjects described in Chapter Four.

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If you steal from one author, it's plagiarism;
if you steal from many, it's research.
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