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Neuroprotective Effects of Long-term Endurance Training on the Cortical Autonomic Network in the Aging Brain

Torri A. Luchyshyn
The University of Western Ontario

Supervisor
Dr. J Kevin Shoemaker
The University of Western Ontario

Graduate Program in Kinesiology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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Torri Alexa Luchyshyn

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CERTIFICATE OF EXAMINATION

Supervisor	Examiners
_____	_____
Dr. Kevin Shoemaker	Dr. Ruth Martin
	_____
Supervisory Committee	Dr. Keith St. Lawrence
_____	_____
Dr. Ruth Martin	Dr. Matthew Heath
_____	
Dr. Peter Lemon	

The thesis by

Torri Alexa Luchyshyn

entitled:

Neuroprotective Effects of Long-term Endurance Training on the Cortical Autonomic Network in the Aging Brain

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Date_____ _____
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ABSTRACT

This study tested whether long-term endurance training in older adults (ET; n = 15, 55 ± 4 years, relative VO_2max = 50 ± 8 ml/kg/min) would alter cardiovagal control and preserve the cortical autonomic network compared to age-matched controls (CON; n = 15, 56 ± 4 years, relative VO_2max = 37 ± 9 ml/kg/min). The hypothesis predicts 1) altered deactivation patterns of the ventral medial prefrontal cortex (vMPFC) in response to isometric hand grip (IHG) and 2) greater indices of cardiovagal control; a) increased baroreflex sensitivity at rest, b) greater heart rate change (ΔHR) and c) reductions in high frequency heart rate variability (ΔHF HRV) in the ET group. Functional magnetic resonance imaging was utilized to observe BOLD signal changes. There was no difference in measured indices of cardiovagal control between groups and both exhibited vMPFC deactivation with IHG. Overall, ET does not preserve cortical functional patterns in the older brain or enhance cardiovagal control compared to age-matched controls.

Keywords: cortical autonomic network, ventral medial prefrontal cortex, cardiovagal control, baroreflex sensitivity, long-term endurance training, aging

CO-AUTHORSHIP STATEMENTS

Katelyn N. Norton: Ms. Norton assisted with neuroimaging analysis and was instrumental in my education of designing an MRI project.

Maria F. Frances: Ms. Frances assisted with the recruitment of several subjects and collection of ultrasound data during laboratory testing sessions. She was also responsible for running stress tests for all of the individuals and interpreting the results.

Dr. Kevin Shoemaker: Dr. Shoemaker helped with the design of this study, provided assistance with data analysis and interpretation, and supervised the writing of this document.

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LIST OF ABBREVIATIONS

ACC	anterior cingulate cortex
Ach	acetylcholine
ANOVA	analysis of variance
AP	action potential
BDNF	brain-derived neurotrophic factor
BOLD	Blood Oxygenation-Level Dependent
BP	blood pressure (mmHg)
bpm	beats per minute
BRS	baroreflex sensitivity (ms/mmHg)
Ca^{2+}	calcium
cAMP	cyclic adenylate monophosphate
CAN	cortical autonomic network
CNS	central nervous system
CSP	carotid sinus pressure
CV	cardiovascular
CVD	cardiovascular disease
CVLM	caudal ventrolateral medulla
dACC	dorsal anterior cingulate cortex
DBP	diastolic blood pressure (mmHg)
dHb	deoxyhemoglobin
ECG	electrocardiogram
EPI	echo planar imaging
ET	endurance training
FDR	false discovery rate
FinAP	finger arterial pressure
fMRI	functional magnetic resonance imaging
FOV	field of view
GLM	general linear model
GM	grey matter
Hb	hemoglobin
HbA1C	glycosylated hemoglobin
HDL	high density lipoprotein
HDR	hemodynamic response
HF	high frequency
HG	hand grip
HR	heart rate (bpm)
HRT	hormone replacement therapy
HRV	heart rate variability (ms^2)
HS CRP	high sensitivity c-reactive protein

Hz	Hertz
IC	insular cortex
IHG	isometric hand grip
IHR	intrinsic heart rate
IML	intermediolateral
K^+	potassium
LDL	low density lipoprotein
LF	low frequency
ln	natural logarithm
MAP	mean arterial pressure (mmHg)
MCC	middle cingulate cortex
MoCA ©	Montreal Cognitive Assessment
MRI	magnetic resonance imaging
MSNA	muscle sympathetic nerve activity
MVC	maximal voluntary contraction
NA	nucleus ambiguous
NE	norepinephrine
NGF	nerve growth factor
NPY	neuropeptide Y
NT	neurotransmitter
NTS	nucleus tractus solitarius
PAG	periaqueductal grey matter
PCC	posterior cingulate cortex
PNS	parasympathetic nervous system
PP	pulse pressure (mmHg)
Q	cardiac output (L/min)
reBAP	reconstructed brachial arterial pressure (mmHg)
RF	radio frequency
RFX	random effects
RTF	return-to-flow
RVLM	rostral ventrolateral medulla
SA	sinoatrial
SBP	systolic blood pressure (mmHg)
SNR	signal to noise ratio
SNS	sympathetic nervous system
sub ACC	subgenual anterior cingulate cortex
SV	stroke volume (ml)
T	Tesla
T_2	spin-spin relaxation time
TE	time to echo

TI	inversion time
TPR	total peripheral resistance
TR	time to repetition
V	Volts
VLF	very low frequency
vMPFC	ventral medial prefrontal cortex
VO_2max	maximal oxygen consumption
WM	white matter

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Physical inactivity contributes to increased prevalence of cardiovascular disease (CVD) which makes it important to understand the mechanisms of how decreased physical fitness affects cardiovascular function from an economic, clinical and public health perspective (232). The autonomic nervous system (ANS) is a crucial component for cardiovascular (CV) system homeostasis. Normal function of the ANS means both parasympathetic and sympathetic branches have certain tonic levels of activity and are constantly interacting with each other (180). This dynamic equilibrium between parasympathetic and sympathetic tone is responsible for the control of numerous CV indices, including blood pressure (BP) and heart rate (HR). Chronic increases in sympathetic tone with decreased vagal tone are hallmark signs of a disturbed ANS and are associated with escalated incidences of morbidity and mortality related to CVD (66).

A recent meta-analysis suggested that in both normal and diseased individuals physical activity benefits neural control of the CV system by increasing parasympathetic tone and attenuating sympathetic activity (58). Those individuals with high levels of exercise training, such as endurance trained athletes, will have a greater parasympathetic-mediated control of their CV system compared to normal or diseased populations. Unfortunately, the physiological process of normal aging contributes to a decline in CV health with a greater frequency of CVD in advancing years (158). This may be due to an altered ANS profile leading to diminished control of the CV with age, as evidenced by a reduction in autonomic control of HR (43; 228).

Recent years have produced large quantities of research investigating the pathogenic role of the ANS in CVD progression, and how ANS dysfunction due to normal aging may amplify the development of CVD (158). Findings suggest that increased physical activity can slow or reverse the detrimental effects of aging on the CV system. For example, older individuals who participate in regular endurance exercise exhibit increased vagal tone as observed by greater baroreflex sensitivity (BRS) (226) and heart rate variability (HRV) at rest and while exercising (44). It is difficult, however to determine if this altered ANS function is due to the physiological processes of aging or if it is a result of the decreased fitness levels that occur naturally with age.

Attention is now focused on a network of forebrain regions responsible for autonomic outflow and CV control, collectively termed the cortical autonomic network (CAN). Researchers are able to study differences in CV responses to a variety of stressors at a central neural level. This is of interest as rodent (232) and human studies (53; 83; 84; 219) have demonstrated that the central nervous system exhibits a physical activity-dependent neural plasticity. To date, this phenomenon has not been studied in the context of human CAN preservation with age due to endurance training.

Because enhanced parasympathetic tone is associated with healthier autonomic function, this study examined areas previously identified in cardiovagal control, such as the ventral medial prefrontal cortex (vMPFC) (362). Using a short term isometric handgrip (IHG) exercise of moderate intensity in healthy subjects, cardiovagal control can be examined by the large tachycardic response that occurs before sympathetic activation (101; 206; 362). By combining a short duration, moderate intensity IHG model with the capabilities of functional magnetic resonance imaging (fMRI), we were able to isolate the structures within the CAN responsible for cardiovagal control.

1.2 PURPOSE

The purpose of this study was to evaluate whether long-term endurance training performed by older adults would have a significant neuroprotective effect on the morphology and function of the CAN. Specifically, this study focused on the vMPFC and how long-term endurance training performed by older adults affected the cortical patterns associated with heart rate responses to an IHG exercise task.

1.3 HYPOTHESIS AND PREDICTED OUTCOMES

Long-term endurance training will preserve parasympathetic control of HR through sustained functional activation patterns of the vMPFC. This hypothesis predicts that endurance trained older adults should have an enhanced HR response, reflecting PNS withdrawal, at the onset of IHG exercise, and decreases in the high frequency component of heart rate variability compared to age-matched controls. The enhanced PNS withdrawal should also be indicated by greater patterns of deactivation in the vMPFC. Baroreflex sensitivity recorded at rest is also expected to be greater in endurance trained individuals compared to controls. The combined outcome measures will point to a pattern of preserved cardiovagal tone and, to extrapolate, a healthier autonomic nervous system, thereby offsetting the effect of physiological aging that occurs in less physically active individuals.

CHAPTER 2: LITERATURE REVIEW

2.1 AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system is responsible for numerous everyday functions and is critical for maintaining homeostasis (see Figure 2.1.1). The CV system is highly regulated by the ANS, which controls variables such as heart rate, blood pressure, and blood flow. The sensitive control of the cardiovascular system by the ANS is divided both anatomically and functionally into the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS).

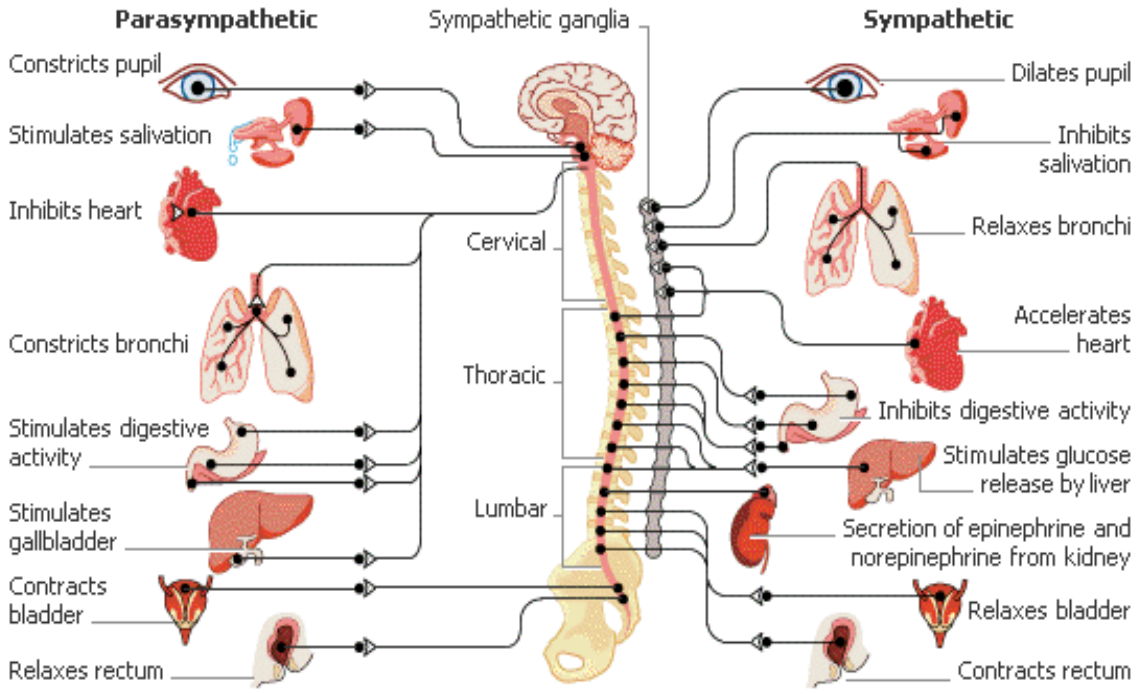

Figure 2.1.1 The two anatomical and functional divisions of the autonomic nervous system: the parasympathetic and sympathetic branches (taken from (105)).

The traditional view that these two centrally controlled neural systems work in opposition to each other is an oversimplification; rather, they work in a dynamic

equilibrium, co-operating to fine tune various processes and maintain homeostasis (312). For example, in humans at rest parasympathetic outflow (or tone) is greater than sympathetic tone but both are present. With age and disease the equilibrium between the overall activities of these neural systems can shift and have detrimental effects on an individual's health. The changes in this equilibrium can bring about changes in control and function of the cardiovascular system at rest and under various stressors such as exercise. Endurance training may help to maintain the sensitive balance between sympathetic and parasympathetic outflow and counteract the physiological effects of aging.

2.1.1 SYMPATHETIC NERVOUS SYSTEM

The sympathetic nervous system has been classically referred to as the body's "fight or flight" system. It acts on the CV system through the effector arm of the ANS via two groups of neurons: pre-ganglionic and post-ganglionic. The axons of these neurons are responsible for transmitting SNS signals to effector organs and tissues. The cell bodies that make up the sympathetic pre-ganglions are located in the intermediolateral (IML) cell column that runs from the level of T1 to L1 in the spinal cord (312), the reason SNS outflow has also been referred to as the thoracolumbar system or thoracolumbar 'outflow' (312). The shorter pre-ganglionic fibres exit the spinal cord through the ventral root (see Figure 2.1.1.1) and are known as the white rami communicantes (195), where they project to a collection of ganglia that lie adjacent to the vertebrae (paravertebral), known as the sympathetic trunk.

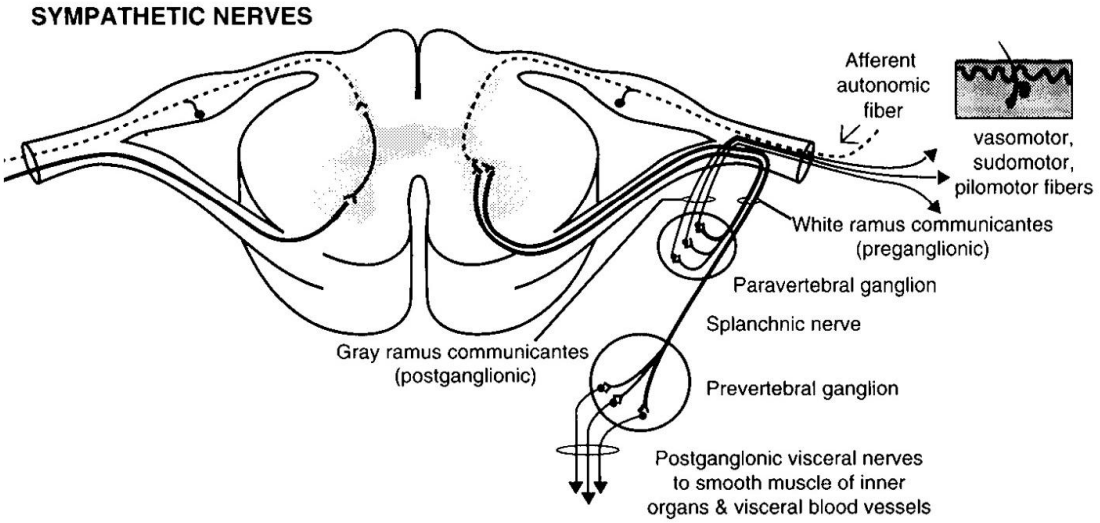

Figure 2.1.1.1 The course of sympathetic nerve fibres as they enter and exit the spinal cord (adapted from (312)).

At the ganglionic synapse acetylcholine (Ach) is released and binds to stimulate the nicotinic cholinergic receptors on the dendrites of the post-ganglionic neuron (312). This causes action potentials to travel down the axons of the much longer post-ganglionic sympathetic neurons. Once the action potential (AP) reaches the distal end of the neuron it signals the release of neurotransmitters (NTs) from an enlarged part of the neuron known as a varicosity. The AP signals an influx of Ca^{2+}, which causes exocytosis of synaptic vesicles containing NTs that stimulate receptors located on the target organ(s) or tissue(s).

The myocardial and pacemaker cells of the heart and the smooth muscle surrounding blood vessels are just a few of the effector targets of the SNS. The SNS acts on these effectors through the release of NTs such as ATP, neuropeptide Y (NPY), and norepinephrine (NE) which then interact with specific receptors. The most studied of these NTs is probably NE and when released from sympathetic varicosities NE can bind

to either alpha or beta-adrenergic receptors. There are two main subtypes of both the alpha (α1 and α2) and beta (β1 and β2) adrenergic receptors, with α1, α2 and β2 located on the endothelial and smooth muscle cells surrounding blood vessels. The β1 receptor is located in the human heart and is the main adrenergic receptor found there. This receptor couples to a stimulating G-protein which activates adenylate cyclase to increase cyclic adenylate monophosphate (cAMP). Through a signalling cascade this ultimately causes an increase in intracellular Ca^{2+} leading to increased chronotropy and inotropy.

2.1.2 PARASYMPATHETIC NERVOUS SYSTEM

The parasympathetic nervous system has been classically referred to as the "rest and digest" system. Just like the SNS, the efferent system of the PNS is organized into two groups of neurons: pre-ganglionic and post-ganglionic. The pre-ganglionic neurons of the PNS utilize acetylcholine as their neurotransmitter but are much longer than the pre-ganglionic neurons of the SNS and thus terminate in close proximity to the target organ. The cell bodies of the pre-ganglionic neurons are found in the IML cell column of the sacral portion of the spinal cord and in these cranial nerve nuclei; cranial nerves III (occulomotor), VII (facial), IX (glossopharyngeal) and X (vagus) (312). For this reason the PNS outflow has been referred to as the craniosacral system. It is also known as "vagal" outflow due to the fact that the vagus nerve carries almost 90% of parasympathetic activity. Neurotransmitter Ach released at the synapse between pre- and post-ganglionic neurons acts on nicotinic receptors to stimulate the post-ganglionic dendrites. Action potentials travel down the much shorter axons of the post-ganglionic neurons and release Ach to stimulate cholinergic muscarinic receptors on the target organ(s) and tissue(s).

Perhaps one of the most important visceral organs acted on by the PNS is the heart. Parasympathetic fibres traversing the left and right vagus nerves are distributed to many different sites, with fibres terminating at both the atria and ventricles. PNS modulates cardiac function through the release of Ach from post-ganglionic nerve terminals, as mentioned above. Acetylcholine diffuses across the synaptic cleft and then binds to a cholinergic muscarinic receptor. There are five subtypes of muscarinic receptors that have been identified; M_1, M_2, M_3, M_4 and M_5 (33), with the predominant receptor found in the heart of most mammalian species, including humans, being the M_2 subtype (45; 138; 263).

In both the atria and ventricles, the activation of the M_2 receptor, which is coupled to an inhibitory G-protein, leads to the inhibition of adenylate cyclase thus inhibiting the increase in intracellular cAMP. The inhibition of cAMP leads to a decrease in the activity of L-type Ca^{2+} current channels (33) therefore decreasing the amount of intracellular Ca^{2+} which is needed for contraction. In atrial myocytes specifically, the activation of the M_2 receptor may also open an inwardly rectifying K^+ channel, allowing K^+ to leave the myocyte and hyperpolarize the cell (33). There are more postulated cellular mechanisms that result from M_2 receptor stimulation but they are beyond the scope of this thesis. The net result of these cellular mechanisms initiated by vagal stimulation ultimately leads to a decrease in heart contractility and heart rate.

2.1.3 HEART RATE REGULATION

The average intrinsic heart rate (IHR) of an individual is based on spontaneous depolarization of the pacemaker cells of the sinoatrial (SA) node without SNS or PNS influence. In a healthy human the IHR is approximately 100 beats per minute (bpm) but

can vary based on traits like age and gender (152). The physiological HR of a healthy human at any given time is characterized predominantly by the combined effects of PNS and SNS on IHR. For a given stable physiological state, the PNS and SNS inputs contribute to a tonic level of activity which determines the HR for that state (180). By varying the relative proportion of SNS and PNS activity, heart rate can be tightly regulated. The reciprocal interaction of the PNS and SNS is what determines heart rate responses to a variety of physiological stressors such as emotion, stress or physical activity and is often described in the literature as "sympathovagal" balance. The SA node, being richly innervated by PNS and SNS fibres, is the primary site where these two systems converge to modulate IHR.

The average resting HR for a healthy adult is approximately 70 bpm. At rest, the SA node is predominantly mediated by parasympathetic tone, which is why average resting HR is lower than IHR. In a human study of nonathletes, pharmacological blockade with atropine (a PNS inhibitor) saw an increase in mean resting HR from 63 bpm to 117 bpm (see Figure 2.1.3.1). Meanwhile blockade with propanolol (an SNS inhibitor, β-adrenergic blocker) only saw a decrease in mean resting HR from 61 bpm to 51 bpm (157). Although this antagonism between both limbs of the ANS is a well known phenomenon, the interaction between the PNS and SNS is far more complex than a simple antagonism and therefore HR control cannot be a simple additive algebraic equation (130).

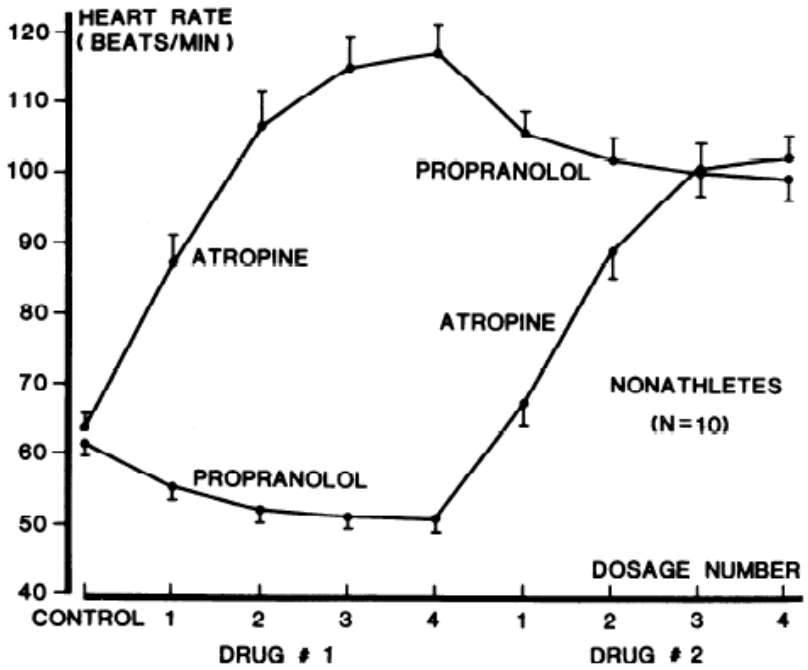

Figure 2.1.3.1 Parasympathetic and sympathetic cardiac control determined with dual drug blockade: atropine (0.04 mg/kg total) a parasympathetic blocker and propanolol (0.2 mg/kg total) a sympathetic blocker in 10 younger nonathletes at rest (taken from (157)).

There are interactions between the parasympathetic and sympathetic limbs that will determine their respective effects on HR (180). A high frequency of vagal stimulation eliminates cardiac responses to sympathetic stimulation (354). In anesthetized dogs the influence of a given level of sympathetic activity on HR became less pronounced with increasing levels of vagal stimulation. Increasing stimulation of sympathetic nerves from 0 to 4 Hz increased HR 80 bpm but had almost no effect on cardiac pacemaker activity when vagal stimulation was high (8 Hz) (190). In contrast, a similar experiment that looked at myocardial ventricular contractility, showed evidence that vagal effects were greater during concurrent sympathetic activity (191).

The majority of research supports vagal domination of HR, however, it is not known whether vagal activity acts to inhibit SNS influence or if sympathetic activity

potentiates the effects of vagal stimulation (190). There are many explanations for how these potential mechanisms would work and only some will be discussed now. It has been suggested that sympathetic potentiation of vagal tone may be due to K^+ fluctuations. Sympathetic stimulation of the SA node causes a brief uptake of K^+ by the pacemaker cells and the responsiveness of the cardiac pacemaker to vagal stimulus is sensitive to extracellular K^+ concentrations (191). Catecholamines released due to SNS activity may act to further stimulate parasympathetic ganglia, although this was only examined in rat skeletal muscle at the end plate motor terminals (176). Also, Ach released from parasympathetic post-ganglionic nerve terminals may cause a partial adrenergic blockade, inhibiting NE release (214). This has been correlated with certain metabolic changes in myocardial cells that act to decrease sympathetic effects on HR (191).

The different kinetics of both the PNS and SNS is another important aspect of the regulation of heart rate control. The parasympathetic limb exerts its effects more quickly than the sympathetic does and is able to control HR on a beat-to-beat basis. This has been previously examined by stimulating both the vagus and sympathetic inputs flowing to the heart of an anesthetized dog. Stimulation of the vagus nerve at both 7 and 10 Hz led to an immediate decrease in HR (and immediate increase in HR when stimulation was removed), while the HR increase to sympathetic stimulation at 20 Hz was more gradual (as was the HR decrease when stimulation was removed) (354). This suggests vagal effects on the heart develop very rapidly, usually within one heartbeat, and they decay nearly as quickly (243). It is hypothesized that vagal effects are this quick due to several factors. First, there is an abundance of Ach that can be released from the vagus nerve and bind to M_2 muscarinic receptors. The M_2 receptors are linked directly to an inward rectifying K^+ channel via a G-protein and thus do not rely on secondary cellular

messenger systems like the adrenergic receptors (173). Finally, any Ach that remains in the synaptic cleft is degraded very quickly by acetylcholinesterase one of the fastest enzymes in the body. It has been suggested that the rate of Ach degradation by acetylcholinesterase is a key factor in the dynamic properties of vagal control of heart rate (233).

2.1.4 BAROREFLEX

The SNS and PNS perform their regulatory functions predominantly through autonomic reflexes. Probably the most carefully studied of these autonomic reflexes is the arterial baroreceptor reflex or baroreflex (312). In humans, it is the primary mechanism through which mean arterial BP is rapidly and tightly controlled despite constant changes to differing physiological conditions.

The baroreflex operates through a negative feedback loop (see Figure 2.1.4.1) which originates anatomically from the baroreceptors (226). The arterial baroreceptors are highly specialized stretch-sensitive receptors located in the walls of the aortic arch and carotid sinus. They relay afferent neural impulses to the central nervous system through their activation/deactivation and are needed for the acute control of BP by initiating appropriate physiological responses that include changes in HR, vascular resistance and myocardial contractility (158).

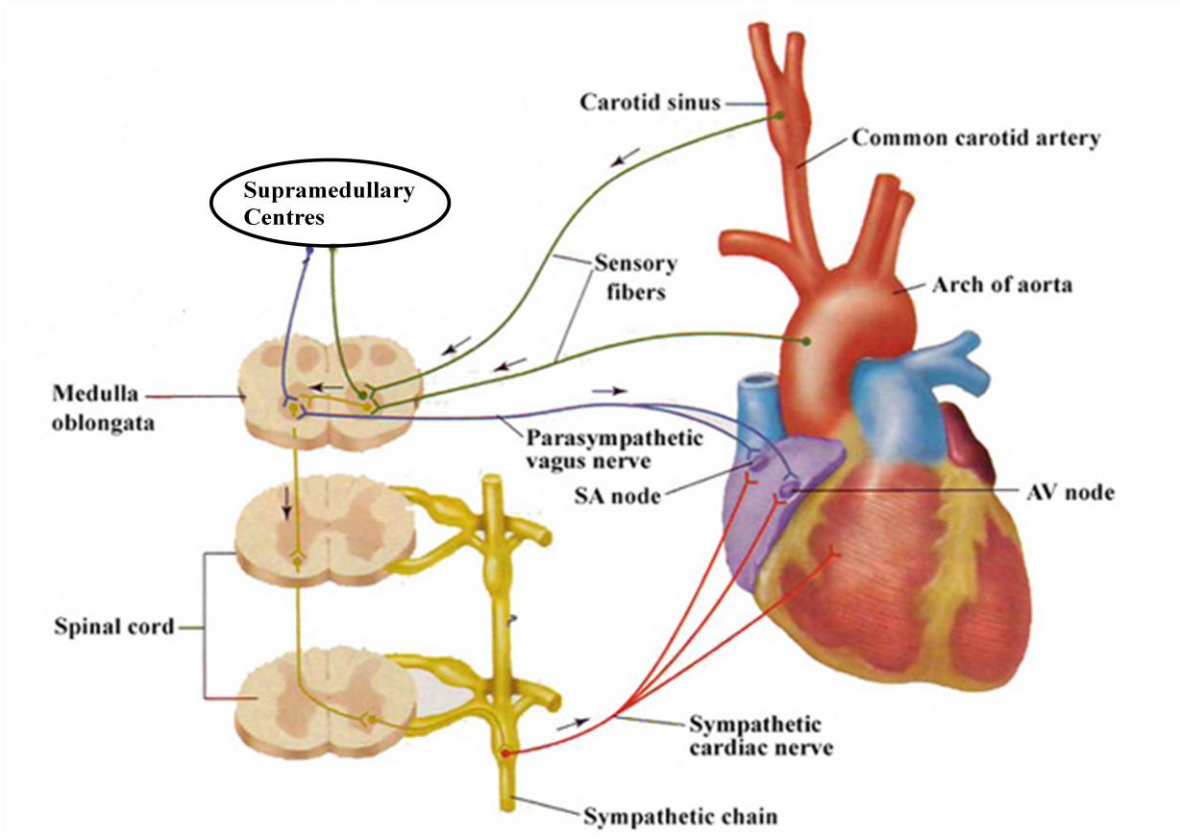

Figure 2.1.4.1 Arterial baroreflex negative feedback loop. Mechanical baroreceptors located in the arch of the aorta and carotid sinus respond to stretch of the vessel walls. Sensory afferent fibres carry information to neurons in the medulla oblongata (adapted from (99)).

With a rise in arterial BP the baroreceptors in the carotid sinus and aortic arch respond to the increased stretch of the arterial walls and transmit action potentials through sensory afferent fibres in the glossopharyngeal and vagus nerves, respectively. These fibres project to the nucleus tractus solitarius (NTS) which is located in the medulla oblongata of the brainstem and is the main integration centre for afferent information as it receives a variety of sensory inputs (151). The increased neural activity of the NTS causes an excitatory response in the caudal ventrolateral medulla (CVLM) and nucleus ambiguous (NA), which increases vagal outflow to the heart. Interneurons from the CVLM inhibit the rostral ventrolateral medulla (RVLM), which has been described as the

tonic vasomotor centre (282). With inhibition of the RVLM there is a decrease in sympathetic outflow which ultimately causes a decrease in arterial BP. With a fall in BP the firing rate of the baroreceptors decreases, reducing the afferent neural information received by the NTS and leading to an increased sympathetic outflow from the RVLM causing an increase in arterial BP.

The inverse relationship between arterial BP and HR provides us with a unique opportunity to study the sensitivity of the baroreflex. After many years and experiments the sigmoidal stimulus-response curve of the baroreflex was finally described (see Figure 2.1.4.2) (277). In normotensive humans the operating or set point of the baroreflex curve is located in the middle, making it more effective in preventing both hyper- and hypotension (202). Because the middle part of the stimulus-response curve is linear, the calculated slope is used to quantify the baroreflex sensitivity (BRS). It is often called cardiovagal BRS to indicate that the measured response is directed toward the heart and is vagally mediated (226).

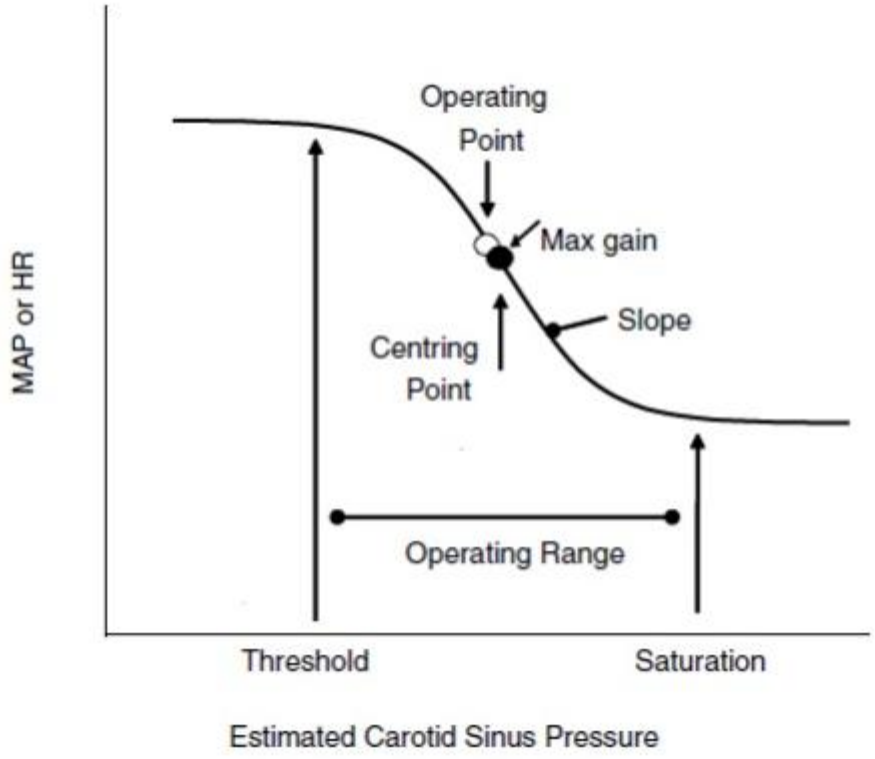

Figure 2.1.4.2 A schematic model of the arterial baroreflex sigmoidal curve and its operational parameters developed by Raven *et al* (2006). It describes the heart rate (HR) and mean arterial pressure (MAP) responses to a given carotid sinus pressure (CSP) which was manipulated using neck suction (stimulates higher CSP due to greater baroreceptor stretch) and inflation (simulates lower CSP due to less baroreceptor stretch). Threshold is the CSP at which no further increases in HR or MAP occur. Saturation is the CSP at which no further decreases in HR or MAP occur. The operating point is the current HR and MAP for a given CSP (taken from (277)).

2.1.5 CHANGES IN ANS FUNCTION WITH ISOMETRIC HAND GRIP EXERCISE

Isometric muscular contractions elicit changes in the cardiovascular system of both animals and humans (95; 114; 222). These responses include alterations in blood pressure and heart rate and are mediated via both limbs of the autonomic nervous system. Generally speaking isometric contractions cause an increase in blood pressure and a modest yet significant increase in heart rate otherwise known as tachycardia. Because the current study tested the hypothesis that cortical cardiovagal control is altered in endurance

trained older adults, isolation of the PNS component of the HR response to exercise was required.

Vagal influence on heart rate responses is most dominant at heart rates less than 100 bpm (310) and previous work suggests that the initial tachycardic response to exercise is due to vagal withdrawal, owing to the relatively short lag time between HR increase after the start of muscular contraction (25; 101; 134; 268). In fact, during sustained isometric handgrips completed to exhaustion at 30% maximal voluntary contraction (MVC), the first minute showed no increases in muscle sympathetic nerve activity (MSNA) (bursts/min) despite large increases in heart rate (204). However, the maximum HR increase due to immediate vagal withdrawal alone is at most 30 bpm and any further increases in HR are due to increased sympathetic activation with or without concurrent PNS withdrawal (266).

It has been a challenge to tease out the independent contributions of both limbs to the rise in HR, but studies using autonomic blockade in both animals and humans have been useful (78; 101; 206; 224; 225). In young adults who performed varying intensities of isometric handgrips (50-100% MVC) during short periods of time (5-10 seconds), administration of atropine (a muscarinic receptor antagonist that blocks parasympathetic outflow) significantly reduced the mean tachycardic response by 17 bpm, compared to pre-drug trials (101) and has been shown to attenuate the heart rate response on other occasions (134; 225). Furthermore, this initial tachycardic response to exercise is not affected by adrenergic receptor blockade (225).

This biphasic autonomic control of HR response can be mediated by the muscle mass, intensity and duration of static exercise performed. Increased pressor reflexes to exercise were observed when large hindlimb muscles were electrically stimulated to

produce isometric contractions via spinal ventral roots in decerebrated cats (146). Human studies have also shown a relationship between greater heart rate responses to static exercise with increased muscle mass (see Figure 2.1.5) (104; 223; 286; 308). For example, CV responses were progressively greater during isometric contractions at 40% MVC by the fingers (digits 2 and 3), forearm (handgrip), knee extension and handgrip with simultaneous knee extension (223).

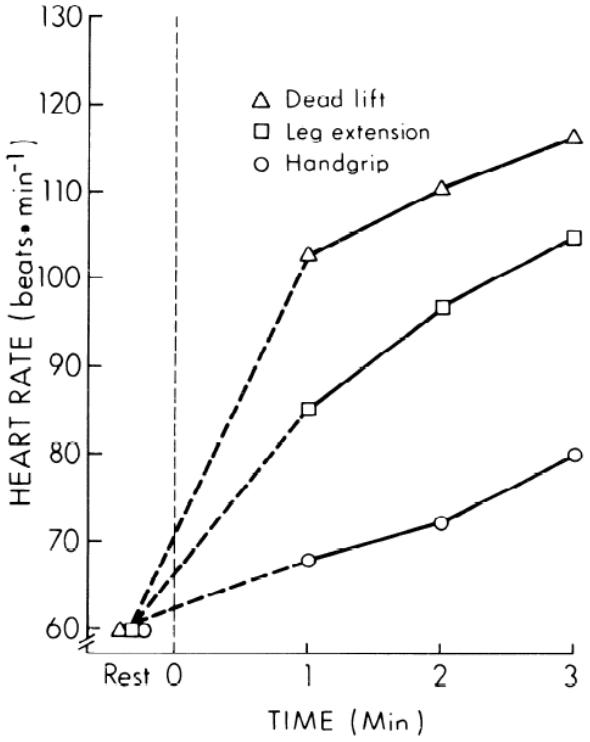

Figure 2.1.5 Heart rate responses to 3 types of sustained isometric contractions performed at 30% of maximal voluntary contraction for 3 minutes in young healthy male subjects. Dashed line demarcates start of contraction (taken from (308)).

Various investigators have also suggested that HR response to isometric exercise is contraction-intensity dependent (78; 104; 189; 192; 305; 308), but it has been difficult to determine an exact relationship as there are a multitude of factors to consider. Generally speaking, a short but intense contraction or a sustained contraction will cause an increase

in sympathetic nerve activity. For example, sympathetic activation will start to contribute to the chronotropic response approximately 30 seconds after the start of a 30% MVC isometric hand grip (206; 222). During a contraction of shorter duration and higher intensity (75% MVC) compared to those of mild (25% MVC) or moderate (50%) intensity there is a significant increase in MSNA that corresponds with increased blood pressure and heart rate (350). Contractions of longer duration (i.e. 2 minutes) and milder intensities (30% MVC) still emphasize PNS withdrawal initially but will have a greater SNS component the longer the contraction is maintained (350). And, if a greater intensity is utilized during a sustained contraction the SNS contribution to the heart rate response would occur earlier in the time course (200) and would progressively increase instead of stabilizing while the contraction was maintained (78; 192).

A mechanism that has been postulated to explain these relationships revolves around the exercise pressor reflex. With this reflex, afferent nerve fibres originating in the contracting muscle(s) respond to chemical and mechanical stimuli produced during contractions such as glycolytic metabolites and increased fibre tension. Thus with a greater muscle mass or increased contraction intensity (i.e. greater number of motor units activated) greater activation of the afferent nerve fibres occurs leading ultimately to a greater CV response (104). In summary, a short term moderate intensity isometric hand grip offers a unique opportunity to study the cortical modulation of PNS outflow during a mild intensity exercise.

2.2 CORTICAL AUTONOMIC NETWORK AND ANS CONTROL

The primary site of autonomic cardiovascular control is the medulla oblongata (or medulla) located in the brainstem, which is the oldest and most primitive region of the

brain. However, a certain network of forebrain sites known as the CAN also modulates control of the autonomic nervous system. Anaesthetized cats that underwent decerebration experienced a 14% fall in resting BP before transection of the vagal and carotid sinus nerves which did not change resting BP further (281). In contrast, animals that underwent transection of the nerves first had a rapid and large increase in resting BP, which then decreased significantly after decerebration (281). It was concluded that this fall in BP could be explained as an interruption of tonic descending excitatory outflow to neurons participating in the reflex response (281). These findings suggest higher cortical structures have a role in cardiovascular control by exerting a tonic influence on some part of the blood pressure regulatory mechanism.

Cortical influence over central cardiovascular control mechanisms has been studied for many years by monitoring HR and arterial BP changes in animals during electrical stimulation of exposed cortical surfaces (133; 155). Certain areas such as the insular cortex and anterior cingulate cortex have been identified to cause depressor and bradycardic responses when electrically stimulated in the rabbit (38; 274), but have also been involved in pressor and tachycardic responses (366) which could be due to a lateralized effect of certain autonomic structures in the forebrain (61). Direct projections from certain cortical areas to the brainstem nuclei and specific spinal regions have also been described. Ricardo and colleagues were the first to discover direct pathways in both the anterograde and retrograde directions between the nucleus tractus solitarius in the brainstem and amygdala in the forebrain of the rat using a horseradish perioxidase labelling technique (285). The ability to conduct a wide array of experiments in different animal species has greatly enhanced our knowledge of cortical influence over the cardiovascular system.

Certain auditory, somatosensory, visual and even emotional stimuli can trigger cardiorespiratory responses. Even in 1949, theories regarding emotion and its autonomic manifestation had been documented (201). Critchley et al were able to show that the association between HR acceleration and emotional facial stimuli was predicted by the level of activity within a matrix of interconnected brain regions including the insular cortex, amygdala, anterior cingulate and brainstem (65). Clinical studies conducted on patients with strokes and epileptic seizures in their prefrontal cortex have shown that autonomic responses are compromised, indicating a relationship between cortical activity and cardiovascular function (51; 56; 252; 254).

Electrical stimulation of cortical surfaces and implantation of depth electrodes were utilized in early human studies to complement the extensive research conducted on animals, supporting a higher cortical role in CV control (49; 50; 127; 193). However, it is often difficult to determine if comparable connections or functional sites exist within humans due to the relatively invasive experimental approaches that have been utilized in animals (339). With the advent of neuroimaging techniques it has been possible to get a better understanding of the functional neuroanatomy of autonomic cardiovascular control sites, allowing researchers to study whole brain response to certain stimuli rather than having to complete multiple investigations in order to determine how many sites are involved. It also permits the study of whether certain sites become more or less active compared to baseline or resting levels.

The vast array of experimental and clinical studies has exposed a variety of potential sites involved in the CAN. Specifically, for the purpose of this thesis, only some sites will be examined in greater detail including the ventral medial prefrontal cortex, insular cortex and anterior cingulate cortex. The medial prefrontal cortex and the insular

cortex have the greatest amount of evidence in terms of central cardiovascular control and are well-established parts of the cortical autonomic network (298; 348). Older studies indicated that areas affecting cardiovascular control were spread over a relatively large cortical area (68) but the study of them all is beyond the scope of this paper. Areas of the brain will also be described using Brodmann's areas based on the cytoarchitectural maps of the cortex published by Korbinian Brodmann (see Figures 2.2.1 and 2.2.2).

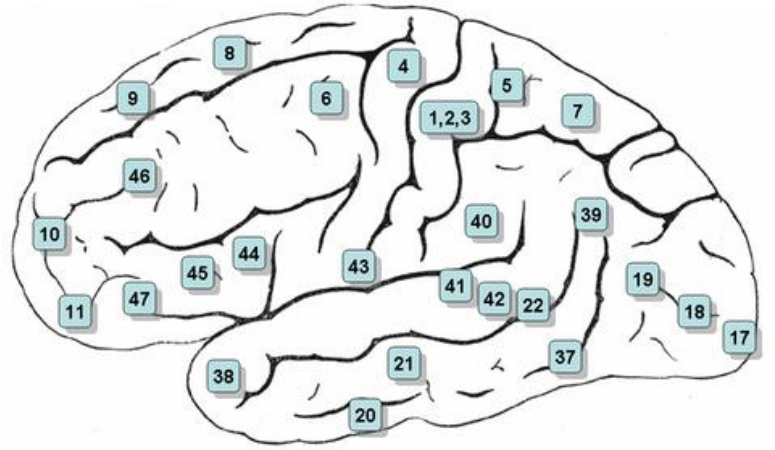

Figure 2.2.1 Brodmann's areas numbered on the lateral side of the brain (adapted from (115)).

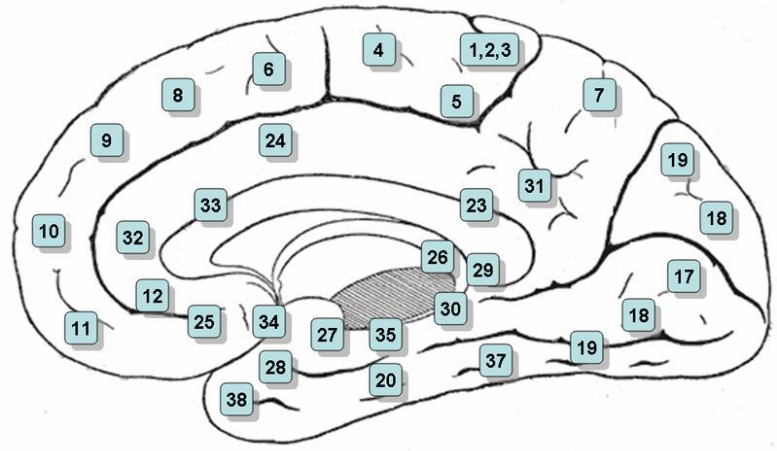

Figure 2.2.2 Brodmann's areas numbered on the medial side of the brain (adapted from (115)).

2.2.1 THE VENTRAL MEDIAL PREFRONTAL CORTEX

The medial prefrontal cortex (MPFC) has been termed a "visceromotor" cortical structure as activity in this area is related to changes in blood pressure, heart rate and breathing (68; 236; 347). It is located anterior to the corpus callosum (Brodmann's areas 10 and 11) and is often anatomically divided into dorsal and ventral divisions (often termed prelimbic and infralimbic cortices, respectively). The dorsal region incorporates the most inferior portion of the anterior cingulate cortex (ACC) whereas the ventral MPFC (vMPFC) is situated below the ventral ACC and includes the orbital frontal gyrus. These anatomical divisions seem to be dependent on the afferent projections to, and the efferent projections from, the MPFC (348). Specifically, the vMPFC has been shown to have connections with the medial dorsal nucleus of the thalamus (207), the amygdala (211), multiple lateral hypothalamic nuclei (139), the periaqueductal grey matter (PAG) of the midbrain (126), the NTS (348), the RVLM (345) and even the intermediolateral

column of the spinal cord (199). Because of the substantial anatomical evidence that the vMPFC projects to a number of key foci involved in autonomic function it is highly likely to be a modulator of cardiovascular function (257).

As mentioned previously there are numerous animal studies that have been conducted to examine cardiovascular responses and the relationship with cortical activity. In 1960, Kaada reviewed experiments conducted in dogs, cats and monkeys to conclude that stimulation of the prefrontal cortex area produced vagally-mediated hypotension and bradycardia (154). In anaesthetized rats electrical stimulation (125; 236; 257; 345) as well as chemical microstimulation with L-glutamate (12) of the vMPFC caused depressor responses. Chemical stimulation mimics the effects of electrical stimulation, indicating the responses are likely due to vMPFC neurons and not stimulation of neurons located in nearby cortical areas (12; 348). Electrical stimulation of the vMPFC in awake rabbits also elicits hypotension as well as bradycardia (38). These depressor responses are accompanied by decreased activity of both the lumbar and splanchnic nerves, in addition to decreased firing of sympatho-excitatory barosensitive neurons located in the rostral ventrolateral medulla (345). This suggests a possible sympathoinhibitory role of the vMPFC in terms of cardiovascular control. In contrast, electrical stimulation of the vMPFC in urethane-anesthetized rats caused a depressor BP response and in unanesthetized animals a pressor response. The authors concluded under normal conditions a pressor response is elicited by vMPFC stimulation due to SNS activation as evidenced by the attenuated pressor response when animals were pretreated with mecamylamine (a long-lasting ganglion blocker) (330).

Enhanced activity in the vMPFC during vegetative states (i.e. sleep) in monkeys (289) and during the resting state in humans (276) suggests that the vMPFC may be more

involved in PNS rather than SNS control (60). The vMPFC may also be involved in parasympathetic control of the cardiovagal baroreflex. Chemical blockade of synaptic transmission within the vMPFC attenuated baroreflex-mediated vagal outflow to the heart without affecting baroreflex gain (284). In conscious humans, baroreceptor unloading through a lower body negative pressure manoeuvre elicited deactivation of the vMPFC in conjunction with an increase in HR (163). Furthermore, a mild IHG exercise produced robust tachycardic and pressor responses without peripheral sympathetic nerve activation, which were strongly correlated with deactivation of the vMPFC (362). It seems more likely that the vMPFC has a modulatory action over cardiovascular control, specifically the baroreflex, rather than a tonic influence because ablation of the vMPFC does not cause changes in mean arterial BP or heart rate (236; 284; 346; 347).

2.2.2 THE INSULAR CORTEX

In humans, the insular cortex (IC) is a portion of the cerebral cortex folded deep within the lateral sulcus (see Figure 2.2.2.1) obscured by the frontal, temporal and parietal lobes (Brodmann area's 13 and 14). There are two distinct cytoarchitectonic divisions of the IC; granular cells in the posterior region and agranular cells in the anterior region. It is an important integration centre of visceral inputs with behavioural and autonomic responses (5) and in regards to cortical structures involved in blood pressure control it has received the most attention.

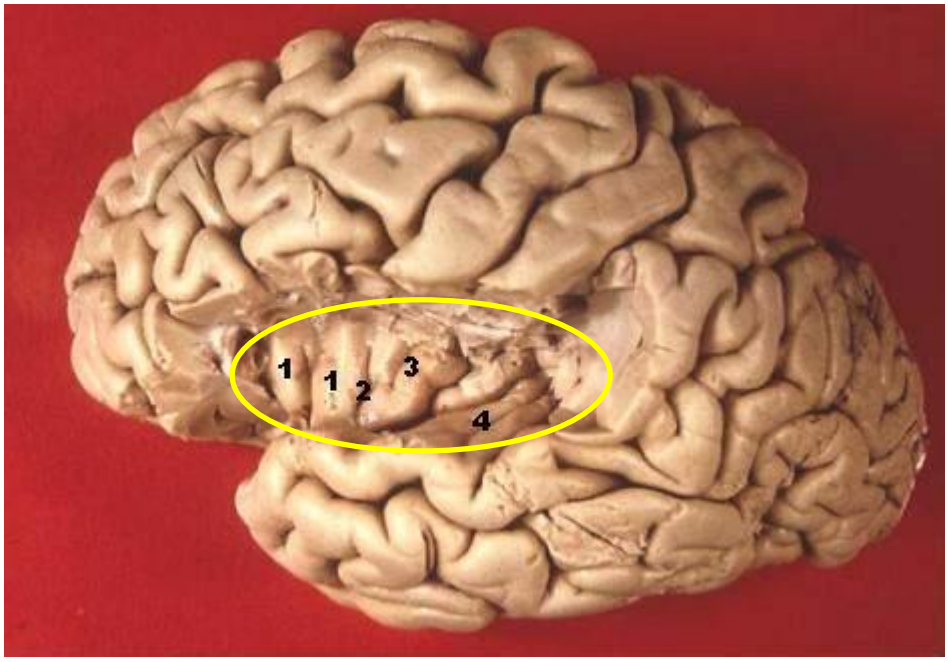

Figure 2.2.2.1 Insular cortex of the left hemisphere, exposed by removal of the opercula. Insular anatomical components: (**1)** Gyri breves insulae **(2)** Sulcus centralis insulae **(3)** Gyrus longus insulae and **(4)** transverse temporal gyri (adapted from (14)).

In animal studies both electrical and chemical stimulation of the insular cortex produced changes in blood pressure, heart rate and respiration (37; 124; 153; 274; 292; 363). Generally it was observed that stimulation of the rostral posterior IC increased BP and caused tachycardic responses and stimulation of the caudal posterior IC decreased BP and caused bradycardic responses. In a study by Oppenheimer and Cechetto, these chronotropic responses were observed independent of BP changes. They can be abolished by atenolol but not atropine suggesting their mediation through increases or decreases in sympathetic activity (253).

There is also evidence that the insular cortex is responsible for the modulation of baroreflex sensitivity in rats and monkeys (47; 297; 365; 366). Zhang and colleagues were able to identify 131 baroreceptor-related neurons in the insular cortex of anesthetized monkeys (365) and stimulation of baroreceptor afferents was shown to

change the firing pattern of neurons within the IC (47). In animal studies lateralization of cardiac control has been observed between the left and right hemispheres. In the study mentioned above by Zhang and colleagues more baroreceptive units were found in the right IC compared to the left (365). Another study conducted by Zhang et al showed that cortex lesions in the left posterior IC significantly increased baroreflex gain while lesions in the right posterior IC did not affect gain but significantly increased HR and BP (366). In a mouse model that utilized middle cerebral artery occlusion, elevated levels of epinephrine causing cardiac dysfunction were found following ischemia of the left IC but not those with right IC ischemia (220).

When examining human patients with ischemic stroke localized to the insula, there is a significant reduction in both low and high frequency heart rate variability and standard deviation of all normal-to-normal R wave intervals, commonly used measures of autonomic function (337). Recent neuroimaging studies conducted in healthy humans have observed activation of the IC in a variety of manoeuvres that elicit autonomic arousal such as mental arithmetic (61), Stroop task (106), and many more. The involvement of the IC in autonomic control is also reported in situations of physical stress such as inspiratory capacity apnea (199) and mild handgrip exercise (362).

Electrical stimulation of the IC has occurred in humans, specifically epileptic patients before undergoing temporal lobectomy for seizure control. A study by Oppenheimer et al also observed lateralization of cardiovascular control with stimulation of the left IC causing bradycardia and depressor responses and stimulation of the right IC causing tachycardia and pressor responses (255). Research conducted previously in our lab has shown with baroreceptor unloading there is a significant increase in the BOLD signal of the right posterior insula and not the left, suggesting the involvement of this

structure in sympathoexcitation (163). In summary, it seems that the right IC plays a predominant role in regulating vasomotor sympathetic tone and the left IC is predominant in establishing vagal tone and sympatho-inhibition (255).

As with the vMPFC, electrophysiological and neuroanatomical tracing studies show that the insular cortex projects to a wide range of brain structures involved in autonomic control. The pressor regions of the posterior IC project to the vMPFC and amygdala while the depressor regions project strongly to the primary somatosensory cortex and ventral basal thalamus (348). There is also reciprocal connectivity of the baroreceptor-related neurons between the left and right insular cortex (364). Of note, is the lack of corticospinal connection and efferent connection between the IC and ventrolateral medulla which was described above for the vMPFC (348). Another contrast is the large amount of afferent projections that converge on the IC compared to the vMPFC. The IC receives significant visceral afferent information by relays through the NTS, parabrachial nucleus and visceral sensory thalamic nuclei (46).

2.2.3 THE ANTERIOR CINGULATE CORTEX

The anterior cingulate cortex (ACC) comprises part of the medial prefrontal cortex located directly in front of the corpus callosum (Brodmann area's 24, 32, and 33) and participates in a diverse range of functions. The dorsal and subgenual (also referred to as ventral) ACC are two distinct structural and functional subdivisions of this area. The dorsal ACC (dACC) has been implicated in the cognitive functions of mediating response inhibition (41) and error processing (42). The ventral ACC (vACC) on the other hand has been implicated in the processing and integration of emotional stimuli (210; 315).

These processes are closely related to autonomic function and both subdivisions of the ACC have numerous connections to structures heavily involved in autonomic control, including the brain sites mentioned above (208). The pyramidal neurons in the ACC also project directly and indirectly to a variety of subcortical structures associated with homeostasis including the hypothalamus (251) and PAG (6). Contributions of afferent somatic and visceral information to ACC activity during contextual modulation of autonomic arousal (62; 64) also indicates a crucial role of the ACC in autonomic control.

Early animal studies using electrical stimulation in both subdivisions of the ACC observed autonomic responses, including changes in heart rate and blood pressure (153; 155). Similar to the insular cortex, dACC activation was correlated with increased sympathetic outflow following a variety of manoeuvres that induced cardiovascular responses such as the Stroop task (208), IHG (63) and baroreceptor unloading (163). In particular, patients with dACC lesions showed less sympathetic outflow and autonomic control of cardiovascular responses during cognitive and motor efforts compared to healthy subjects (63). There is some evidence that the vACC may contribute to the modulation of PNS activity as activation of the area correlated to increased high frequency heart rate variability (208). Critchley and colleagues argue for a central role of the ACC, amongst the CAN framework, in the production and control of behaviourally integrated patterns of autonomic activity (63).

2.3 AGE AND ANS FUNCTION

The normal aging process invokes a variety of changes within the human body including complex structural and functional changes to the heart and blood vessels of the cardiovascular system. Reduced cardiac output, a lower maximal heart rate and reduced

aerobic work capacity occur generally with age. One of the many changes in the CV system is arterial stiffening which leads to increased systolic blood pressure and pulse pressure (158). This arterial stiffening is generally thought to be brought on by increased sympathetic activity with advancing age which reflects significant changes to the autonomic control of the CV system, specifically the shift in equilibrium between sympathetic and parasympathetic tone.

The effect of aging on human sympathetic activity has been a much studied topic due to its high correlation with CVD, specifically increased incidences of essential hypertension, cardiac failure, and ventricular arrhythmias that occur with elevated sympathetic tone (159; 217; 301). Neurohumoral methods of examining sympathetic activity, such as plasma norepinephrine spillover, have indicated age-related increases in sympathetic activity due to elevated levels of whole-body norepinephrine concentration and spillover rate (132; 231).

To complement neurochemical methods, electrophysiological recordings of sympathetic nerve firing rates using microneurography have also been utilized. Increased MSNA recorded from the peroneal and tibial nerves in older adults under resting conditions was observed several decades ago (230; 325) and has been verified in more recent studies (150; 234). Peroneal nerve MSNA outflow was doubled in older compared to younger adults similarly matched for physical fitness levels (242). The attenuation of α-adrenergic responsiveness known to occur in older men (76) may explain the elevated MSNA activity at rest which is compensating for and providing sympathetic constrictor support for blood pressure (349). On the other hand, down-regulated receptor responsiveness may compensate due to an already elevated sympathetic drive.

Although advancing age does not significantly affect resting heart rate per se (182; 183; 321), it does affect the heart rate response to exercise (158; 306; 322; 331). Evidence from neurochemical observations suggests that sympathetic drive to the heart increases with age (86; 307), but older individuals generally produce a smaller tachycardic response to isometric (85; 258; 294; 331) and dynamic exercise (169; 294; 332). Decreased β-adrenergic responsiveness causes this diminished response (see Figure 2.3.1) which contributes to an attenuated left ventricular contractile response to exercise (307) despite older individuals having larger cardiac norepinephrine spillover (85).

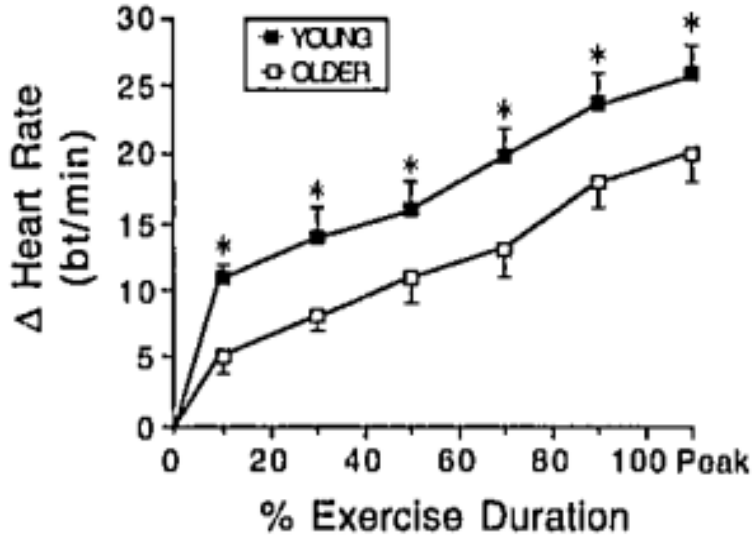

Figure 2.3.1 Heart rate response to isometric hand grip exercise at 40% MVC to exhaustion, are greater in younger male controls compared to healthy older men (adapted from (307)). Data are mean ± SE. Peak exercise = final 10% of exercise period. * $P <$ 0.05 vs. older men.

As mentioned in a previous section the heart rate response to exercise relies on the interaction between both the sympathetic and parasympathetic systems. Over several decades there have been many human studies that have looked at the decline in

parasympathetic control of the heart with age (80; 119; 181) with less withdrawal of cardiac vagal tone causing decreases in heart rate response to exercise (307). Decreased vagal control of the aging heart also compromises baroreflex gain (see Figure 2.3.2) (158; 226). Unfortunately, in humans, we are limited in our ability to dissect out points throughout the baroreflex loop which are most affected by physiologic aging but some are presented here.

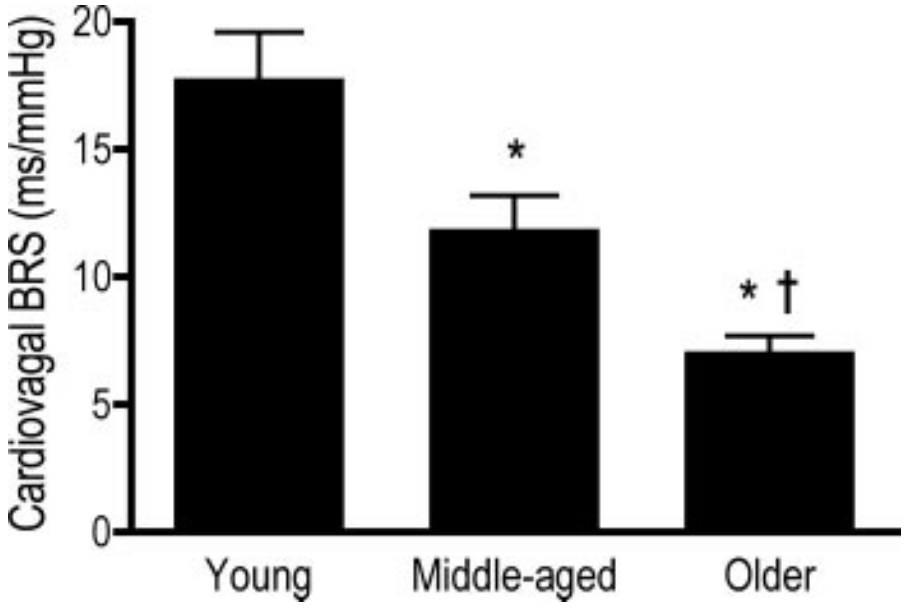

Figure 2.3.2 Declining cardiovagal BRS values from young (18–37 years old), middle-aged (38–56 years old), and older (57–79 years old) adults (taken from (226)). * $P < 0.05$ vs. young subjects. † $P < 0.05$ vs. middle-aged.

It is unknown whether a given baroreceptor stimulus induces the same afferent signals in young adults as it does with older adults (226). In a study conducted on rats, older animals required more of a vessel wall distortion to reach the same neural discharge patterns observed in younger animals (7). The increased arterial stiffening, mentioned earlier, that occurs with age may impair baroreflex function due to structural (i.e. atherosclerosis) or functional (i.e. reduced nitric oxide activity) changes in the vessels

(287). Because arterial stretch is a key component of baroreflex activation (9), stiffening within the barosensory segments of the reflex loop such as the aortic arch and carotid arteries may reduce the stimulus applied to the baroreceptors during a given change in blood pressure (226). This in turn would lead to a blunted baroreflex-mediated change of heart rate in older adults.

In regards to another point in the baroreflex arc, the actual end organ response (i.e. changes in R-R interval) could be interrupted via a host of factors. For example, in a canine heart failure model, a defect was observed in the pre-junctional parasympathetic ganglion which could result in diminished vagal control if neural impulses cannot be transmitted efficiently (16). Additionally, it has also been suggested that the aging heart has decreased M_2 muscarinic receptor density (32), altered post-junctional cholinergic signalling mechanisms, decreased cardiac responsiveness to muscarinic receptor activation (188; 272), and potential changes in pre-junctional regulation of Ach activity (158). Any of these changes would be enough to cause an impaired ability of vagal nerve activity to elicit an end organ heart rate response.

These changes could also explain the reduced heart rate variability that occurs with aging. Even though aging does not necessarily alter resting heart rate, it does decrease resting heart rate variability and is discernible even after 10 years of age (171; 245; 316). Spectral analysis studies have shown specifically that the high frequency power in the heart rate variability spectra and the high-to-low frequency power ratio (both used more often as a measure of PNS control than total power) is diminished with age (117; 293). In summary, older individuals seem to have a reduced range of parasympathetic and sympathetic responses to physiological stressors as well as a less adaptive and responsive cardiovascular system due to declines in autonomic control with age. Interestingly, these

declines may be explained by alterations in central processing pathways from higher cortical centres. Robinson and colleagues determined that baroreflex gain can be altered acutely by stroke (287), pointing to another site in the baroreflex arc that may be affected by age, the brain.

2.4 EFFECTS OF AGE ON THE BRAIN

Unfortunately, the cardiovascular system is not the only system in the body affected by physiologic aging. The brain is also negatively affected by age (34), which in turn may contribute to the dysfunction of the ANS previously discussed, as the cortical autonomic network is a modulator of CV control (48). Much of the research that has been conducted in this area examines age-related structural alterations with less of a focus on functional changes. As early as 1928 scientists had noticed age-related neural deterioration (336) and it can be summarized succinctly in this quote by neurobiologist Frederick Tilney, *"The senile brain is always small and atrophied. Its weight and volume are much diminished. The atrophy is predominant in the frontal lobe...The white matter of each individual convolution is decreased."* To complement this, more recent widespread findings have also found ventricular enlargement (30; 90; 93; 270) total brain atrophy (91), loss of cortical thickness (90) and decreased grey and white matter volumes (280) (see Figure 2.4.1).

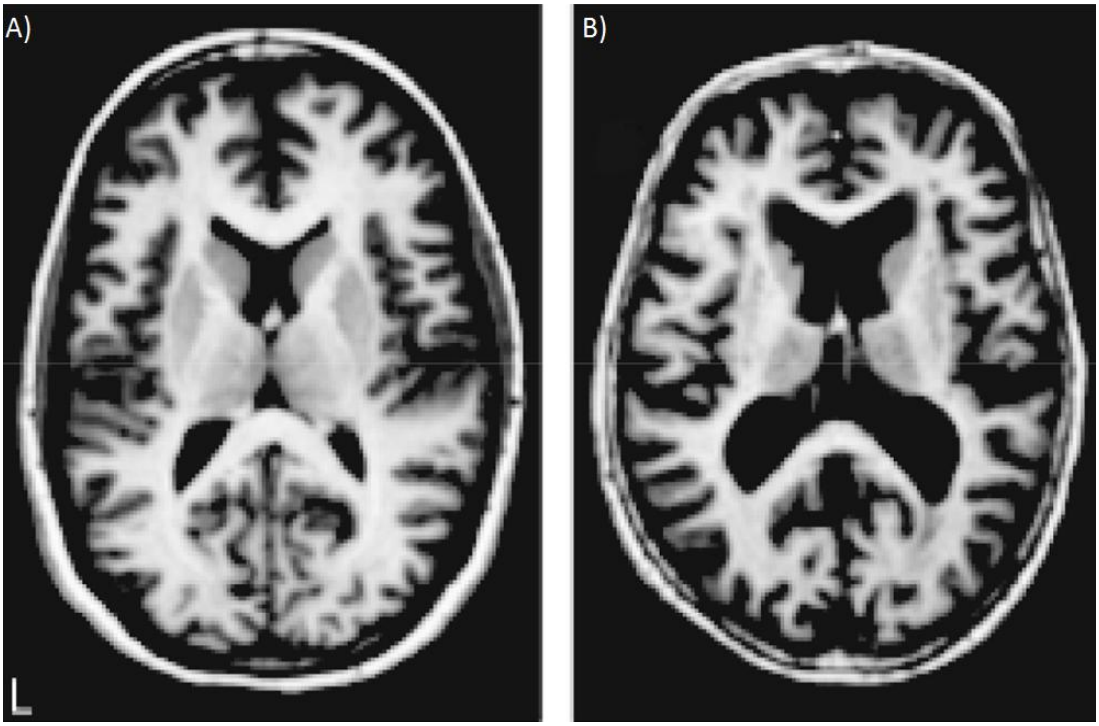

Figure 2.4.1 Sagittal MRI images, presented in neurological convention, of A) young adult brain and B) 94 year old brain free of dementia. Notice the enlarged ventricles and total tissue atrophy (adapted from (136)).

The general scientific consensus is that age influences total brain volume negatively but there are large differences between specific structures with some structures declining substantially in old age and others being better preserved. In addition, different structures appear to have different age trajectories with some declining linearly with advancing age and others following a quadratic path (90). To date there are more than 50 cross sectional magnetic resonance imaging studies that have tested the effects of age on the volume and thickness of various brain structures (90). Grey matter (GM) specifically is reduced with age and its decline can begin relatively early in life (59; 113; 148; 279; 283; 295; 324; 352). In an extensive review, the caudate and putamen (components of the basal ganglia) were the subcortical structures most severely affected by age with generally larger effects for the putamen than the caudate (90). Grey matter loss in the cortex is somewhat greater

than in subcortical structures (352; 353) with the effects of age most prominent in the frontal and prefrontal areas (1; 31; 92; 113; 295; 327). These results support the "last in, first out" hypothesis meaning the last areas of the brain to develop phylogenetically and ontogenetically are the first to be affected by normal aging (90). The fact that numerous studies have found decreased volumes in these areas also corresponds well with neuropsychological studies that show executive processing skills (which depend heavily on frontal circuitry) are the cognitive functions most affected by age (303).

The effects of age on white matter (WM) volume are different compared to those seen for GM. White matter consists largely of myelinated long distance axonal projections that connect many areas of the brain (90). Typically WM volume continues to increase until 40 to 50 years of age before rapid accelerating volume reductions occur (141; 149; 280; 296). Jernigan and colleagues discovered that despite the later onset of decline, WM loss actually exceeded that of GM (149). However, similar to GM the strongest effects of WM volume-age dependent relationships were found in the frontal and temporal areas (296). Post-mortem studies in both humans and primates have confirmed WM decreases observed with magnetic resonance studies and shown loss and shrinkage of myelinated fibres (205; 267; 271).

Recently, there has been great interest in determining the cellular and molecular mechanisms behind the neurobiological processes that are responsible for these morphometric changes. There are many hypotheses with one of the more popular centred around neurotrophins. Neurotrophins are a class of proteins that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5). Neurotrophins are especially important for brain plasticity and development, and may explain regional differences in structural decline as well as

individual variations (90). Neurotrophins are involved in regulation of neuronal survival (3; 221), axonal growth (52), synaptogenesis (256) and neurotransmission (198).

BDNF has received particular attention because it is expressed highly in the prefrontal cortex and hippocampus (209) and reduced levels of BDNF in various regions of the brain have been correlated with neurodegenerative and psychiatric pathologies (269). Serum and plasma BDNF levels have also shown declines with advancing age (110; 194). Driscoll and colleagues were the first to show in a recent longitudinal study that decreases in plasma BDNF were associated with steeper rates of age-related volumetric decline (79) and may also be associated with neuronal loss (97). BDNF has also received much attention for its single nucleotide polymorphism known as Val66Met. Compared to the Val-BDNF carriers, individuals with the Met-BDNF gene show larger age-related reductions of prefrontal cortical (241) and amygdala volume (323).

There is ample evidence that normal aging causes morphometric changes in the brain and that a regionally heterogeneous pattern occurs. The difficult part is determining to what degree these structural changes lead to altered neural function and cognitive abilities; the cause and effect relationship is difficult to pinpoint. Scientists generally agree that aging is associated with reduced cognitive functions in the areas of mental speed, episodic memory, executive and flexible cognition, and non-verbal problem solving (90). There is major motivation to study areas in the brain responsible for the control of these functions (such as the hippocampus), specifically in the context of neurodegenerative pathologies like Alzheimer’s disease. Comparatively, less attention is paid to areas in the brain that form the CAN and are responsible for autonomic cardiovascular control.

2.5 ENDURANCE TRAINING AND ANS FUNCTION

Endurance training (ET) contributes to a healthier cardiovascular system with some of the many adaptations including increased maximal cardiac output, stroke volume, hemoglobin concentration, greater capillary density, and decreased resting heart rate, blood pressure and systemic vascular resistance. It has been suggested that the beneficial effects on the CV system through increased physical activity occurs via alterations in neural control of circulation (17; 58; 367). In 1977, Scheuer and Tipton suggested in a review of literature that resting bradycardia due to ET may be due in part to both a decrease in sympathetic and an increase in parasympathetic influence (299). This shift in autonomic tone that occurs with ET has been the focus of much research over the past few decades.

Evidence from rodent studies has consistently supported reductions in resting and reflex-mediated sympathetic outflow due to increased physical activity (232). Exercise training in rats (174; 239) and rabbits (74; 75) has shown suppression of resting sympathetic activity and baroreflex-mediated outflow of renal sympathetic nerve activity. There is also indirect evidence that treadmill training in both rats (175; 240) and mice (72) can decrease cardiac sympathetic nerve activity.

In humans, the effects of exercise training have been less consistent in regards to sympathetic activity. Some suggest endurance training decreases resting sympathetic tone (58) due to a 40% reduction in whole-body plasma norepinephrine levels after a training program. Others suggest it may just influence the sympatho-excitatory response to exercise and other stressors, such as baroreceptor unloading (232; 278). Earlier studies, some with the use of autonomic blockade, have shown that endurance training does decrease efferent sympathetic outflow directly to the sinoatrial node (19; 317).

Alternatively, peripheral adaptations to endurance training that act to decrease systemic vascular resistance, such as decreased arterial stiffness, may contribute to lower resting HR and BP in more fit individuals (216; 328; 340). The effects of exercise training may be more pronounced in populations that have elevated sympathetic activity, such as heart failure patients, compared to normal individuals. Roveda and colleagues demonstrated that patients who completed a 4 month exercise training program had dramatic reductions in resting MSNA compared to a sedentary group of patients (291).

While the results of training on sympathetic activity are less in agreement, most researchers conclude that endurance training does act to increase parasympathetic tone. Human studies that have utilized autonomic blockade to examine PNS control of the heart have shown after an endurance training regimen there is increased vagal tone (310; 317). Importantly, these studies saw large increases in the VO_{2max} of the trained individuals, whereas studies that have reported smaller increases in maximal oxygen uptake after training also saw no change in parasympathetic control of the heart (82). Many studies that have used spectral analysis approaches to study changes in autonomic function with exercise report increases in PNS tone at rest (see Figure 2.5.1). The linear relationship between heart rate variability and aerobic power has been studied since the 1970s in anaesthetized dogs (156) and humans (98; 160) with strong correlations observed.

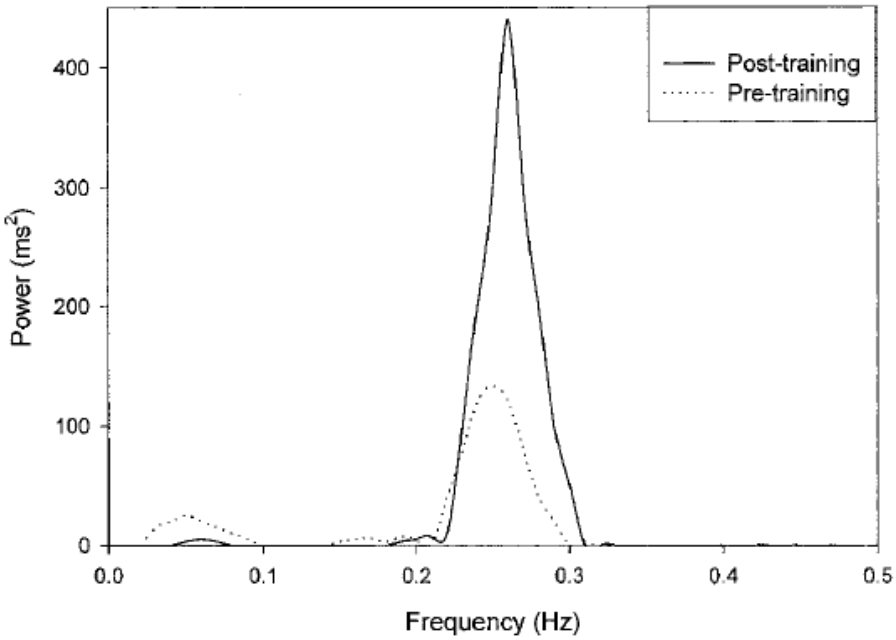

Figure 2.5.1 The power spectrum for heart rate variability of a 20-year old female comparing pre-training (dashed line) to post-training (solid line) in supine position breathing at fixed rate of 15 breaths/min (taken from (44)).

In cross-sectional studies that compared autonomic profiles of endurance athletes to non-athletes, the endurance athletes had a significantly greater high frequency power spectrum of R-R interval variability compared to non-athletes, suggesting increased cardiovagal control (77; 313). In longitudinal studies of professional endurance athletes completing aerobic exercise programs leading up to professional events, heart rate variability measurements were elevated (128; 275). Even in normal young adults who underwent a 12-week endurance training program showed significant improvement in heart rate variability at rest in both the frequency (total power) and time (SD) domains (44). Other longitudinal studies conducted in older adults have also come to the same conclusions (304; 320).

Another aspect of autonomic control of the heart that has been mentioned previously is baroreflex function. In numerous studies examining a variety of populations,

endurance training has led to increased baroreflex sensitivity which is usually indicative of increased vagal control of the heart (57; 140; 170; 178; 184; 212; 227-229; 290). In rabbits an 8-week treadmill training program was able to increase the range and sensitivity of baroreflex control after chemical and mechanical manipulations of blood pressure (74). In younger humans both cross sectional (170) and longitudinal studies ranging from 4 to 10 weeks (57; 212) have shown that endurance training is able to increase cardiovagal baroreflex function. In one longitudinal study, baroreflex slope at rest increased by as much as 50 ± 6.3% after endurance training (212). In middle-aged humans with coronary artery disease (140; 178) or hypertension (184) endurance training programs of varying lengths have been just as effective at enhancing baroreflex cardiovagal control.

In older adults, endurance exercise training also seems capable of maintaining BRS despite the age-associated declines that occur (as mentioned in section 2.3). In cross sectional studies of both older men (228; 229) and postmenopausal women (69; 70), there was evidence of greater BRS in endurance trained individuals compared to sedentary age-matched controls. In a longitudinal study utilizing a 13-week aerobic intervention program, 13 older previously sedentary men showed increased cardiovagal BRS compared to pre-intervention conditions (227). Regular exercise also increased carotid arterial compliance in these older men and this was strongly correlated with the increases observed in cardiovagal BRS ($r = 0.72$; $p < 0.01$). Increased vessel wall compliance of the carotid artery may be one mechanism responsible for exercise-induced increases in BRS (170) and has been observed before in older populations (227; 229). Another possible mechanism may be neural alterations in the baroreflex loop. Evidence for this has been difficult to discern in studies of older adults but has been demonstrated in younger men

using carotid lumen diameter with corresponding R-R interval and reporting no differences in carotid artery compliance between trained and untrained groups (170).

It should be mentioned however, that an emerging body of literature suggests that very high levels of endurance training can have detrimental effects on the human body. In some individuals long-term excessive ET may lead to adverse CV structural and functional changes that diminish the benefits conferred by a more moderate training program. A fifteen year observational study following 52,000 adults found a 19% reduction in all-cause mortality compared to non-runners but the mortality curve was U-shaped based on speed, distance and frequency. Higher mileage, more frequent runs and a faster pace was not associated with better survival (187). Also, a randomized crossover trial of coronary heart disease patients, which assigned subjects to either a 30 or 60 minute ET exercise session, showed that the 30 minute session was more beneficial. The 60 minute session worsened oxidant stress and increased vascular stiffness as measured by pulse wave velocity in the older patients (218). Chronic restructuring of the heart, such as dilated right ventricle/atria and myocardial fibrosis, due to sustained and cumulative ET sessions may result in serious electrical remodelling causing ventricular arrhythmias and atrial fibrillation (246). These alterations may have a serious and negative impact on the autonomic neural control of the CV system.

2.6 EFFECTS OF ENDURANCE TRAINING ON THE BRAIN

It was long thought that brain plasticity, defined as functional or structural changes that occur in response to perturbations in the external environment or internal milieu (219), occurred strictly during critical periods of development. Over the past few decades it has become widely recognized that brain remodelling also occurs in the mature brain,

suggesting that plasticity is an inherent property of the adult brain (262). There is an increasing body of evidence that physical activity and exercise training, including endurance training, are powerful catalysts that stimulate neuroplastic changes in the brain and central nervous system.

In both humans and rodents physical activity enhances cognition (288; 326; 342; 360), attenuates age-related memory decline (172; 186; 343), delays onset of neurodegenerative diseases (102; 185; 335) and can enhance recovery of brain injury (22; 107; 116). The areas involved in cardiovascular and autonomic control have received considerably less attention and so the involvement of a neuroplastic mechanism in exercise-induced improvements of cardiovascular function has not yet been fully elucidated.

There are numerous beneficial structural changes that occur in the brain due to increased aerobic fitness. One of the first studies to examine the structural preservation of the brain in older adults (mean age 66.5 years) due to increased cardiovascular fitness noticed a substantial sparing effect on the GM in the prefrontal, superior parietal and temporal cortices and the greatest conservation of WM was the anterior tracts and the transverse tracts between the frontal and posterior parietal lobes (53). Cross sectional studies in healthy older adults showed increased cardiorespiratory fitness resulted in increased gray and white matter volumes in the prefrontal cortex (54), and prevented atrophy in the prefrontal and medial temporal lobes of patients with Alzheimer's disease (40; 135).

A 9-year longitudinal study that was part of the Cardiovascular Health and Cognition Study determined that older adults who walked greater distances were able to preserve more GM volume in the frontal, temporal and occipital lobes as well as the

entorhinal and hippocampus, compared to adults who reported walking less distance over the nine year period (83). Even a moderate intensity aerobic fitness program carried out over one year was effective at increasing anterior hippocampal volume by 2% on top of mediating the average 1-2% loss that would have occurred due to age within the same time period (84). There was however no volume increase in the posterior hippocampus, thalamus or caudate nucleus (84) indicating a regional selectivity of the beneficial effects of exercise.

Animal studies have been able to complement human research and explain possible reasons for the increased brain volumes that occur with increased aerobic fitness. Researchers were able to determine a significant positive correlation between neuron cell proliferation and survival in the dentate gyrus of the hippocampus and the distance run on a wheel (4). Voluntary exercise on a running wheel enhanced the survival of new neurons and increased cell division in mice (342). According to a review by van Praag in 2008, enhancement of neurogenesis in the hippocampus is a robust phenomenon but literature regarding other areas in the brain remains controversial (341). Physical activity has been found to enhance proliferation of microglia in superficial cortical layers and astrocytes in the motor cortex of rodents (81) and increased voluntary running was associated with elevated numbers of both astrocytes and oligodendrocytes in the rat prefrontal cortex (203). Michelini and Stern, in a recent review summarized that an increasing body of evidence shows exercise training does induce neuroanatomical changes through neurogenesis, synaptic plasticity and dendritic remodelling (see Figure 2.6.1).

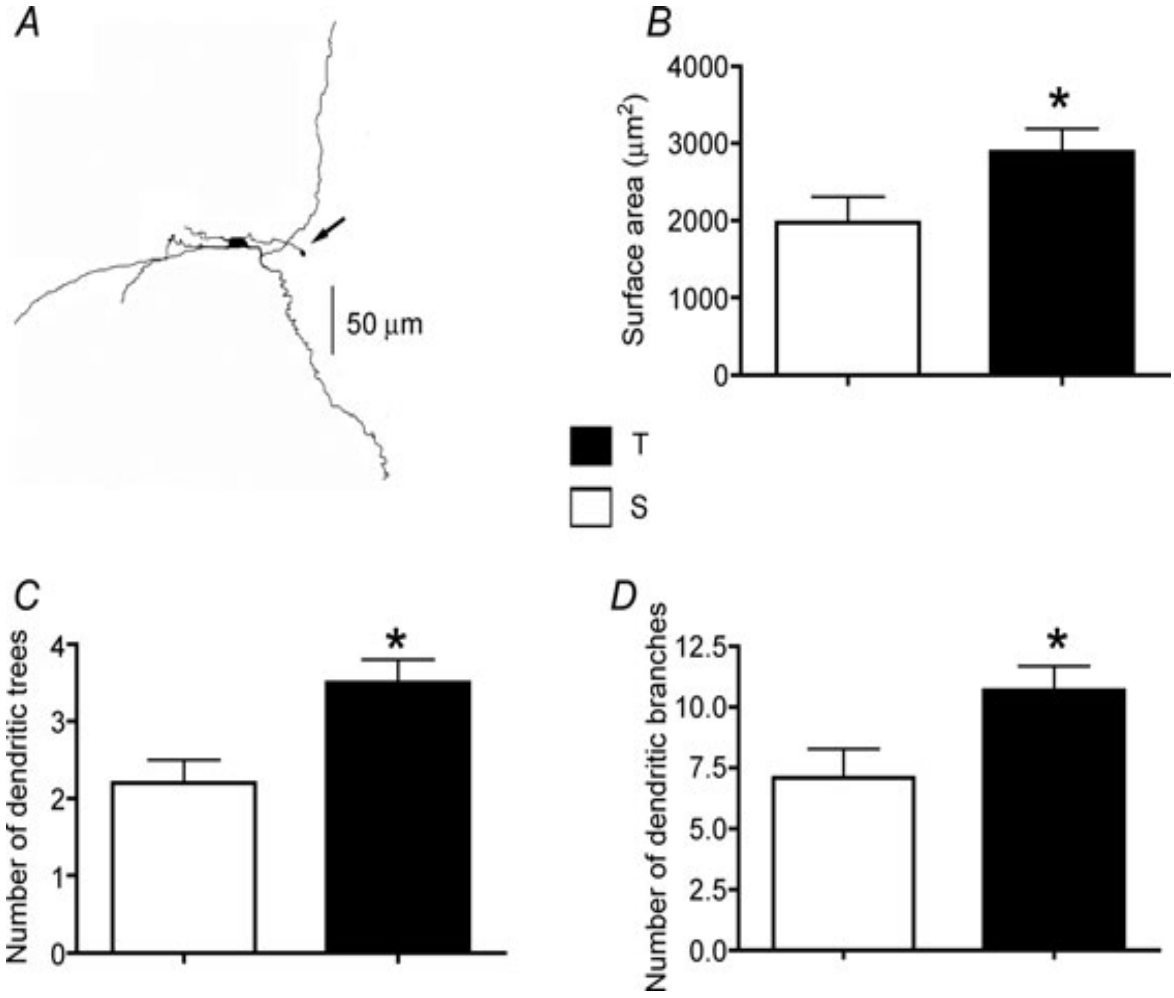

Figure 2.6.1 Example of the structural neuroplasticity observed with exercise training taken from the paraventricular nucleus and nucleus tractus solitarius in rats. **(A)** An example of a retrogradely labelled PVN-NTS neuron, with the arrow pointing to the axon. **(B)** Neuronal surface area, **(C)** number of dendritic trees, and **(D)** number of dendritic branches between trained (T) and sedentary (S) rats. * denotes significant difference ($P < 0.05$) between trained and sedentary animals (taken from (219)).

Despite the amount of literature that has described the beneficial effects of aerobic fitness on the structure and morphology of the brain it is more difficult to ascertain its effects on function. Even small changes in aerobic fitness can lead to improved executive function, a task predominantly carried out by the frontal and prefrontal cortices (172). Improved executive function was seen in a group of younger adults even after an acute bout of cardiovascular exercise, without an aerobic training program (131). In a

longitudinal randomized trial over six months Colcombe and colleagues found that during a focused attention task, aerobically trained older adults showed increased neural activity in frontal and parietal regions and reduced activity in the dACC compared to control subjects (55).

Until we better understand the molecular mechanisms and signalling pathways that control cortical function, it will be difficult to determine how exercise training ameliorates CNS deterioration. Previous studies in adult animals concluded that metabolic alterations such as angiogenesis in the cerebellar (18) and motor cortices (167) and neurochemical changes such as elevations in neurotrophic factors (237; 238) occur with increased aerobic fitness. In humans they have also observed increased vascularization (39; 264) but the majority of studies have been interested in changes of neurotrophic factors with aerobic exercise. Although there are many neurotrophic factors involved in promoting neural plasticity, in terms of the brain and exercise, BDNF has been the centre of most of the research in this particular area.

The release and expression of BDNF, unlike other neurotrophins, seems to be especially susceptible to regulation by physical activity (196; 300). In healthy humans, even short-term exercise increases circulating BDNF levels (89). This activity dependence of BDNF is especially prominent in hippocampal neurons where there is a high expression of BDNF mRNA (344; 358) but has been noted in other central nervous system structures such as the spinal cord, cerebellum and cerebral cortex (111; 112; 168; 238; 358).

In a recent longitudinal study of young sedentary males, three months of endurance training was able to significantly increase resting levels of venous BDNF compared to the untrained group (309). Because BDNF can cross the blood brain barrier in both

directions, peripheral concentrations of BDNF may represent an important reserve of the brain (259). This longitudinal study was complimented by a training study conducted in mice to localize where in the brain might account for the increased levels of BDNF. The 5-week treadmill training program increased BDNF mRNA expression in the hippocampus by 317 ± 38% with no significant change of expression in the cerebral cortex (see Figure 2.6.2) (309).

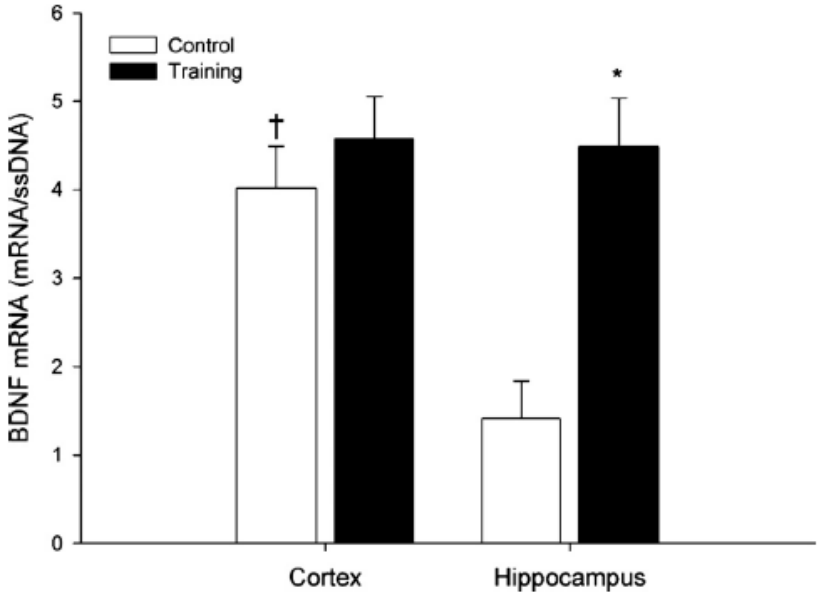

Figure 2.6.2 BDNF mRNA expression in the cortex and hippocampus of trained and control mice. Control mice had greater mRNA expression in the cortex compared to the hippocampus. mRNA levels between the cortex and the hippocampus were not significantly different in trained animals, but BDNF expression in the trained hippocampus was 317% greater than in controls. There was only a slight increase in mRNA in the cortex with training. * denotes significant ($P < 0.05$) vs. untrained and † denotes significant ($P < 0.05$) vs. hippocampus. All values are means ± SE (taken from (309)).

Although there are not many studies in older adults, a study by Erickson and colleagues demonstrated that an aerobic training program in men (mean age 66 years) showed an increase in serum levels of BDNF compared to a control group, which was

associated with greater hippocampal volume (84). Because BDNF is key in the regulation of synaptic proteins that are needed for things such as axonal elongation, formation and maintenance of presynaptic structure, and neurotransmitter release, exercise-induced increases in BDNF may lead to new synaptic formation and efficacy of synaptic transmission (344). Thus the role of cardiovascular fitness as a protector and enhancer of neural function and CNS integrity in older adults appears to have a strong biological basis.

2.7 SUMMARY

In summary, the physiological impacts of aging are widespread and detrimental to many systems in the body. It impacts autonomic function by generally decreasing parasympathetic activity and thereby shifting the dynamic equilibrium between SNS and PNS tone. This increased sympathetic function that occurs with age has been correlated with increased morbidities of the cardiovascular system. Aging is harmful to the brain causing morphological changes consisting of total tissue atrophy, decreased white and grey matter volume and reductions in cortical thickness. Functional declines most often studied revolve around cognitive abilities that are associated with the prefrontal cortex, including the loss of executive processing skills and episodic memory. In terms of the function of other structures found in the prefrontal cortex, including those of the cortical autonomic network, much less attention has been given to how they may be affected by age.

There may be a way to ameliorate the effects of aging through endurance training. Endurance training has many benefits including increasing cardiac output, capillary density, decreasing resting heart rate and reducing blood pressure amongst many others.

Many of these benefits may be the direct result of alterations in autonomic neural control of the cardiovascular system. Many cross sectional and longitudinal studies examining the impacts of endurance training specifically on autonomic function have observed increased indices of parasympathetic control including improved cardiovagal BRS and greater HF HRV. The heart rate response to an exercise task of short duration and moderate intensity is predominantly due to vagal withdrawal so the heart rate change itself can also be used as an index of parasympathetic function.

So the question becomes, does increased parasympathetic function caused by endurance training stem from the beneficial effects endurance training has on the cortical autonomic network? Endurance training has been shown to have positive effects on forebrain morphology and cognitive function. To date however, no one has examined the impacts of long-term endurance training on the structures of the cortical autonomic network which are also found in the forebrain. Perhaps if endurance training can preserve the function of these structures involved in autonomic cardiovascular control this would extend and translate into improvements in baroreflex function and may contribute to an improved heart rate response to exercise.

CHAPTER 3: TOOLS & TECHNIQUES

3.1 BRACHIAL ARTERY BLOOD PRESSURE

Continuous non-invasive beat-to-beat blood pressure measurements were recorded using the Finometer® (Finapres Medical Systems, Amsterdam, Netherlands). The Finometer® measures finger arterial blood pressure (FinAP) and, using several predefined methods, permits the reconstruction of brachial arterial blood pressure (reBAP) (120). Making use of the volume clamp method first described by Czech physiologist Penaz (20), continuous and accurate FinAP can be measured. The Finometer® uses a finger cuff with an inflatable bladder and an infrared plethysmograph with a light detector. The air bladder is inflated so that the diameter of the artery is kept constant (clamped) at a specific diameter known as the "set-point" despite changes in arterial pressure with each heart beat (21). Any changes in diameter are detected by the infrared plethysmograph and through a servo-controller system the air bladder is either rapidly inflated (during systole) or deflated (during diastole) to maintain a constant diameter and keep the transmural pressure across the arterial wall at zero (21). Keeping the transmural pressure at zero is important as this represents the unloaded diameter of the artery (357). As a result, the finger cuff pressure provides an indirect measurement of the intra-arterial pressure and an arterial brachial waveform can be calculated each cardiac cycle.

It is not a simple calculation converting the arterial BP waveform obtained from the finger into a recalculated brachial BP waveform. There are physiological differences that need to be accounted for such as differences in pulse shape and pressure levels (120). There are several methods that the Finometer® uses to correct for these physiological

differences. First, an inverse transfer function or waveform filter is applied to the finger pressure waveform. The systolic and diastolic pressures of the filtered waveform are averaged over 30 seconds (prior to the return-to-flow calibration explained below) and these averages are then used in the calculation of the correction formulas (121). A level correction is then applied automatically, owing to the fact that finger BP is usually lower than brachial BP so the waveforms must be shifted upwards (121). The final step is a return-to-flow (RTF) calibration (26) based on the systolic pressure measured using an arm cuff. This way a subject's individual RTF shift can be permanently applied to beat-to-beat measurements (121). One RTF calibration in the supine position is sufficient to meet the requirements of the Association for the Advancement of Medical Instrumentation (AAMI) for automated BP monitoring (121). The resulting waveforms after all of the above corrections have been applied are then labelled RTF intrabrachial arterial pressure (reBAP).

There have been numerous articles that have examined the accuracy of non-invasive blood pressure measurements compared to results obtained using intra-arterial methods (26; 121; 143). Non-invasive BP measurements have been validated in healthy older adults (142), whose mean age was older than the subjects in this study. Some potential limitations have been mentioned, for example brachial SBP may be overestimated due to PP amplification (143) but as long as the corrections are applied when reconstructing brachial BP waveforms the differences in pressure can be reduced substantially (121). It is also possible to obtain manual BP measurements from the opposite arm to be used for calibration. Regardless, the majority of findings in a variety of groups suggest that the Finometer® is an appropriate method for obtaining beat-to-beat BP measurements that comply with AAMI requirements. This continuous monitoring of arterial BP was an

important tool for calculating variables associated with PNS activity, such as baroreflex sensitivity.

3.2 BAROREFLEX SENSITIVITY

Baroreflex sensitivity (BRS) has long been used as a tool for assessing the autonomic control of the CV system, particularly PNS control. Recently, non-invasive methods for examining BRS have been developed and offer clear advantages over more traditional methods such as simplifying test procedures, minimizing subject risk, lowering costs and allowing testing under a broader range of conditions (179; 356).

A non-invasive method for measuring cardiovagal baroreflex gain known as the sequence method involves examining spontaneous beat-to-beat covariations in SBP and R-R interval and extracting the magnitude of the changes across sequential beats (260). This technique is based on the identification of three or more sequential beats in which progressive increases/decreases in SBP are followed by progressive lengthening/shortening of the R-R interval. To be included in the sequence, changes in SBP and R-R interval must meet specified threshold values, 1 mmHg and 6 ms respectively (179). The mean slope of the regression line between all sequential changes in SBP and R-R interval is taken as the measure of baroreflex sensitivity.

The sequence method for determining BRS has been validated using more traditional pharmacological methods, often described as the modified Oxford technique, with positive correlations between BRS estimates (261; 355) in various populations including the elderly (147) and hypertensive individuals (356). The sequence method is also capable of detecting significant differences between groups that have differing degrees of autonomic function. For example, in one study comparing diabetics with no

autonomic neuropathies and age-matched controls the sequence method determined a significant decrease in BRS in the diabetics compared to the controls where a classic laboratory test had failed to determine any autonomic dysfunction (100). This suggests that the sequence method for determining baroreflex function may be a more sensitive measure for determination of autonomic dysfunction and can detect differences in groups that may not have been noticed with traditional methods. There are still more advantages to the sequence method, including the fact that computations using an algorithm developed for specific software programs are automatic and standardized which helps to eliminate intra- and inter-subject variability.

3.3 HEART RATE VARIABILITY FREQUENCY DOMAIN ANALYSIS

Heart rate variability (HRV) is the term commonly used to describe the beat-to-beat oscillations in R-R interval. There are several methods that have been developed to analyze HR variations which generally fall under two broad categories of “time domain” or “frequency domain”. Frequency domain measures of HRV use spectral analysis of a sequence of R-R intervals, and utilize Fast Fourier Transform, to provide information on how the variance (or power) is distributed as a function of frequency (180).

Numerous studies have utilized power spectrum methods to analyze HR fluctuations and have shown that in short term recordings the power is concentrated in three spectral peaks with one of these peaks centered around the respiratory frequency (2). The three peaks are characterized as very low frequency (VLF; 0.003 to 0.04 Hz), low frequency (LF; 0.04 to 0.15 Hz), and high frequency (HF; 0.15 to 0.4 Hz) (180). It is this HF component that coincides with the respiratory frequency and it is thought that this reflects the modulation of vagus nerve charge with respiration (180).

A study using glycopyrrolate to block muscarinic parasympathetic receptors in the cardiovascular systems of conscious dogs showed complete abolition of the mid- to high-frequency spectral peak (2) suggesting that PNS control operates at higher frequencies. Similar results were observed in a study that examined both supine and standing positions in young healthy humans using atropine to block parasympathetic muscarinic receptors (273). In short even though it is an indirect method, analysis of HF HRV power seems to be a good indicator of PNS control of the heart.

3.4 FUNCTIONAL MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) is a medical imaging technique that has become very popular in the field of radiology. It has evolved from its humble beginnings in the 1970s to become the "imaging method of choice" for a large number of radiological examinations. Considering the breadth and depth of scientific disciplines that have contributed to the evolution of MRI, the physical properties of the technique are beyond the scope of this thesis. However, I will highlight some of the basic principles to provide brief background knowledge.

In MRI we are only interested in the nucleus of an atom, particularly the hydrogen atom. Approximately 70-90% of the human body is composed of water where the majority of the hydrogen atoms are found. Each hydrogen nucleus contains one positively charged proton that continuously spins on its own axis and basic electromagnetism states that a moving charge has its own associated magnetic field. Therefore each hydrogen nucleus creates its own tiny field known as a magnetic moment. When a human body is placed in the middle of a strong external magnetic field, the magnetic moments ("spin") of the hydrogen nuclei experience torque. This causes a shift towards equilibrium where

the once randomly spinning nuclei become aligned with the external magnetic field producing a net magnetization within the body (213).

Unfortunately, this net magnetization is virtually undetectable when in equilibrium compared to the strength of the external magnet used to induce it. Magnets used in MRI scanners typically produce magnetic fields measureable in Teslas (T) whereas the net magnetization of the human body is on a microtesla (μT) scale. This alone will not produce an image of biological tissue and so MRI uses radiofrequency (RF) pulses to excite the protons and essentially "tip" the net magnetization into a perpendicular frame of reference. The voltage that is produced by the vector sum of all the magnetic moments is measured by a receiver coil which is sensitive only to magnetization perpendicular to the external magnetic field (see Figure 3.4.1). Once the RF pulse ends there is an exponential decay of the signal caused by the random interaction of the hydrogen nuclei. This is known as *spin-spin relaxation time* (T_2) and describes the time it takes the tissue to return to equilibrium (213).

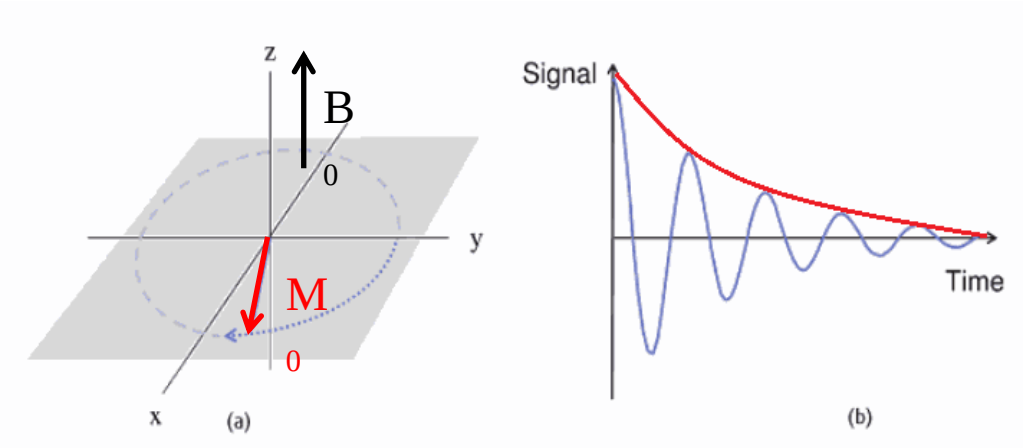

Figure 3.4.1 (A) Precession of the tipped net magnetization (M_0) in the transverse plane (perpendicular frame of reference to the main magnetic field (B_0). (B) Voltage signal induced in the receiver coil by M_0 undergoes exponential decay due to random interactions of hydrogen molecules (adapted from (213)).

Not only is it a valuable diagnostic tool, allowing us to examine anatomical structure and pathologies *in vivo*, but at its most advanced MRI allows us to investigate organ function and even visualize the brain "thinking". Functional magnetic resonance imaging (fMRI) is a relatively recent development in MRI and has become quite popular in both research and clinical settings. Functional magnetic resonance imaging is based on the Blood Oxygenation-Level Dependent (BOLD) effect. This effect was observed at the start of the 1990s in animal-based experiments by Ogawa and co-workers (247-249) and is dependent on the different magnetic properties of oxygenated and deoxygenated blood, as well as the concept of neurovascular coupling (311).

Neurovascular coupling is the process by which neural activity influences the hemodynamic properties of the surrounding vasculature (67). With neuronal activity there is a local hemodynamic response (HDR) (see Figure 3.4.2) consisting of changes in cerebral blood flow and blood volume with changes in metabolism (177). When nonmagnetic (i.e. diamagnetic) oxyhemoglobin (Hb) releases oxygen molecules to neurons, the resulting deoxyhemoglobin (dHb) becomes magnetic (i.e. paramagnetic) due to an unpaired electron. A magnetically inhomogeneous environment for tissues surrounded by deoxygenated blood is thus created, translating into a lower BOLD signal (ratio of Hb:dHb) due to a shorter T_2. Activation of neurons in the brain causes a disproportionate increase in fresh oxygenated blood at the downstream site (i.e. the draining veins) due to the fact that cerebral blood flow changes exceed blood volume changes by approximately 2-4 times, with blood oxygen extraction increasing only slightly (177). This decrease in the relative concentration of dHb increases the Hb:dHb ratio and increases the BOLD signal compared to resting values.

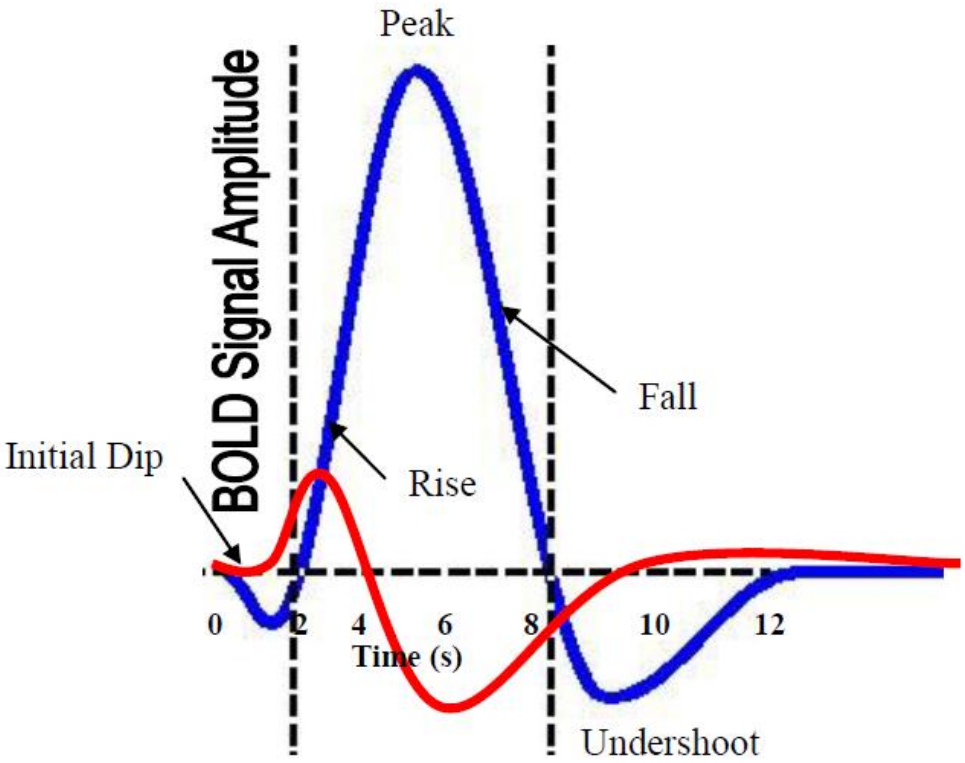

Figure 3.4.2 Graphical representation of the deoxygenated hemoglobin (red line) and accompanying hemodynamic response (blue line) which causes the BOLD signal, following neuronal stimulation (0s) (taken from (243)). The BOLD signal reaches its peak amplitude approximately 5-6s after increased neuronal activity.

Alterations in cerebrovascular function that affect neurovascular coupling could influence characteristics of the BOLD signal and make it difficult to compare across various populations. Modifications in cerebrovascular dynamics can result from changes to the vessel ultrastructure, cerebral metabolic rate of oxygen consumption, cerebral blood flow, and vascular reactivity (67). Both direct alterations in cerebral vasculature and alterations in complex neurochemical control over blood flow might affect the BOLD response (67). Hence any disorder that affects vascular structure or interferes with neurochemical functions could result in changes to vascular physiology, and unfortunately many of these disorders are common in the aging brain. Several studies

have examined the effect of normal aging on the BOLD signal (129; 137; 329). Despite having average HDR amplitudes to younger individuals, older subjects had higher noise levels in activated voxels resulting in lower signal-to-noise ratios and a decreased spatial extent of the BOLD signal (see Figure 3.4.3) (137). Taoka and colleagues also noticed an age-associated lag in the time for the BOLD signal to reach its half maximum level in the motor cortex after the start of a 10 second hand grasping task, which they speculated could be attributable to vascular stiffening (329).

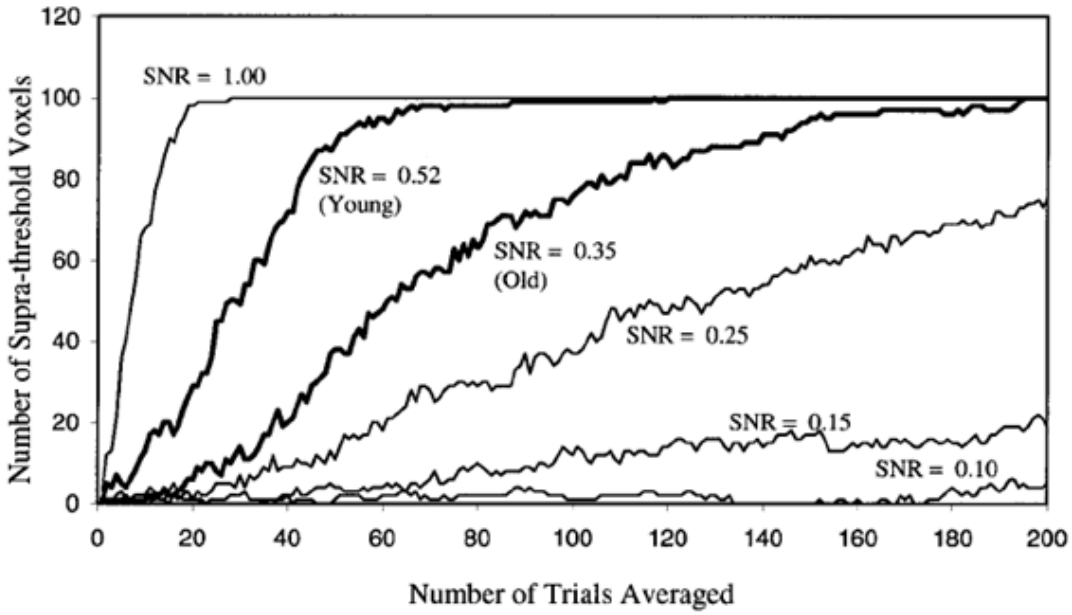

Figure 3.4.3 The effects of signal averaging, commonly done to decrease noise levels, on the detection of active voxels. Because of the lower signal to noise ratio (SNR) at any given number of trials averaged, older adults would tend to have less activated voxels compared to young (taken from (137)).

Even though neuroimaging has the capability to revolutionize our understanding of the brain and neural function, caution must be taken when comparing between various populations that may have differing BOLD signals as a result of altered neurovascular coupling.

CHAPTER 4: METHODOLOGY

4.1 SUBJECTS

Recruitment of participants was accomplished through emails sent to local (London, Ontario) running clubs and The University of Western Ontario community, poster distribution across campus and numerous local stores, malls, and apartment buildings, and advertisements placed in local newspapers. Subjects who had participated in previous experiments with our laboratory who had expressed interest in becoming involved in future studies were contacted directly. Participants were screened medically and had no previous personal history of cardiovascular, neurological and metabolic diseases. Subjects were not taking any medications contraindicative to participation. Participants were given MRI questionnaires to ensure safe compatibility within the MRI environment.

Fifteen long-term endurance trained (ET) older adults (5 female; 55 ± 4 years; SBP: 110 ± 10 mmHg, DBP: 66 ± 7 mmHg) and fifteen untrained (CON) older adults (6 female; 56 ± 4 years; SBP: 115 ± 11 mmHg, DBP: 70 ± 8 mmHg) provided informed written consent before participation in the study, which was approved by The University of Western Ontario Health Sciences Research Ethics Board. For this study, long-term endurance training was defined as five or more years of running distances equal to or longer than 25 km/week. Subjects that did not meet these criteria were excluded from the ET group. Note, it is difficult to determine what constitutes *"long-term endurance training"* with no standard definition available and a relatively limited number of studies conducted in this particular area. Many cross sectional studies do not utilize running (265) and longitudinal studies that examined ET effects were most times limited to 12 months or less (275; 310). Currently there is no formal training theory to suggest the pattern, duration and intensity of exercise that will cause a specific physiological adaptation (43).

Runners of the ET group ended up reporting an average of 14.6 years of running (range 5 to 38 years) 46 kilometres per week (range 25 to 90). This duration corresponds with studies that have examined the impacts of long-term ET on variables other than autonomic function such as mechanical cardiac properties (244) and the immune and endocrine systems (10). In each of these studies the *"long-term endurance training"* was defined as 22 ± 5 years and 23 ± 2 years, respectively.

Participants in the CON group were recreationally active but not participating in any known endurance training program. All female participants involved in the study were postmenopausal with no hormone replacement therapy (HRT) except three women in the CON group. One was postmenopausal taking HRT (combination of 0.3 mg conjugated estrogen and 2.5 mg medroxyprogesterone acetate), one was perimenopausal and one was eumenorrheic in her early follicular phase (i.e. third day of menstrual cycle) during testing.

4.2 STUDY DESIGN

All participants recruited for this study completed four different testing sessions on separate days. Session one consisted of a visit to the Laboratory for Brain and Heart Health located in the Health Sciences Building at The University of Western Ontario. Participants were instructed to fast for 12 hours and abstain from nicotine, alcohol, caffeine and strenuous exercise 12 hours prior to testing. When participants first arrived they were instructed to lie quietly for 30 minutes before undergoing a blood draw. The blood draw was performed by a registered nurse and 47 ml was taken from the antecubital vein and analyzed for fasting glucose, cholesterol (total, LDL and HDL), triglycerides, glycosylated hemoglobin, high-sensitivity C-reactive protein (HS CRP), renin,

epinephrine and norepinephrine. After the blood draw participants were given a standardized meal while they completed the Edinburgh Handedness Inventory (250), Montreal Cognitive Assessment (MoCA©) (235) and health history questionnaire. The Edinburgh Handedness Inventory is used to determine left or right hand dominance and the MoCA© was used to determine normal cognitive function. The MoCA© has been validated against the Mini-Mental State Examination (MMSE) (94) in both healthy older populations and older patients with mild cognitive impairments (in this case mild Alzheimer's Disease) and had a high degree of sensitivity and specificity (235). Basic anthropometric measurements including height, weight, waist and hip circumference were collected and participants were acquainted with lab set-up and testing protocol.

Heart rate was collected using a standard three-lead electrocardiogram (ECG). Respiration was monitored continuously with a respiratory belt using the respiratory inductance plethysmography method (measuring movement of the chest and abdominal wall). Arterial BP was monitored continuously using the middle finger of the right hand by photoplethysmographic methods from which pulsatile brachial BP was determined (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands). A cuff was also placed around the brachial artery of the right arm and after a two minute recording period, a RTF calibration was performed to calibrate brachial pressure with the finger pressure. Testing did not commence until the difference in pressure was 5mmHg or less between the finger and upper-arm. Automated BP measurements were verified and adjusted accordingly using the average of three manual sphymogameter BP measurements taken from the left arm before testing. The participant rested quietly in a supine position for 10 minutes after set up was complete to record resting ECG, finger and brachial BPs, Q and SV. Analog signals for these hemodynamic variables were acquired using an on-line data

acquisition system and software (PowerLab and Lab Chart v. 7.0, ADInstruments; Colorado Springs, CO, USA) and sampled at a rate of 1000 Hz with a 5 V sampling range. After the 10 minutes of supine rest participants completed the HG exercise protocol described in section 4.3 below.

The second session consisted of a visit to Robarts Research Institute at the University of Western Ontario where the HG protocol (see section 4.3) performed in the lab session was completed during the MRI scan. The order of these two visits was not randomized, with participants always completing the lab session first, in order to become familiarized with the HG device and protocol before undergoing the MRI scan. Again, participants were instructed to refrain from nicotine, alcohol, caffeine and strenuous exercise 12 hours prior and to consume a light meal 3 hours before the MRI session. All imaging data was collected using a whole-body 3-Tesla imaging system (Magnetom TRIO TIM, Siemens Medical Solutions, Enlargen, Germany) with a maximum gradient strength of 45 mT/m and slew rate up to 200 T/m/s. Scanning sequences for neuroimage acquisition will be discussed in further detail in section 4.4 below. During the MRI session, HR was calculated from pulse intervals recorded with an MRI-compatible oximeter (Nonin Medical Inc, 8600FO MRI, Plymouth, MN, USA) placed over the middle finger of the non-exercising right hand. The analog signal was acquired using an on-line data acquisition system and software (PowerLab and Lab Chart v. 7.0, ADInstruments; Colorado Springs, CO, USA) and sampled at a rate of 1000 Hz with a 5 V sampling range.

The third and fourth visits consisted of either an echocardiogram or stress test. The echocardiogram was performed by a licensed echocardiography trained ultrasound technician. The stress test was performed on a treadmill in the attendance of a physician

to determine maximal oxygen consumption (VO_2max). For the CON group the standard Bruce protocol was utilized (35; 36). For the ET group, each stress test was tailored to the individual's average marathon running speed. After a light intensity 5 minute warm-up the test was started at this speed, with speed increases of 0.5 km/hr every minute (grade 0%) until the subject indicated it was a comfortable pace. The incline was then increased by 1% every 2 minutes until the subject indicated they had reached maximal exertion described as a score of 19-20 on the Borg Scale (24). Oxygen and carbon dioxide gas exchange was measured and heart rate was measured with 12-lead a wireless heart rate monitor.

4.3 HAND GRIP PROTOCOL

The hand grip protocol developed for this study used an isometric hand dynamometer device (BSL – SS25LA model; Biopac Systems Inc., CA, USA) to measure the force produced during the hand grip exercise (see Figure 4.3.1). It is lightweight and ergonomically designed with an isometric range of 0 – 90 kg, excellent for experiment accuracy and repeatability. It was also non-magnetic and therefore compatible with the MRI. The device was attached in series to a bridge amplifier which was connected to the on-line data acquisition system (Powerlab, ADInstruments; CO, USA) via a BNC cable which sampled the analog signal at 1000 Hz with a sampling range of 200 mV. For the MRI session these devices were located outside of the MRI suite with the HG device cable passing through an insulated copper pipe to the inside of the suite to prevent RF interference with scanning.

Figure 4.3.1 The hand grip device utilized for testing in the lab and MRI sessions.

The IHG protocol involved two different intensities, 30% and 40% of the individual's maximal voluntary contraction (MVC). For the MVC, participants were instructed to squeeze the HG device (using their left hand) to their maximal ability. The MVC was performed before starting any of the IHG trials and repeated to ensure accurate measurement with at least thirty seconds of rest in between the first and second MVC. During the lab session the participants were given practice trials before completing each IHG trial to ensure they understood the instructions of achieving the desired intensities as quickly and accurately as possible. This was not done at the MRI session due to time constraints and because subjects had already been familiarized at the lab session. Participants were able to determine the intensity of their IHG using visual feedback that displayed their force production in real-time, in both the lab and MRI sessions. The actual IHG protocol was a boxcar design consisting of three 30 second isometric contractions per trial (see Figure 4.3.2).

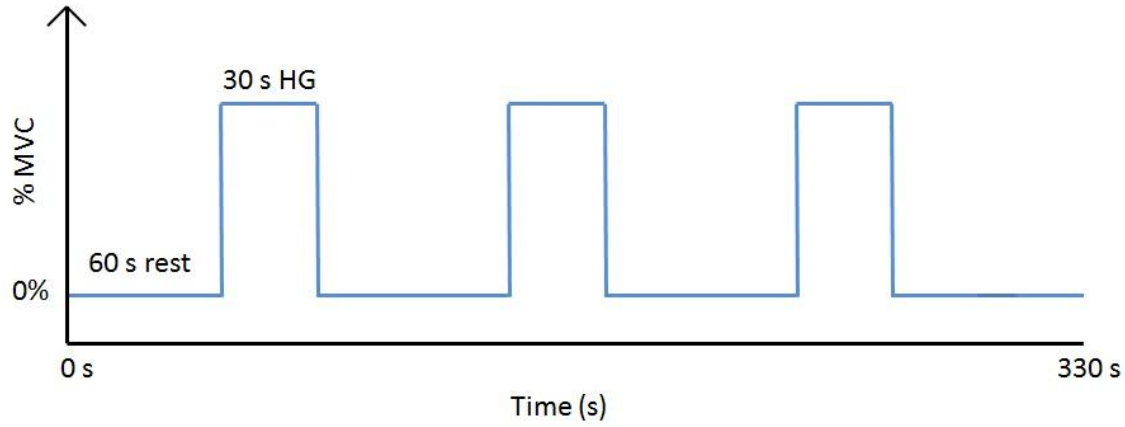

Figure 4.3.2 Hand grip protocol schematic of one trial. Three 30s isometric hand grips were performed at either 30 or 40% of the individual's MVC following a one minute rest period. Hemodynamic data was averaged using the last 30s of rest before each IHG and the last 10s of each HG.

There were two IHG trials completed at the lab session (one at 30% and one at 40%) and four IHG trials completed at the MRI session so that each intensity could be repeated. The order of the HG trials was randomized for each participant during both the lab and MRI sessions. For the lab session participants were given verbal cues instructing them when to start and stop the IHG. For the MRI session visual cues were used. Subjects were instructed to continue to breathe throughout the entire IHG protocol in order to discourage the Valsalva maneuver which would have influenced physiologic recordings of BP and HR. At the end of each IHG trial participants were asked to rate their perceived exertion using the 6 – 20 Borg Scale (24).

4.4 NEUROIMAGING DATA ACQUISITION

Participants were supine on the scanning bed with foam pads placed on either side of the head to minimize movement during scanning. Participant hearing was protected using ear plugs. A standard 32-channel transmit-receive cylindrical hybrid birdcage head coil was used to detect BOLD contrast signal (13). Prior to imaging a global shimming

procedure (RASTAMAP) was performed using first- and second-order shims to optimize the magnetic field over the volume of interest (166). The first scan was a high resolution gray/white matter contrast T_1-weighted anatomical image acquired using 3D MPRAGE sequence with the following parameters: 192 slices, sagittal field of view (FOV) = 256 mm, flip angle = 90°, TE = 2.98 ms, TI = 900 ms, TR = 2300 ms with an isotropic voxel resolution of 1.0 x 1.0 x 1.0 mm. The following functional images were collected using a multi-shot T_2*-weighted gradient echo planar imaging (EPI) pulse sequence (FOV = 240 mm, flip angle = 90°). A total of 147 volumes were collected per functional scan. Each volume consisted of 45 interleaved axial slices with the following parameters: TE = 30 ms, TR = 2.5 s with an in-plane voxel resolution of 3.0 x 3.0 mm. Five steady-state volumes were acquired before actual data collection to allow for magnetization equilibrium and discarded before data analysis.

4.5 PHYSIOLOGICAL DATA

4.5.1 DATA ANALYSIS

RHR, SBP, DBP, MAP, Q and SV were averaged during the last five minutes of supine rest. Pulse pressure (PP) was calculated using the equation SBP – DBP and total peripheral resistance (TPR) was calculated using the equation MAP/Q. BRS was analyzed across approximately 300 cardiac cycles at rest using the sequence method (described in section 3.2). A program to perform sequence analysis was developed for MatLab (R2007b©, The MathWorks Inc; Natick, MA, USA) previously in our lab.

The HR response to HG was determined for each IHG trial by averaging the last 30 s of rest before each IHG contraction and averaging the last 10 s of each IHG per trial (see Figure 4.3.2). The change in HR from rest to exercise was then calculated using these

averages. Overall change in HR for both IHG intensities was determined for each group by averaging all individual HR changes.

Frequency-specific levels of variability in beat-by-beat R-R interval during each HG trial was assessed using a wavelet-based spectral analysis approach (338). This HRV analysis focused on respiratory frequency modulations (averaged from 1 min of rest) in the R-R intervals which varied across individuals but were typically in the HF range of 0.15 to 0.3 Hz. The HF HRV change with IHG was determined by averaging the frequencies of all three 1 min rest periods and subtracting this from the averaged frequencies of all three IHGs per trial. This was done for each individual at each contraction intensity. Overall change in HF HRV for both intensities was determined for each group by averaging all individual HF HRV changes.

4.5.2 STATISTICAL ANALYSIS

The effect of group on age, RHR, SBP, DBP, MAP, Q, SV, PP, TPR, BRS, relative VO_2max and all blood work factors was determined using a two-tailed independent Student's t-test. The main effect of group and IHG intensity on change in HR (ΔHR) and change in HF HRV (ΔHF HRV) were determined using a mixed one-way analysis of variance (ANOVA). High frequency HRV data was normalized to the natural logarithm (ln) prior to statistical analysis. Shapiro-Wilk tests were performed to determine if data were normally distributed. For variables that were significantly non-normal the parametric independent Student's t-test was still used as it is quite robust against violations of normality (23). Levene's test for equality of variances was performed for all independent Student's t-test calculations and if significant the degrees of freedom were adjusted. Cohen's *d* was used as a measure effect size (standardized difference between

two independent groups) for BRS, ΔHR, and ΔHF HRV. All statistical analysis was calculated using SPSS (IBM v. 19; Armonk, NY, USA). All data are reported as mean ± standard deviation (SD) unless otherwise stated.

4.6 NEUROIMAGING DATA

4.6.1 DATA ANALYSIS

All raw fMRI data were analyzed using BrainVoyager QX software (v. 2.4.1, Brain Innovation B.V., Maastricht, The Netherlands; (108)). Preprocessing included interscan slice acquisition time correction, linear trend removal, temporal high-pass filtering to remove low-frequency drifts, and rigid-body transformation of data to the first acquired image to correct for motion. The functional data were not smoothed spatially; reported data were unsmoothed throughout all analyses. Functional scans were co-registered with the T_1-weighted anatomical scans, and subsequently transformed to Talairach space.

A two-level statistical paradigm was used for all functional imaging data. First, individual design matrices were created to analyze participant-session interactions. The epochs of each IHG trial were modelled by a boxcar design and convolved with a canonical hemodynamic response function. This resulted in subject specific contrast images containing whole brain information of BOLD signal changes during the IHG task. The General Linear Model (GLM) was used to create a statistical parametric map on a voxel-by-voxel basis (103). Corrections for multiple comparisons were made using cluster level threshold estimation (96; 123) with 1000 iterations of Monte Carlo simulation setting a statistical threshold of $p < 0.001$ for the main task effects. To ensure generalizability of the results, all analyses were performed at a random effects (RFX) level.

Second level group analysis was performed using each individual's functional runs and associated single-run design matrix to create a multi-subject design matrix. Each of the CON and ET group were analyzed at 30% and 40% IHG with a general linear model performed at a random effects level. Corrections for multiple comparisons were made using a false discovery rate (FDR) and $p < 0.05$. The BOLD signal changes in each group were also regressed against the observed average HR time course response during HG exercise, determined by a separate design matrix of HR time course for each individual. Clusters of significant activation/deactivation and colour coded for T-score were overlaid on representative anatomical images of one ET and one CON individual (each male and 58 years of age). All fMRI data are presented in radiological convention (i.e. subject's left appears on the right). Differences between groups were determined by examination of whether *a priori* areas were (de)activated compared to rest or whether they were absent.

CHAPTER 5: RESULTS

5.1 SUBJECT CHARACTERISTICS

Group anthropometrics, resting hemodynamic variables and cognitive scores are presented in Table 5.1.1, with no significant differences between groups. Blood work profiles are presented for each group in Table 5.1.2. The CON group had significantly greater levels of cholesterol, total triglycerides, LDL cholesterol and HS CRP compared to the ET group and lower levels of epinephrine ($p < 0.05$). The ET group had significantly greater absolute and relative VO_2max values, 3.7 ± 0.8 L/min and 50 ± 8 ml/kg/min compared to the CON group, 2.7 ± 0.8 L/min and 37 ± 9 ml/kg/min respectively ($p < 0.05$). It should be noted that stress tests were performed on only 14 of 15 ET subjects, as one individual elected to forgo the assessment due to health issues.

Table 5.1.1 Endurance trained and control group subject characteristics collected during lab session.

	ET	CON
N (female)	15 (5)	15 (6)
Age (years)	55 ± 4	56 ± 4
Weight (kg)	72.2 ± 11.2	74.6 ± 13.6
BMI (kg/m^2)	23.7 ± 2.5	24.6 ± 2.4
SBP (mmHg)	110 ± 10	115 ± 11
DBP (mmHg)	66 ± 7	70 ± 8
MAP (mmHg)	81 ± 8	85 ± 9
PP (mmHg)	44 ± 5	46 ± 9
RHR (bpm)	53 ± 4	59 ± 12
Q (L/min)	5.5 ± 1.4	6.5 ± 1.8
SV (ml)	103 ± 23	111 ± 22
TPR (mmHg/L/min)	15.3 ± 2.9	13.6 ± 3
MVC (V)	0.18 ± 0.32	0.07 ± 0.04
MoCA©	28 ± 2	27 ± 2

Values are mean ± standard deviation (SD). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; RHR, resting heart rate; Q, cardiac output; SV, stroke volume; TPR, total peripheral resistance; MVC, maximal voluntary contraction (from lab session). * indicates $p < 0.05$ between ET and CON groups.

Table 5.1.2 Blood profiles of endurance trained and control groups.

	ET	CON
Fasted blood glucose (mmol/L)	4.5 ± 0.8	5.7 ± 2.7
Cholesterol (mmol/L)	3.7 ± 1.1	4.8 ± 0.6*
Triglycerides (mmol/L)	0.67 ± 0.2	1.1 ± 0.7*
HDL (mmol/L)	1.4 ± 0.3	1.5 ± 0.4
LDL (mmol/L)	2.03 ± 0.8	2.8 ± 0.5*
HbA1C (%)	6 ± 0.2	6 ± 2
HS CRP (mg/L)	0.65 ± 0.5	1.7 ± 1.6*
Renin (ng/L/S)	0.15 ± 0.1	0.16 ± 0.09
Norepinephrine (nmol/L)	1.4 ± 0.5	1.5 ± 0.5
Epinephrine (nmol/L)	0.23 ± 0.08	0.14 ± 0.05*

Values are mean ± standard deviation (SD). HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1C, glycosylated hemoglobin; HS CRP, high-sensitivity C-reactive protein. * indicates $p < 0.05$ between ET and CON groups.

5.2 PHYSIOLOGICAL DATA

Resting cardiovagal baroreflex sensitivity was not different between ET and CON groups (25 ± 17ms/mmHg and 20 ± 12 ms/mmHg, respectively) ($p > 0.05$, $d = 0.36$). All individual BRS values were regressed against age and a very weak correlation was observed (see Figure 5.2.1). Heart rate increases due to IHG at both intensities were not different between groups ($p > 0.05$), but within groups the heart rate change at 40% IHG was greater than at 30% IHG intensity (main effect of IHG intensity; $p < 0.05$) (see Figure 5.2.2). No effect of group or IHG intensity on ΔHF HRV (ln) ($p > 0.05$) was observed (see Figure 5.2.3).

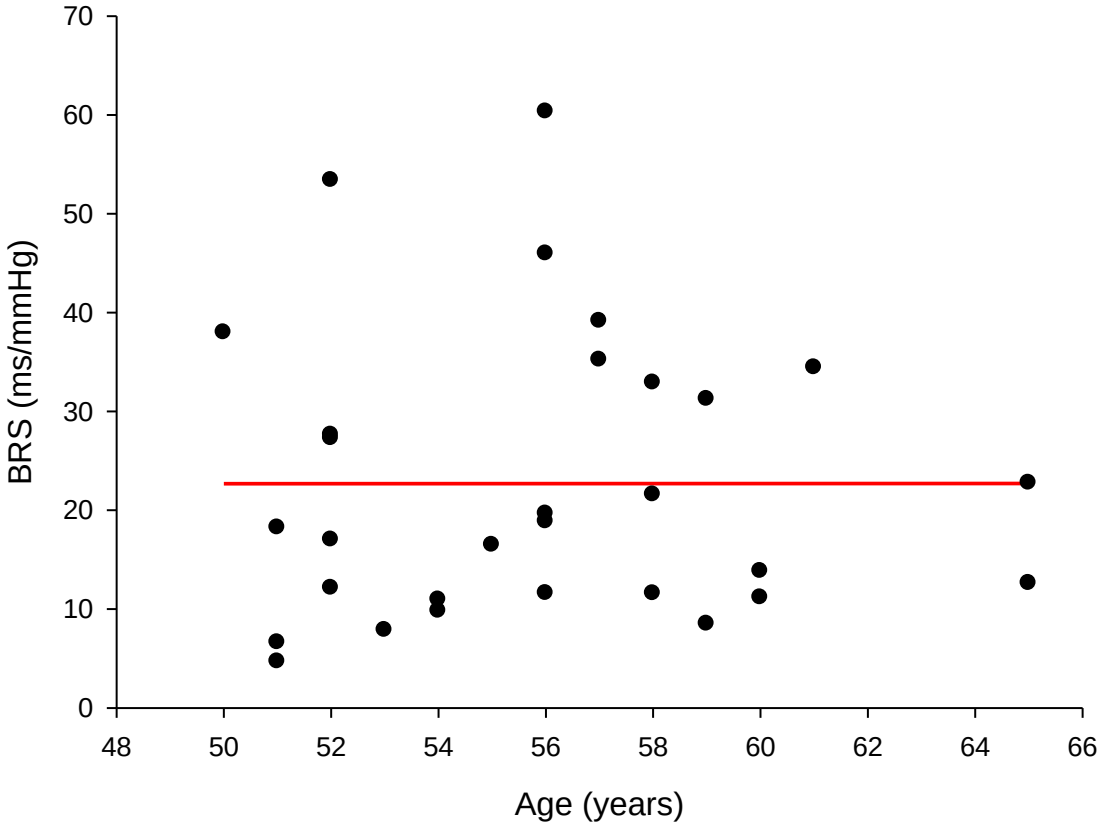

Figure 5.2.1 Individual BRS values (ms/mmHg) regressed against age (years). Regression equation is y = *0.081x* + 22.67 with r^2= 0.001 ($p > 0.05$).

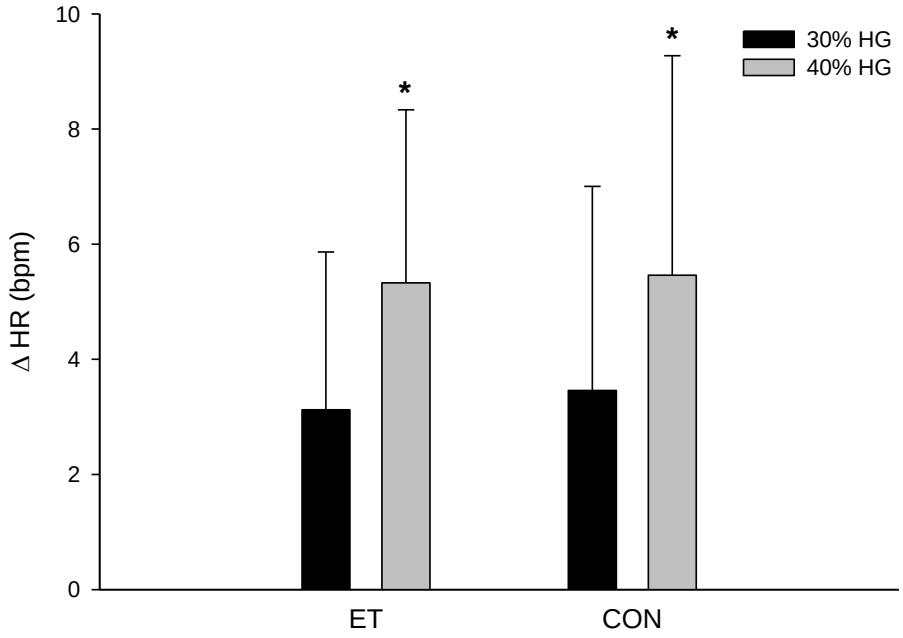

Figure 5.2.2 Change in HR with IHG exercise during lab session at 30% and 40% intensity in both ET and CON groups. ET 30% IHG ΔHR: 3.1 ± 2.7 bpm and CON 30% IHG ΔHR: 3.5 ± 3.5 bpm (d = -0.11); ET 40% IHG ΔHR: 5.3 ± 3 bpm and CON 40% IHG ΔHR: 5.5 ± 3.8 bpm (d = -0.04). All values are mean ± SD. * indicates $p < 0.05$ between ΔHR of 30% and 40% IHG intensity.

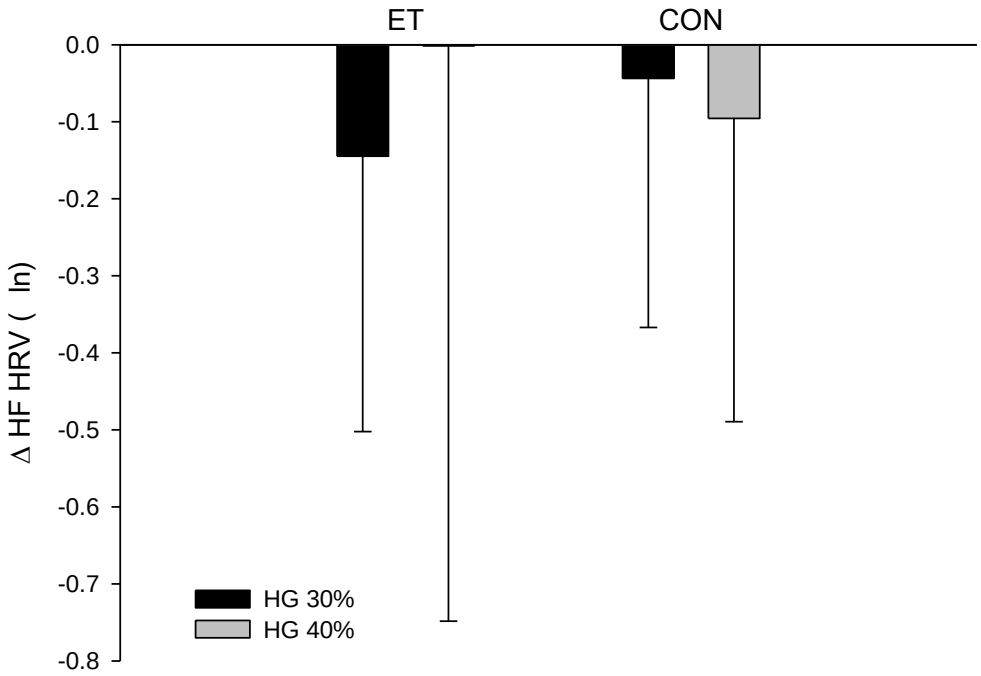

Figure 5.2.3 Change in HF HRV (ln ms^2) with IHG exercise at 30% and 40% intensity in both ET and CON groups. ET 30% IHG: -0.14 ± 0.36 and CON 30% IHG (*d* = -0.3): -0.04 ± 0.32; ET 40% IHG: -0.001 ± 0.75 and CON 40% IHG: -0.1 ± 0.39 (*d* = 0.16). All values are mean ± SD.

To investigate the lack of between-group ΔHR difference for both IHG intensities individual heart rate responses were regressed against age and cardiovagal BRS (see Figures 5.2.4 and 5.2.5, respectively). Age was a significant predictor ($p < 0.05$) of ΔHR at 40% IHG intensity and accounted for 15% of the variance between groups. Cardiovagal BRS was not a significant predictor of ΔHR at either IHG intensity.

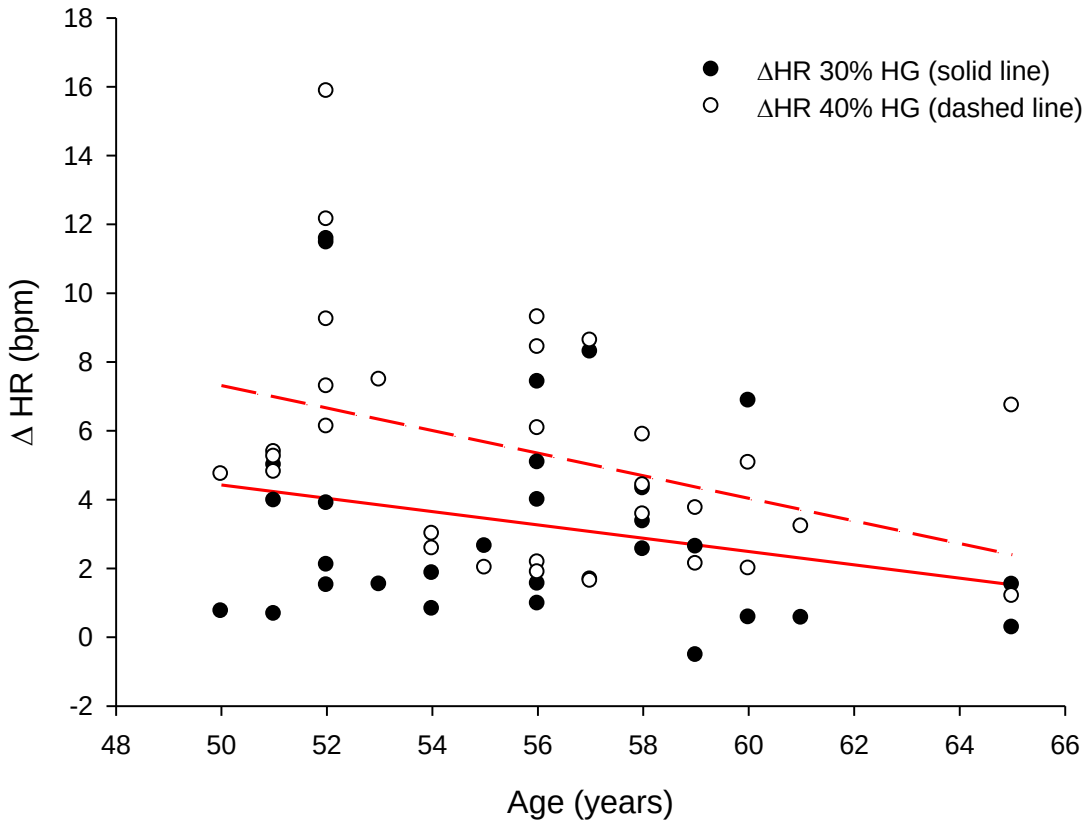

Figure 5.2.4 Individual heart rate changes (bpm) for 30% IHG and 40% IHG regressed against age (years). Solid regression line represents 30% IHG ($y = -0.22x + 15$, $r^2 = 0.075$, $p > 0.05$) and dashed regression line represents 40% IHG ($y = -0.33x + 24$, $r^2 = 0.15$, $p < 0.05$).

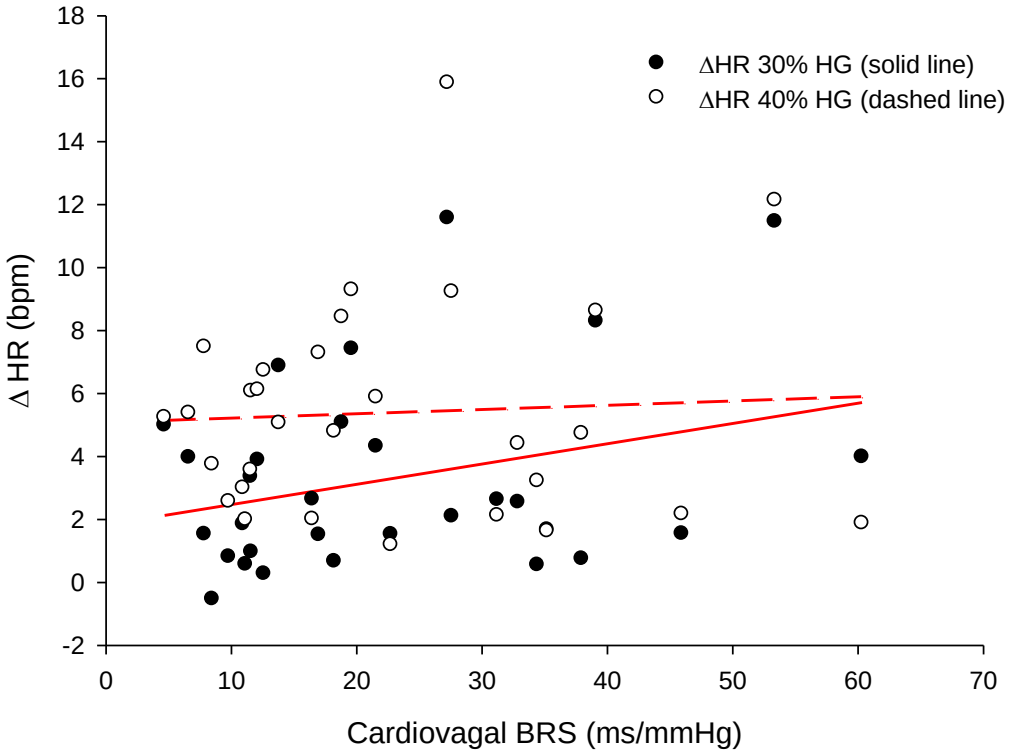

Figure 5.2.5 Individual heart rate changes (bpm) for 30% IHG and 40% IHG regressed against cardiovagal BRS (ms/mmHg). Solid regression line represents 30% IHG ($y = 0.07x + 1.4$, $r^2 = 0.1$, $p > 0.05$) and dashed regression line represents 40% IHG ($y = 0.02x + 5$, $r^2 = 0.004$, $p > 0.05$).

Because one of the main purposes of this project was to determine if increased aerobic fitness due to ET would result in enhanced markers of PNS activity (i.e. enhanced cardiovagal BRS at rest, greater HR responses with IHG and decreased HF HRV due to the exercise task) regression analyses were performed using individual relative VO_2max values for both groups (See Figures 5.2.6, 5.2.7, 5.2.8). Relative VO_2 max was a significant predictor ($p < 0.05$) of ΔHR at 40% IHG intensity and accounted for 18% of the variance between groups. At 30% IHG intensity relative VO_2max did not significantly predict ΔHR but did approach significance ($p = 0.065$) and would account for 13% of the variance. Relative VO_2 max did not predict cardiovagal BRS or ΔHF HRV ($p > 0.05$).

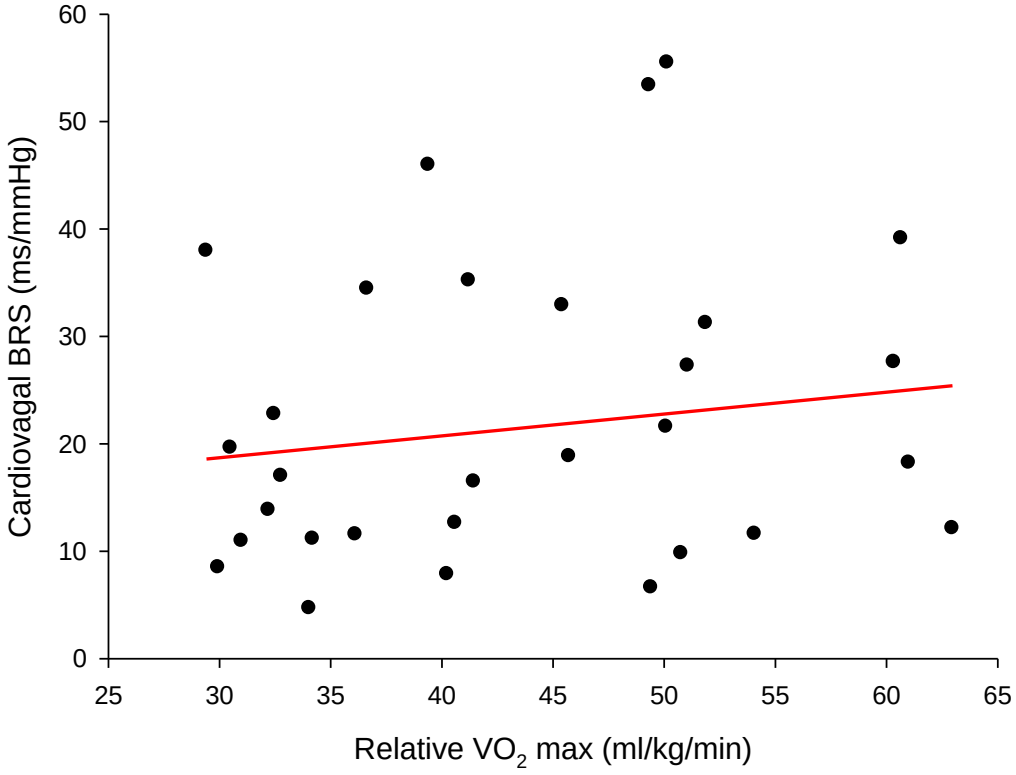

Figure 5.2.6 Individual cardiovagal BRS (ms/mmHg) calculated from supine rest regressed against relative VO_2 max (ml/kg/min) values. Regression equation is $y = 0.21x + 12.4$ with $r^2 = 0.017$ ($p > 0.05$).

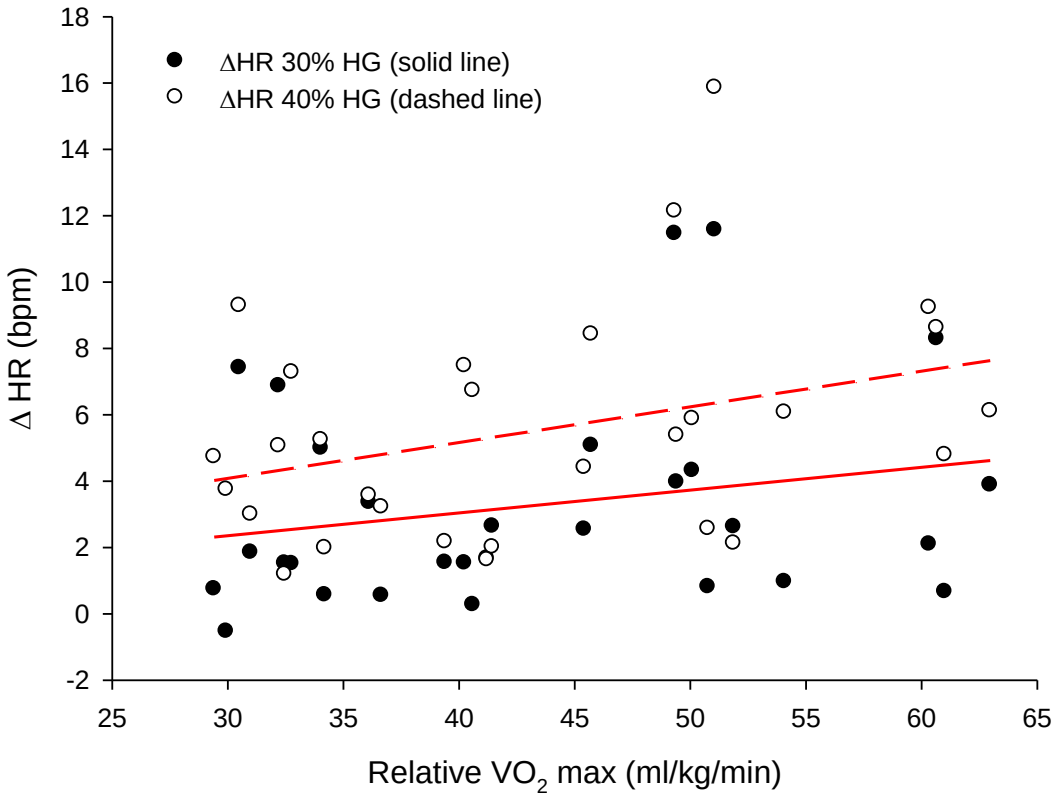

Figure 5.2.7 Individual ΔHR (bpm) for 30% IHG and 40% IHG values regressed against relative VO_2 max (ml/kg/min). Solid regression line represents 30% IHG ($y = 0.14x - 4$, $r^2 = 0.13$, $p = 0.065$) and dashed line represents 40% IHG ($y = 0.18x – 3.4$, $r^2 = 0.18$, $p < 0.05$).

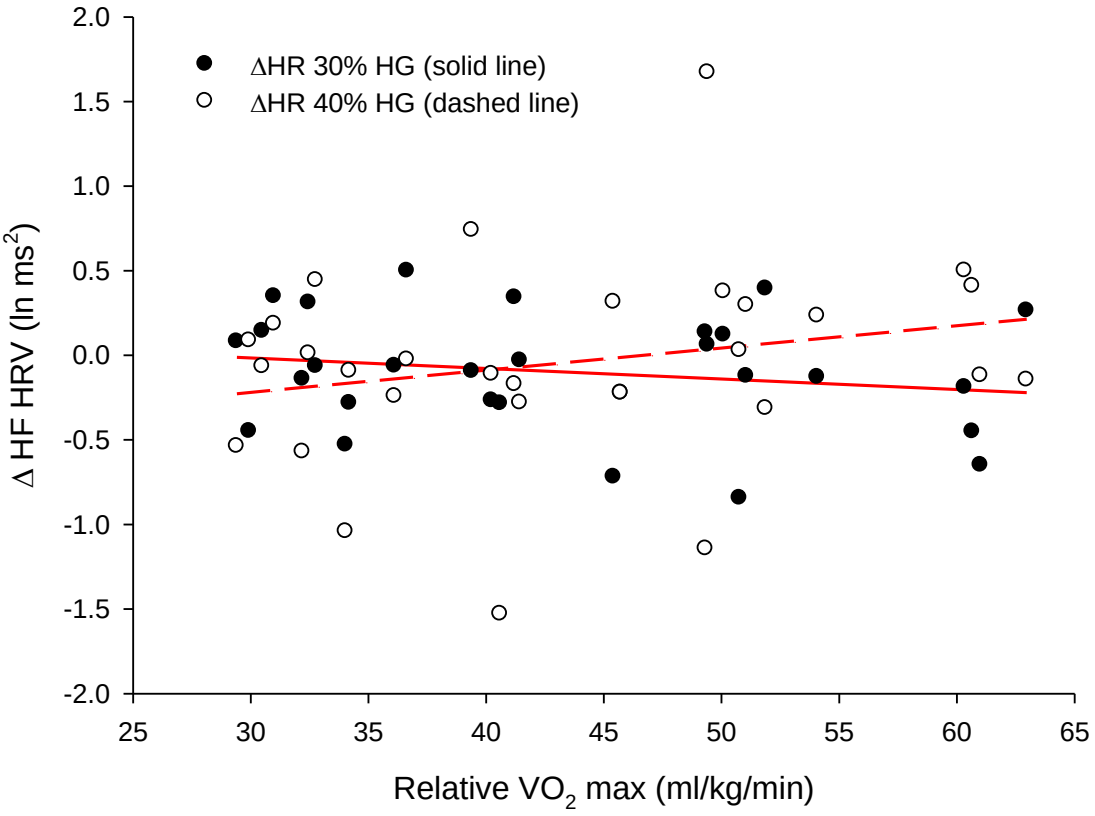

Figure 5.2.8 Individual ΔHF HRV (ln ms^2) for 30% IHG and 40% IHG values regressed against relative VO_2 max (ml/kg/min). Solid regression line represents 30% IHG ($y = -0.01x + 0.08$, $r^2 = 0.012$, $p > 0.05$) and dashed line represents 40% IHG ($y = 0.02x – 0.85$, $r^2 = 0.05$, $p > 0.05$).

5.3 NEUROIMAGING DATA

At the group level, 30% IHG task was associated with activation in the bilateral insula and middle superior cingulate cortex (MCC), and deactivation in vMPFC and subgenual ACC (sub ACC) regions for both groups. Additionally, both groups had bi-lateral activation in the pre-central gyrus, otherwise known as the primary motor cortex, and the right post-central gyrus known as the primary somatosensory cortex. The ET

group also had bilateral activation in the thalamus and deactivation in the posterior cingulate cortex (PCC). Certain basal ganglia nuclei also had activation such as the caudate (bilateral) and putamen (right). Both the ET and CON groups had hippocampus deactivation but in opposite hemispheres, right and left respectively. These global BOLD responses to 30% IHG task are summarized in Tables 5.3.1 and 5.3.2 for ET and CON groups, respectively.

At the group level, 40% IHG task was associated with activation in the right thalamus, right post-central gyrus, bilateral insula and bilateral pre-central gyrus. Deactivation was again observed in the vMPFC for both groups. Additionally, the ET group specifically had left thalamus activation and the CON group had deactivation of the sub ACC, the PCC and the left hippocampus. These global BOLD responses to 40% IHG are summarized in Tables 5.3.3 and 5.3.4 for ET and CON groups, respectively.

Table 5.3.1 Brain region BOLD responses for ET group during 30% IHG task versus rest.

Region	Side	Activation	Talairach Co-ordinates			T-score	# of voxels
			x	*y*	*z*		
vMPFC	L	↓	-8	26	-10	-4.32	45
dACC	-	-	-	-	-	-	-
sub ACC	L	↓	-7	35	-9	-4.11	13
MCC	L	↑	2	3	46	4.92	637
	R	↑	-6	-6	48	4.39	159
PCC	R	↓	3	-53	20	-4.24	284
Anterior Insula	L	↑	-31	24	9	4.42	148
	L	↑	-33	12	13	4.4	236
	R	↑	37	18	9	4.48	554
Mid Insula	R	↑	37	0	14	4.52	376
	R	↑	45	8	1	4.24	99
Posterior Insula	-	-	-	-	-	-	-
Pre-central gyrus	L	↑	-48	-3	36	4.41	266
	R	↑	34	-15	49	4.83	523
	R	↑	28	-11	54	4.36	147
Post-central gyrus	R	↑	39	-21	49	5.46	773
	R	↑	44	-19	56	5.07	615
	R	↑	47	-28	54	4.3	412
Hippocampus	R	↓	27	-16	-17	-4.49	130
Thalamus	L	↑	-12	-16	8	4.51	346
	R	↑	8	-16	8	4.59	350
Caudate	L	↑	-16	6	21	4.54	23
	R	↑	17	11	18	4.32	116
Putamen	R	↑	28	-1	-2	4.65	207

$P < 0.05$, FDR corrected. vMPFC, ventral medial prefrontal cortex; dACC, dorsal anterior cingulate cortex; sub ACC, subgenual anterior cingulate cortex; MCC, middle superior cingulate cortex; PCC, posterior cingulate cortex; L, left; R, right. ↑=activation; ↓=deactivation.

Table 5.3.2 Brain region BOLD responses for CON group during 30% IHG task versus rest.

Region	Side	Activation	Talairach Co-ordinates			T-score	# of voxels
			x	*y*	*z*		
vMPFC	L	↓	-4	32	-12	-3.95	109
	L	↓	-6	40	-7	-4.42	332
dACC	-	-	-	-	-	-	-
sub ACC	L	↓	-4	43	-5	-4.41	274
MCC	L	↑	-4	1	43	4.07	239
	R	↑	5	22	29	4.28	35
	R	↑	6	3	43	4.28	496
PCC	-	-	-	-	-	-	-
Anterior Insula	L	↑	-31	19	13	4.09	234
	R	↑	41	15	2	4.74	623
Mid Insula	L	↑	-44	-4	7	3.92	103
	R	↑	39	6	6	4.23	358
	R	↑	39	-3	11	4.03	200
Posterior Insula	-	-	-	-	-	-	-
Pre-central gyrus	L	↑	-40	-7	46	3.81	275
	R	↑	36	-8	52	4.17	597
	R	↑	40	-14	52	4.19	692
	R	↑	34	-24	49	5.21	886
Post-central gyrus	R	↑	41	-22	52	4.6	661
Hippocampus	L	↓	-26	-14	-15	-3.99	120
Thalamus	R	↑	5	-17	8	4.46	544
Caudate	-	-	-	-	-	-	-
Putamen	-	-	-	-	-	-	-

$P < 0.05$, FDR corrected. vMPFC, ventral medial prefrontal cortex; dACC, dorsal anterior cingulate cortex; sub ACC, subgenual anterior cingulate cortex; MCC, middle superior cingulate cortex; PCC, posterior cingulate cortex; L, left; R, right. ↑=activation; ↓=deactivation.

Table 5.3.3 Brain region BOLD responses for ET group during 40% IHG task versus rest.

Region	Side	Activation	Talairach Co-ordinates			T-score	# of voxels
			x	*y*	*z*		
vMPFC	L	↓	-3	40	-5	-4.67	165
dACC	-	-	-	-	-	-	-
sub ACC	-	-	-	-	-	-	-
MCC	-	-	-	-	-	-	-
PCC	-	-	-	-	-	-	-
Anterior Insula	L	↑	-31	23	10	4.77	269
	R	↑	32	21	14	4.7	635
	R	↑	33	13	14	4.74	564
Mid Insula	L	↑	-39	-8	15	4.95	37
	R	↑	35	-2	17	4.53	153
	R	↑	42	5	10	4.74	281
Posterior Insula	-	-	-	-	-	-	-
Pre-central gyrus	L	↑	-48	-3	36	4.62	179
	R	↑	33	-17	47	5.35	536
Post-central gyrus	R	↑	46	-22	52	4.81	413
	R	↑	36	-33	59	4.52	367
Hippocampus	-	-	-	-	-	-	-
Thalamus	L	↑	-12	-18	9	4.2	13
	R	↑	6	-17	6	4.56	24
Caudate	-	-	-	-	-	-	-
Putamen	-	-	-	-	-	-	-

$P < 0.05$, FDR corrected. vMPFC, ventral medial prefrontal cortex; dACC, dorsal anterior cingulate cortex; sub ACC, subgenual anterior cingulate cortex; MCC, middle superior cingulate cortex; PCC, posterior cingulate cortex; L, left; R, right. ↑=activation; ↓=deactivation.

Table 5.3.4 Brain region BOLD responses for CON group during 40% IHG task versus rest.

Region	Side	Activation	Talairach Co-ordinates			T-score	# of voxels
			x	*y*	*z*		
vMPFC	L	↓	-5	27	-12	-4.43	405
dACC	-	-	-	-	-	-	-
sub ACC	L	↓	-4	34	-10	-4.69	650
	L	↓	-5	34	-5	-4.61	671
	L	↓	-5	17	-9	-4.16	209
MCC	-	-	-	-	-	-	-
PCC	L	↓	2	-51	20	-4.28	492
	R	↓	-4	-57	20	-4.43	371
Anterior Insula	R	↑	37	15	10	4.32	292
Mid Insula	L	↑	-43	-3	10	4.19	31
	R	↑	36	7	12	4.07	65
	R	↑	39	4	8	4.31	128
Posterior Insula	-	-	-	-	-	-	-
Pre-central gyrus	L	↑	-56	2	36	4.13	78
	R	↑	37	-23	53	4.85	482
	R	↑	35	-8	53	4.26	73
Post-central gyrus	R	↑	34	-24	46	5.42	723
Hippocampus	L	↓	-28	-16	-16	-4.18	61
Thalamus	R	↑	4	-18	9	4.26	35
Caudate	-	-	-	-	-	-	-
Putamen	-	-	-	-	-	-	-

$P < 0.05$, FDR corrected. vMPFC, ventral medial prefrontal cortex; dACC, dorsal anterior cingulate cortex; sub ACC, subgenual anterior cingulate cortex; MCC, middle superior cingulate cortex; PCC, posterior cingulate cortex; L, left; R, right. ↑=activation; ↓=deactivation.

When the BOLD signal was regressed against the average HR response of both groups at each hand grip intensity there was activation of the right pre-central gyrus (the primary motor cortex) and deactivation of the vMFPC (Figures 5.3.1 to 5.3.2 and Figures 5.3.3 to 5.3.4, respectively). Activation of the bilateral anterior insula also occurred with the HR response in all cases except the CON group at 40% IHG (Figure 5.3.5 and 5.3.6). The observed deactivation in the PCC was apparent at 30% IHG but not 40% in both groups (Figure 5.3.7). In Figures 5.3.8 to 5.3.9, the time course of the BOLD signal change in the vMPFC for both groups is displayed with their respective average HR responses. The vMPFC activity mirrored the magnitude of the HR response but had no progressive decreases in activity throughout the contraction period. The HR response for the most part, increased progressively during the exercise period and reached its zenith at the end of each IHG.

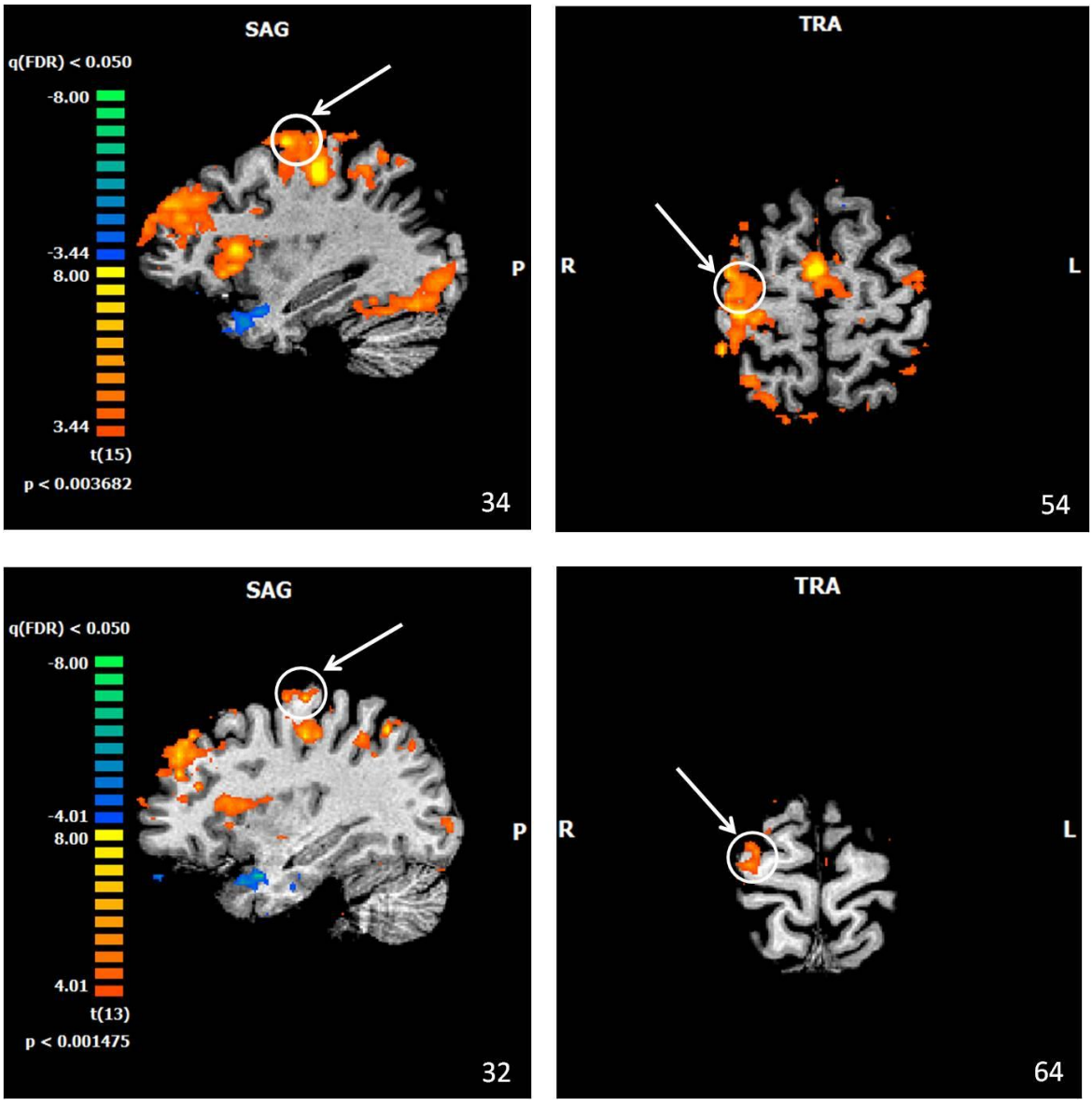

Figure 5.3.1 Right precentral gyrus or primary motor cortex activation (surrounded by white circle) in CON (top panel; T-score 5.49, # of voxels = 741) and ET group (bottom panel; T-score 4.62, # of voxels = 263) for 30% HR response. Sagittal slice presented on the left and transverse slice on the right. $P < 0.05$, FDR corrected.

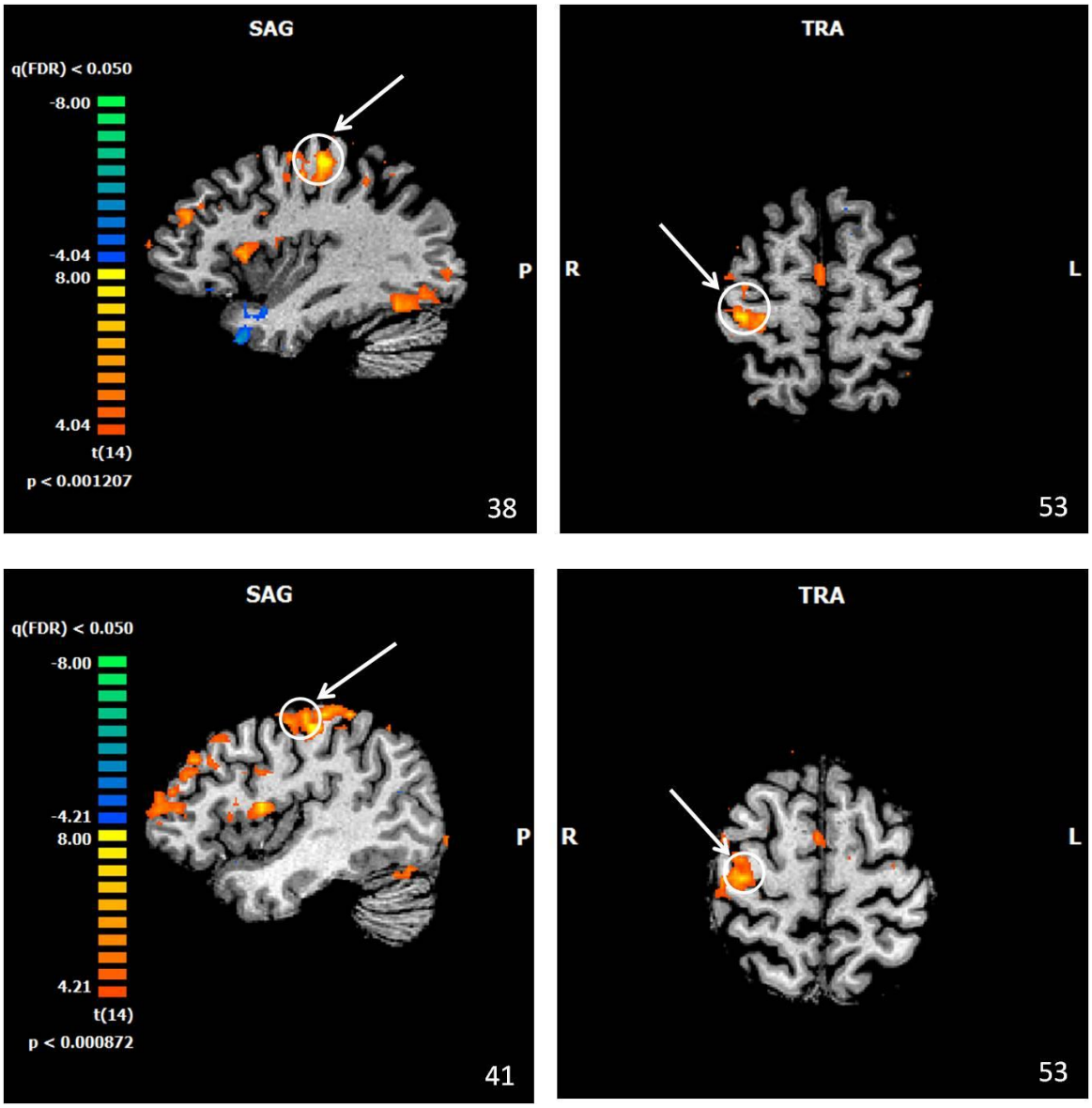

Figure 5.3.2 Right precentral gyrus or primary motor cortex activation (surrounded by white circle) in CON (top panel; T-score 5.87, # of voxels = 744) and ET group (bottom panel; T-score 5.82, # of voxels = 723) 40% HR response. Sagittal slice presented on the left and transverse slice on the right. $P < 0.05$, FDR corrected.

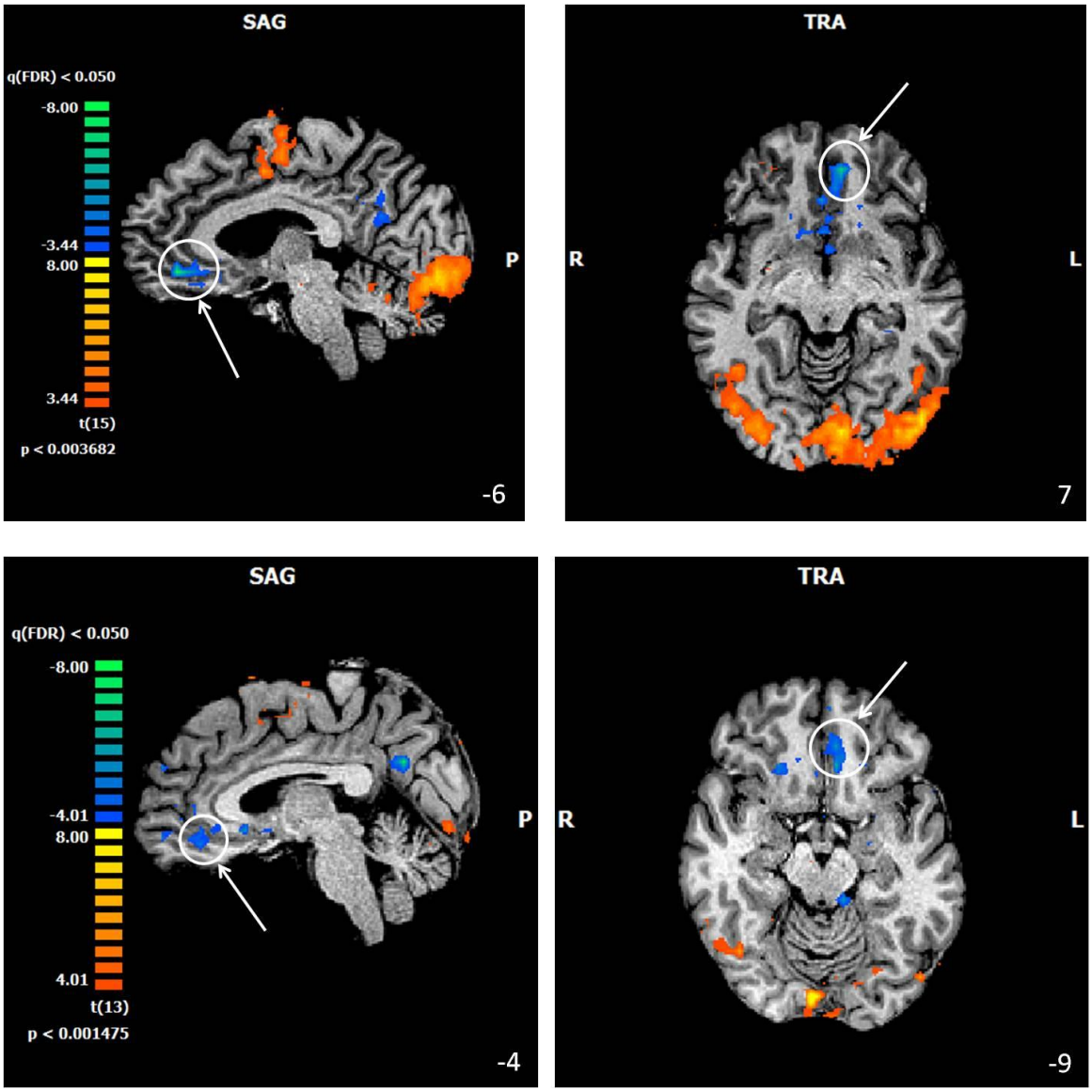

Figure 5.3.3 Left vMPFC deactivation (surrounded by white circle) in CON (top panel; T-score -4.62, # of voxels = 434) and ET group (bottom panel; T-score -4.62, # of voxels = 285) for 30% HR response. Sagittal slice presented on the left and transverse slice on the right. $P < 0.05$, FDR corrected.

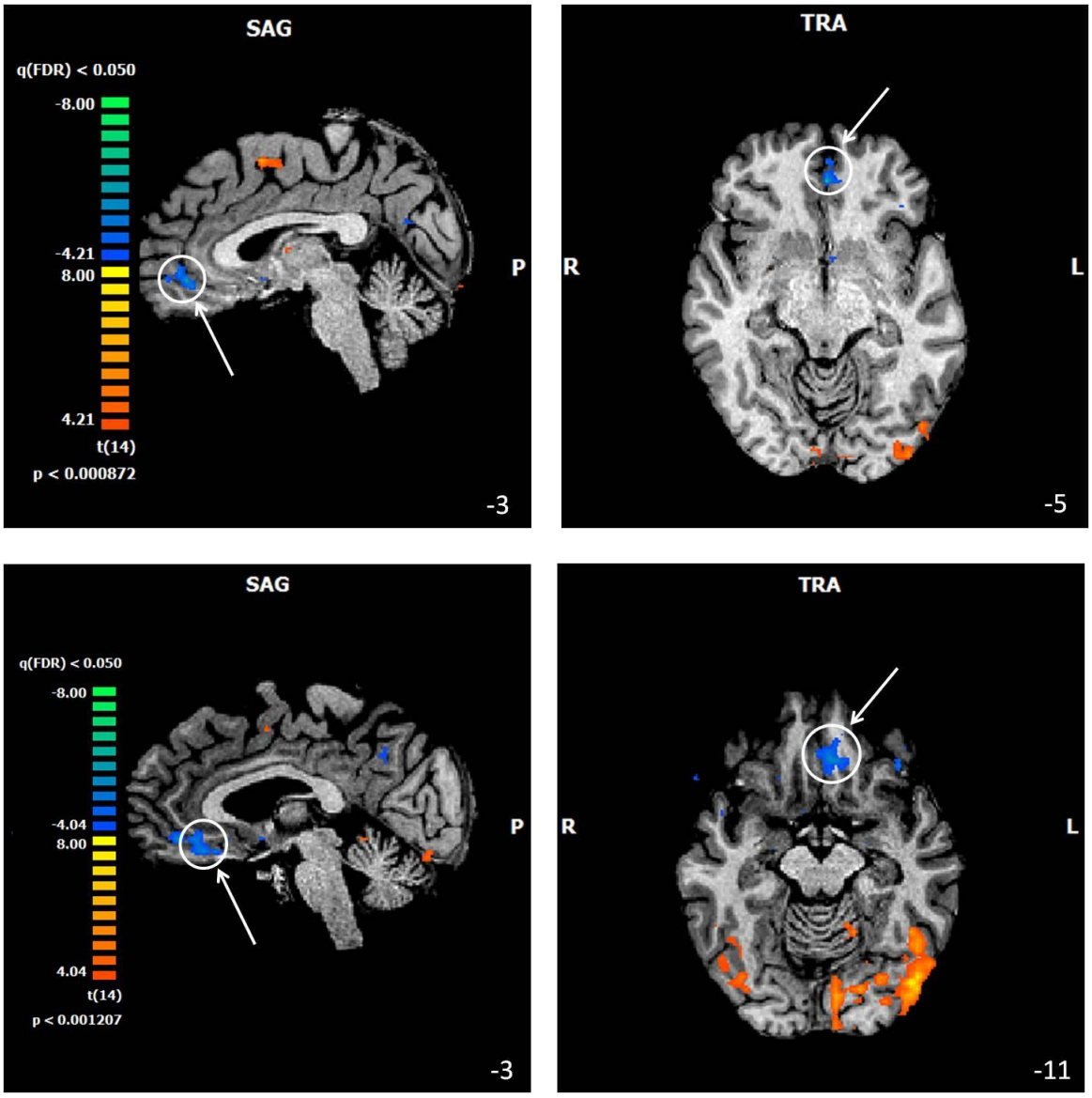

Figure 5.3.4 Left vMPFC deactivation (surrounded by white circle) in CON (top panel; T-score -4.68, # of voxels = 453) and ET group (bottom panel; T-score -4.9, # of voxels = 364) for 40% HR response. Sagittal slice presented on the left and transverse slice on the right. $P < 0.05$, FDR corrected.

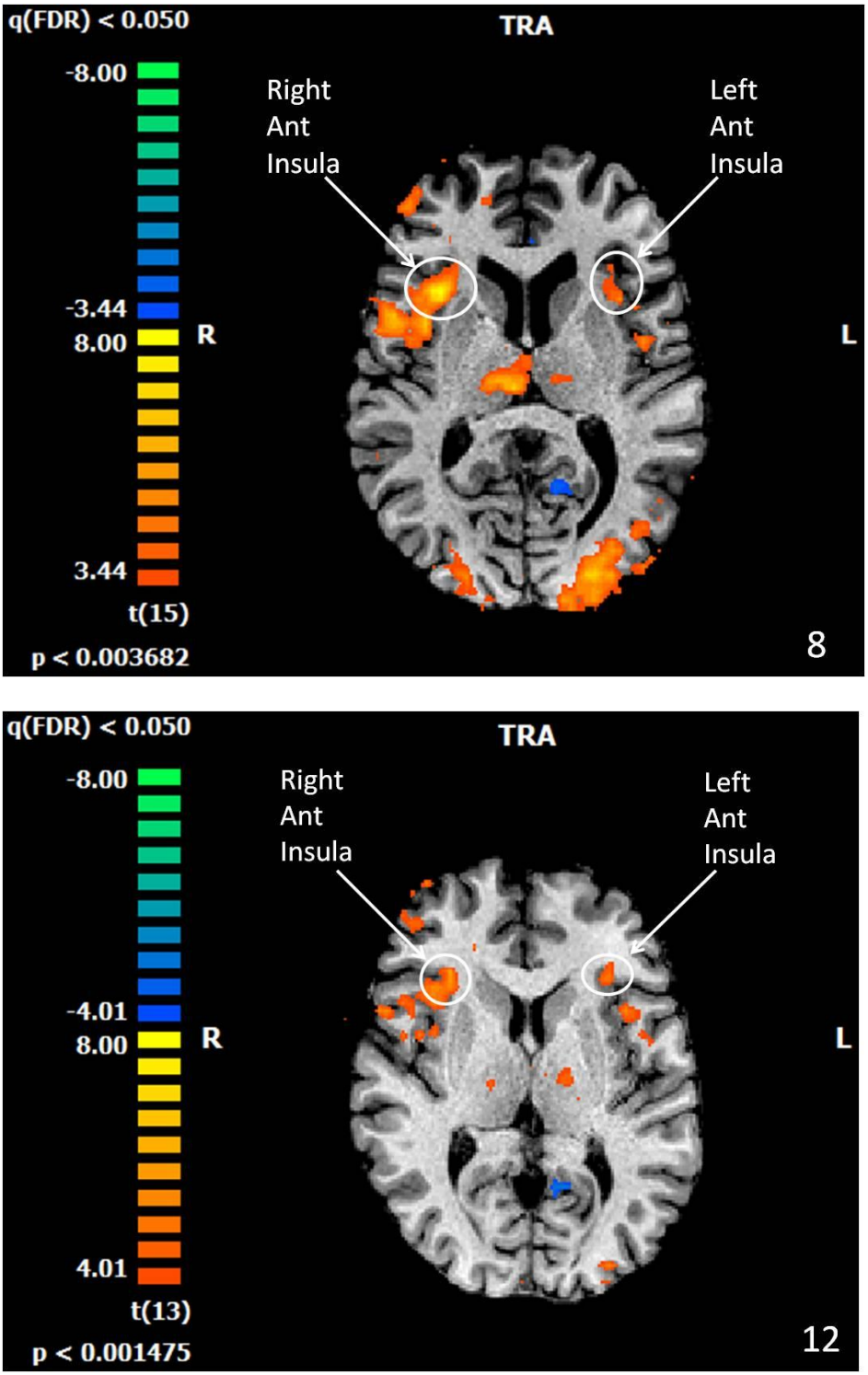

Figure 5.3.5 Bilateral anterior insular cortex activation (surrounded by white circles) in CON (top; **L**: T-score 4.07 and # of voxels = 303, **R**: T-score 5.15 and # of voxels = 868) and ET group (bottom; **L**: T-score 4.54 and # of voxels = 143, **R**: T-score 4.92 and # of voxels = 670) for 30% HR response. Presented using transverse slice. $P < 0.05$, FDR corrected.

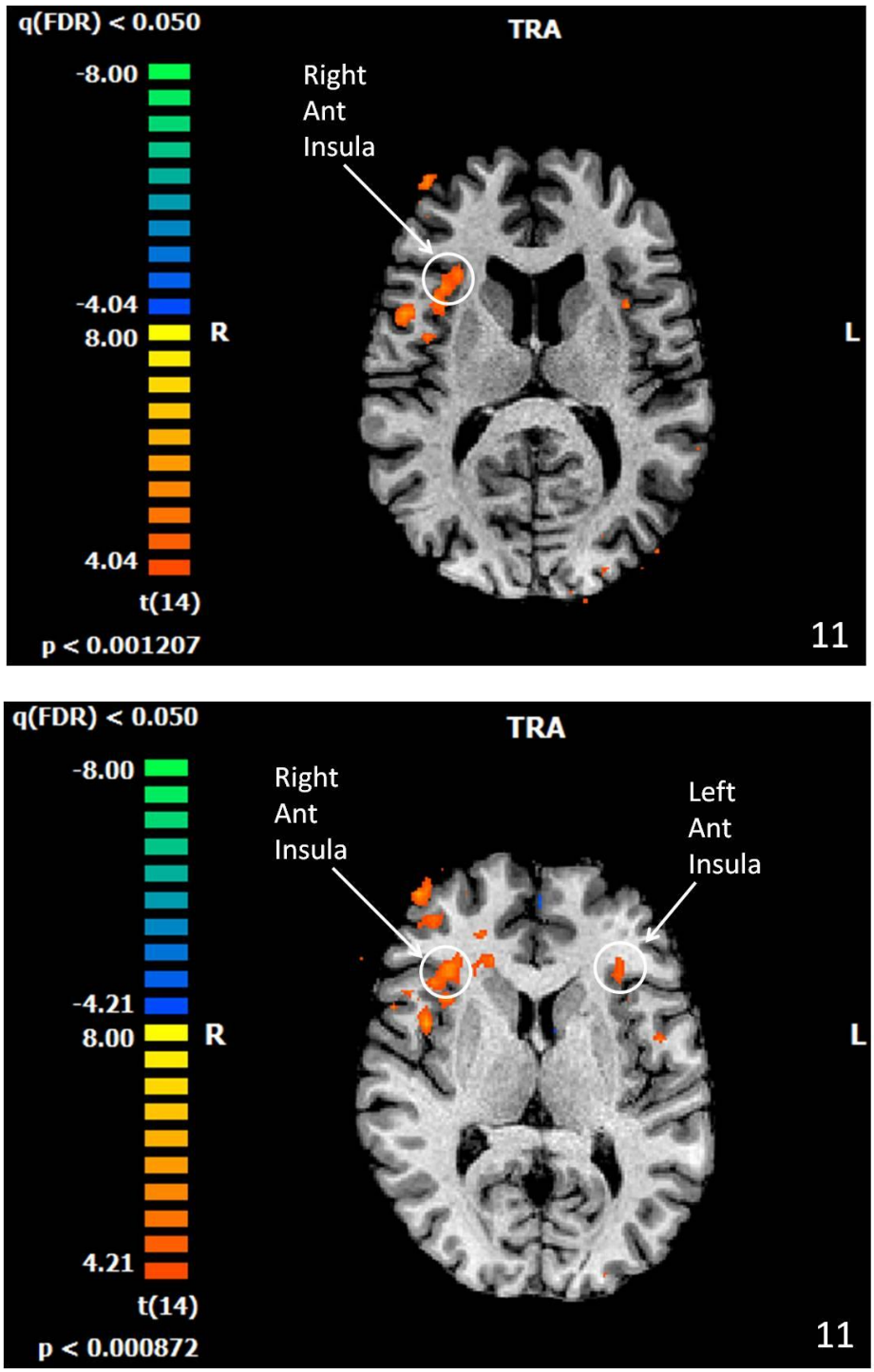

Figure 5.3.6 Right anterior insular cortex activation (surrounded by white circles) in CON (top; **R**: T-score 4.77 and # of voxels = 334) and bilateral anterior insular cortex activation in ET group (bottom; **L**: T-score 4.61 and # of voxels = 97, **R**: T-score 5.13 and # of voxels = 586) for 40% HR response. Presented using transverse slice. $P < 0.05$, FDR corrected.

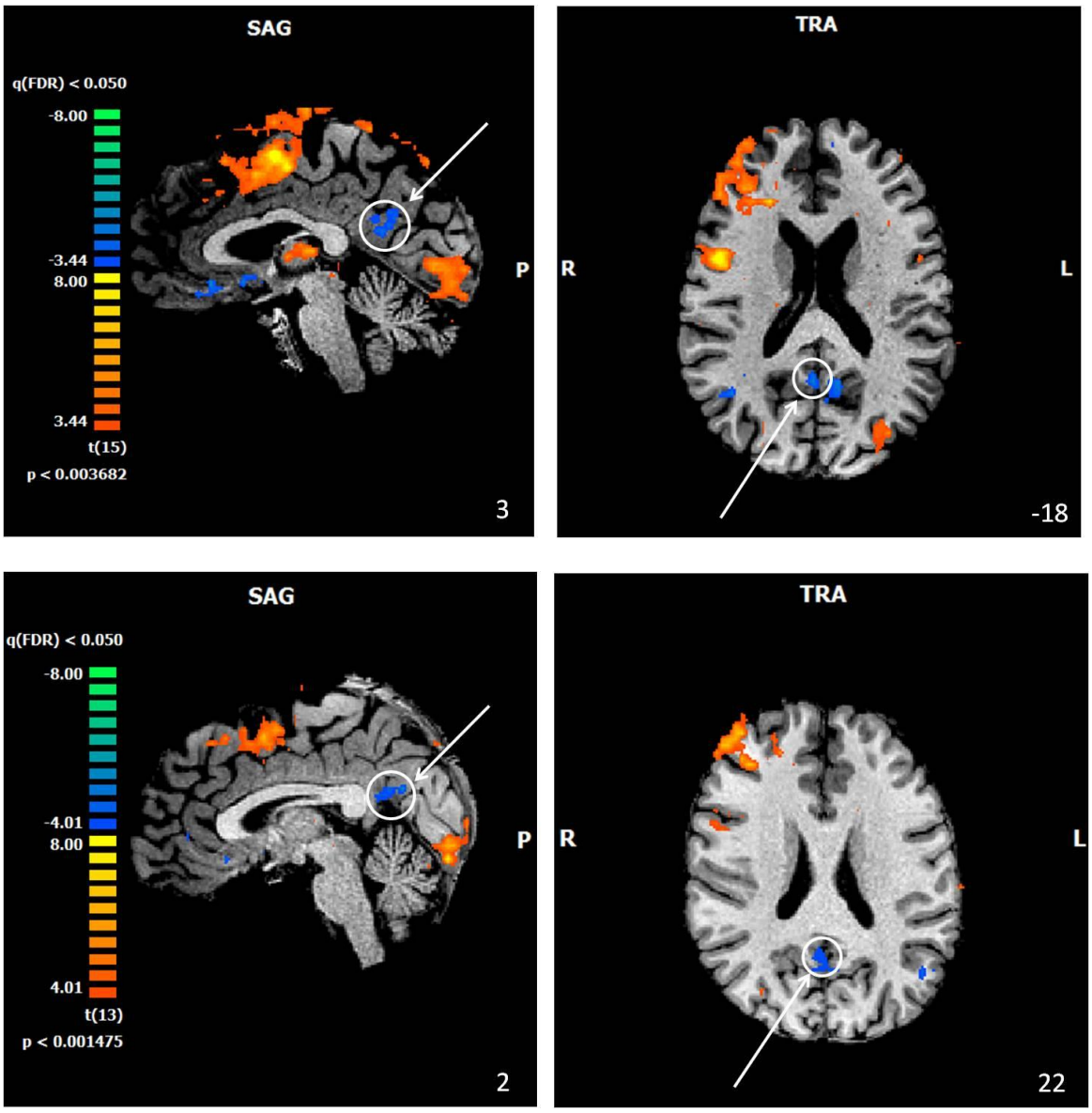

Figure 5.3.7 Right PCC deactivation (surrounded by white circle) for 30% HR response in CON (top panel; T-score -3.86, # of voxels = 253) and ET group (bottom panel; T-score -4.33, # of voxels = 257). Sagittal slice presented on the left and transverse slice on the right. $P < 0.05$, FDR corrected.

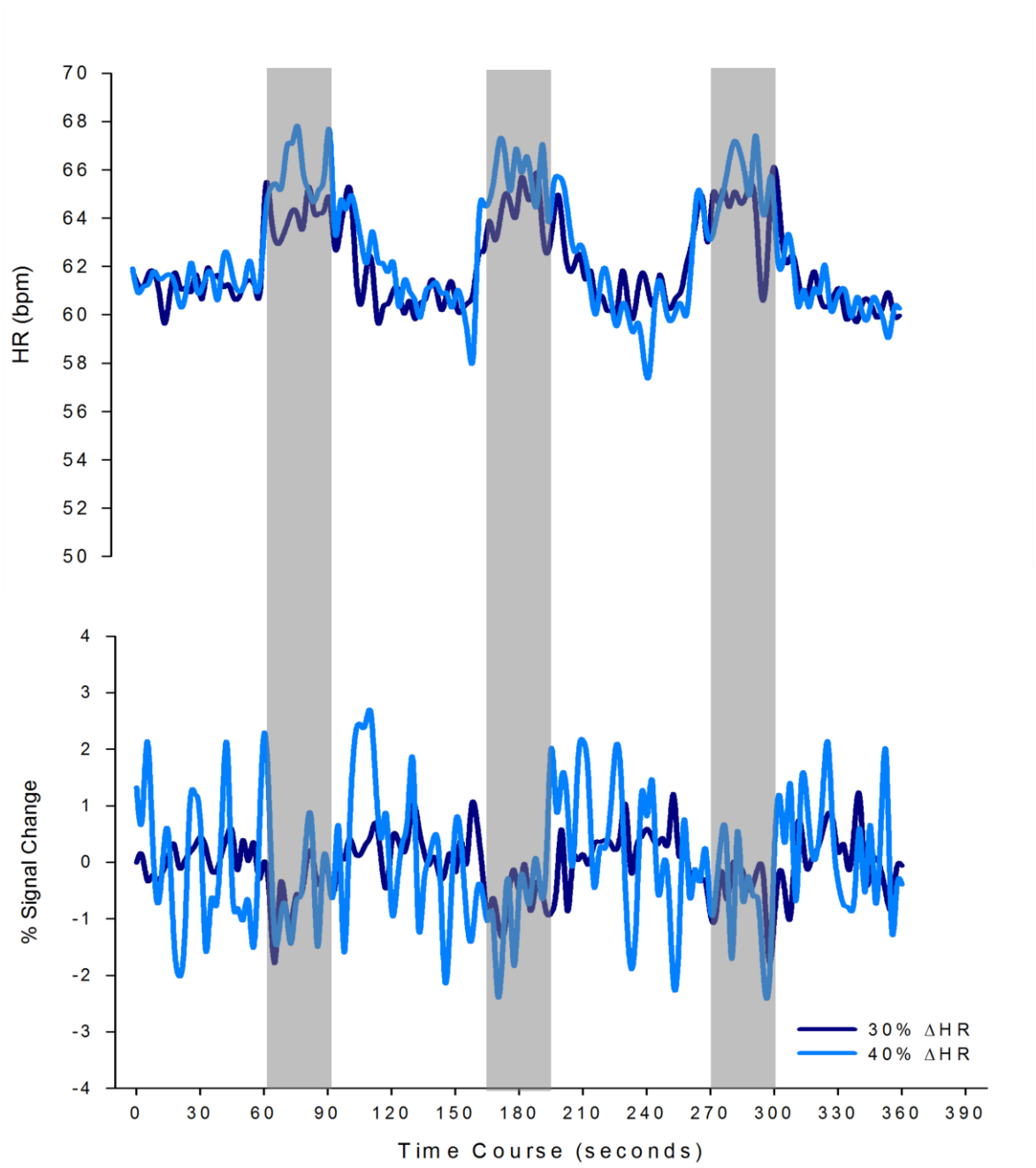

Figure 5.3.8 Average time course of the HR and vMPFC BOLD response during 30% and 40% IHG in CON group. Top: average group HR response during MRI session. Bottom: % signal change of vMPFC. Gray bars represent contraction periods.

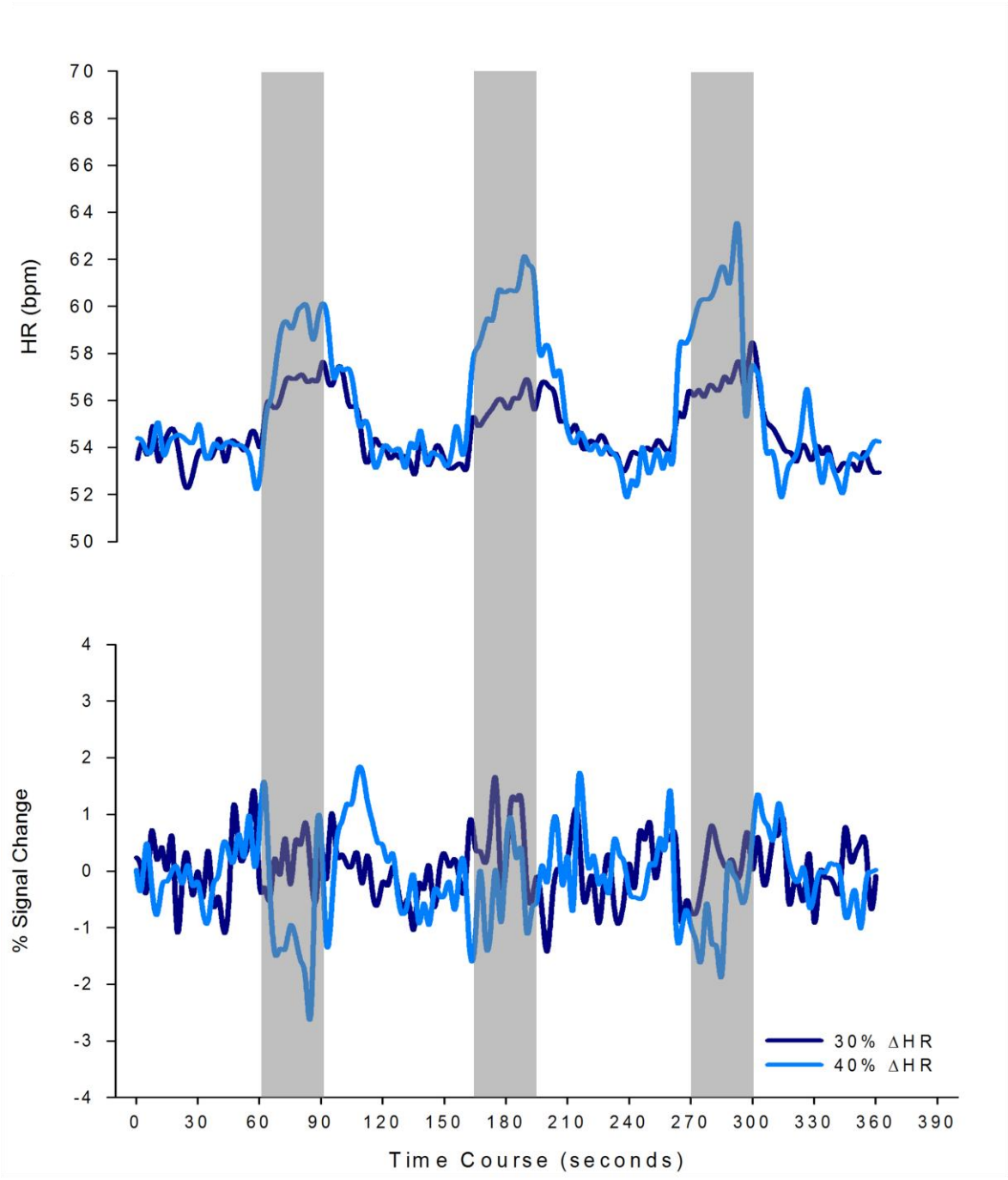

Figure 5.3.9 Average time course of the HR and vMPFC BOLD response during 30% and 40% IHG in ET group. Top: average group HR response during MRI session. Bottom: % signal change of vMPFC. Gray bars represent contraction periods.

CHAPTER 6: DISCUSSION

The present study examined the differences in cardiovascular responses and associated forebrain (de)activation patterns during two intensities of short term moderate IHG between endurance trained individuals and age-matched untrained controls. The results suggest that long-term endurance training does not significantly alter the cardiovascular responses to exercise and indices of PNS control or the functional responses of the CAN to the IHG tasks and HR responses compared to untrained controls. This was unexpected as previous literature supports the idea that endurance training preserves cardiovagal control in older adults. An improvement in vagal control would be induced by ET if the following had been observed 1) increased HR response to exercise tasks, 2) increased cardiovagal BRS, 3) a greater decrease in HF HRV with IHG and 4) greater vMPFC deactivation.

6.1 PHYSIOLOGICAL RESULTS

6.1.1 BAROREFLEX SENSITIVITY

Baseline BRS values were not significantly different between the two groups, but the mean BRS value was approximately 5 ms/mmHg greater in the ET group than the CON group. The fact that they were not statistically different may account for the observation that both groups produced similar HR responses for both the 30% and 40% IHG intensities (approximately 3 bpm and 5 bpm, respectively). If HR response to IHG is due to PNS withdrawal and BRS, a marker of baseline cardiovagal control, was similar between groups, they should have similar HR responses to exercise and this was the case. An examination of individual relationships, revealed a weak correlation between individual HR responses (30% $r^2 = 0.1$ and 40% $r^2 = 0.004$) and BRS slope in this group

of participants, suggesting that even though we use BRS as a marker of resting cardiovagal control in older populations it may not predict CV responses to exercise tasks.

Because BRS has been shown to decline with age (158; 181; 227; 228), BRS values were regressed against individual ages regardless of endurance training status. A strong correlation was expected as previous studies have observed correlations of r = -0.65 (healthy subjects aged 23 – 77 years) and -0.69 (healthy sedentary men aged 19 – 76 years) (181; 227). Rather, a high degree of variability was observed in BRS (ET SD = 16.6 ms/mmHg and CON SD = 11.9 ms/mmHg) and a weak relationship between age and BRS was established (Figure 5.2.1). The relationship between age and BRS is exemplified by two of the youngest individuals (51 years of age each, one from each group) who had two of the lowest BRS values (6.61. and 4.68 ms/mmHg). The age range of individuals involved in this study may be too narrow (50 to 65 years) to generate a broad enough independent variable for regression analysis. This becomes more apparent when compared to previous work that examined a sample of seventy normotensive subjects aged 22 – 82 years using several methods (71). Up until the fourth decade, age was the dominant factor of decreasing BRS with little further decline afterwards (71). These results could indicate that a basement level of BRS may have been reached in both groups (both past their fourth decade) and long-term ET was not effective in preventing its decline. This seems unlikely, however, as previous literature reports lower BRS values than those reported in this study, approximately 10 – 12 ms/mmHg, for a similar age range (181; 227; 228).

Regression of BRS values against relative VO_2max data also resulted in a very weak correlation (r^2 = 0.017). These data suggest endurance training has not enhanced

baroreflex function at least in the group of individuals tested for this study. A possible explanation is that, in some cases, chronic endurance exercise can be detrimental to autonomic function and depress baroreflex control by decreasing baroreceptor sensitivity. In a study of young adults, the very fit group had a decreased arterial BRS compared to a moderately fit group indicating that long-term ET may not markedly increase BRS in comparison to an age-matched untrained group (318). In these cases, baroreceptor function may be decreased due to increased arterial stiffening meaning a greater change in blood pressure is required for the same level of stretch in the arterial wall. There is speculation that high levels of shear stress caused by repeated endurance efforts induce fibrotic changes and decrease arterial wall elasticity (246). Compared to controls, veteran ultra-endurance athletes have been observed with greater aortic stiffness and arterial pulse wave velocity (351). Therefore, high levels of aerobic training may actually be harmful to the stimulus portion of the baroreflex loop resulting in blunted BRS.

At the other end of the baroreflex loop, cardiac responsiveness may also be diminished with chronic training (164) regardless of the function of the efferent neural portion of the baroreflex loop. Whether this diminished responsiveness was due to structural or functional changes of the heart remains to be fully understood. An elegant rat model demonstrated that chronically exercised animals (60 minutes of strenuous running for 16 weeks) developed adverse cardiac remodelling including left and right ventricle hypertrophy and dilation of left and right atria. They also had increased collagen deposition and fibrosis in both atria and ventricles (15). Therefore, even if afferent neural information was being transmitted effectively, the heart may not respond to neural inputs if constrained by accumulated structural and functional remodelling caused by long-term ET.

6.1.2 HIGH FREQUENCY HEART RATE VARIABILITY

The change in HF HRV due to the exercise task was also similar between groups but did decrease with IHG regardless of intensity. It is difficult to draw any conclusions from the HF HRV data due to the high inter-subject variability even after normalization using the natural log. In a study that examined autonomic control under a wide range of conditions it was found that none of the HRV measurements, in both the time and frequency domains, responded to the stimuli as consistently as HR (109). The authors concluded that HRV may not be an appropriate index of cardiovagal control which may also apply to this study.

Evidence also indicates that HRV may not benefit from endurance training. In elderly subjects, an eight week aerobic training program increased the VO_2peak values but did not change any HRV parameters (265). Although this population is different from the long-term ET group included in this study, these earlier results are consistent with and support the current observations. Other studies have also supported the idea that HRV measures do not increase in a dose-dependent manner with increasing levels of fitness (215). This effect may be limited to older adults as a short-term endurance training program in young adults (26 ± 2 years) was able to significantly increase HF HRV power over a 24-hour period (165). In other words an increase in aerobic fitness per se in older individuals does not imply modifications in HRV parameters.

6.1.3 HEART RATE CHANGE

There were no significant differences between groups when examining ΔHR at 30% and 40% IHG tasks. As explained in the literature review, the initial tachycardic response to the HG task should be solely mediated by withdrawal of PNS independent of

sympathetic activation (134; 204; 225). Thus, because both groups have similar indices of PNS tone they should have similar HR responses to HG exercise. One issue with regards to this assumption is that our understanding of the relationship between HR responses to HG and PNS levels comes from data observed in young individuals. For example, the HR responses in the current older groups were less than what has previously been reported in young adults. Specifically, compared to healthy young subjects who exhibited a HR increase of 10 ± 2 bpm in response to a 35% IHG task (362) a HR response of approximately 5 ± 3 bpm for both groups at 40% IHG was observed in this study.

This is probably due to greater PNS tone in younger individuals but may also result from decreased cardiac responsiveness that occurs with age. In a study that examined the parasympathomimetic effects of low dose atropine, a smaller decrease in heart rate was observed in older adults compared to young individuals independent of fitness level (188). This suggests that inevitable age-related declines in cardiac muscarinic receptor function cannot be prevented by regular physical activity even in highly trained endurance athletes. This is also supported by the regression analysis of ΔHR versus age for both IHG intensities (Figure 5.2.4). At 40% IHG there was a significant correlation (r^2 = 0.15) between the inverse relationship of advancing age and ΔHR. Therefore, regardless of fitness level, advancing age appears to have detrimental effects on heart rate control.

6.2 NEUROIMAGING RESULTS

Neuroimaging analysis showed BOLD signal changes in CAN structures and motor regions as well as various basal ganglia structures in both groups. These relationships were maintained when BOLD responses were regressed against the actual exercise task or the average group heart rate time course. Group level analysis showed significant

deactivation in the vMPFC at both IHG intensities for both groups. These data did not support the hypothesis, as it was expected that, the ET group would have different patterns of (de)activation in the CAN structures, specifically the vMPFC, compared to the CON group. However, because both groups exhibited a similar HR response to the exercise tasks they should have similar BOLD responses. This pattern of deactivation does follow previously established work in young healthy controls (362) and in older adults (regardless of blood pressure or pharmacologic status) who were classified as HR responders (243). Both these studies showed that IHG produced a HR response as well as deactivation in the vMPFC in an intensity-dependent manner and that the deactivation time course of the vMPFC mirrored the HR response, each reaching their peak at the end of each IHG. Similar intensity-dependent patterns were observed in the current study as observed in the time course Figures 5.3.8 and 5.3.9.

As mentioned previously, pharmacological studies have demonstrated that the tachycardic response at the onset of exercise cannot be altered with beta-adrenoreceptor blockade but could with vagal blockade (87; 225) suggesting it is PNS-mediated. Even though this study did not utilize inter-dwelling nerve recordings other studies have shown no increases in peripheral muscle sympathetic nerve activity during hand grip exercises of similar duration and intensity (362), again suggesting that the observed HR responses are dominated by vagal control. This study thus provides further evidence for vMPFC involvement in cardiovagal control of HR responses to exercise tasks. Regardless of age, fitness level, or even blood pressure status, it seems that if a HR response occurs, deactivation of the vMPFC will be observed.

Previously, it has been suggested that the vMPFC may be associated with baseline brain function or a “default brain network” (122). Researchers have found that the

vMPFC was deactivated during an active task regardless of what that task was (314) and also that high levels of activity were exhibited in this area just during supine rest (276). The relatively high levels of activity at rest may exert a tonic inhibitory drive suggesting that the vMPFC has a dominant role in autonomic activity at rest (362). This idea may be supported by the numerous direct connections observed in the rat brain between the vMPFC and nucleus tractus solitarius in the brainstem (333; 334), a structure that is involved in PNS outflow to the heart. More studies are needed, however, to elucidate if this relationship can be seen in other populations and determine if it is indeed part of a default network.

Interestingly activation of the dACC, a fairly consistent BOLD response to elevated HR which has occurred across numerous studies (61; 63; 161; 199), was absent in this study. These earlier studies, however, focused on increasing HR using a variety of manoeuvres such as mental stress, baroreceptor unloading, and effortful exercise that amplified sympathetic outflow. Similar to another study conducted by Wong et al (2007) the exercise task used in this study was designed to avoid increases in sympathetic outflow. Critchley and colleagues have proposed that the dACC modulates sympathetic outflow (63) and therefore a lack of sympathetic outflow in the IHG protocol would lead to no BOLD signal changes in this structure. Both this study and Wong et al (2007) found no increased activity in the dACC perhaps indicating there was no peripheral sympathetic activation.

The observed deactivation of the PCC in response to increased HR has also been observed in young healthy adults performing an IHG task but was not intensity-dependent like the vMPFC (61; 362). The PCC is thought to be part of the default brain network (276) and play a role in monitoring and representing the external environment at rest, and

its decreased activity is likely attributed to suspending these activities during a goal-directed IHG task (362). However, this deactivation was only present in both groups at 30% IHG relative to rest and not at 40% IHG. This contrasts previous work that concluded if a task was not sufficiently challenging, activity in the default mode network (which the PCC is part of) may persist through both the experimental and rest epochs (118). At this point, it is not understood why no deactivation would occur with the HR response to 40% IHG but perhaps the greater effort was able to flush out underlying age-related dysfunctions in the PCC.

As mentioned the PCC, along with the vMPFC, is part of the default brain network and is a prominent hub in intrinsic functional connectivity (276). There are some studies that have reported normal age-related alterations in the default brain network and resting functional connectivity (8; 197; 302; 319) which may impact the BOLD responses of certain CAN structures, such as the PCC, to different stimuli. In cognitively intact older adults it was shown that the PCC is highly susceptible to early amyloid deposition which can lead to disrupted default activity (319). Another recent study observed an age-related decrease in inflow (i.e. a measure of how strong the activity in a region is influenced by the activity in other regions) to the PCC using magnetoencephalography (MEG) which has a higher temporal resolution than fMRI (302). Thus the PCC seems to be highly susceptible to the effects of age, more so than other structures in the default brain network which could affect the PCC's BOLD responses to certain tasks.

The IC is an important integration centre for visceral inputs and resulting autonomic responses and has received considerable attention as being one of the dominant structures in the CAN. Its involvement in autonomic states has been well established from electrophysiological and stimulation studies in animals (37; 124; 153; 274; 292; 363). In

the current study, the bilateral anterior insula showed significant activation above baseline in both groups for the HR response at both IHG intensities. This relationship, however, was not observed at 40% IHG for the CON group where activation occurred in the right anterior insula but not in the left. Activation of the anterior insular cortices could be explained by the significant role these structures play in somatosensory processing (11; 88) as they are involved in somatosensory mapping of the body (5). Work of Cechetto and Saper (47) suggested that the IC represents viscerotopic sensory aspects of cardiovascular arousal and that it merely reflects the body's constant internal state.

However as the above animal studies – and more recent human work – have demonstrated, increased insular activity is noted whenever cardiovascular responses occur (61; 63; 161; 359; 362). Also, damage to this structure causes various cardiovascular dysfunctions, such as more frequent and complex arrhythmias (56; 337). This evidence, along with its neural connections to the vMPFC (348), suggests that the insula has an active role in feedback modulation of CV responses (361).

Generally speaking, the left insula has been associated with parasympathetic effects and the right insula with sympathetic effects; however, this is likely an oversimplification. Both insulae have been implicated in the complex process of modulating cardiovagal BRS (161; 297; 365) and evoked potential recordings have demonstrated that the IC receives input indirectly from the vagal C- and A-fibre afferents (144; 145). Furthermore, there also seems to be baroreflex-related inter-neuronal connections between both insulae suggesting that they may interact to integrate circulatory control via the cardiovagal baroreflex loop (364) with both IC receiving markedly greater afferent inputs even than the vMPFC by way of the NTS, parabrachial nucleus and visceral sensory thalamic nuclei (46).

In summary, the neuroimaging results of this study indicate that cortical responses in older individuals who provide a HR response to IHG are consistent with previous work in younger individuals. Deactivation of the vMPFC and activation of the expected primary motor cortex with a significant HR response to IHG response was observed. Bilateral anterior insula activation for the most part was consistent throughout the study except during the 40% IHG trial in the CON group where only right anterior insula activation was observed. It is uncertain why the PCC would be deactivated during 30% IHG in both groups but not in the 40% task but could occur due to altered default brain function with age. It is unknown whether CAN structures exhibit lateralization such as the motor cortices but the vMPFC deactivation consistently appeared in the left hemisphere and the PCC always appeared in the right.

6.3 LIMITATIONS AND FUTURE DIRECTIONS

Due to lack of familiarization with the MRI environment some subjects may have suffered from mild anxiety and/or claustrophobia which could have influenced the BOLD responses to the IHG task. However, HR responses to IHG were similar between the lab session and MRI sessions for each group, so it seems unlikely that cardiovascular responses manifested from anxiety occurred during the neuroimaging session.

Because we did not include a method for directly measuring sympathetic activity we can only speculate as to what actually occurred. Comparisons to previous studies that utilized similar methodology and made inter-dwelling nerve recordings of peripheral sympathetic activation were made instead. In the future, it would be useful to make direct measures of sympathetic activity when parasympathetic activity is analyzed to better ensure physiological results are PNS-mediated.

Even though each group contained 15 individuals they were heterogeneous in the male to female ratio. This may be an important factor when analyzing group differences as there are small differences between males and females in autonomic profiles and cortical (de)activation patterns. However, the attempt was made to recruit post-menopausal women to eliminate cycling levels of estrogen as a variable. Of the 11 women involved in the study eight were post-menopausal. Previous fMRI studies examining sex differences in CAN function observed altered, but still measureable, BOLD responses in females compared to males (162; 361). It was also imperative to recruit enough individuals for each group in order to compare fMRI results. Generally, 14 to 16 subjects per group is the minimum requirement to obtain significant results after correcting for multiple comparisons (i.e. false discovery rate, FDR). In fact to address the problem of multiple comparisons in a voxel-wise analysis previous research has suggested a minimum of 20 participants for between-group analysis (73). Therefore, future studies may benefit from specifically recruiting and testing either strictly males or females and utilizing a larger sample size.

For future studies, it may prove useful to include another contraction intensity, such as 50% MVC, to determine if there would have been a significant difference between group HR responses to exercise. It must be cautioned though that at this intensity sympathetic activation may start to occur before handgrip is finished and therefore an adjustment to the hand grip duration might be required.

Additionally, future cross-sectional studies examining endurance trained individuals should collect physical activity records. Even though VO_2max tests were utilized to distinguish fitness levels between groups and ensure one group could be classified as ET, a significant part of an individual's VO_2max trainability is genetic (27) and there are

noted genomic predictors that determine an individual's maximal O_2 uptake response to training programs (28). This genetic component of maximal oxygen uptake makes it difficult to use as the only index for endurance training status. Collecting physical activity records would also help to expose any large training differences amongst the ET group. Even though ET subjects were recruited based on the criteria of running 25 km/week for five years or greater there was no set maximum amount. This produced a mixed array of ET individuals with number of years running ranging from 5 to 38 and number of kilometres per week ranging from 25 to 90.

Another subject characteristic that may have limited the results of this study is age. Even though older adults were the intended population, the subject group could potentially be classified as middle-aged (approximate mean age of 55 years in each group). For example, when Monahan and colleagues examined the effects of aging on cardiovagal BRS they examined three age groups: young, middle-aged and older adults (226). The middle-aged group ranged from 38 – 56 years of age and the older group ranged from 57 – 79 years of age with a significant reduction in BRS in the older group compared to middle-aged adults. Also, other studies that have examined the effects of endurance training on autonomic control in older adults utilized populations greater than sixty years of age (29; 265). As a follow-up to this study and to address this limitation, if the same group of people could be assessed after five years when the mean age would be approximately 60 perhaps significant differences would then be observed between groups.

Finally, utilizing other imaging techniques such as arterial-spin labelling and diffusion tensor imaging might allow for detection of ET related differences between groups. Even though there were no functional differences based on the observed BOLD

responses the addition of further imaging techniques would enable assessment of potential differences in cerebral blood flow or white matter connectivity. Analysis of anatomical data might also be more informative than functional data for between-group differences as there is strong evidence to support the positive effects ET has on the age-related declines in GM volume, cortical thickness and total tissue atrophy.

6.4 SIGNIFICANCE OF FINDINGS

The major finding of this study is that long-term endurance training in 55 year old adults does not seem to offer any discernible neuroprotective effects nor does it offer any benefits for autonomic cardiovascular control. It did not increase parasympathetic activity or alter BOLD responses to IHG or HR in the structures associated with the CAN compared to age-matched untrained controls. It is important to point out that all of the results were internally consistent. Because there were no significant differences between physiological variables it was expected that there would be no differences between cortical activation patterns. Thus, aging appears to have an important and perhaps irreversible effect on CAN function. Further analysis however, may reveal significant differences between long-term endurance athletes and age-matched controls with respect to total brain anatomy, the morphology of certain structures and white matter connections between different areas of the brain.

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APPENDIX I

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Kevin Shoemaker
Review Number: 17828
Review Level: Full Board
Approved Local Adult Participants: 60
Approved Local Minor Participants: 0
Protocol Title: The neuroprotective effects of long term endurance training on the aging brain
Department & Institution: Kinesiology,University of Western Ontario
Sponsor: Canadian Institutes of Health Research

Ethics Approval Date: June 10, 2011 **Expiry Date:** August 31, 2013

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
UWO Protocol	(including instruments noted in section 8.1)	
Letter of Information & Consent	(version - April 2011)	
Advertisement		

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer to Contact for Further Information

This is an official document. Please retain the original in your files.

The **University *of* Western Ontario**
Office of Research Ethics
Support Services Building Room 5150 • London, Ontario • CANADA - N6A 3K7
PH: 519-661-3036 • F: 519-850-2466 • ethics@uwo.ca • www.uwo.ca/research/ethics

CURRICULUM VITAE

Torri Alexa Luchyshyn

EDUCATION:

2010 – Present **Masters of Science in Kinesiology**
School of Kinesiology, The University of Western Ontario, London, ON

Thesis: Neuroprotective Effects of Long-term Endurance Training on the Cortical Autonomic Network in the Aging Brain

2006 – 2010 **Honours Specialization Bachelor of Science in Kinesiology**
Department of Kinesiology, The University of Western Ontario, London, ON

ACADEMIC AWARDS:

2011-2012	CIHR Canada Graduate Scholarship – Masters	17500/yr
2010-2011	Western Graduate Research Scholarship	5500
2011	Ontario Graduate Scholarship (declined)	15000
2010	NSERC Undergraduate Student Research Award	4500
2010	CSEP Undergraduate Student Award	
2006-2010	UWO Continuing Entrance Scholarship	2500/yr
2006-2010	The University of Western Ontario Dean's Honour List	

RESEARCH INTERESTS:

Autonomic Network; Aging; Cardiovascular Control; Neuroimaging

RELEVANT EXPERIENCE:

05/2010 – present **Research Assistant** – Supervised by Dr. Kevin Shoemaker and Charlotte Usselman (PhD), School of Kinesiology, The University of Western Ontario, London, ON

Topic: Impact of sex hormones on autonomic cardiovascular reflex responses
- Lab experimental set-up and data collection
- Stroke volume velocity ultrasound, phlebotomy, LBNP

09/2011 – 12/2011 **Graduate Teaching Assistant** – Supervised by Dr. Kevin Shoemaker, School of Kinesiology, The University of Western Ontario, London, ON

Course: Physiology of Exercise and Reflex Cardiovascular Physiology (4th year)
• Offering weekly office hours and answering student questions
• Attending review sessions and marking examinations
• Taught two guest lectures

09/2010 – 12/2010 **Graduate Teaching Assistant** – Supervised by Dr. J. Kevin Shoemaker, School of Kinesiology, The University of Western Ontario, London, ON

Course: Physiology of Exercise and Reflex Cardiovascular Physiology (4th year)
• Offering weekly office hours and answering student questions
• Attending review sessions and marking examinations
• Taught three guests lectures

05/2010 - 08/2010 **Research Assistant** – Supervised by Dr. Kevin Shoemaker, Department of Kinesiology, The University of Western Ontario, London, ON

Topic: Baroreflex Sensitivity across Various Populations
• Baroreflex sensitivity analysis (Matlab)
• Consolidated results from database of past subjects

09/2008 - 11/2008 **Teaching Assistant** – Supervised by Dr. Michelle Mottola, Department of Kinesiology, The University of Western Ontario, London, ON

Course: Functional Human Anatomy (2nd year)
• Assisting with cadaver anatomy laboratory sessions
• Answering student questions and running review sessions

PUBLICATIONS:

Peer Reviewed Abstracts: Poster Presentations

Luchyshyn, T., Norton, K., Corkal, J., & Shoemaker, JK. Long-term endurance training preserves cardiovagal control but not heart rate response to exercise. *FASEB J.* 26: 1091.29, 2012.

Usselman, C., Nielson, C., **Luchyshyn, T.**, Gimon, T., & Shoemaker, JK. Sex-specific sympathetic responses to chemoreflex stress in healthy young men and women. *FASEB J.* 26: 1091.29, 2012.

Usselman, C., **Luchyshyn, T.**, Gimon, T., Nielson, C., & Shoemaker, JK. Sympathetic responses to chemoreflex stress across the menstrual cycle in young eumenorrheic women. *Canadian Society of Exercise Physiology*, Quebec City, QC, 2011.

Non-Peer Reviewed Abstracts: Poster Presentations

Luchyshyn, T., Shoemaker, JK., Usselman, C., Rothwell, A., & Wong, S. Rapid functional malleability of cortical organization associated with cardiovascular arousal. *The University of Western Ontario Faculty of Health Sciences Research Day*, London, ON, March, 2011.

Non-Peer Reviewed Abstracts: Oral Presentations

Luchyshyn, T. & Shoemaker, JK. The neuroprotective effects of exercise on the cortical autonomic network in the aging brain: a prospectus. *Ontario Exercise Physiology Conference*, Barrie, ON, January, 2011.