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The neuroprotective effects of endurance training on the aging brain

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Abstract

Dysregulation of autonomic control often develops with advancing age, favoring a chronic state of heightened sympathetic outflow with parasympathetic withdrawal. However, the mechanisms of this age-related autonomic impairment are not known and may relate to alterations in brain structure (e.g. cortical atrophy) and/or altered neural function, particularly in regions related to the cortical autonomic network, namely, the medial prefrontal cortex (MPFC), insula cortex (IC), and hippocampus (HC). Exercise exerts beneficial effects on brain structure and, in the case of cognition, neurologic function; however, how exercise affects regions of the brain related to autonomic function are not known. This thesis tested the hypothesis that changes in autonomic outflow across the adult age-span are related to cerebral cortex atrophy and function, and are sensitive to the effects of physical fitness. Study 1 demonstrated that advancing age impairs the heart rate (HR) response and modifies the cortical patterns associated with cardiovascular control during isometric handgrip (IHG), and is further exacerbated with coronary artery disease. The utility of aerobic exercise to prevent these age-related changes is not known. Study 2 revealed that lifelong, sustained aerobic training builds cortical reserve early in life, and sustains this benefit over the 40-70 year age span, but did not alter the rate of age-related cortical or subcortical decline. Study 3 demonstrated that cardiorespiratory fitness correlated strongly with whole-brain cortical thickness, while markers of autonomic outflow were specifically associated with cortical mass at the MPFC. Importantly, the strength of the relationship between autonomic variables and cortical thickness was determined by age, and was not altered following adjustments for cardiorespiratory fitness. Study 4 revealed a positive effect of high fitness on MPFC activation, yet did not affect absolute HR responses to IHG in this age range. Therefore, this series of studies implicates cortical atrophy in the frontal lobe as a contributor to the dysregulation of autonomic outflow associated with advancing age, and suggests that high cardiorespiratory fitness delays the age-related decline in cortical circuitry associated with cardiovascular control.

Keywords

cortical autonomic network, cardiorespiratory fitness, age, handgrip exercise, Masters athletes, cortical thickness, insula, medial prefrontal cortex

Co-Authorship Statement

Katelyn N. Norton was the first author on the four papers that compose the body of this thesis and J. Kevin Shoemaker was the senior author. The co-authors on Chapter 2 were Mark B. Badrov, Carly C. Barron, Neville Suskin, and Armin Heinecke. The co-author on Chapter 3 was Robert Nikolov. The co-author for Chapter 4 was Torri A. Luchyshyn.

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ACC	Anterior Cingulate Cortex
ACh	Acetylcholine
AChE	Acetylcholinesterase
ANS	Autonomic Nervous System
AV	Atrioventricular
BDNF	Brain-derived neurotrophic factor
BMI	Body Mass Index
BOLD	Blood-oxygen-level-dependent
BP	Blood Pressure
BRS	Baroreflex Sensitivity
CAD	Coronary Artery Disease
CO	Cardiac Output
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
fMRI	Functional Magnetic Resonance Imaging
FDR	False Discovery Rate
HA	Healthy Active
HC	Hippocampus
HR	Heart Rate

HRV	Heart Rate Variability
IC	Insula Cortex
IGF-1	Insulin-like Growth Factor 1
IHG	Isometric Handgrip
K	Potassium
LVEF	Left-ventricular Ejection Fraction
MA	Master's Athletes
MAP	Mean Arterial Pressure
MPFC	Medial Prefrontal Cortex
MSNA	Muscle Sympathetic Nerve Activity
MVC	Maximal Voluntary Contraction
NTS	Nucleus Tractus Solitarus
PCC	Posterior Cingulate Cortex
PNS	Parasympathetic Nervous System
PPI	Psychophysiological Interaction
RFX	Random Effects
ROI	Region-of-Interest
RVLM	Rostral Ventrolateral Medulla
SA	Sinoatrial
SBP	Systolic Blood Pressure

SDNN	Standard Deviation of Normal-to-Normal RR Intervals
SNS	Sympathetic Nervous System
VEGF	Vascular Endothelial-derived Growth Factor
VO_{2max}	Maximal Oxygen Consumption

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Chapter 1

1 Introduction

1.1 Overview

As a major risk factor for dementia and other neurologic impairments, the current trajectory of our aging population will produce an unsustainable health and economic burden for future generations. Advancing age is often associated with cortical atrophy (Raz, Lindenberger et al. 2005), changes in brain functional responses (Nyberg, Salami et al. 2010), and declines in cognitive performance (Ronnlund, Nyberg et al. 2005). Autonomic dysregulation represents a further insidious age-related neurological impairment that threatens morbidity and mortality (Nicolini, Ciulla et al. 2014) through detrimental effects on blood pressure control (Izzo, Smith et al. 1987, Izzo and Taylor 1999), exacerbation of atherosclerosis (Zhang and Faber 2001), cardiac tissue damage, and heightened risk of arrhythmias (Hachinski, Wilson et al. 1992). Nonetheless, substantial inter-individual differences exist with some individuals showing resistance to major age-related brain pathologies (Nyberg, Lovden et al. 2012, Pudas, Persson et al. 2013). The large heterogeneity in brain aging represents a major scientific challenge that has led researchers to examine what factors contribute to this protection against imminent age-related decline. In the last decade, it has frequently been suggested that physical activity may have positive global influences on brain health, including spared brain volume (Erickson, Prakash et al. 2009, Erickson, Voss et al. 2011, Niemann, Godde et al. 2014), improved task-related functional responses (Colcombe, Kramer et al. 2004, Voelcker-Rehage, Godde et al. 2010), increased white matter integrity (Johnson, Kim et al. 2012, Voss, Heo et al. 2013), and cognitive performance (Josefsson, de Luna et al. 2012). However, whether exercise has a similar impact on autonomic function has only recently been explored (Carter, Banister et al. 2003) showing the most benefit in aged individuals or those with pathological conditions that raise baseline sympathetic outflow, such as heart disease. Further, existing data are difficult to interpret in terms of the overall exercise benefit. Prospective training studies normally are of short duration (<12 months) and are employed in a narrow age range of senescent individuals with a starting

point of cognitive impairment or cardiovascular disease. Therefore, there is need to understand the importance of intervention timing and the exercise dose response patterns across the adult age range during which brain atrophy occurs.

Coronary artery disease (CAD) increases the risk for stroke, cognitive impairment and autonomic dysregulation (Martins, Hone et al. 2006, Zulli, Nicosia et al. 2008, Roberts, Knopman et al. 2010, Barekatain, Askarpour et al. 2014). Impaired autonomic outcomes of CAD may exacerbate the disease pattern though tissue damage and/or a diminished ability to affect rapid adjustments in response to stress and, thereby, limit the benefits that can be derived from exercise rehabilitation. Moreover, our laboratory previously reported accelerated age-related cortical atrophy in CAD patients (Anazodo, Shoemaker et al. 2013). However, data are limited regarding the impact of CAD on the brain-heart connection and the utility of aerobic exercise to prevent these insidious changes.

Experimental models in rodents (Cechetto and Chen 1990, Dampney 1994, Verberne 1996), as well as clinical (Norris, Froggatt et al. 1978, Critchley, Mathias et al. 2003, Woo, Macey et al. 2003, Soros and Hachinski 2012, Woo, Yadav et al. 2014), and observational studies in humans (Critchley, Corfield et al. 2000, Cechetto and Shoemaker 2009, Thayer, Sollers et al. 2009, Shoemaker and Goswami 2015), indicate that several cortical sites modulate autonomic cardiovascular control. In fact, cognitive (Critchley, Mathias et al. 2003, Critchley, Wiens et al. 2004, Critchley, Rotshtein et al. 2005, Critchley, Nagai et al. 2011) and autonomic network regions (Cechetto and Shoemaker 2009, Shoemaker and Goswami 2015) appear to overlap in critical cortical sites including the medial prefrontal cortex (MPFC), hippocampus (HC), anterior cingulate cortex (ACC), and insula cortex (IC) (Barron and Chokroverty 1993, Soufer, Bremner et al. 1998, Critchley, Corfield et al. 2000, Gianaros, Van Der Veen et al. 2004, Gianaros, Derbyshire et al. 2005). The prefrontal areas of the brain, which are the last to fully mature in the early twenties, are the first to show signs of decline as early as age forty (Raz, Lindenberger et al. 2005) with degradation of fiber myelination (Northoff, Richter et al. 2000), and a loss of structural connectivity (O'Sullivan, Jones et al. 2001). Significant but more moderate declines are seen in the temporal, parietal and occipital cortices, respectively (Raz and Rodrigue 2006). In addition, the hippocampus, amygdala

and cerebellum all show signs of age-related atrophy (Raz and Rodrigue 2006). Consequently, alterations in cerebral cortex structure may lead to generalized impairment across several neurological outcomes. Thus, prevention or treatment of cortical degradation with age may yield significant social and clinical outcomes.

1.2 Study Approach

Moderate intensity isometric handgrip (IHG) exercise of short duration produces a rapid tachycardia in young and healthy individuals (Mancia, Iannos et al. 1978, Mark, Victor et al. 1985, Wong, Masse et al. 2007), offering a unique opportunity to explore the cortical representation of autonomic cardiac control. Pharmacologic evidence indicates that a decrease in parasympathetic dominance accounts for much of this rapid heart rate (HR) change (Hollander and Bouman 1975, Fagraeus and Linnarsson 1976, Mitchell, Reeves et al. 1989). In young individuals, the magnitude of this rapid increase in HR with IHG exercise is correlated with reduced activity within the MPFC (Gianaros, Van Der Veen et al. 2004, Wong, Masse et al. 2007) and the HC (Norton, Luchyshyn et al. 2013). Age and coronary artery disease (CAD) are associated with impaired autonomic outcomes including diminished parasympathetic modulation of HR (Mancia, Cleroux et al. 1991, Seals, Taylor et al. 1994, Ford 1999). It follows that these regions are among those associated with cardiovagal control and form our *a priori* cortical regions of interest including the IC and ACC.

T1-weighted structural acquisition at 3-Tesla will enable quantification of cortical thickness and subcortical gray and white matter volumes, while T2-weighted functional neuroimaging will examine blood-oxygen-level-dependent (BOLD) activation patterns in response to the IHG task.

Age, cardiorespiratory fitness, and markers of autonomic outflow will be treated as possible covariates.

1.3 Purpose

The purpose of this study was to test the interaction between cardiorespiratory fitness and cortical structure and functional responses related to the neural control of the circulation. The **working hypothesis** is that differences in autonomic outflow across the adult age-span are related to cerebral cortex atrophy and function, and are sensitive to the effects of physical fitness. We suggest that brain structure and functional responses are modified by negative (pathology) and positive (cardiorespiratory fitness) behaviors and that such impairment, or improvement, is associated with HR control, particularly emphasizing activity patterns within discrete regions of the cortical autonomic network, namely the MPFC, IC, and HC.

Study 1. Coronary artery disease affects cortical circuitry associated with brain-heart integration during volitional exercise.

Purpose: To determine if CAD negatively impacts the cortical and HR response to volitional IHG.

Hypothesis: Coronary artery disease is associated with a reduced HR response to volitional exercise, which is accompanied by dysregulation of the cortical autonomic network.

Study 2. Impact of long-term endurance training vs. guideline-based physical activity on brain structure in healthy aging.

Purpose: To determine the maximal benefit of exercise training on cortical and subcortical gray matter.

Hypothesis: Maximal levels of aerobic exercise training will provide benefits to brain structure beyond those achieved through guideline-based fitness.

Study 3. Regional cerebral cortical thickness correlates with autonomic outflow.

Purpose: To examine the relationship between cortical structure and autonomic outflow in aging adults.

Hypothesis: Cortical atrophy predicts age-related changes in autonomic outflow.

Study 4. High cardiorespiratory fitness in middle-age preserves the cortical circuitry associated with brain-heart integration during volitional exercise.

Purpose: To examine how cardiorespiratory fitness affects the cortical circuitry associated with cardiovascular arousal.

Hypothesis: High cardiorespiratory fitness prevents the age-related decline in cortical circuitry associated with isometric handgrip and cardiovascular arousal.

1.4 The Autonomic Nervous System

The autonomic nervous system (ANS) is responsible for virtually every aspect of involuntary physiologic control. It is structurally and functionally positioned to interface between the internal and external environments, coordinating bodily functions to ensure homeostasis, and generate adaptive responses. Thus, the ANS has the crucial task of overseeing system regularity and ultimately ensuring our survival. The ANS is comprised of three divisions: the sympathetic and parasympathetic nervous systems, as well as the enteric nervous system. It is through the sympathetic and parasympathetic centrally-orchestrated neural systems that the body achieves regulation over the cardiovascular system. A dynamic balance occurs between these two effectors such that the development and control of cardiovascular regulation at any given level depends upon the balance between their respective activities or dominance of functional outcome.

1.4.1 Sympathetic Nervous System

Pre- and post-ganglionic adrenergic axons ensure transmission of signals through the sympathetic nervous system (SNS), which work to mobilize the body's resources for action under stress. The shorter preganglionic neurons of the SNS originate in the interomedial cell column of the thoracic and lumbar spinal cord (T1 - L2), and receive input signals from multiple transmitter systems in the hypothalamus, brainstem, and autonomic control centers within the cortex (Shields 1993). Axons from the preganglionic neurons project to a chain of ganglia located near the spinal cord (collectively referred to as the sympathetic trunk) where they synapse with much longer postganglionic neurons. At these synapses, preganglionic neurons release acetylcholine (ACh), a neurotransmitter that binds to nicotinic cholinergic receptors on postganglionic dendrites. Upon stimulation of postganglionic neurons, action potentials descend along their axons to peripheral targets where various neurotransmitters are released, thereby activating receptors on the peripheral tissues. Targets of the SNS include the vascular smooth muscle, myocardial and pacemaker cells of the heart, as well as smooth muscle cells surrounding the vasculature in skeletal muscle, visceral organs, and skin (Shields 1993). It is the activation of these target tissue receptors that causes end-organ sympathetic effects on the blood vessels and the heart.

Within the blood vessels, small enlargements along the nerve fibers, called varicosities, are the site of neurotransmitter storage and release. There are several neurotransmitters released from sympathetic nerve terminals during sympathetic discharge. Norepinephrine, neuropeptide Y and adenosine triphosphate all exert effects on vascular smooth muscle when they bind to their corresponding receptors. Generally, the overall effect of sympathetic innervation on the vascular system is vasoconstriction causing an increase in vascular resistance and cardiac output, and a decrease in peripheral blood flow (Robertson, Waller et al. 1988).

Direct recordings of sympathetic outflow in humans are made with the use of microneurographic techniques (Hagbarth and Vallbo 1968) from the populations of postganglionic sympathetic neurons innervating the skeletal muscle vasculature (muscle sympathetic nerve activity; MSNA). The measured bursts of activity reflect periods of efferent action potential synchronization (Delius, Hagbarth et al. 1972). The size of any given burst is determined by the proximity of the recording electrode to the individual neurons within the respective bundle of sympathetic axons (Tompkins, Melling et al. 2013), as well as the number (Ninomiya, Malpas et al. 1993) and size (Salmanpour, Brown et al. 2011) of action potentials.

Sympathetic activation of the heart through the cardiac nerves results in tachycardia and increased contractility. Neural activation of the sinoatrial (SA) and atrioventricular (AV) nodes acts to increase HR. When the SA node receives sympathetic stimulation, norepinephrine is released from the nerve endings and binds to adrenergic receptors on the pacemaker cell membrane. Innervation of the myocardial fibers causes an increase in the force of contraction at the atria and ventricles. As a result of these combined effects, enhanced sympathetic activity causes blood pressure and HR to rise and is particularly important during periods of excitement and stress.

1.4.2 Parasympathetic Nervous System

Similar to the SNS, the parasympathetic nervous system (PNS) follows a two-neuron efferent system that has both preganglionic and postganglionic neurons. The preganglionic fibers of the PNS originate from the craniosacral regions of the spinal cord,

including the medial medullary sites of the nucleus ambiguus, nucleus tractus solitarius, and dorsal motor nucleus (Olshansky, Sabbah et al. 2008). In the medulla, the cranial nerves III (oculomotor nerve), VII (facial nerve), IX (glossopharyngeal nerve), and X (vagus nerve) form the preganglionic parasympathetic fibers (Guyenet 2006), which project to ganglia very close to their visceral targets. The most important cranial nerve responsible for cardiovascular control is the vagus nerve, containing nearly 90% of the preganglionic parasympathetic fibers in the body (Marieb E 2004). As indicated in the SNS, preganglionic innervation in the PNS is cholinergic with these terminals releasing ACh at the ganglion synapse onto nicotinic receptors.

The parasympathetic influence on cardiac function occurs predominantly through the binding of ACh to muscarinic receptors in the heart. There are three main types of muscarinic receptors that are well characterized: M_1, M_2 and M_3. M_2 is the isoform most frequently found in the heart. ACh binding here inhibits adenylate cyclase activity, resulting in a decrease in the excitability of the pacemaker cells, which reduces contractile forces of the atrial cardiac muscle, and decreases conduction velocity of the SA and AV nodes. The net effect of vagal stimulation is therefore a decrease in HR (Bebbington and Brimblecombe 1965).

1.5 Neural Control of the Heart

In normal adults, the average HR at rest is approximately 70 beats per minute (bpm). During emotional excitement, muscular activity, or stress, it may accelerate to rates considerably higher than 100 bpm (Berne 2001). The SA node is the pacemaker of the heart, responsible for setting rate and rhythm. The SA node is under the tonic influence of both the sympathetic and parasympathetic nervous systems, such that changes in HR involve a reciprocal action of the two divisions. In healthy resting individuals, parasympathetic tone predominates, which is why the average resting HR is 70 bpm or less. Abolition of parasympathetic influences by administration of atropine increases HR substantially (~40 bpm), whereas inhibition of sympathetic effects by administration of propranolol decreases HR only slightly (~9 bpm) (Katona, McLean et al. 1982). Levy and colleagues further proved that parasympathetic influences prevail at the SA node through constant, simultaneous vagal and sympathetic stimulation in the anesthetized dog (Levy and Zieske 1969). As the frequency of sympathetic stimulation was increased from 0-4Hz, HR increased by about 80 bpm in the absence of vagal stimulation. However, when the vagus nerve was stimulated at 8Hz, increasing the sympathetic stimulation frequency had a negligible influence on HR. This vagal domination in the regulation of HR is mediated mainly by functional interactions between the SNS and PNS. Presynaptic interneuronal and postsynaptic intracellular mechanisms have been shown to exist between the terminal postganglionic vagal and sympathetic fibres, which lie in close proximity to one another within the heart. The release of ACh from neighbouring nerve endings causes the effective blockade of the release of norepinephrine from the sympathetic nerve endings thus facilitating the antagonizing activity of the vagus nerve on any concomitant sympathetic activity (Kulbertus 1988).

There is consistent evidence for a reduction in parasympathetic influence as the primary mediator of the increase in HR at the onset of exercise, however the characteristics of sympathetic activity have yet to be clarified. An early study by Robinson et al. (Robinson, Epstein et al. 1966) reported that at low-to-moderate exercise intensity, an increase in HR was mediated primarily by parasympathetic withdrawal, and that sympathetic activity increased initially at 60% of VO_{2max}. In support of this finding,

Nakamura et al. (Nakamura, Yamamoto et al. 1993) later reported that parasympathetic activity decreased significantly up to 60% VO_{2max}, and sympathetic activity increased significantly after 60% VO_{2max}. Yamamoto and Hughson (Yamamoto, Hughson et al. 1991) further reported parasympathetic activity decreased until exercise intensity reached 60% of ventilatory threshold. These authors also reported that sympathetic activity remained unchanged up to 100% of ventilatory threshold and then increased abruptly at 110%. Arai et al. (Arai, Saul et al. 1989) reported a reduction in parasympathetic activity with increasing cycle ergometer intensity, but with an absence of change in sympathetic activity. Therefore, it is important to note that the time course kinetics and relative contribution of the two autonomic divisions differs substantially.

Vagal effects on the heart develop rapidly, often within one heartbeat, and they decay nearly as quickly. Hence, the vagus nerve can exert beat-by-beat control of cardiac function. Conversely, the onset and decay of sympathetic effects are much more gradual; only small changes are affected within the time of one cardiac cycle. For example, during sympathetic cardiac nerve stimulation, the HR and force of contraction increase after a latent period of about 1-3s, approaching an increased steady state level in about 30s (Robertson, Waller et al. 1988). Once stimulation has ended, the return back to baseline takes place much more gradually than at the onset owing to the relatively slow rate of norepinephrine metabolism by the cardiac tissue. Conversely, the changes in HR produced by vagal stimuli appear after a brief latent period (about 50-100ms), reach a steady state response within a few beats, and decay rapidly back to baseline levels (Warner and Cox 1962). As a result, PNS activation is associated with immediate reductions in arterial pressure and HR due to the characteristics of acetylcholinesterase (AChE) and special potassium (K^+) channels in the cardiac cells. AChE is an enzyme that regulates synaptic ACh concentrations through the hydrolysis of ACh. The molecular mechanisms responsible for the rapid action of the enzyme have yet to be elucidated (Nair, Seravalli et al. 1994). However, it is well known that the release of ACh activates specialized K^+ channels causing them to open rapidly and effect changes in the pacemaker potential. Thus, very rapid changes in HR are mediated exclusively by the PNS due to the abundance of AChE and consequent rapid clearance of synaptic ACh.

Taken into context of the current study, moderate intensity IHG exercise of short duration produces a rapid tachycardia in young and healthy individuals at the onset of exercise (Mancia, Iannos et al. 1978, Mark, Victor et al. 1985, Wong, Masse et al. 2007), which pharmacologic evidence indicates is mediated by a decrease in parasympathetic dominance (Hollander and Bouman 1975, Fagraeus and Linnarsson 1976, Mitchell, Reeves et al. 1989). Therefore, the characteristics of the vagal system, in relation to a task known to elicit large and rapid changes in HR, offers a unique opportunity to explore the cortical representation of autonomic cardiovascular control.

1.5.1 The Baroreflex

Arterial blood pressure (BP), the product of cardiac output and systemic vascular resistance, is regulated acutely and chronically through various local, humoral, and neural factors. In humans, the major neural pathway by which BP is rapidly and reflexively modulated is called the baroreflex. The baroreflex is a neurocardiovascular reflex that operates in a negative feedback loop to maintain BP homeostasis (Monahan, Dinenno et al. 2001). The purpose of the baroreflex is to regulate mean BP, preventing large transient changes that may arise from sudden stressors (Cowley, Liard et al. 1973). This loop anatomically begins at the level of the carotid and aortic baroreceptors, which are highly specialized stretch-sensitive receptors (a multitude of free nerve endings) located within the wall of the carotid sinus and the aortic arch. These arterial baroreceptors play a key role in the acute control of BP, initiating physiological responses including the modification of HR, vascular resistance, and myocardial contractility (Kaye and Esler 2008). In response to an increase in systemic BP, baroreceptors in the carotid sinus excite fibers in the sinus branch of the glossopharyngeal nerve which project to the nucleus tractus solitarius (NTS), resulting in the inhibition of efferent sympathetic outflow from the rostral ventrolateral medulla (RVLM; primary SNS generator) and excitation of the nucleus ambiguous which initiates vagal outflow. Preganglionic parasympathetic fibers then project to the ganglion cells of the posterior heart where they act to reduce pacemaker activity, thus reducing HR and therefore BP (Berne 2001). When BP decreases, disinhibition of the RVLM leads to an increase in pre- and postganglionic

sympathetic activity to the peripheral arterioles, thus increasing the arterial resistance, and effectively increasing HR.

The study of the relationship between changes in BP and HR is used as a measure of the sensitivity of the arterial baroreflex (Monahan, Dinenno et al. 2000). Acute changes in BP reflexively elicit inverse changes in HR via the baroreceptors. This relationship is sigmoidal and the maximum slope of the curve is a key index of baroreflex sensitivity (BRS). Most importantly, the slope of this portion of the curve is principally mediated by PNS activity and accordingly is often termed cardiovagal BRS (Kaye and Esler 2008).

Thus, the baroreflex mechanisms finely tune HR, AV node conduction, myocardial contractility and electrophysiological properties, and peripheral resistance on a beat-by-beat basis, and dampen the effects of perturbations (Eckberg 1992).

1.5.2 Heart Rate Variability

As previously mentioned, variation in HR, whether at rest or in response to a stimulus, is mediated by the combined effects of cardiac vagal and sympathetic nerves acting upon the SA node. Heart rate variability (HRV) is a simple and non-invasive measure representing one of the most promising quantitative markers of autonomic balance. HRV describes the oscillations in the interval between consecutive heart beats (RR interval), as well as the oscillations between consecutive instantaneous heart rates. Sinus arrhythmia, or HRV, is enhanced during beta-adrenergic blockade (Coker, Koziell et al. 1984) and is abolished by atropine (Wheeler and Watkins 1973). Although the precise mechanisms responsible for increased variability are not fully understood, the efferent pathway is largely via cardiac vagal fibres.

HRV simultaneously reflects the status of the heart and the reaction of the ANS in compensating for hemodynamic changes and provides a sensible and advanced indicator of health. Thus, HRV can be used to assess the ANS modulation under physiological conditions such as wakefulness and sleep conditions, different body positions, physical training and pathological conditions. High HRV is a signal of efficient adaptation and characterizes competent autonomic mechanisms, while low HRV is frequently an

indicator of abnormal and insufficient adaptation of the ANS, provoking poor physiological function. Low resting HRV has been related to an increased risk of all-cause mortality (Tsuji, Venditti et al. 1994) and to the incidence of ventricular arrhythmias and coronary artery disease (Algra, Tijssen et al. 1993, Tsuji, Larson et al. 1996, Dekker, Schouten et al. 1997, Liao, Cai et al. 1997).

1.5.3 Statistical Analysis

The recognition that both the HR and BP responses to a variety of stressors exhibit variations on a beat-by-beat scale has led to their evaluation by mathematical analyses, most notably power spectral analyses (Akselrod, Gordon et al. 1985) and the sequence method (Bertinieri, Di Rienzo et al. 1988). These techniques provide an indirect guide to both sympathetic and parasympathetic control of HR and may also be applied to the autonomic control of BP. Using spectral analysis, the HR power spectrum can be divided into low- and high-frequency components. Previous studies using beta-blockade, and atropine demonstrated that the low-frequency oscillations (0.05-0.1Hz) in BP reflect sympathetic modulation of vasomotor tone, and in HR reflect a combination of baroreflex-mediated sympathetic and parasympathetic influences (Smyth, Sleight et al. 1969, Pickering and Davies 1973). High-frequency portions of the power spectrum (0.15-0.5Hz) are under the primary influence of parasympathetic control; however, the high frequency variability of RR interval is mediated not only by direct modulation of vagal efferent activity resulting from baroreceptor responses to respiratory BP fluctuations (Piepoli, Sleight et al. 1997), but also by mechanical effects of sinus node stretch from respiration-related changes in venous return (Bernardi, Rossi et al. 1989).

The spontaneous cardiovagal baroreflex can also be calculated using the sequence method, which scans BP recordings for sequences of systolic blood pressure (SBP) that are characterized by either increases (or decreases) in SBP of at least 1 mmHg during each of three or more BP waves, and pulse intervals lengthening (or shortening) at least 4 ms/beat. The sequences are then analyzed as linear regressions between the SBP values and the subsequent pulse intervals. The coefficient of determination (r^2) is taken as the measure of gain or sensitivity of the changes in HR induced by the BP changes, as it is done by the baroreceptor reflex (Bertinieri, Di Rienzo et al. 1988).

1.6 The Cortical Autonomic Network

Although it has been established that the medulla oblongata is the primary cardiovascular control site (Guyenet 2006), studies in rodent models have highlighted the importance of certain forebrain structures involved with the modulation of efferent autonomic outflow (Cechetto and Saper 1987, Dampney 1994, Verberne and Owens 1998). In addition, clinical neuroimaging research has revealed that basal levels of autonomic tone may be disrupted in patients with stroke or epileptic seizures in higher regions of the brain such as the prefrontal and insular cortices, suggesting that cortical regions are involved in cardiovascular regulation (Oppenheimer, Gelb et al. 1992, Cheung and Hachinski 2000, Colivicchi, Bassi et al. 2004). Functional magnetic resonance imaging (fMRI) techniques have enabled the non-invasive assessment of cortical autonomic correlates in healthy, young conscious humans (King, Menon et al. 1999, Critchley, Corfield et al. 2000, Harper, Bandler et al. 2000, Henderson, Macey et al. 2002). A network of forebrain regions, termed the 'cortical autonomic network', has been shown to exert influence over autonomic outflow and cardiovascular control. These regions include the bilateral insular cortex (IC), amygdala, thalamus, medial prefrontal cortex (MPFC), anterior cingulate cortex (ACC), and most recently, the hippocampus (HC).

The IC and ACC are activated during a variety of cognitive maneuvers that elevate autonomic stimulation such as gambling (Critchley, Mathias et al. 2001), Stroop task (Gianaros, Derbyshire et al. 2005), mental arithmetic (Critchley, Corfield et al. 2000) and many more. The involvement of the ACC and IC in influencing sympathetic nerve activity are not only observed during cognitive tasks that induce mental arousal, but are also reported in situations of physical stress such as baroreceptor unloading (Kimmerly, O'Leary et al. 2005), and isometric exercise (Wong, Masse et al. 2007). Also, neuronal responses have been recorded in the IC when HR and BP changes were elicited by stimulation of the vagus nerve (Barnabi and Cechetto 2001) and baroreflex afferents (Cechetto and Saper 1987). Furthermore, studies in humans have supported a lateralization of insula effects on autonomic cardiovascular control, which is consistent

with evidence provided from anesthetized rodents. Specifically, HR and BP increase upon stimulation of the right IC, while left IC stimulation produces depressed cardiac and pressor responses (Oppenheimer, Gelb et al. 1992). Experimental studies indicate further that electrical stimulation of the posterior IC, increases HR and BP in anesthetized rats (Ruggiero, Mraovitch et al. 1987), with a large predominance of sympathoexcitatory neurons in the right posterior insula (Oppenheimer and Cechetto 1990, Zhang, Dougherty et al. 1999). Furthermore, during direct stimulation of the rodent brain, the superior IC is associated with tachycardia, whereas inferior portions produce bradycardia (Oppenheimer and Cechetto 1990).

The MPFC has been shown to have strong efferent connections with structures involved in autonomic function including the amygdala, hypothalamus, HC, periaqueductal gray, the NTS and the caudal and rostral ventrolateral medulla (Neafsey 1990, Chiba and Semba 1991, Hurley, Herbert et al. 1991, Verberne and Owens 1998, Vertes 2004). Recent studies conducted in animals and humans have revealed depressor sites within the ventral region of the MPFC that are heightened during periods of relaxation, including sleep and rest (Barnabi and Cechetto 2001, Critchley, Mathias et al. 2001). Furthermore, pharmacological blockade studies (Hollander and Bouman 1975, Mitchell, Reeves et al. 1989, Victor, Pryor et al. 1989) indicate that parasympathetic withdrawal represents the primary determinant of the rapid adjustments in HR that are initiated immediately at the onset of IHG exercise (Mitchell, Reeves et al. 1989, Wong, Masse et al. 2007), and has been shown to be associated with deactivation in the MPFC (Wong, Masse et al. 2007). These observations suggest that the MPFC is involved in mediating behavioural mechanisms that reduce the cardiovascular response to psychological or physical stress, while augmenting vagal efferent control of HR (Critchley, Wiens et al. 2004), thus supporting a direct relation between MPFC activity and cardiovagal control in young, healthy adults.

Emphasis on the role of the HC in cardiovascular dynamic responses to stress has received relatively little attention despite early studies which noted anatomical visceral sensory connections to the HC (Maclean 1952). More recently, retroviral tracing techniques have established the neuronal linkages and relays that connect brainstem

autonomic nuclei with the HC (Westerhaus and Loewy 2001, Castle, Comoli et al. 2005). Previously, Ruit and Neafsey (Ruit and Neafsey 1988) illustrated the ability of electrical stimulation of the ventral HC to depress cardiovascular activation, exposing an inverse relationship between HC activation and cardiovascular arousal. Of note, the cardiovascular outcomes in this electrical stimulation model required an intact MPFC. These findings confirm early evidence that electrical stimulation of the HC in anesthetized rats elicits a variety of visceral or autonomic modifications, such as decreases in HR and increases in pulse pressure (Kaada 1951, Kaada and Jasper 1952, Andy and Akert 1955, Liberson and Akert 1955, Anand and DUA 1956). These earlier findings are supported further by recent electroencephalography results from conscious rats that point to entrainment of theta rhythms between the HC and MPFC (Hyman, Hasselmo et al. 2011). Moreover, the necessity of an intact MPFC for electrical stimulation of the HC to elicit cardiovascular changes (Ruit and Neafsey 1988) supports data from conscious humans where cardiac dynamics are inversely related to MPFC activation state in functional neuroimaging studies (Gianaros, Van Der Veen et al. 2004, Wong, Kimmerly et al. 2007, Wong, Masse et al. 2007, Goswami, Frances et al. 2011, Norton, Luchyshyn et al. 2013).

Overall, these observations confirm a network of regions involved in mediating the cardiovascular response to psychological or physical stress, thus supporting a direct relation between cortical activity and cardiovagal control in young, healthy adults. The modulation of this autonomic network through the influence of positive and negative lifestyle factors such as fitness and age, respectively, is not known.

1.7 The Effect of Age

1.7.1 Effect of Age on the Heart

The aging process is accompanied by a complex series of changes favoring heightened cardiac sympathetic tone with reduced parasympathetic influence (Meredith, Broughton et al. 1991, Schlaich, Lambert et al. 2004). Within the context of cardiovascular health, chronic elevations in sympathetic outflow, and/or reductions in parasympathetic cardiac control, contribute to the pathogenesis and progression of atherosclerosis, vascular wall thickening, cardiac damage, and disturbed cardiac rhythms (Amiya, Watanabe et al. 2014, Chistiakov, Ashwell et al. 2015, Pellman and Sheikh 2015), which combine to increase the risk of morbidity and mortality (Schmidt, Muller-Werdan et al. 2005).

Age-related changes in maximal exercise HR, and HR responsiveness, have been reported in the literature (Astrand 1960, Bruce, Blackmon et al. 1963, Lester, Sheffield et al. 1968, Dauchot and Gravenstein 1971, Kino, Lance et al. 1975, Seliger, Macek et al. 1978, Sheffield, Maloof et al. 1978, Yin, Spurgeon et al. 1979, Lalande, Sawicki et al. 2014), and represent a sensitive indicator of autonomic dysfunction. Substantial research has shown that HRV decreases with age (O'Brien, O'Hare et al. 1986, Schwartz, Gibb et al. 1991, Agelink, Malessa et al. 2001, Bonnemeier, Richardt et al. 2003, Russoniello, Zhirnov et al. 2013), which has been observed in both cross-sectional comparisons and longitudinal studies (Sinnreich, Kark et al. 1998). HRV is greater in subjects with the lowest HR and decreased when sympathetic activity increases and vagal activity decreases (Van Hoogenhuyze, Weinstein et al. 1991). It has been shown that the decline in HRV with age is mainly, but not exclusively, attributable to the decline in parasympathetic function (Pfeifer, Weinberg et al. 1983, Shannon, Carley et al. 1987). In addition, respiratory induced changes in HR are reduced in the elderly (Collins, Exton-Smith et al. 1980, Smith, Smith et al. 1981, Smith 1982, Wieling, van Brederode et al. 1982, Taylor, Myers et al. 2001), which may be due to altered vagal activity, since the chronotropic response to atropine is also reduced in older subjects (Dauchot and Gravenstein 1971). However, several other components involved in the genesis of HRV could also be specifically affected by aging including the beta-adrenergic modulation of cardiovascular function (Lakatta 1993, Seals, Taylor et al. 1994, Taylor, Myers et al.

2001), activity of the renin-angiotensin system (Anderson 1997), and thermoregulation (Kerckhoffs, Blaak et al. 1998).

Advancing age has also been associated with diminished cardiovagal BRS (Robertson, Waller et al. 1988, Ebert, Morgan et al. 1992, Monahan, Dinenno et al. 2001, Monahan 2007, Kaye and Esler 2008), leading to functional changes in BP control and an impaired ability to reflexively engage the cardiovascular system. That is, the degree of HR slowing that occurs with increasing BP is blunted with aging. Traditional thinking has been that increased blood vessel stiffening impairs the function of the afferent baroreceptors in the carotid arteries and aortic arch, through either structural (atherosclerosis) or functional (reduced nitric oxide activity) changes (Robertson, Waller et al. 1988, Hunt, Farquhar et al. 2001). However, Robinson and colleagues found that BRS can be altered acutely by stroke, suggesting that altered central processing can also occur (Robinson, James et al. 1997).

In addition, advancing age often (Ebert, Morgan et al. 1992), but not always (Greaney, Schwartz et al. 2013), increases baseline MSNA. Decreased BRS accounts for some of the decrease in parasympathetic control of HR, as inferred by measures of cardiovagal BRS (Ebert, Morgan et al. 1992, Monahan, Dinenno et al. 2001). However, age exerts little effect on baroreflex control of MSNA whether levels of sympathetic burst activity are elevated (Ebert, Morgan et al. 1992) or not (Greaney, Schwartz et al. 2013).

Combined, these outcomes suggest that advancing age is associated with a chronic state of heightened peripheral sympathetic tone and parasympathetic withdrawal (Meredith, Eisenhofer et al. 1993, Monahan, Dinenno et al. 2001, Thayer and Lane 2007, Lambert, Dawood et al. 2008, Freeling and Li 2015). The mechanisms affecting this autonomic dysregulation are not clear; however, alterations in cerebral cortex structure may contribute to these age-related autonomic changes.

1.7.2 Effect of Age on the Brain

The human brain begins to lose tissue early in the third decade of life, with average losses estimated at roughly 15% of the cerebral cortex and 25% of the cerebral white matter

between ages 30 and 90 (Jernigan, Archibald et al. 2001). Major findings include widespread ventricular enlargement (Bradley, Bydder et al. 2002, Fleisher, Sun et al. 2008) and whole brain atrophy (Grubb, Fox et al. 2000), as well as a loss of brain volume in both gray and white matter (Raz, Lindenberger et al. 2005). The prefrontal areas of the brain, which are the last to fully mature in the early twenties, are the first to show signs of decline as early as age forty (Raz, Lindenberger et al. 2005) with degradation of fiber myelination (Northoff, Richter et al. 2000), and a loss of structural connectivity (O'Sullivan, Jones et al. 2001). Significant but more moderate declines are seen in the temporal, parietal and occipital cortices, respectively (Raz and Rodrigue 2006). In addition, the HC, amygdala and cerebellum all show signs of age-related atrophy (Raz and Rodrigue 2006). It is, therefore, noteworthy that those regions associated with autonomic cardiovascular control (e.g. MPFC, IC, HC) exist within regions that display high sensitivity towards age-related cortical atrophy (Raz, Williamson et al. 2000, Anazodo, Shoemaker et al. 2013). In fact, age-related autonomic dysregulation represents one of the many consequences to declines in the integrity of the aged brain (Brody 1955, Cechetto and Shoemaker 2009, Hart, Joyner et al. 2009).

The link between brain structure and function has yet to be fully elucidated. However, advancing age is often associated with changes in brain functional responses (Nyberg, Salami et al. 2010), and declines in cognitive performance (Kramer, Erickson et al. 2006, Prakash, Voss et al. 2011, Voss, Heo et al. 2013) (Ronnlund, Nyberg et al. 2005). Atrophy of the prefrontal cortices in particular, predict a decrease in performance on age-sensitive tests of executive function (Raz, Gunning et al. 1997, Gunning-Dixon and Raz 2003) and other cognitive operations dependent on executive control (Head, Rodrigue et al. 2008). This is in line with neuropsychological studies showing that executive functions, which are heavily dependent on frontal neural circuits, are among the cognitive functions most susceptible to advancing age (Connelly, Hasher et al. 1991, Schretlen, Pearlson et al. 2000).

However, although the current literature suggests a consistent age-related decline in cortical structure and function, the rate and extent to which individuals express this decline is highly variable. Erickson et al. (Erickson, Miller et al. 2012) suggest that, at

least in the context of the HC, exercise as a lifestyle may contribute to this variability. Therefore, despite the overlapping relationship between cognitive and cardiovascular control sites in the brain, the extent to which the inter-individual variation in cortical structure predicts age-related changes in autonomic outflow is not known. If elucidated, details related to cortical structure may form a preliminary basis for explaining age-related autonomic dysregulation.

1.8 The Effect of Exercise

Over the past several decades, numerous scientific reports have examined the relationship between physical fitness and cardiovascular health. A sedentary lifestyle is one of the five major risk factors for cardiovascular disease, along with high BP, abnormal blood lipids, smoking and obesity (Blair and Brodney 1999). Disturbances in autonomic function are associated with many of the potential mechanisms linked to physical inactivity, including increased BP (Duncan, Farr et al. 1985) and reduced cardiorespiratory fitness (Blair and Brodney 1999). Moreover, the vast majority of patients with autonomic disorders have a blunted or abnormal cardiovascular response to exercise. These patients have a low VO_{2max}, indicating reduced physical fitness and exercise capacity (Hilsted, Galbo et al. 1979, Bottini, Tantucci et al. 1995); however, it is likely that the disorder prevents an active lifestyle making direct connections between exercise behavior and autonomic outcomes difficult to interpret and unclear. Physical activity has been shown to improve autonomic balance in older individuals and in patients with cardiovascular disease (Carter and Ray 2015) with altered baseline autonomic function as indicated by heightened SNS and diminished PNS. Thus, understanding how physical (in)activity impacts autonomic control is crucial for sustained cardiovascular health.

1.8.1 Effect of Acute Exercise on the Heart

The ANS plays a fundamental role in the cardiovascular response to acute (dynamic) exercise in animals and humans. Successful initiation and persistence of elevated physical activity requires neurally-mediated cardiovascular adjustments that elevate cardiac output, redirect blood flow to the working muscles, and counter the vasodilation in skeletal muscle to prevent hypotension. Input from three neural mechanisms are requisite to precisely match systemic oxygen delivery with metabolic demand: central command, the exercise pressor reflex and the arterial baroreflex (Rowell and O'Leary 1990).

The arterial baroreflex, as previously mentioned, is one of the body’s homeostatic mechanisms that works to maintain BP at nearly constant levels. In acute exercise, the operating point of the baroreflex is “reset” to a higher operating systemic pressure

(Rowell and O'Leary 1990), which allows the baroreflex to operate at the prevailing BP evoked by the exercise (Potts, Shi et al. 1993, Norton, Boushel et al. 1999, Fadel, Ogoh et al. 2001, Ogoh, Fadel et al. 2003, Ogoh, Fisher et al. 2005, Raven, Fadel et al. 2006). There is evidence that both central command and the peripheral feedback from muscle contribute to reset the baroreflex (Iellamo, Legramante et al. 1997, Raven, Fadel et al. 2006).

The term "central command" was coined by Krogh and Lindhard (Krogh and Lindhard 1913) who hypothesized that specific regions of the cortex provided coordinated parallel and concurrent drive to the skeletomotor, respiratory and autonomic neural systems to support muscular activity. At the onset of exercise, central command initiates vagal withdrawal to increase HR and cardiac output (CO), causing an increase in arterial BP. As work intensity increases and HR approaches 100 beats/min, the demand from active skeletal muscle vasodilation is greater than the increase in CO and BP, thus initiating a rise in sympathetic nerve activity. A cascade of events involving a further increase in BP and vasoconstriction of inactive vascular beds is initiated through the exercise pressor reflex from sensory afferents of active skeletal muscle (Coote, Hilton et al. 1971, McCloskey and Mitchell 1972, Kaufman, Longhurst et al. 1983, Mitchell, Kaufman et al. 1983).

Therefore, our current understanding of the mechanisms that enable the continuation of high intensity exercise involve central command as the primary regulator of baroreflex resetting, and the feedback mechanism of the exercise pressor reflex as a modulator of this resetting.

1.8.2 Effect of Exercise Training on the Heart

The effect of exercise training on cardiac autonomic regulation has been extensively studied in both humans and animals. Several studies provide strong evidence that, under conditions of altered baseline activity, endurance exercise training increases cardiac parasympathetic regulation and decreases sympathetic activation (Gregoire, Tuck et al. 1996, Carter, Banister et al. 2003, Zanesco and Antunes 2007, Billman 2009). These exercise training-induced changes in cardiac autonomic regulation are believed to be

largely responsible for training-induced decreases in resting HR (Carter, Banister et al. 2003, Zanesco and Antunes 2007). However, the rationale for this exercise-induced resting bradycardia remains controversial. Most, but not all, studies have shown that exercise training consistently increases parasympathetic dominance at rest (Seals and Chase 1989, Scott, Eberhard et al. 2004, Billman and Kukielka 2006, Billman and Kukielka 2007, Billman 2009), whereas intrinsic HR, as revealed by pharmacological blockade, is similar in exercise-trained and sedentary humans or animals (Shi, Stevens et al. 1995, De Angelis, Wichi et al. 2004, Zanesco and Antunes 2007). However, in contrast to longitudinal studies that largely support a predominant autonomic component (Seals and Chase 1989, Shi, Stevens et al. 1995), cross-sectional studies comparing elite athletes with sedentary subjects tend to support an important role for intrinsic HR changes in training bradycardia (Lewis, Nylander et al. 1980, Katona, McLean et al. 1982, Smith, Hudson et al. 1989, Bonaduce, Petretta et al. 1998, Stein, Medeiros et al. 2002).

Endurance training-associated changes in baroreflex control of HR have also been proposed (Stegemann, Busert et al. 1974, Bedford and Tipton 1987, Mack, Shi et al. 1987, Jingu, Takeshita et al. 1988). However, the experimental findings to date have been inconsistent. Chronotropic responsiveness to arterial baroreflex perturbations have been reported to be greater (Monahan, Dinenno et al. 2000), not different (Hudson, Smith et al. 1987, Vroman, Healy et al. 1988, Sheldahl, Ebert et al. 1994, Bowman, Clayton et al. 1997, Davy, Willis et al. 1997), and blunted (Stegemann, Busert et al. 1974, Smith and Raven 1986, Smith, Graitzer et al. 1988) in endurance-trained vs. untrained humans. Importantly, however, regular aerobic exercise has been shown to increase cardiovagal BRS in previously sedentary middle-aged and older adults (Davy, Miniclier et al. 1996, Davy, DeSouza et al. 1998, Monahan, Dinenno et al. 2000). Furthermore, based on the results of a cross-sectional study (Monahan, Dinenno et al. 2000), moderate intensity exercise appears to be an adequate stimulus to attenuate the age-related decline in cardiovagal BRS - no additional benefit was demonstrated in middle-aged and older men who performed more strenuous and prolonged endurance training beyond that of moderate intensity exercise (Monahan, Dinenno et al. 2000). Therefore, there appears to be a dose of training at which the impact of aerobic exercise is transferred to

improvements in BRS. Three previous intervention studies in middle-aged and older adults found no change in cardiovagal BRS in response to regular aerobic exercise (Sheldahl, Ebert et al. 1994, Bowman, Clayton et al. 1997, Davy, Willis et al. 1997). One potential explanation for this difference is the varied intensity of the exercise stimulus. Specifically, the total volume of the exercise performed in the study by Monahan et al. (5-6 days/week for ~45 min session for 13.5 weeks at >70% maximal HR), was greater than that performed in prior investigations (< 3 days/week for ~20-45 min session for 6-12 weeks at 70% maximal HR). Indeed, there is currently no formal training theory that quantitatively and accurately prescribes the pattern, duration and intensity of exercise required to elicit a specific physiological adaptation. In addition, research also indicates that there is individual variability in the response to exercise (Bouchard and Rankinen 2001), and that the training history of an individual will also influence their physiological response.

Considered together, individual differences in physical activity and training-related changes in autonomic outflow suggest that cardiorespiratory fitness is a critical mediator of autonomic adaptation.

1.8.3 Effect of Exercise on the Brain

The role of the cortex in the acute adjustment to exercise was first suspected by Krogh and Lindhard (1913) when an anticipatory rise in both HR and respiration was observed prior to the onset of exercise. Pharmacological blockade studies have since supported the idea that the cortex has an important role in adjusting autonomic cardiovascular variables in a manner related to the rapid HR adjustments at the onset of volitional work (McCloskey and Mitchell 1972, Mitchell, Reeves et al. 1989, Victor, Pryor et al. 1989, Mitchell 1990). Subsequently, neuroimaging methods have enabled more detailed studies using volitional exercise with evidence for involvement of the MPFC and IC in modulation of the autonomic nervous system (Williamson, Friedman et al. 1996, Williamson, McColl et al. 1999, Williamson, McColl et al. 2001, Williamson, McColl et al. 2002, Williamson, Fadel et al. 2006, Wong, Masse et al. 2007).

Considerable change in cortical activation occurs at the onset of moderate intensity exercise where cardiovascular changes appear to be dominated by reductions in parasympathetic contributions. These changes emphasize increased activation within the IC and decreased activation relative to baseline in the MPFC and HC that correlate with HR (Wong, Masse et al. 2007, Norton, Luchyshyn et al. 2013). Importantly, as mentioned previously, these cardiovascular centers are found in regions of the brain most vulnerable to the effects of advancing age (Raz, Gunning et al. 1997, Raz, Lindenberger et al. 2005, Nyberg, Salami et al. 2010, Salami, Eriksson et al. 2012). However, substantial inter-individual differences exist in the rate and extent of age-related cortical atrophy leading researchers to examine the potential modifiable risk factors for improved brain health into senescence.

Physical activity has emerged as a potent stimulus for improving neural health and has been shown to have positive global influences in aging, including spared brain volume (Erickson, Prakash et al. 2009, Erickson, Voss et al. 2011, Niemann, Godde et al. 2014), improved task-related functional responses (Colcombe, Kramer et al. 2004, Voelcker-Rehage, Godde et al. 2010), increased white matter integrity (Johnson, Kim et al. 2012, Voss, Heo et al. 2013, Voss, Weng et al. 2016), and cognitive performance (Josefsson, de Luna et al. 2012). Therefore, the possibility that physical fitness can impact cortical health is of particular importance given that the functional architecture of the cardiovascular control sites, in particular, are negatively altered in advancing age. However, interpretation of the literature remains difficult as studies have previously focused on individuals whose baseline condition, including cognitive impairment or a sedentary lifestyle, represent an effect of such conditions as much as an exercise effect. Further, whether a physically active lifestyle can affect cortical circuitry related to autonomic control and exercise outcomes remains unknown.

A critical aspect of understanding how aerobic activity is protective for brain aging is identifying the fundamental principles by which exercise positively affects brain health. In humans, several studies suggest that physical exercise leads to improvements at both the structural and functional levels in the aging brain (Kramer, Erickson et al. 2006, Hillman, Erickson et al. 2008, Erickson, Prakash et al. 2009) (Voelcker-Rehage, Godde et

al. 2010, Liu-Ambrose, Nagamatsu et al. 2012). Using voxel-based morphometry, Colcombe et al. (Colcombe, Erickson et al. 2003) reported that a higher cardiorespiratory fitness (VO_{2max}) was associated with attenuation of cortical decay to both gray and white matter in the frontal, prefrontal, and temporal regions in older adults. In another study, Erickson et al. (Erickson, Prakash et al. 2009) performed a region-of-interest analysis in 165 non-demented older adults and found that higher fitness levels were associated with an increased volume of the bilateral HC. Moreover, Voss et al. (Voss, Prakash et al. 2010) observed that 12 months of aerobic training leads to increased functional connectivity in regional connections that support both the default-mode network and the frontal executive network, suggesting that physical exercise has a restorative effect on large-scale brain circuitry. The evidence supporting a positive influence of fitness on the maintenance and delayed decline of cognitive function is well documented (Hillman, Erickson et al. 2008, Josefsson, de Luna et al. 2012, Bherer, Erickson et al. 2013). Evidence from cross-sectional studies indicates that age-related differences in cognitive performance are observed when older adults are compared to younger adults, and are reduced if the comparisons involve higher-fit individuals rather than sedentary older adults (Spirduso 1975, Clarkson-Smith and Hartley 1989, Hillman, Weiss et al. 2002, Renaud, Bherer et al. 2010). In a meta-analytic review of randomized-control trials of aerobic exercise on neurocognitive functions, Smith et al. found that individuals who were randomly assigned to aerobic exercise training showed modest improvements in attention, processing speed, executive function, and memory (Smith, Blumenthal et al. 2010). Such exercise-induced structural and functional changes have been documented in various brain regions, but the plethora of data has supported alterations in the prefrontal cortex and medial temporal lobes. Thus, the regions of the cortex most vulnerable to the precipitous effects of aging are those same regions where physical activity promotes improvements in cognitive performance. It is noteworthy that these same regions are also responsible for cardiovascular control. Nonetheless, little is known about concurrent autonomic consequences, despite similar, and often times overlapping, regional specificity in the cortex.

1.9 Determinants of Cortical Thickness and Brain Function

The cardiovascular fitness hypothesis suggests that cardiovascular (aerobic) fitness is the key physiological mediator that explains the positive relationship between physical exercise and brain health, with the implication that gains in cardiovascular fitness are necessary for gains in brain health to be observed (Dustman, Ruhling et al. 1984, Barnes, Santos-Modesitt et al. 2013, Brickman, Khan et al. 2014). However, the extent to which exercise can benefit the brain, its impact on autonomic neurologic outcomes, and the mechanisms through which it modulates brain structure and function, remain to be elucidated.

The latest research in both human and non-human animal research demonstrates that exercise targets direct and indirect aspects of neural health leading to broad-based effects on overall brain health. An increase in cell proliferation and cell survival in the dentate gyrus of the HC is one of the most consistently observed direct effects of exercise training (van Praag, Christie et al. 1999, van Praag, Kempermann et al. 1999, Trejo, Carro et al. 2001, Brown, Cooper-Kuhn et al. 2003, Eadie, Redila et al. 2005). In mice, voluntary wheel running enhanced neurogenesis in the dentate gyrus and was associated with an increased number of new hippocampal cells (van Praag, Kempermann et al. 1999). The functional significance of hippocampal neurogenesis and the survival of new neurons is not clear; however, dementias such as Alzheimer's disease are characterized by a marked reduction in the number of neurons in the HC, which may be alleviated, in part, by increased neurogenesis resulting from aerobic activity (Hillman, Erickson et al. 2008). This proliferation of new cells in the brain is accompanied by an increased demand for nutrients, which is met by the stimulation of new blood vessel growth (angiogenesis) in the cortex (Kleim, Cooper et al. 2002), the cerebellum (Black, Isaacs et al. 1990), and the HC (Lopez-Lopez, LeRoith et al. 2004). A recent imaging study in humans (ages 21–45) showed that 12 weeks of aerobic training increased blood flow in the HC, and this increase was correlated with an improved rate of learning (Pereira, Huddleston et al. 2007). Further, after completion of a 6-month exercise-based cardiac

rehabilitation program, Anazodo et al. (Anazodo, Shoemaker et al. 2013) observed an increase in cerebral blood flow in the bilateral ACC of CAD patients; an area known to be involved in cognitive processing and regulation of cardiovascular autonomic control.

The predominant theory as to how exercise directly mitigates these effects in the brain is through an increased cortical availability of several classes of growth factors that modulate nearly all of the functional endpoints enhanced by exercise. At present, brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1) and vascular endothelial-derived growth factor (VEGF) are the principal neurotrophins known to mediate the effects of exercise on the brain. These growth factors work together to produce complementary functional effects, modulating both overlapping and unique aspects of exercise-related benefits in brain plasticity, function, and health. Of all the neurotrophins, BDNF appears to be the most susceptible to regulation by exercise and physical activity (Cotman and Berchtold 2002, Knaepen, Goekint et al. 2010). BDNF has consistently been demonstrated to be necessary for long-term memory formation, and for the growth and survival of new neurons (Vaynman, Ying et al. 2004). In humans, BDNF concentrations are increased after an acute exercise bout (Ferris, Williams et al. 2007) in both young adults and patients with multiple sclerosis (Gold, Schulz et al. 2003). Increases in BDNF in response to an exercise stimulus could be clinically relevant, as serum and cortical concentrations of BDNF are reduced in Alzheimer's and Parkinson's disease, as well as depression, anorexia and many others (Adlard, Perreau et al. 2005, Adlard, Perreau et al. 2005). Therefore, aerobic activity may be neuroprotective through the regulation of growth factor secretion.

In addition, exercise-induced increases in microglia and astrocytes (Ehninger and Kempermann 2003), observed in several brain regions, also might help to maintain enhanced brain health and function with exercise. Astrocytes and associated neuroglia are involved in the maintenance and homeostasis of the central nervous system, and act as secretory cells releasing neurotransmitters and growth factors, which affect various aspects of plasticity and information processing in the central nervous system (Malarkey and Parpura 2008, Reichenbach A. 2010, Parpura, Grubisic et al. 2011, Kettenmann, Kirchhoff et al. 2013, Verkhratsky, Rodriguez et al. 2013, Martineau, Parpura et al.

2014). Morphometric studies have demonstrated that two types of glia, the oligodendrocytes and the microglia, are most affected by physiological aging with reductions of ~11% in white matter tissues and ~3% in cortical volume (Haug and Eggers 1991). The impact of aging on astrocyte number remains controversial however, with some observing no change in the aging human brain (Pelvig, Pakkenberg et al. 2008, Fabricius, Jacobsen et al. 2013) and others presenting an age-dependent increase in expression and hypertrophy (Rodriguez, Terzieva et al. 2013, Sampedro-Piquero, De Bartolo et al. 2014). Exposure of aged mice and rats to physical activity or an enriched environment increased astrocyte expression in hippocampal regions and led to astrocytic hypertrophy (Rodriguez, Terzieva et al. 2013, Sampedro-Piquero, De Bartolo et al. 2014). Importantly, these changes in astroglia coincided with cognitive improvement (Sampedro-Piquero, De Bartolo et al. 2014). Therefore, the significance of changes in glia and astrocytes in response to exercise has not been clearly defined and merits further study.

Despite offering some mechanistic insights, a direct effect of the exercise itself may not be the only, or the primary, mechanism through which brain health is improved. As suggested by Spirduso et al. (Spirduso 2005), exercise may also enhance brain health indirectly by improving related conditions such as stress and sleep, diet, and social exposure. Indeed, factors that reduce neurogenesis, such as insomnia, stress and aging, are associated with diminished performance on spatial learning tasks (Lucassen, Meerlo et al. 2010, Lucassen, Oomen et al. 2015, Mueller, Meerlo et al. 2015). Exercise has also been shown to interact with dietary interventions – increasing the positive effects on brain functioning, and decreasing the unhealthy effects of a high-fat diet. In animals, a high-fat diet reduced the hippocampal concentration of BDNF, but this dietary increase was reversed with exercise (Molteni, Wu et al. 2004). In addition, diets rich in sugar, saturated fats, and calories are considered deleterious for neural function, as they act to elevate levels of oxidative stress and reduce synaptic plasticity and cognitive function (Gomez-Pinilla 2011).

Furthermore, exercise reduces peripheral risk factors such as obesity, diabetes, hypertension and cardiovascular disease, which converge to cause brain dysfunction and

neurodegeneration. Raji et al. (Raji, Ho et al. 2010) used fMRI to assess gray and white matter atrophy in 94 elderly adults (mean age of 77 years). Results showed that body mass index, fasting plasma insulin, and type 2 diabetes were strongly associated with atrophy of the frontal, temporal, and subcortical regions of the brain. In addition, animal studies have revealed a neuroendocrine and/or metabolic role for BDNF in the periphery (Knaepen, Goekint et al. 2010), suggesting a reduction in food intake, an increase in the oxidation of glucose, lower blood glucose levels, and increased insulin sensitivity with higher concentrations of BDNF (Araya, Orellana et al. 2008).

Taken together, these data indicate that the indirect effects of a positive lifestyle associated with improved fitness and increased physical activity may be associated with marked improvements in brain structure and function, and provide a greater understanding of the underlying mechanisms and consequences of high physical activity.

Finally, specific associations between cardiorespiratory fitness and brain health independent of physical activity need to be considered. One possibility is the strong genetic influence (up to 50%) on cardiorespiratory fitness and the capacity for training-induced changes in fitness (Bouchard, An et al. 1999, Bouchard, Sarzynski et al. 2011). Similarly, there may be overlapping genetic predictors of the responsiveness of central and peripheral cellular and vascular systems to regular moderate-to-vigorous physical activity and training. For example, increased distribution of blood flow to the muscle is a very important predictor of cardiorespiratory fitness, and this is influenced by the heart's ability to generate more CO (i.e. by increases in stroke volume) and to recruit and form new capillaries (angiogenesis) in the muscle. Another important training-related adaptation includes changes in the oxidative capacity of mitochondria in active muscle fibers. Angiogenesis and mitochondrial function also increase in the brain in response to exercise training (Marques-Aleixo, Oliveira et al. 2012, Voss, Heo et al. 2013), and so it is possible that individuals that are genetically predisposed to have relatively higher levels of cardiorespiratory fitness or have greater training-induced increases in fitness will experience the most protection against adverse effects of aging on the brain through these vascular and cellular pathways.

In conclusion, despite its promise, the ways in which physical activity impacts the rate and prevalence of neural decline is still under investigation. Furthermore, several open issues call for further research, such as the dose-response relationship, the level of change or protection provided by physical activity, the biological and/or psychological mechanisms by which these effects occur and whether physical activity can be beneficial despite chronic medical conditions and neurological syndromes such as dementia. Although recent advancements in neuroimaging techniques and genetics have opened new research avenues, more research is required to provide definitive answers to these important questions.

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Chapter 2

2 Coronary Artery Disease Affects Cortical Circuitry Associated with Brain-Heart Integration during Volitional Exercise

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2.1 Introduction

Coronary artery disease (CAD) increases risk of stroke, cognitive impairment and autonomic dysregulation (Martins, Hone et al. 2006, Zulli, Nicosia et al. 2008, Roberts, Knopman et al. 2010, Barekatain, Askarpour et al. 2014). In turn, impaired autonomic outcomes of CAD include diminished parasympathetic modulation of HR (Mancia, Cleroux et al. 1991, Seals, Taylor et al. 1994, Ford 1999). Moreover, adverse outcomes in autonomic cardiovascular control may exacerbate the disease pattern through tissue damage and diminished ability to affect rapid HR adjustments in response to stress and, thereby, limit the benefits that can be derived from exercise rehabilitation.

A role for the forebrain and brain stem in cardiac autonomic function has been established in both experimental studies in rodents (Cechetto and Saper 1990, Yasui, Breder et al. 1991) and clinical studies in patients with stroke or epileptic seizures in the prefrontal cortex (Cheung and Hachinski 2000). Recently, neuroimaging techniques have enabled investigation into a network of cortical regions associated with the autonomic nervous system and cardiovascular control in conscious humans (Critchley, Corfield et al. 2000, Gianaros, Van Der Veen et al. 2004, Williamson 2010, Basnayake, Green et al. 2012, Macey, Wu et al. 2012, Norton, Luchyshyn et al. 2013, Cechetto 2014, Shoemaker, Norton et al. 2014). These regions include the bilateral IC, ACC, posterior cingulate cortex (PCC), thalamus, MPFC, and HC. Importantly, the combined results indicate close homology between cortical sites identified experimentally in lower animals, and those observed in humans (Cechetto 2014). These experimental studies indicate that the IC, MPFC and HC are of particular relevance to HR control (Burns and Wyss 1985, Ruggiero, Mraovitch et al. 1987, Cechetto and Chen 1990, Yasui, Breder et al. 1991,

Oppenheimer, Gelb et al. 1992, Verberne 1996, Fisk and Wyss 1997, Owens and Verberne 2001). Anatomically, the MPFC and HC have a large number of direct connections with subcortical structures (Verberne and Owens 1998) and have been linked in connectivity analyses of functional magnetic resonance imaging data in humans (Norton, Luchyshyn et al. 2013). Thus, the MPFC and HC form regions of interest for the current study.

Despite the mounting clinical evidence that vagal activity is an important predictor of cardiovascular prognosis in humans (Curtis and O'Keefe 2002), data are limited regarding the impact of CAD on the brain-heart connection. Volitional IHG contractions offer a unique opportunity to explore the cortical representation of autonomic cardiac control. More specifically, moderate intensity IHG exercise of short duration produces a rapid tachycardia in young and healthy individuals (Mancia, Iannos et al. 1978, Mark, Victor et al. 1985, Wong, Masse et al. 2007) and pharmacologic evidence indicates that a decrease in parasympathetic dominance accounts for much of this rapid HR change (Hollander and Bouman 1975, Fagraeus and Linnarsson 1976, Mitchell, Reeves et al. 1989). In young individuals, the magnitude of this rapid increase in HR with IHG exercise is correlated with reduced activity within the MPFC (Gianaros, Van Der Veen et al. 2004, Wong, Masse et al. 2007) and the HC (Norton, Luchyshyn et al. 2013). It follows that these regions are associated with cardiovagal control.

The purpose of this study was to test the hypothesis that CAD impairs HR responses to volitional handgrip and that such impairment is related to dysregulation of the cortical autonomic network associated with HR control, particularly emphasizing activity patterns within the MPFC and HC.

2.2 Methods

2.2.1 Participants

A total of 40 individuals participated in this study. Observations were made in 17 patients with coronary artery disease (CAD) and 23 similarly-aged healthy control subjects (Control). Anthropometric and baseline cardiovascular data for each group are provided in Table 2.1. All subjects were non-smokers for longer than 10 years. Control subjects

were free of any medications, and did not have diagnosed hypertension, vascular disease or diabetes. CAD patients were recruited from the London Health Sciences Centre for Cardiac Rehabilitation and Secondary Prevention Program following recent diagnosis of one of the following: admission for acute coronary syndrome (ST elevation or non ST elevation myocardial infarction), angina, per cutaneous coronary intervention, or coronary artery bypass graft. Thirteen of the patients were considered to be in functional Class I (as described by the New York Heart Association Functional Classification of heart failure), and four in functional Class II. Drug therapy included cholesterol lowering statins (94%), beta-blockers (94%), ACE-inhibitors/angiotensin II receptor blockers (82%), calcium channel blockers (18%), diuretics (6%), and anti-platelets including aspirin (94%). Patients were excluded if they had uncontrolled hypertension or a history of diabetes for more than 5 years. Both CAD patients and Controls were free of any neurological condition or disease. Each participant provided informed, written consent before participating in the study, which was approved by The University of Western Ontario Health Sciences Ethics Review Board and adhered to the Declaration of Helsinki.

2.2.2 Experimental Design

Participants completed two separate experimental sessions: 1) physiological recording (LAB session) and 2) a functional magnetic resonance neuroimaging session (fMRI; Robarts Research Institute Centre for Functional and Metabolic Imaging). The sessions were performed at the same time of day and separated by a minimum period of 1 week. Participants were familiarized with the experimental procedures prior to their first test session. Participants were instructed to arrive at the laboratory following a 12h fast and to refrain from nicotine, alcohol, caffeine, and intense physical exertion for the same duration. Each session began with a maximal voluntary contraction (MVC) handgrip calibration, in which the participant was instructed to squeeze a non-magnetic handgrip device connected in series to a pressure transducer (Edwards Lifesciences, PX272, Irvine CA) to their maximal ability while in the supine position. This was repeated twice with the larger value calibrated as 100%. Isometric handgrip (IHG) exercise was performed with the right hand in all subjects, regardless of handedness (n=37 right-handed). During each recording session, visual feedback was provided to the participant of their achieved

force in real-time. Baseline data were collected over 5 min of quiet supine rest. Four repeated bouts each of 30%, 40% and 50% of MVC force (LAB session), and seven repeated bouts of IHG at 40% MVC force (fMRI session) were performed, with each contraction lasting 20 sec and separated by 40 sec of rest. The number of trials was increased in the fMRI session to increase the signal-to-noise ratio. The level of perceived exertion produced by the exercise was monitored after each trial on a scale from 6-20 (Borg 1982).

2.2.3 Cardiorespiratory Fitness Test

Breath-by-breath measurements of oxygen consumption (VO_2), HR and BP were recorded throughout the test. Maximal oxygen consumption (VO_{2max}) is an established marker of cardiorespiratory fitness and a clinically accepted surrogate marker for left ventricular function (Fletcher, Balady et al. 2001). Each subject's VO_{2max} was estimated from a graded treadmill exercise test to volitional exhaustion under standard clinical observation (ACSM 1995).

2.2.4 Physiological Data Analysis

Analog signals were sampled at 1000Hz with an on-line data acquisition and analysis system (PowerLab, ADInstruments, Mountain View, CA, USA). HR was calculated from successive R-R intervals obtained from the ECG signal. BP from the Finometer was converted to mean arterial pressure (MAP) using the formula MAP = 1/3 SBP + 2/3 DBP. Beat-by-beat HR data were averaged over 2.5s bins (the TR interval for functional scans) and time aligned to ensure a corresponding mean value for each functional scan obtained during the fMRI collection period. The HR response (ΔHR) to the IHG was determined by averaging the response over the last 10s of each rest and IHG interval. HR responses for each participant were averaged over the four repeated blocks in the three separate trials (30%, 40% and 50%).

The effect of group and IHG intensity on HR response was assessed using a two-way mixed ANOVA with an alpha level of $p < 0.05$. Statistical analyses were performed using SigmaPlot (version 12.5, 2011). The Shapiro-Wilk test for normal distribution, as well as

the Holm-Sidak method for pairwise multiple comparisons were used. All data are presented as mean ± standard deviation.

2.2.5 Neuroimaging Recording Session

All imaging data were collected using a whole body 3-Tesla imaging system (Magnetom Prisma, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil (Barberi, Gati et al. 2000). A high-resolution T_1-weighted structural volume was acquired with a 3D MPRAGE sequence at the beginning of the scanning session (sagittal, matrix 256 X 240 mm, voxel resolution 1.0 X 1.0 X 1.0 mm, 1 mm slice thickness, no gap, flip angle 9°, TE = 2.98 ms, TI = 900 ms, TR = 2.3 ms). Transmission and detection of the BOLD contrast signal were acquired by T_2-weighted gradient echo-echo planar imaging pulse sequence with the following parameters: TE = 30 ms; FOV = 240 x 240 mm, flip angle = 90°. Forty-five interleaved axial slices (3.0 X 3.0 mm in-plane voxel resolution, TR = 2.5 s) were acquired in each volume. Five volumes were acquired in the resting participant prior to actual data collection to allow for magnetization equilibrium; these were discarded prior to data analysis. Head movement was limited during the experimental session within a head cradle packed with foam padding, and each subject was instructed to avoid head movements during the scanning period. Beat-by-beat HR was calculated from the continuous signal derived from an MRI-compatible pulse Oximeter (Nonin Medical Inc, 8600FO MRI, Plymouth, MN) placed over the index finger of the non-exercising left hand. In each session, analog signals for pulse recordings and IHG contraction force were sampled at 1000 Hz with an on-line data acquisition and analysis system (PowerLab, ADInstruments, Mountain View, CA, USA). Respiratory frequency was monitored continuously during the exercise period.

2.2.6 Neuroimaging Data Analysis

The HR response (ΔHR) to the handgrip was determined by averaging the response over the last 10s of each rest and IHG interval. Individual HR time courses were determined using 2.5s averages of the beat-by-beat HR measures to generate time-aligned data with the BOLD imaging acquisition. For both the ΔHR and the HR time course, responses for each participant were averaged over the seven repeated blocks at 40% MVC.

All fMRI data were analyzed using Brain Voyager QX 2.8.2 (Brain Innovation, Maastricht, Netherlands) (Goebel, Esposito et al. 2006). At the first (individual) level, 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. Individual functional data were co-registered to their respective anatomical template, and subsequently transformed to Talairach space (Talairach and Tournoux 1988). The change in BOLD signal over the exercise period was modeled with a boxcar function convolved with a canonical haemodynamic response function and regressed with the individual movement parameters generated during preprocessing. This resulted in subject-specific contrast images containing whole brain information related to sites of both increased and decreased BOLD signal, relative to baseline, during the IHG task as a function of the task itself and the individual HR correlation. The General Linear Model was used to calculate the parameter estimates for all brain voxels (Friston, Holmes et al. 1995).

To make valid population inferences, a second-level, two-group, random effects (RFX) analysis was performed both in response to the task and the HR regression to assess the consistency of effects between individuals based on the variability of the first-level estimates across subjects. Subsequently, a subtraction analysis of the group mean parameter estimates was performed to assess significant differences between Control and CAD groups. Corrections for multiple comparisons were made using the false discovery rate ($p<0.05$), as well as cluster level threshold estimation (Hagler, Saygin et al. 2006), with 1000 iterations of Monte Carlo simulation and a statistical threshold of $p<0.05$ for the main task effects. Due to the abundance of neural activity, both corrections were performed sequentially, such that the final results represent only clusters > 10 voxels in size (unless otherwise specified). Based on earlier data in young individuals performing the same IHG protocol (Wong, Masse et al. 2007, Norton, Luchyshyn et al. 2013), an *a priori* region-of-interest analysis was performed for relevant cortical autonomic network regions including the IC, ACC, PCC, thalamus, MPFC, and HC. All fMRI data are represented in radiologic convention (i.e. subject's right appears on the left).

Probabilistic functional maps were created for each group to investigate the spatial consistency of activation patterns across subjects. These maps represent the relative number of subjects leading to significant task activity at each spatial location.

2.3 Results

2.3.1 Physiological Responses

The groups were not statistically different in age, mean arterial pressure, resting HR or maximal handgrip strength (Table 2.1). The heart rate response (ΔHR) to the 40% MVC contraction was the same during the physiological (LAB) and neuroimaging (fMRI) session in both groups (Table 2.1). There was a significant effect of group ($p=0.03$) on ΔHR across all IHG tasks (Figure 2.1). None of the participants reported feeling any significant degree of aversive emotional stress or forearm fatigue as indicated by the Borg scale outcomes (Table 2.1)(Borg 1982). Further, the ΔHR at maximal exertion (stress test) in Control participants was greater than CAD patients (Table 2.1, $p<0.001$). All patients exercised to maximum effort (Control: 19 ± 1, CAD: 19 ± 1 on 6-20 Borg Scale) and the tests were not discontinued due to medical reasons (angina, ST depression, arrhythmia or abnormal BP response). In addition, left ventricular ejection fraction (LVEF) was normal (>= 50%) in 11/17 patients, mild (35-49%) in 3/17 patients, moderate (20-34%) in 2/17 patients, and severe (<20%) in one patient.

2.3.2 Functional (BOLD) Imaging Data: First-level (Individual) Response to 40% IHG Task

A-priori region-of-interest analysis revealed high inter-subject variability in both groups, with bilateral IC activation observed in 22/23 Control subjects and 15/17 CAD patients, ACC deactivation observed in 17/23 Control and 14/17 CAD, PCC deactivation observed in 18/23 Control and 16/17 CAD, thalamus activation observed in 20/23 Control and 7/17 CAD, MPFC deactivation observed in 16/23 Control and 12/17 CAD, and HC deactivation observed in 8/23 Control and 8/17 CAD ($p<0.05$, FDR).

2.3.3 Functional (BOLD) Imaging Data: First-level (Individual) Response Correlated with Heart Rate

Bilateral IC activation was observed in 18/23 Control subjects and 13/17 CAD patients, ACC deactivation was observed in 11/23 Control and 5/17 CAD, PCC deactivation was observed in 5/23 Control and 2/17 CAD, thalamus activation was observed in 16/23 Control and 11/17 CAD, MPFC deactivation was observed in 16/23 Control and 12/17 CAD, and HC deactivation was observed in 2/23 Control and 2/17 CAD ($p<0.05$, FDR).

2.3.4 Functional (BOLD) Imaging Data: Second-level (Group) Response to 40% IHG Task

A-priori region-of-interest analysis revealed common increases in BOLD signal in the primary motor cortex (precentral cortex), bilateral anterior IC and occipital lobe (Tables 2.2 and 2.3; Figure 2.2). In addition, common deactivation was observed in the PCC ($p<0.05$, FDR; Tables 2.2 and 2.3, Figure 2.2). In Control subjects, activation was observed in the ACC and thalamus; and deactivation was observed in the HC. No signal change was observed in the HC of the CAD group. No signal change was observed in the MPFC in either Control or CAD patients at the group level.

2.3.5 Contrasting BOLD Responses between Control and CAD to 40% IHG Task

Comparisons of activated regions between Control and CAD during the 40% IHG task are shown in Figure 2.3. In subtraction analyses for CAD>Control, greater activation (or less deactivation) was observed in the PCC and MPFC. The contrast CAD<Control showed activation in the right anterior insula, bilateral precentral cortex, and occipital lobe ($p<0.05$).

2.3.6 Functional (BOLD) Imaging Data: Second-level (Group) Response Correlated with Heart Rate

Extensive activation patterns were revealed in both Control and CAD groups, but lacked significant deactivation in expected autonomic regions (Figure 2.4). Specifically, the bilateral IC and precentral gyrus were activated in both Control and CAD groups while deactivation in the MPFC and HC were absent (FDR $p<0.05$).

2.3.7 Contrasting BOLD Responses between Control and CAD Correlated with Heart Rate

Comparisons of activated regions between Control and CAD during the 40% IHG task correlated with the individual HR time courses are shown in Figure 2.5. In subtraction analyses for CAD>Control, greater activation was observed in the perigenual anterior cingulate cortex. The contrast CAD<Control demonstrated activation in the bilateral insula and posterior cingulate cortex ($p<0.05$).

2.3.8 Probability Mapping

Probabilistic maps were created for Control and CAD groups to provide a general means to evaluate the spatial consistency of task-specific brain activation across subjects. We plotted the cross-subject (Control and CAD) overlap probability maps for 40% IHG at a range of 0-100% (Figure 2.6). Control subjects (yellow/orange) displayed greater anatomical consistency compared to CAD patients (blue) who showed much greater variability in activation responses (cluster threshold=15 voxels). Probability percent values (overlap) between Control and CAD in expected CAN regions include: the left anterior IC (8.43% overlap; x, y, z coordinates: -40, 14, 5), right anterior IC (11.02%; x, y, z coordinates: 34, 20, 9), PCC (0.08%; x, y, z coordinates: -8, -55, 14), precentral gyrus (31.97%; x, y, z coordinates: 34, -21, 52), and MPFC (2.81%; x, y, z coordinates: 4, 37, -3).

Table 2.1 Anthropometric and baseline cardiovascular data during baseline and isometric handgrip exercise (mean ± SD).

Group	Age	Sex	MAP (mmHg)	MVC (mV)	Resting HR (bpm)	40% ΔHR (LAB)	40% ΔHR (fMRI)	40% RPE	Stress Test ΔHR (LAB)
CTRL (n=23)	63 ± 11	15M, 8F	90 ± 9	62 ± 28	58 ± 8	4 ± 2	2 ± 2	13 ± 2	105 ± 16
CAD (n=17)	59 ± 9	13M, 4F	87 ± 10	63 ± 31	59 ± 5	3 ± 2	3 ± 2	11 ± 2*	78 ± 24*

CTRL, healthy older controls; CAD, coronary artery disease patients; MAP, mean arterial pressure; MVC, maximal voluntary contraction (average of LAB + fMRI sessions); HR, heart rate (beats/min); fMRI, neuroimaging session; LAB, physiological recording session; Stress test, voluntary maximal exertion; Borg rate of perceived exertion (RPE) scale: 6-20. RPE=11, "light" exercise; 13, "somewhat hard". There was a main effect of group such that CAD patients had less of a HR response during all conditions than Control. *Different from Control ($p<0.05$).

Table 2.2 BOLD signal changes to 40% MVC handgrip in Control subjects.

Location		Side	Coordinates			T-score	Number of Voxels
			x	***y***	***z***		
Insula	(↑)	L	*-38*	*22*	*9*	4.39	986
Insula	(↑)	R	*31*	*23*	*9*	5.44	1000
Dorsal ACC	(↑)	R	*5*	*40*	*14*	2.93	398
Mid-superior CC	(↑)	R	*1*	*-1*	*30*	3.78	766
PCC	(↓)	R	*3*	*-50*	*20*	-3.05	363
Precentral gyrus	(↑)	L	*-31*	*-6*	*50*	4.03	809
Precentral gyrus	(↑)	R	*31*	*-6*	*50*	6.24	1000
Postcentral gyrus	(↑)	R	*31*	*-27*	*50*	6.61	1000
Thalamus	(↑)	L	*-9*	*-17*	*12*	4.18	935
Thalamus	(↑)	R	*8*	*-17*	*12*	4.61	896
Hippocampus	(↓)	L	*-28*	*-27*	*-3*	-3.26	655
Occipital	(↑)	R	*0*	*-87*	*9*	4.46	823

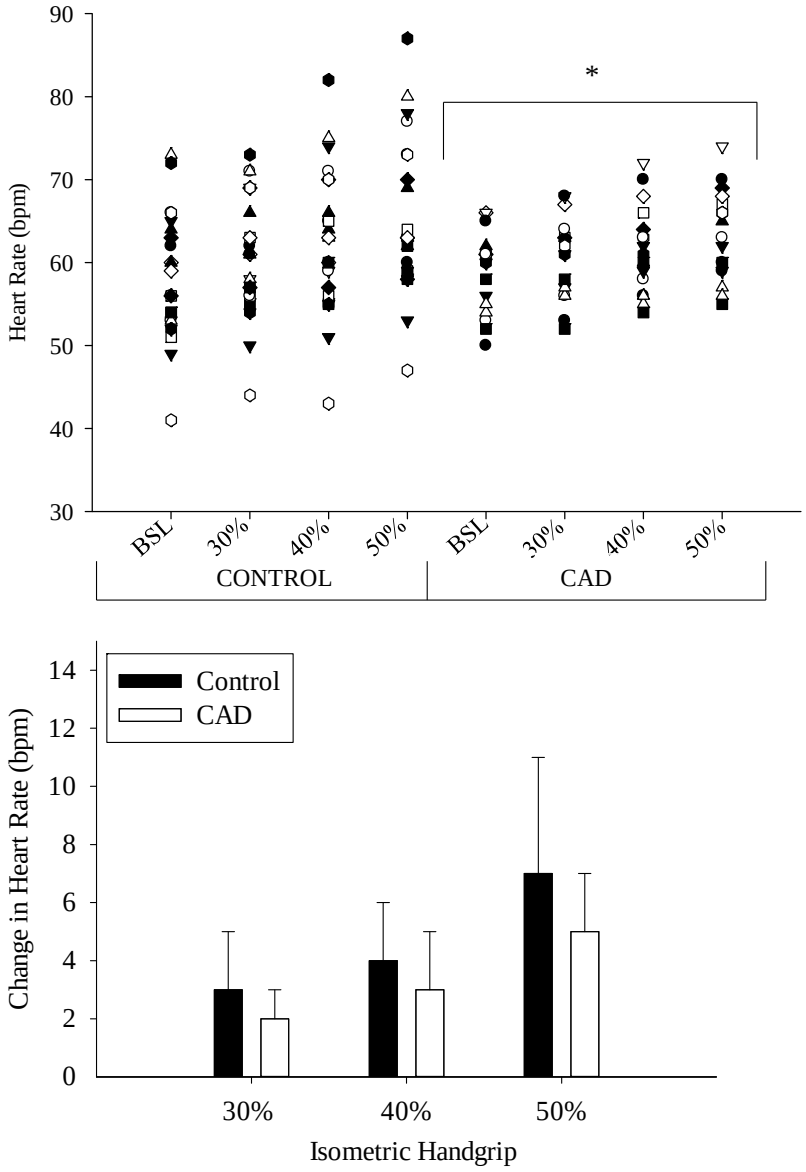
ACC = anterior cingulate cortex, PCC = posterior cingulate cortex. (↑) = activation; (↓) = deactivation. (Talairach coordinates represent voxel of maximum response: *x* represents position in brain on horizontal axis, *y* represents position on vertical axis, *z* represents the depth position).

Table 2.3 BOLD signal changes to 40% MVC handgrip in CAD subjects.

Location		Side	Coordinates			T-score	Number of Voxels
			x	***y***	***z***		
Insula	(↑)	L	*-33*	*18*	*11*	4.40	236
Insula	(↑)	R	*34*	*13*	*11*	4.33	313
Mid-superior CC	(↓)	L	*-8*	*-32*	*33*	-4.29	532
PCC	(↓)	L	*-8*	*-48*	*11*	-4.22	362
Precentral gyrus	(↑)	R	*34*	*-23*	*44*	5.29	525
Precentral gyrus	(↑)	L	*-46*	*1*	*33*	4.04	750
Occipital	(↑)	R	*0*	*-87*	*9*	4.06	792

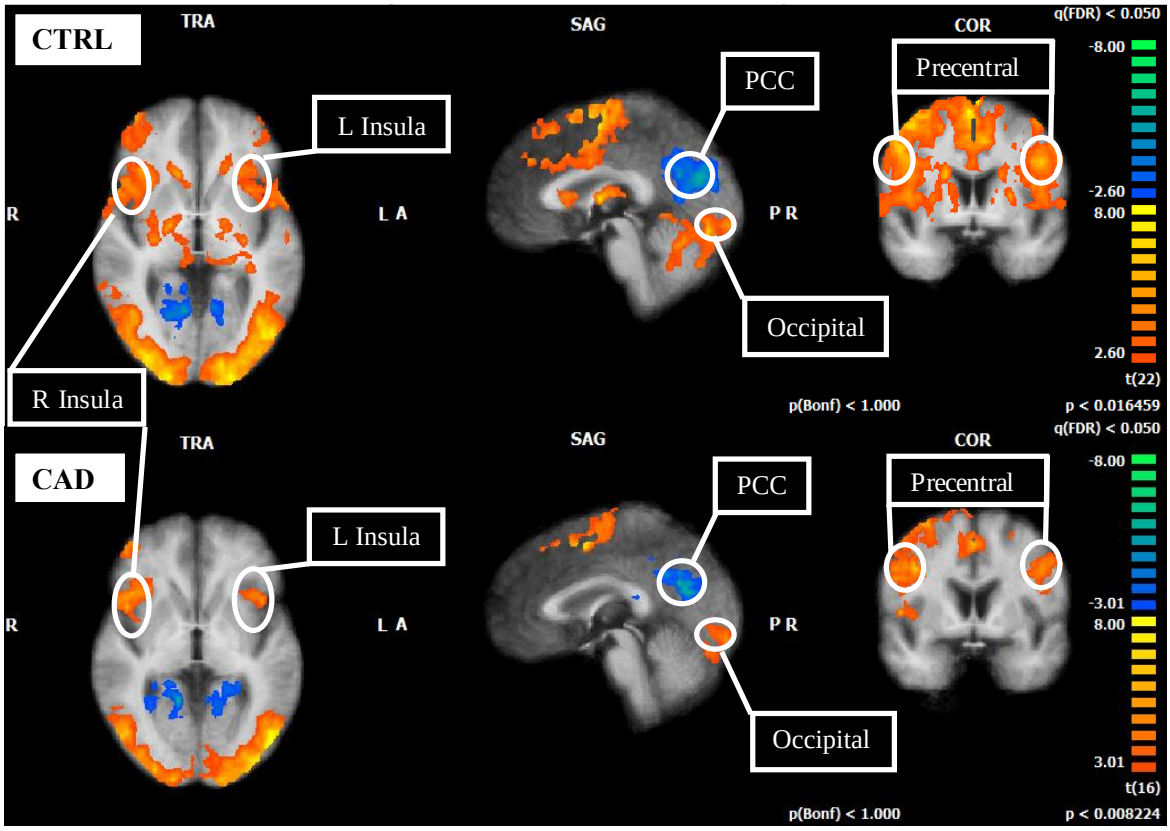
ACC = anterior cingulate cortex, PCC = posterior cingulate cortex. (↑) = activation; (↓) = deactivation. (Talairach coordinates represent voxel of maximum response: *x* represents position in brain on horizontal axis, *y* represents position on vertical axis, *z* represents the depth position).

Figure 2.1 Graded heart rate response to isometric handgrip.



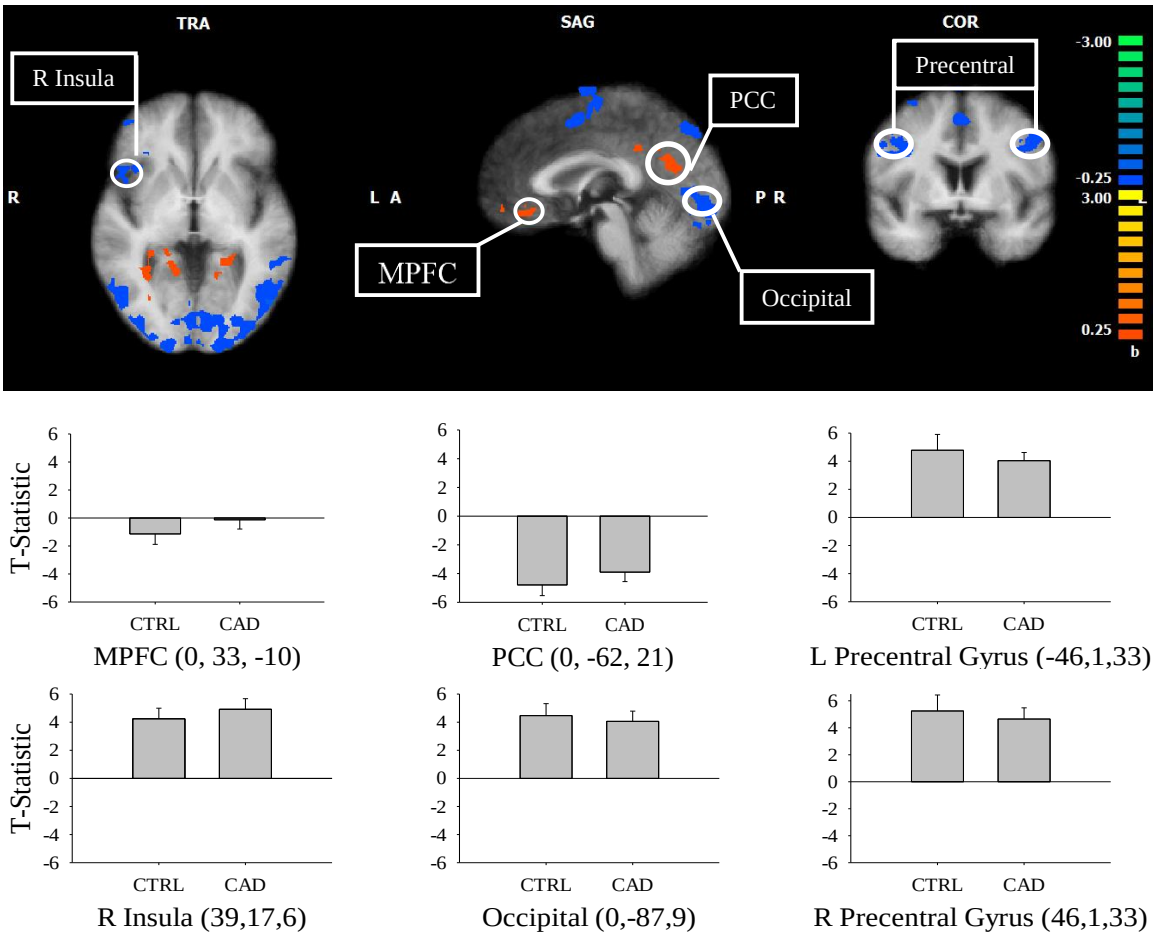
Control and coronary artery disease patients (CAD) represented as individuals (top panel) and as a group (bottom panel). BSL=Baseline. *Different from Control; main effect of Group ($p<0.05$).

Figure 2.2 Cortical functional response to 40% IHG task in Control (CTRL; top three images) and CAD (lower three images).



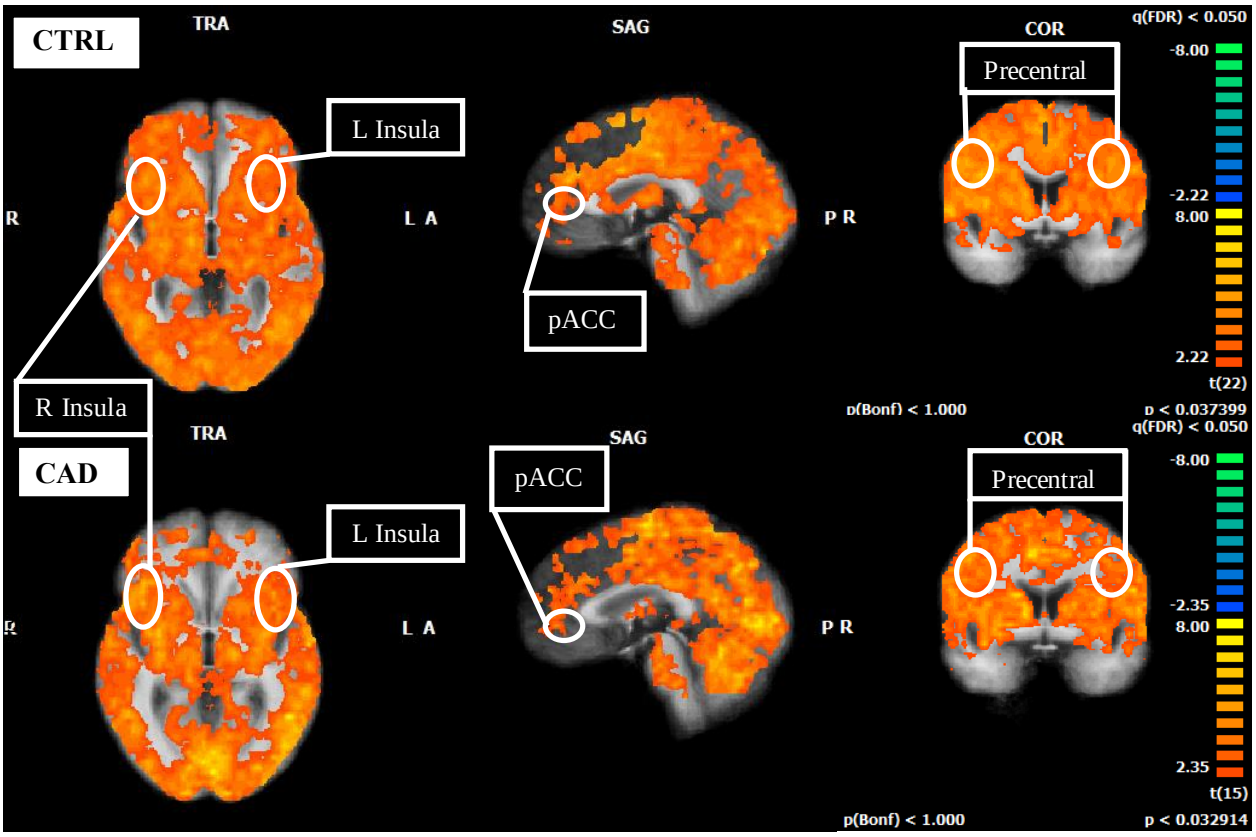
L: left, R: right, PCC: posterior cingulate cortex. T-statistics (beta values) at specified regions (Talairach coordinates, circled) are represented as an average for each group. FDR, $p<0.05$, corrected for multiple comparisons. Color scheme identified by scale at right. Red/warm colors denote regions of activation above baseline levels, blue/cold colors denote regions of deactivation below baseline levels (exact values given in beta graphs, Figure 2.3). Note the absence of deactivation at the medial prefrontal cortex and hippocampus in both groups.

Figure 2.3 Subtraction Result for Group 1 average (CAD) vs Group 2 average (Control) to 40% IHG boxcar analysis.



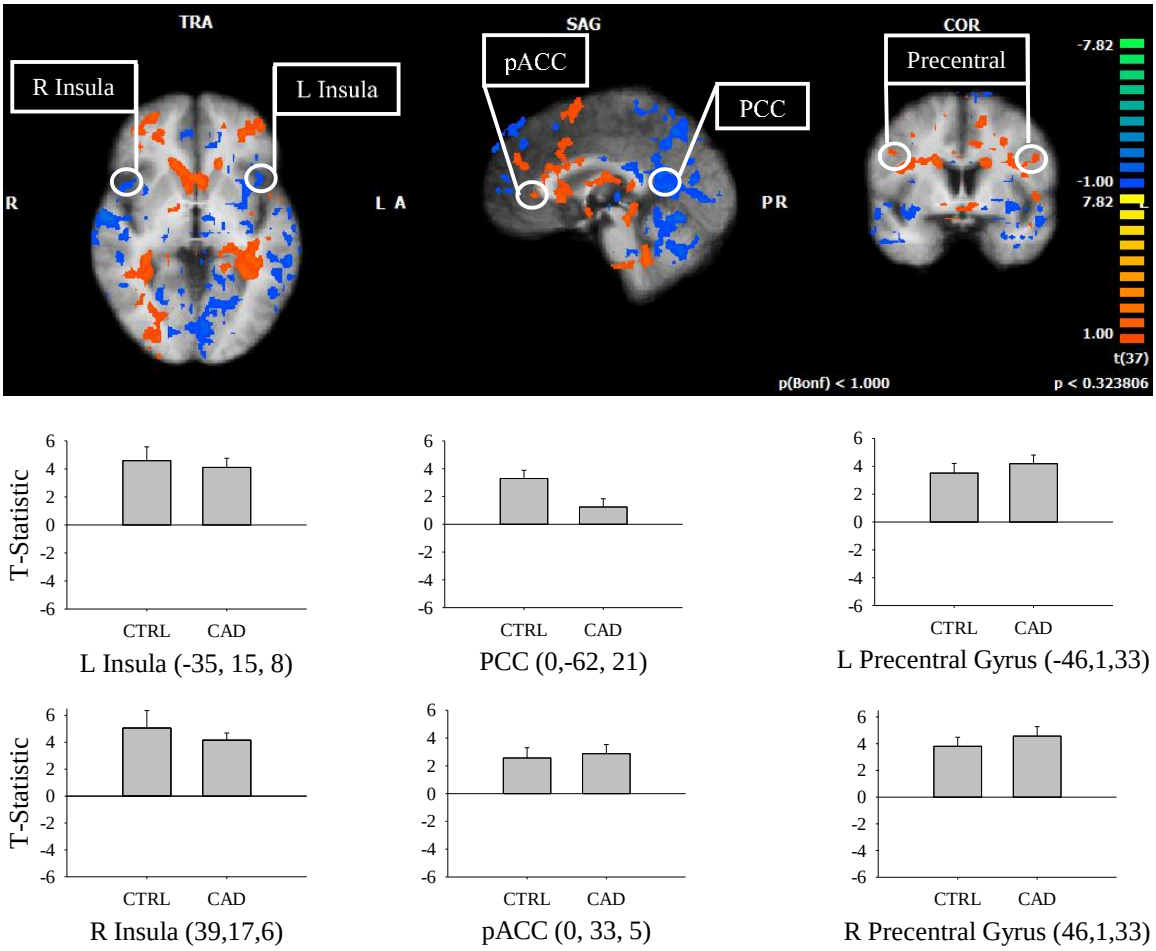
L: left, R: right, MPFC: medial prefrontal cortex, PCC: posterior cingulate cortex. Warm colors show areas where there is a positive difference with respect to group 1 (CAD > Control), and cold colors show areas where there is a negative difference with respect to group 1 (CAD < Control). T-statistic (beta value) at specified regions (Talairach coordinates, circled) are represented as an average for each group and denoted by color scale at right, threshold = 2; $p<0.05$. Cluster threshold = 10 voxels. Error bars represent standard deviation.

Figure 2.4 Cortical functional response correlated to individual heart rate time course during 40% IHG task in Control (CTRL; top three images) and CAD (lower three images).



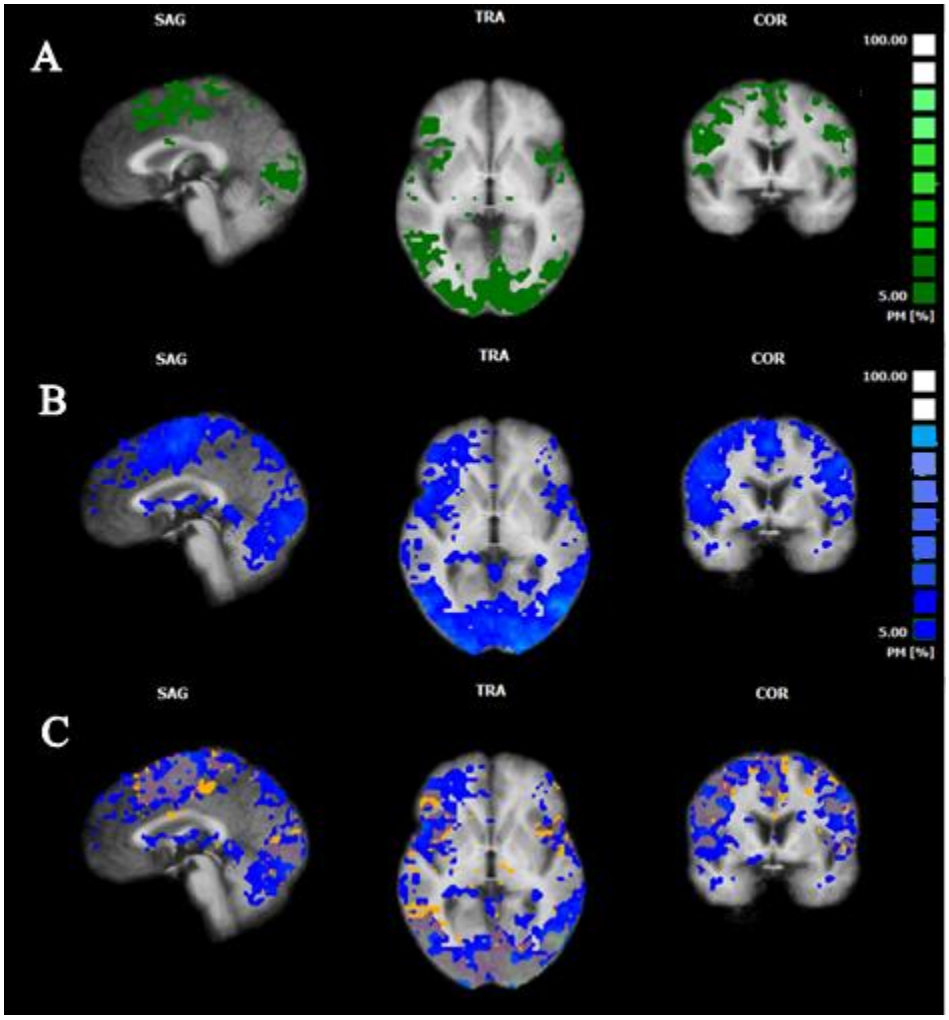
L: left, R: right, pACC: perigenual anterior cingulate cortex. T-statistics (beta values) at specified regions (Talairach coordinates given, circled) are represented as an average for each group. FDR, $p<0.05$, corrected for multiple comparisons. Color scheme identified by scale at right. Red/warm colors denote regions of activation above baseline levels, blue/cold colors denote regions of deactivation below baseline levels (exact values given in beta graphs, Figure 2.5). Note the absence of deactivation at the medial prefrontal cortex and hippocampus in both groups.

Figure 2.5 Subtraction result for Group 1 average (CAD) vs Group 2 average (Control) correlated to individual heart rate time course during 40% IHG task.



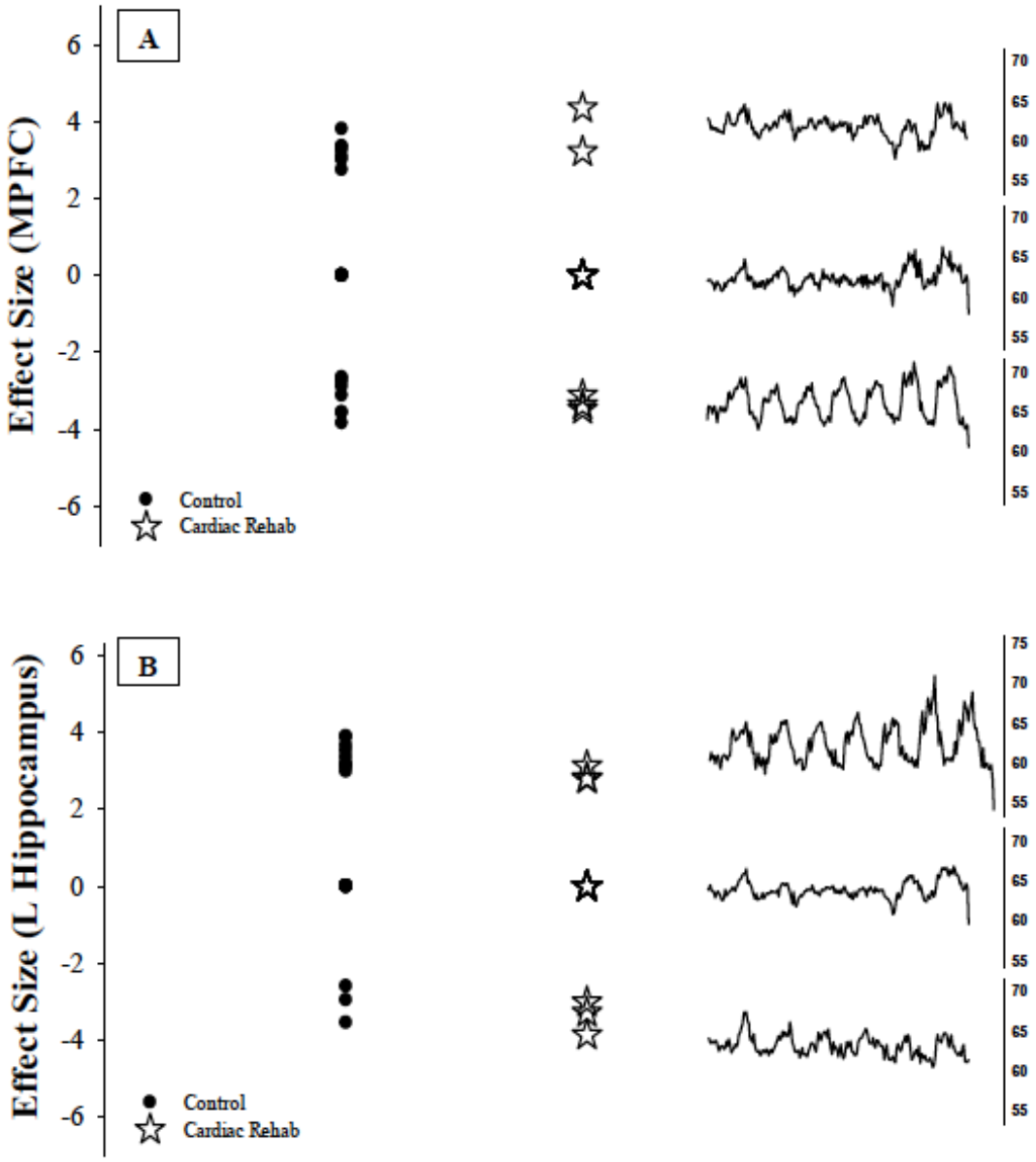
L: left, R: right, PCC: posterior cingulate cortex; pACC: perigenual anterior cingulate cortex. Warm colors show areas where there is a positive difference with respect to group 1 (CAD > Control), and cold colors show areas where there is a negative difference with respect to group 1 (CAD < Control). T-statistic (beta value) at specified regions (Talairach coordinates, circled) are represented as an average for each group and denoted by color scale at right, threshold = 2; $p<0.05$. Cluster threshold = 10 voxels. Error bars represent standard deviation.

Figure 2.6 Probability Mapping.



(A) Control subjects (green), 0% minimum threshold. (B) Coronary artery disease patients (CAD; blue), 5% minimum threshold. Each colored cluster represents the relative percentage of subjects leading to significant task activity during a 40% MVC handgrip task based on the bar graph at right. (C) Probability map overlap of both groups. Control = orange; CAD = blue (voxel threshold = 15 voxels; 0% minimum threshold). CAD patients (blue) had much greater variability in activation responses than Control who indicated greater anatomical consistency.

Figure 2.7



The effect size (left) in *a priori* regions (panel A: medial prefrontal cortex; panel B: left hippocampus) to the 40% isometric handgrip task with the average heart rate response (bpm) of those individuals (right). Marked individual variability was observed in neural and heart rate responses in both groups.

2.4 Discussion

In contrast to healthy Controls, the CAD patient group demonstrated diminished HR responses across all exercise workloads and high variance in activation patterns amongst regions of the cortical autonomic network. These cortical patterns appear to be consistent with the overall suppressed HR response despite the ability to perform the IHG task adequately. Therefore, the current data supports the hypothesis that CAD alters the cortical circuitry associated with exercise and patients exhibit accelerated age-related dysregulation of the brain-heart connection.

An initial important observation of the current study was the difference in HR responses to IHG between the current participants and those of younger individuals reported earlier from our laboratory (Wong, Masse et al. 2007, Goswami, Frances et al. 2012, Norton, Luchyshyn et al. 2013). This difference was statistically significant, as determined by an independent group's t-test that contrasted the present data with those published earlier. Specifically, young individuals (25±4 years), when compared to the current participants (61±10 years), generate a much larger HR response (6-15bpm) to a similar relative IHG tension ($p<0.0001$). Mechanistically, this IHG protocol is designed to engage exercise-onset reflexive increases in HR that predominantly reflect reduced dominance of parasympathetic control (Hollander and Bouman 1975, Fagraeus and Linnarsson 1976, Mitchell, Reeves et al. 1989). Therefore, the smaller HR responses in Control, and smaller yet in CAD, are likely a consequence of age-related impairment of parasympathetic outflow (Seals, Taylor et al. 1994, Monahan, Dinenno et al. 2001) that is further negatively impacted by CAD (Gribbin, Pickering et al. 1969, Eckberg, Drabinsky et al. 1971). Recently, our laboratory reported the cortical activation patterns and HR responses in healthy individuals ranging from 21-80 years of age, with conclusions that age alone does not determine a smaller ΔHR response (Norton, Luchyshyn et al. 2013) in that several older adults still generated similar responses to young individuals. Thus, inter-individual variability in HR responses was augmented with increasing age. The current study further supports a depressed HR response overall, as well as enhanced variability in HR responses, as an effect of age (Figure 2.1).

A second observation of the current study was the marked and unexpected differences in brain activation patterns associated with both the IHG task and the HR response in both the Control and CAD patients compared to previous studies from our laboratory performed by young and healthy individuals (Wong, Masse et al. 2007, Goswami, Frances et al. 2012, Norton, Luchyshyn et al. 2013). Specifically, the current participants exhibited a large and widespread pattern of enhanced brain activation relative to baseline when correlated with both the IHG task and the HR response. This widespread activation pattern was different from the discrete pattern observed in young individuals specifically in regions related to autonomic control. Within this pattern of higher overall activation, however, there was a marked absence of deactivation within the MPFC and HC in the current participants (at least at the group level) which are consistently present in young healthy subjects (Wong, Masse et al. 2007, Goswami, Frances et al. 2012, Norton, Luchyshyn et al. 2013). Yet, group-level activation was observed in the bilateral insula, and deactivation was observed in the posterior cingulate cortex, observations that are consistent with previously published results in young subjects.

Although this apparent "overactivation" identified above is unique in the context of cardiovascular control, it has been reported earlier in the context of perceptual or cognitive tasks performed by aged individuals. The mechanism(s) of these patterns is yet unknown. They may reflect alterations in the coupling between regional blood flow and oxygen extraction. However, some hypothesize these patterns to reflect compensatory neural responses (Reuter-Lorenz 2002, Cabeza, Daselaar et al. 2004) where, in the aging brain, previously connected networks are disrupted such that alternative patterns emerge which must "work harder" to make up either for its own declining efficiency or for processing deficiencies elsewhere in the brain. The current observations appear to be the first to report a similar phenomenon related to volitional tasks such as IHG exercise. If this observation reflects neural compensation, the smaller response in CAD versus Control participants becomes the third important observation of the current study (Figure 2.4). The degree of compensatory activation is thought to be related to a sense of effort required to perform the task (Reuter-Lorenz and Park 2010). In this context, the smaller amount of compensation in CAD patients may reflect lower perceived effort required to perform the IHG. However, the similar Borg scores of perceived effort and identical

absolute and relative workloads produced (MVC, Table 2.1) argue that the smaller brain activation patterns in CAD patients are not related to perceived effort. Previously, our laboratory reported accelerated age-related cortical atrophy in CAD patients (Anazodo, Shoemaker et al. 2013). Thus, it may be that age-related compensatory responses to IHG are also related to accelerated brain atrophy and/or impaired local flow-metabolism coupling.

The probability mapping analysis indicated that CAD patients exhibited greater regional variability in activation responses to the 40% IHG task than Control. Probability percent values, which reflect overlapping patterns of activation between Control and CAD participants, were highest in the primary motor cortex (31.97%), but significant variability existed in expected CAN regions such as the left anterior IC (8.43%), right anterior IC (11.02%), PCC (0.08%), and MPFC (2.81%). Thus, it appears that brain regions required for motor activity are retained in CAD, but that those regions believed to be required for explicit autonomic homeostatic functions, such as the MPFC and HC, are dysregulated more in CAD patients.

A notable outcome of the current study was the absence of deactivation associated with the HR response to IHG in the MPFC and HC, in both Control and CAD patients when studied at the group level (Figure 2.4). The MPFC region was of particular interest in the present study as it has been associated with cardiovagal control across many stimuli that elicit cardiovascular arousal (Critchley, Corfield et al. 2000, Williamson, McColl et al. 2003, Gianaros, Van Der Veen et al. 2004, Matthews, Paulus et al. 2004, Resstel, Fernandes et al. 2004, Kimmerly, O'Leary et al. 2005, Thayer, Sollers et al. 2009, Norton, Luchyshyn et al. 2013). Previous studies have further suggested that the MPFC is involved in the processes of integrating sensory information during the resting default state and its activity is hence attenuated during goal-directed behaviours (Raichle, MacLeod et al. 2001). This interpretation is consistent with repeated observations of decreased MPFC activation, relative to baseline, during volitional IHG (Wong, Masse et al. 2007, Goswami, Frances et al. 2011, Norton, Luchyshyn et al. 2013). Despite high inter-individual variability, the MPFC factored importantly into the subtraction analyses when correlated with the task alone (Figure 2.3) illustrating that Control subjects had

more deactivation (or less activation) than CAD in response to the exercise task. To investigate this outcome further, a secondary analysis of individual brain activation patterns was pursued. This analysis indicated that 16/23 Control and 12/17 CAD patients exhibited a reduction in MPFC activation relative to baseline during the IHG period. However, the number of participants who demonstrated a reduction in MPFC activation and produced a HR response of ≥ 3 bpm to IHG was 9/23 (39%) in Control and 3/17 (18%) in CAD. Thus, the inter-individual variability minimized group-level statistical power in both the specified MPFC regional activation and in the HR response to IHG and, consequently, reflects the reduced brain activation evident in the subtraction analysis in Control subjects.

The variability in brain deactivation patterns outlined above seems to exert a dominant impact on the current results. The high cortical variability in these groups is clear in Figure 2.7, which illustrates that both groups had a lack of consistent MPFC deactivation in response to the IHG task, with some subjects having activation in expected autonomic regions and many individuals having no response at all. In earlier studies from our laboratory, and reports from several other laboratories (Critchley, Corfield et al. 2000, Gianaros, Van Der Veen et al. 2004, Norton, Luchyshyn et al. 2013), HR variations in young, healthy subjects most strongly correlate inversely with deactivation within the HC and MPFC. Moreover, HR-associated effective connectivity exists between the MPFC and HC in healthy individuals promoting a much larger HR response when both regions are deactivated in concert rather than each region alone (Norton, Luchyshyn et al. 2013). However, HR responses in the current study were much smaller suggesting an effective consequence of cortical "de-coupling". For example, a small HR response was observed in some individuals who did not express deactivation within the MPFC or HC. This pattern was dominated by Control subjects (9/12). To our knowledge, no data exist to address this critical component of changes in cardiovascular control that emerge in aging and diseased individuals.

2.5 Limitations

All CAD patients were on a combination of drug therapies including cholesterol lowering statins, beta-blockade, angiotensin-converting enzyme (ACE) inhibitors/angiotensin II

receptor blockers, calcium channel blockers, diuretics, and anti-platelets including aspirin. ACE-inhibitors and angiotensin II receptor blockers affect vasomotor control but exert minimal effect on cardiac function and should not have impacted the current results. Beta-blockers can reduce baseline HR and interfere with sympathetically-driven changes in HR. However, these effects are largely seen at maximal workloads, which is supported by our data (stress test data, Table 2.1) and are not expected to affect HR responses to the 40% IHG task where vagal control dominates below 100bpm (Rowell and O'Leary 1990). Furthermore, the heart rate response to the 40% IHG was not different between groups suggesting that beta-blockade did not influence HR responses at the level of the heart. Finally, as outlined above, CAD patients were capable of mounting a significant HR response during the cardiorespiratory fitness test indicating that the heart's ability to respond to volitional effortful tasks was not altered by the medication.

2.6 Conclusion

Overall, the current results indicate that relative to similarly-aged, and apparently healthy individuals, vascular disease impairs functional outcomes in the brain in response to moderate intensity IHG. In particular, the enhanced variability of cortical responses and diminished total cortical activation patterns in CAD are consistent with an overall lower HR response, promoting the hypothesis that CAD patients appear to exhibit dysregulation of the brain-heart connection.

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Chapter 3

3 Impact of Long-Term Endurance Training Versus Guideline-Based Physical Activity on Brain Structure in Healthy Aging

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3.1 Introduction

Atrophy of cortical gray matter is a hallmark of aging, with tissue loss beginning early in the third decade of life (Good, Johnsrude et al. 2001, Raz, Lindenberger et al. 2005). Importantly, strong inverse relationships exist between cortical thickness and age-related dementias (Lerch, Pruessner et al. 2005, Du, Schuff et al. 2007). Furthermore, parallel declines also occur between cognitive function and structural changes in the medial prefrontal cortex, dorso-lateral prefrontal cortex, hippocampus, anterior cingulate, amygdala, and insula (Coffey, Wilkinson et al. 1992, Raz, Gunning et al. 1997, Good, Johnsrude et al. 2001, Raz, Lindenberger et al. 2005). Given the economic, social, and personal burden associated with age-related neural deterioration, identifying strategic mechanisms to prevent declines in structural and functional brain health *before they begin* represents an imperative public health goal.

Exercise has emerged as a potent stimulus for cognitive improvement in older adults who are sedentary and experience cognitive decline (Kramer, Colcombe et al. 2005, Voss, Nagamatsu et al. 2011, Chaddock, Erickson et al. 2012). However, the *extent* to which exercise training can modify cortical thickness or subcortical gray matter volume remains relatively unstudied, with considerable variability across studies. For example, Erickson et al. observed rather rapid increases in HC volume in just 6 weeks of a walking intervention performed by sedentary, elderly individuals (Erickson, Voss et al. 2011). This study shows that exercise is neuroprotective when applied in a late-onset model after atrophy likely has begun, as suggested by cognitive impairment. In a whole brain analysis, Rovio et al. reported that people who were physically active during middle age

(~50 years of age) expressed greater frontal lobe cortical thickness at a 21-year follow-up assessment, compared to an inactive control group (Rovio, Spulber et al. 2010). Similarly, Erikson et al. showed a threshold effect of walking distance over a 9-year follow-up period on cortical thickness in the prefrontal, temporal and hippocampus regions (Erickson, Raji et al. 2010). These studies show that activity during the middle age period may exert neuroprotective benefits to a limited portion of the brain later in life. This idea stands in contrast to the knowledge that overall risk of disease and mortality relates to cardiorespiratory fitness (Defina, Willis et al. 2013, Barry, Baruth et al. 2014) or daily energy expenditure (Booth, Roberts et al. 2012) suggesting that higher gains may be possible than those achieved with mild to moderate interventions such as those provided through published guidelines for active living (ACSM 1995). Also, the research linking individual differences in physical activity, and training-related changes in fitness to brain health, has led to a hypothesis that cardiorespiratory fitness is a critical mediator of these benefits (Etnier, Nowell et al. 2006, Angevaren, Aufdemkampe et al. 2008, Smith 2012). If so, then a dose-response pattern should exist in the building of cortical mass reserve through the life-long pursuit of high fitness levels.

Master's Athletes (MA) provide a unique model to assess the extent to which long-term training can affect brain metrics. Compared to a cohort of inactive elderly controls, Tseng et al. observed greater posterior cortical thickness in the cuneus and precuneus in a small group of older (72 years) MA (Tseng, Uh et al. 2013). These results are interesting when considering previous evidence for greater sensitivity of anterior brain regions for age-related brain atrophy (Raz, Gunning et al. 1997, Good, Johnsrude et al. 2001, Raz, Lindenberger et al. 2005). The relative lack of exercise effect in these MA in frontal brain regions is of importance because it suggests either a transient effect of exercise on frontal brain mass, or a minimized effect of high training loads on neuroplastic outcomes. Variations in the above studies may also reflect issues such as small sample size, focus on narrow age ranges, varying baseline conditions, short versus long-term training periods, timing of the exercise onset, and/or emphasis on univariate group-based or region-of-interest analysis. In addition, the use of sedentary older individuals as the control condition in many studies introduces a higher probability of undetected nuisance variables such as latent cerebrovascular damage, endothelial dysfunction, and

subthreshold neurological impairments. These possibilities raise questions regarding the maximal exercise-induced benefit possible across the adult age span, as well as the appropriate control group for such studies. In addition, it may be that adhering to accepted guidelines for active living (ACSM 1995) achieves benefits that are not enhanced by additional training loads.

This study attempts to expose the maximal benefit to cortical and subcortical gray matter possible in the context of exercise training. To avoid concerns about subthreshold age-related nuisance variables related to physical inactivity, and to directly relate the differences between guideline-based training versus high training loads, world-class MA as well as regularly active, but non-competitive, healthy volunteers were studied. We tested the overall hypothesis that gray matter adaptations to exercise follow a dose-dependent pattern in each of the cortical and subcortical gray matter regions.

3.2 Methods

3.2.1 Participants

A total of 32 individuals participated in this study. Observations were made in 16 elite-level middle-aged and older Masters Athletes (MA) who trained and raced in the sport of triathlon at the professional or national level for >30 years (53 ± 6 years of age (4 female), training >15 h/wk; VO_{2max}=55±10 ml/kg/min), and 16 similarly-aged healthy, active (HA) control subjects who met the age-appropriate health recommendations for exercise for at least 5 years (58 ± 9 years, (6 female); VO_{2max}=38±7 ml/kg/min). Table 3.1 provides group characteristics. All subjects were right-handed, non-smokers, free of medications, and did not have diagnosed hypertension, diabetes, vascular or neurological/psychological impairments. Testing of menstruating females occurred during days 1-14 of the menstrual cycle, with day one representing the first day of menstruation. The post-menopausal women were not taking hormone replacement therapy. The University of Western Ontario Health Sciences Ethics Review Board approved this study and each participant provided informed, written consent. The study adhered to the Declaration of Helsinki.

3.2.2 Assessment of Cardiorespiratory Fitness

A graded treadmill exercise test, conducted under standard clinical observation, provided information regarding each subject's peak oxygen uptake (VO_{2max}). During this test, analysis of expired air samples occurred over 3-second intervals until the point of volitional exhaustion. Based on the American College of Sports Medicine guidelines (ACSM 1995), VO_{2max} was determined by meeting at least three of the following criteria: (1) VO_2 ceased to increase with increasing workloads (plateau); (2) heart rate reached the age-predicted value (220-age); (3) respiratory exchange ratio > 1.0; and (4) blood lactate > 8.0mmol/L. These methods have been tested and validated extensively in previous studies of older subjects (Levine 2008, Fujimoto, Prasad et al. 2010).

3.2.3 Physiological Data Acquisition

Participants completed two separate experimental sessions: 1) physiological laboratory recording, and 2) magnetic resonance neuroimaging session (MRI; Robarts Research Institute Centre for Functional and Metabolic Imaging). The two sessions were performed at the same time of day and separated by a minimum period of 1 week. Participants practiced the experimental procedures prior to their first test session. Participants reported to the laboratory following a 12h abstinence from nicotine, alcohol, caffeine and intense physical exertion. Venous blood sampling occurred following 30mins of quiet, supine rest. Analysis of these samples assessed and confirmed baseline levels of blood-borne acute phase inflammatory markers (hsCRP) and glycemic status. Heart rate was measured using a standard three-lead electrocardiogram. Finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands) provided continuous measures of arterial blood pressure following calibration to manual sphygmomanometer blood pressure measurements obtained throughout the protocol. Data were collected using LabChart7 and PowerLab data acquisition system (ADInstruments). Neurological screening was also completed during the laboratory session to exclude dementia (The Montreal Cognitive Assessment) and confirm intact executive function (Trail making tests A and B).

3.2.4 Neuroimaging Data Acquisition

Participants completed a structural MRI session following a 12h abstinence from nicotine, alcohol, caffeine and intense physical exertion. All imaging data were collected using a whole body 3-Tesla imaging system (Magnetom Prisma, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil (Barberi, Gati et al. 2000). A high-resolution T1-weighted structural volume was acquired with a 3D MPRAGE sequence at the beginning of the scanning session (sagittal, matrix 256x240mm, voxel resolution 1.0x1.0x1.0mm, 1mm slice thickness, no gap, flip angle 9°, TE = 2.98 ms, TI = 900 ms, TR = 2.3 ms). Head movement was limited during the experimental session within a head cradle packed with foam padding, and each subject received instruction to avoid head movements during the scanning period.

3.2.5 Neuroimaging Data Analysis

We used the analysis approaches best suited to measure gray and white matter cortically and subcortically, from high-resolution MRI images.

3.2.5.1 Subcortical Level

Semiautomatic software quantified ventricular and subcortical volumes, as well as total intracranial volume, total gray matter and white matter hypointensities (Freesurfer Image Analysis Suite, http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications (Dale and Sereno 1993, Dale, Fischl et al. 1999, Fischl and Dale 2000, Fischl, Liu et al. 2001, Fischl, Salat et al. 2002, Fischl, Salat et al. 2004). Briefly, this processing included motion correction and averaging (Reuter, Schmansky et al. 2012) of T1-weighted images, removal of non-brain tissue (Segonne, Dale et al. 2004), automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures of interest (including hippocampus, amygdala, anterior cingulate cortex, lateral ventricles) (Fischl, Salat et al. 2002, Fischl, Salat et al. 2004), intensity normalization (Sled, Zijdenbos et al. 1998), delineation of the gray and white matter boundary, automated topology correction (Fischl, Salat et al. 2004, Segonne, Dale et al. 2004) and surface deformation following intensity gradients for optimal placement of gray/white and gray/cerebrospinal fluid

borders at the location where the greatest shift in intensity defines the transition to the other tissue (Dale and Sereno 1993, Dale, Fischl et al. 1999, Fischl and Dale 2000). Measurement of ventricular volume is particularly amenable to segmentation due to the high signal intensity contrast between cerebrospinal fluid and surrounding brain tissue in T_1-weighted MRI. Of note, ventricle volume segmentation included only the lateral ventricles. Adjusting regional volumes to intracranial volume accounted for the potential impact of sex and height on structural outcomes. Subcortical results were corrected for multiple comparisons at FDR, $p<0.05$.

Cognitive and age-related cortical decline appear to overlap in cortical sites such as the MPFC and IC; as well at specific subcortical sites including the HC, ACC, and amygdala (Coffey, Wilkinson et al. 1992, Raz, Gunning et al. 1997, Good, Johnsrude et al. 2001, Raz, Lindenberger et al. 2005). Thus, we used a region-of-interest (ROI) analysis to focus on these most vulnerable deep gray matter sites (HC, amygdala and ACC) as well as the fluid-filled lateral ventricles (Good, Johnsrude et al. 2001, Resnick, Pham et al. 2003, Raz, Lindenberger et al. 2005, Kennedy, Erickson et al. 2009, Raz, Ghisletta et al. 2010). White-matter hypointensities provided an index of cerebrovascular disease and cognitive decline (Debette and Markus 2010). In this case, Freesurfer provides reliable sensitivity in measuring white matter damage in non-demented older adults (Leritz, Shepel et al. 2014).

3.2.5.2 Cortical Level

Cortical thickness analysis was performed using Brain Voyager 2.8.4 (BVQX, Brain Innovation, Maastricht, Netherlands). The Lapalace method provided measures of cortical thickness for each subject's right and left hemisphere (Jones, Buchbinder et al. 2000). An inter-subject cortical alignment procedure reduced the effect of anatomical variability and improved the spatial correspondence of cortical areas between individual brains. Spatial intensity inhomogeneities in the original T_1-weighted scans were corrected, converted into volumes with 1-mm isotropic voxel resolution using sinc-interpolation, and transformed spatially into standard Talairach space. Once normalized, an automatic segmentation of white-matter and gray-matter boundaries was applied, and images were resampled to 0.5mm isovoxel resolution. Manual correction removed

topological errors such as “bridges” and remaining fragments of dura mater or cerebellum on a slice-by-slice and individual basis. The reconstructed cortical hemispheres were morphed into a folded three-dimensional mesh. Cortex-based-alignment followed, producing a spherical representation of the folded cortex and finally for each hemisphere. The curvature information of individual brains enabled cortex-based inter-subject alignment (Goebel, Esposito et al. 2006), and resulted in average curvature maps for each hemisphere, for each group. Each mesh resulted from averaging 16 individual datasets (16 MA and 16 HA).

3.2.6 Statistical Analysis

A two-tailed unpaired t-test assessed group-level hemodynamic and anthropometric characteristics, as well as the effect of group on cortical thickness ($p<0.05$; Systat Software 12.5, 2011). Data are presented as mean ± standard deviation. Overlap existed in the fitness levels of the MA and HA group members. Therefore, following a combining of groups, multiple linear regression analysis assessed the independence of the relationship between subcortical volumes and cortical thickness after adjusting for age, VO_{2max}, body mass index (BMI), and left cardiac ventricular mass (indexed to body surface area), as they exert strong effects on the morphology of the cortex (Table 3.2) (Barnes, Yaffe et al. 2003). A threshold for significance was set at $p<0.05$. All regression analyses passed the normality test, as assessed by the Shapiro-Wilk test for normal distribution. The effect of high cardiorespiratory fitness on the age-related decay of cortical and subcortical mass at the specified regions of interest was determined using linear regression and was compared using Student’s *t*-test.

To assess the effects of fitness and age on regional brain tissue health, we performed a full cortex correlation analysis in Brain Voyager using each individual’s VO_{2max} and age in years. The final correlation maps were adapted by switching off the positive or negative correlation values respectively, increasing the minimum threshold value ($p<0.005$), and adding a cluster threshold ($10mm^2$).

3.3 Results

Table 3.1 provides descriptive group information. Participant ages ranged from 45 to 73 years in HA and 45 to 67 years in MA, with a mean age of 58 years and 53 years, respectively. Overall, the sample was 31% female. Groups did not differ with respect to age, sex, years of education, blood pressure, heart rate and/or BMI. The HA group demonstrated near 100% of age-predicted VO_{2max}, consistent with self-report confirmation of adherence to age-appropriate physical activity guidelines (ACSM 1995). Compared to HA participants, MA had a greater VO_{2max}, and greater left cardiac ventricular mass. The MoCA and Trail Making Test B scores were not different between groups. HA participants expressed a slightly longer time to completion in Trail Making Test A. Plasma glucose, circulating triglycerides, and systolic blood pressures were greater in HA than MA, but all values were within healthy ranges. Circulating hsCRP and lipid levels, as well as total brain volume indicators, were similar between groups.

3.3.1 Subcortical Gray Matter

When indexed to total intracranial volume, average subcortical gray matter volumes were not different between HA and MA (Table 3.2).

The mean rate of ventricular volume enlargement was 0.03% per year in HA and 0.04% per year in MA ($P = 0.75$; Figure 3.1). The mean annual rate of atrophy at the ACC was -0.0006% in HA and -0.0002% in MA, which was not different between groups ($p=0.31$). The mean rate of atrophy at the right HC (HA: -0.0009%; MA: -0.0006%) was not different between groups ($p = 0.57$), nor was it different at the left HC ($p = 0.25$) in either HA (-0.0016%) or MA (0.0002%). The right amygdala also showed no difference in rate of volume change ($p= 0.44$) in either HA (-0.0007%) or MA (-0.0003%), which was consistent with the left amygdala ($p = 0.11$; HA: -0.0006%; MA: 0.0002%). Pearson Correlation analysis confirmed linear dependence between age and ventricle volume ($r = 0.49$, $p < 0.001$), and an inverse correlation between age and VO_{2max} ($r = -0.71$, $p < 0.001$).

3.3.1.1 Region of Interest Analysis

Average volumes at the lateral ventricles, ACC, the right and left HC, and the right amygdala were similar in the MA and HA groups (Table 3.2). However, the left amygdala was larger in MA versus HA ($p<0.05$).

3.3.1.2 Covariate Analysis

Pearson correlation analysis revealed that ventricle volume correlated inversely, albeit weakly, with VO_{2max} ($r = -0.28$, $p=0.05$). Multiple linear regression revealed age as the strongest predictor of lateral ventricle volume ($p<0.001$), right HC ($p<0.05$), right amygdala ($p<0.05$), and ACC volumes ($p<0.05$, Table 3.3).

3.3.2 Cortical Gray Matter

Total gray matter was greater in MA than in HA ($p<0.05$). An unpaired t-test revealed group differences in cortical thickness throughout a wide range of cortical areas ($p<0.005$, corrected for multiple comparisons by cluster threshold = 10 voxels; Figure 3.2, Tables 3.4-3.5). The most prominent differences (>1.5mm) were observed in occipital and temporal regions. Smaller clusters of group differences in cortical thickness (>1mm) were also detected within the frontal lobe and medial plane. Additionally, there was a dominant lateralization pattern to the left hemisphere, which had greater cortical thickness in many regions, than the right hemisphere.

The annual rate of cortical atrophy across the age span (Figure 3.3) was not different between HA and MA at the right IC (HA: -0.53%; MA: -0.69%; $p = 0.82$), left IC (HA: -0.52%; MA: -1.79%; $p = 0.32$), the right MPFC (HA: -1.23%; MA: -1.37%; $p = 0.96$), or the left MPFC (HA: 0.044%; MA: 0.29%; $p = 0.88$). Also, the mean annual rate of change did not differ between groups at the left precentral gyrus (HA: -0.01%; MA: -0.61%; $p = 0.33$), the right postcentral gyrus (HA: -0.17%; MA: 0.11%; $p = 0.95$) and the left postcentral gyrus (HA: -0.009%; MA: 0.82%; $p = 0.11$). The effect of age on cortical thickness at the right precentral gyrus was different between groups ($p<0.05$) where little change was observed in HA (-0.28%) but a progressive increase in cortical thickness was observed in MA (0.12%).

In a whole-brain, voxel-wise negative correlation analysis, age correlated negatively with cortical thickness in the parietal, temporal and frontal lobes (Figure 3.4). Further, a negative correlation analysis revealed regions of cortical thickness that correlated negatively (i.e., was thinner) with VO_{2max} (Figure 3.5) in MA versus HA; these regions were few in number and were concentrated around the occipital and parietal cortices.

3.3.2.1 Region of Interest Analysis

Cortical thickness was greater in MA than HA at each region of interest including the bilateral IC, MPFC, precentral and postcentral gyri (Figure 3.6).

3.3.2.2 Covariate Analysis

As depicted in Figure 3.7, cortical thickness and VO_{2max} exhibited strong correlations across much of the cerebral cortex. Specific results from the multiple linear regression indicated that across all regions of interest, VO_{2max} was the strongest predictor of cortical thickness (Table 3.6) with the exception of the right precentral gyrus and left postcentral gyrus.

Table 3.1 Anthropometric and baseline hemodynamic data (mean ± SD).

	Healthy Active	Masters Athletes
Age (years)	58±9 Range: 45-73	53±6 Range: 45-67
Resting Blood Pressure (mmHg)	118/73±9	118/78±9
Resting Heart Rate (bpm)	58±8	55±9
BMI (kg/m^2)	26±4	23±3
VO_{2max} (mL/kg/min)	38±7 Range: 26-51	55±10* Range: 33-67
Age-predicted VO_{2max} (%)	94±19	125±26*
LVM (g)	64±14	77±17*
Education (years)	12±2	10±3
Trail Making Score A (secs)	31±8	23±10*
Trail Making Score B (secs)	59±21	53±23
MoCA Score	28±2	27±2
Fasting Glucose (mmol/L)	5.16±0.35	4.89±0.31*
hsC-Reactive Protein (mg/L)	1.54±3.22	1.49±1.17
Cholesterol (mmol/L)	4.46±0.70	4.23±0.76
Triglycerides (mmol/L)	0.92±0.45	0.61±0.22*
High Density Lipoprotein (mmol/L)	1.50±0.40	1.53±0.35
Low Density Lipoprotein (mmol/L)	2.54±0.60	2.42±0.79
HbA1c	0.06±0.002	0.06±0.002
Gray Matter Volume (mL)	655±51	691±55*
White Matter Hypointensities (mm^3)	1924±1221	1501±708
Total Intracranial Volume (mm^3)	$1.6x10^6$±$1.5x10^5$	$1.6x10^6$±$1.6x10^5$

BMI, body mass index; VO_{2max}, maximal oxygen consumption; percent of age-predicted VO_{2max} based on ACSM guidelines, 1995; LVM, left ventricular mass at the heart; education in years including high school; MoCA: Montreal Cognitive Assessment (scored out of 30 possible points); hs, high sensitivity. Cardiac output and LVM indexed to body surface area. *different from healthy active, $p<0.05$.

Table 3.2 Whole-brain subcortical volumes.

Subcortical Site	Mean MA (mm^3)	Mean HA (mm^3)	Difference (MA-HA)	P value	Corrected P value
Lateral Ventricles	0.0119	0.0136	-0.0017	0.41	0.55
Brain Stem	0.0301	0.0140	0.0161	0.27	0.54
CSF	0.0477	0.0007	0.0470	0.71	0.74
L Cerebellum WM	0.0097	0.0092	0.0005	0.15	0.38
R Cerebellum WM	0.0276	0.0094	0.0181	0.29	0.54
L Cerebellum C	0.0367	0.0320	0.0047	0.14	0.38
R Cerebellum C	0.0603	0.0338	0.0265	0.47	0.58
L Thalamus	0.0464	0.0054	0.0411	0.63	0.68
R Thalamus	0.0197	0.0045	0.0152	0.24	0.54
L Caudate	0.0236	0.0021	0.0215	0.33	0.54
R Caudate	0.0085	0.0022	0.0063	0.09	0.38
L Putamen	0.0471	0.0030	0.0441	0.11	0.38
R Putamen	0.0210	0.0029	0.0182	0.28	0.54
L Pallidum	0.0273	0.0008	0.0264	0.39	0.55
R Pallidum	0.0078	0.0010	0.0068	0.10	0.38
L Hippocampus	0.0342	0.0026	0.0316	0.48	0.58
R Hippocampus	0.0097	0.0020	0.0077	0.12	0.38
L Amygdala	0.0034	0.0009	0.0024	0.03*	0.35
R Amygdala	0.0109	0.0010	0.0099	0.15	0.38
L Accumbens	0.0014	0.0002	0.0012	0.02*	0.35
R Accumbens	0.0235	0.0003	0.0232	0.35	0.54
L Ventral DC	0.0046	0.0023	0.0024	0.04*	0.35
R Ventral DC	0.0053	0.0022	0.0031	0.05*	0.35
Posterior CC	0.0256	0.0006	0.0250	0.38	0.55
Mid-Posterior CC	0.0497	0.0003	0.0494	0.74	0.74
Central CC	0.0358	0.0003	0.0355	0.53	0.59
Mid-Anterior CC	0.0225	0.0003	0.0223	0.33	0.54
Anterior CC	0.0347	0.0006	0.0342	0.51	0.59

All values indexed to total intracranial volume. L: left; R: right; WM: white matter; GM: gray matter; C: cortex; DC: dorsal column; CC: cingulate cortex. *Different from HA, $p<0.05$.

Table 3.3 Results of multiple linear regression of ROI subcortical volumes against anthropometric covariates.

		Std. Coefficient	Std. Error	P value	Power (α 0.05)
Lateral Ventricles	Age	0.000483	0.000138	**0.001**	0.997
	VO_{2max}	0.000146	0.000117	0.218	-
	Body Mass Index	-0.000273	0.000277	0.329	-
	L Ventricular Mass	-0.000122	0.000058	0.041	-
L Hippocampus	Age	-0.000016	0.000009	0.072	-
	VO_{2max}	-0.000004	0.000008	0.597	-
	Body Mass Index	0.000006	0.000018	0.751	-
	L Ventricular Mass	-0.000002	0.000004	0.675	-
R Hippocampus	Age	-0.000015	0.000008	**0.049**	0.894
	VO_{2max}	0.000002	0.000006	0.705	-
	Body Mass Index	0.000019	0.000015	0.184	-
	L Ventricular Mass	-0.000005	0.000003	0.135	-
L Amygdala	Age	-0.000003	0.000003	0.373	-
	VO_{2max}	0.000005	0.000002	0.073	-
	Body Mass Index	0.000003	0.000006	0.606	-
	L Ventricular Mass	-0.000001	0.000001	0.349	-
R Amygdala	Age	-0.000008	0.000003	**0.009**	0.937
	VO_{2max}	-0.000001	0.000002	0.742	-
	Body Mass Index	0.000008	0.000006	0.173	-
	L Ventricular Mass	-0.000001	0.000001	0.414	-
Anterior CC	Age	-0.000005	0.000002	**0.033**	0.675
	VO_{2max}	-0.000002	0.000002	0.249	-
	Body Mass Index	0.000001	0.000004	0.872	-
	L Ventricular Mass	-0.000001	0.00001	0.433	-

Note: all individuals were entered into regression, irrespective of group. L ventricular mass indexed to body surface area. Std: Standardized; L: left; R: right; CC: cingulate cortex.

Table 3.4 Right hemisphere brain regions showing significant group differences in cortical thickness in a whole-brain analysis.

Right Hemisphere Region	Mean MA (mm)	Mean HA (mm)	Difference (MA-HA)	StdError	t value
Calcarine S	3.1	2.6	0.5	0.13	3.80
Central S	2.3	2.0	0.3	0.04	8.60
Cingulate G	3.2	2.7	0.5	0.08	5.40
Cingulate S	2.7	2.4	0.3	0.04	7.30
Collateral S	3.5	2.8	0.7	0.14	5.30
Cuneus	2.4	2.0	0.4	0.06	6.40
Rectus G	3.0	2.8	0.2	0.09	2.20
Inf FG	2.7	2.4	0.3	0.06	5.70
Inf FS	2.8	2.3	0.5	0.09	5.60
Inf OG	3.1	2.4	0.7	0.12	6.00
Inf PL	2.7	2.3	0.4	0.07	5.60
Inf TG	3.5	2.9	0.6	0.09	7.10
Inf TS	3.3	2.8	0.5	0.10	5.40
Insula	3.4	2.7	0.7	0.07	9.00
Intraparietal S	2.5	2.2	0.3	0.05	5.60
Lat OTG	3.5	2.9	0.6	0.11	5.30
Lat S	2.7	2.4	0.3	0.04	8.50
Med OTG	2.8	2.3	0.5	0.10	5.20
Middle FG	2.6	2.3	0.3	0.05	6.80
Middle OG	2.9	2.3	0.6	0.09	6.50
Middle TG	3.0	2.6	0.4	0.08	5.00
OTS	3.5	2.9	0.6	0.14	4.20
Olfactory S	3.9	2.5	1.4	0.24	5.90
Orbital G	3.5	2.7	0.8	0.11	7.10
Orbital S	3.2	2.5	0.7	0.10	6.70
Parahippocampal G	4.1	3.3	0.8	0.18	4.30
POS	2.5	2.2	0.3	0.05	7.30
Postcentral G	2.2	1.9	0.3	0.04	7.80
Postcentral S	2.4	2.1	0.3	0.05	6.70
Precentral G	2.4	2.1	0.3	0.04	5.80
Precentral S	2.6	2.2	0.4	0.05	7.20
Precuneus	2.7	2.3	0.4	0.06	5.30
Sup FG	2.7	2.5	0.2	0.04	5.20
Sup FS	2.7	2.3	0.4	0.04	7.70
Sup OG	2.8	2.2	0.6	0.10	6.10
Sup PL	2.3	2.1	0.2	0.04	5.50
Sup TG	2.7	2.5	0.2	0.05	4.40
Sup TS	3.0	2.5	0.5	0.07	6.20
Supramarginal G	2.7	2.4	0.3	0.05	6.00
Trans OS	2.6	2.2	0.4	0.07	4.70
Med PreF	3.9	3.1	0.8	0.17	4.50

MA: masters athletes; HA: healthy active. S: sulcus; G: gyrus; Inf: inferior; Lat: lateral; Med: medial; Sup: superior; Trans: transverse; F: frontal; O: occipital; P: parietal; T: temporal, L: lobe. All $p<0.005$.

Table 3.5 Left hemisphere brain regions showing significant group differences in cortical thickness in a whole-brain analysis.

Left Hemisphere Region	Mean MA (mm)	Mean HA (mm)	Difference (MA-HA)	StdError	t value
Calcarine S	2.9	2.0	0.9	0.14	6.60
Central S	3.2	1.8	1.4	0.16	8.80
Cingulate G	3.9	2.7	1.2	0.14	8.60
Cingulate S	3.8	2.4	1.4	0.22	6.30
Collateral S	4.7	2.9	1.8	0.25	7.10
Cuneus	4.3	2.1	2.2	0.37	6.00
Rectus G	4.4	2.6	1.8	0.23	7.30
Inf FG	2.7	2.2	0.5	0.08	6.30
Inf FS	2.8	2.3	0.5	0.10	5.80
Inf OG	3.0	2.2	0.8	0.14	5.60
Inf PL	2.7	2.2	0.5	0.09	5.90
Inf TG	4.2	2.9	1.3	0.23	6.10
Inf TS	3.3	2.5	0.8	0.12	6.70
Insula	3.2	2.2	1.0	0.10	9.70
Intraparietal S	2.8	2.1	0.7	0.13	5.60
Lat OTG	3.9	2.8	1.1	0.39	2.90
Lat S	2.7	2.2	0.5	0.08	7.20
Med OTG	2.6	1.8	0.8	0.12	6.00
Middle FG	2.8	2.3	0.5	0.08	7.30
Middle OG	3.0	1.9	1.1	0.18	6.40
Middle TG	3.5	2.3	1.2	0.17	7.20
OTS	4.2	2.6	1.6	0.39	4.00
Olfactory S	3.6	2.6	1.0	0.62	1.70
Orbital G	3.9	2.1	1.8	0.26	6.80
Orbital S	3.7	2.7	1.0	0.18	5.40
Parahippocampal G	4.0	3.2	0.8	0.58	1.40
POS	2.5	2.0	0.5	0.08	5.70
Postcentral G	2.7	2.1	0.6	0.06	9.70
Postcentral S	2.5	1.9	0.6	0.09	6.50
Precentral G	3.0	2.1	0.9	0.13	6.20
Precentral S	2.8	2.2	0.6	0.08	6.60
Precuneus	3.5	2.5	1.0	0.17	5.70
Sup FG	3.3	2.4	0.9	0.08	10.00
Sup FS	3.0	2.3	0.7	0.12	5.90
Sup OG	3.0	1.9	1.1	0.18	6.40
Sup PL	2.4	2.0	0.4	0.07	5.90
Sup TG	2.8	2.2	0.6	0.10	6.00
Sup TS	3.8	2.3	1.5	0.22	6.70
Supramarginal G	2.6	2.1	0.5	0.09	5.70
Trans OS	3.3	2.2	1.1	0.18	5.90
Med PreF	3.9	2.8	1.1	0.20	5.60

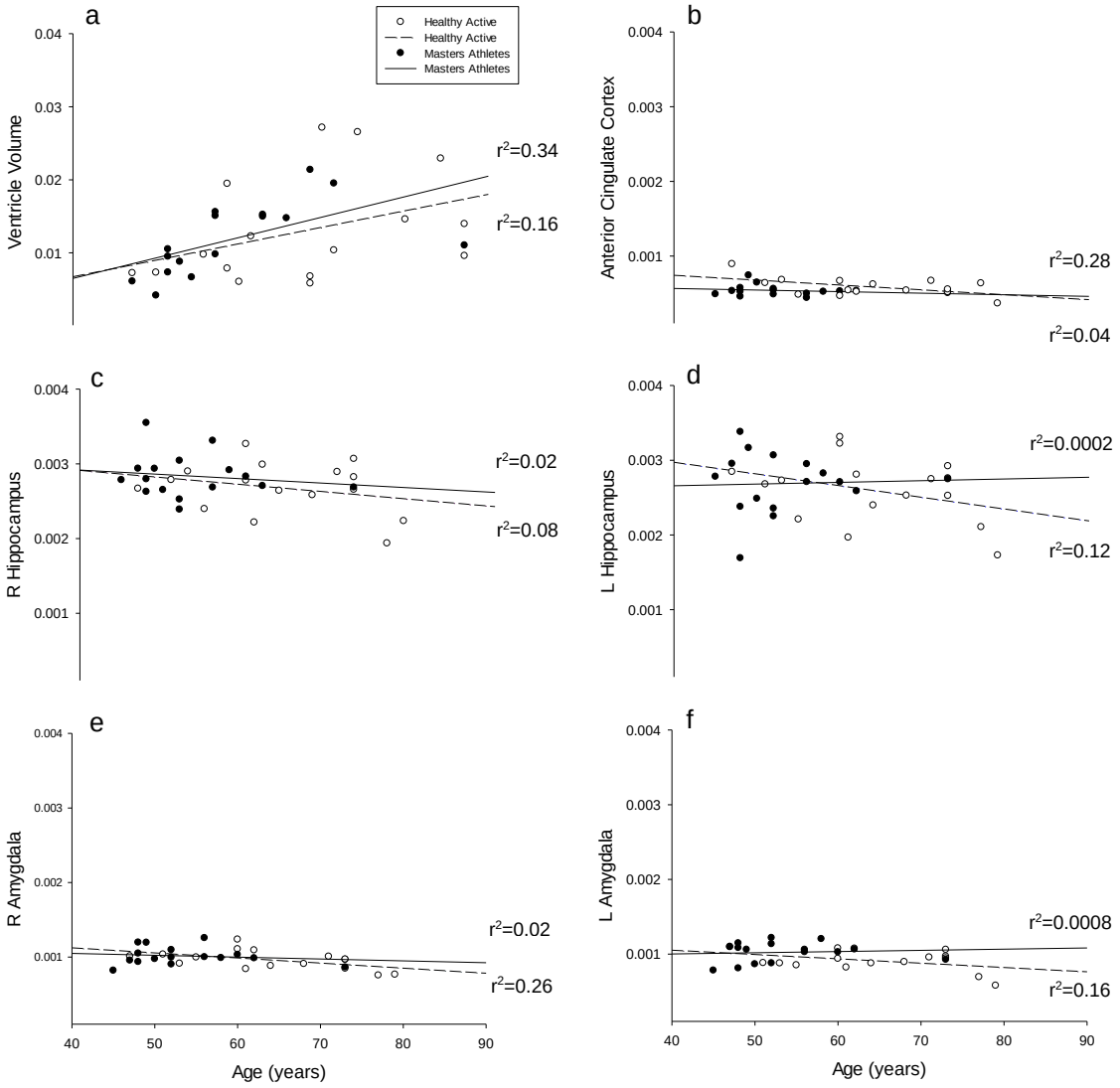
MA: masters athletes; HA: healthy active. S: sulcus; G: gyrus; Inf: inferior; Lat: lateral; Med: medial; Sup: superior; Trans: transverse; F: frontal; O: occipital; P: parietal; T: temporal, L: lobe. All $p<0.005$.

Table 3.6 Results of multiple linear regression of ROI cortical thickness against anthropometric covariates.

		Std. Coefficient	Std. Error	P value	Power (α 0.05)
R Insula	Age	-0.0014	0.008	0.862	-
	VO_{2max}	0.0169	0.007	**0.031**	0.999
	Body Mass Index	-0.0116	0.015	0.453	-
	L Ventricular Mass	0.0016	0.004	0.682	-
L Insula	Age	0.0009	0.011	0.935	-
	VO_{2max}	0.0318	0.010	**0.005**	0.999
	Body Mass Index	0.0024	0.021	0.911	-
	L Ventricular Mass	-0.0035	0.005	0.515	-
R Med PreF	Age	-0.0008	0.019	0.968	-
	VO_{2max}	0.0353	0.018	**0.047**	0.973
	Body Mass Index	0.0198	0.036	0.591	-
	L Ventricular Mass	0.0001	0.009	0.991	-
L Med PreF	Age	0.0134	0.009	0.133	-
	VO_{2max}	0.0227	0.008	**0.008**	0.999
	Body Mass Index	-0.0031	0.016	0.852	-
	L Ventricular Mass	0.0070	0.004	0.096	-
R Precentral	Age	0.0007	0.006	0.903	-
	VO_{2max}	0.0044	0.005	0.420	-
	Body Mass Index	-0.0094	0.011	0.406	-
	L Ventricular Mass	0.0006	0.003	0.829	-
L Precentral	Age	-0.0002	0.004	0.964	-
	VO_{2max}	0.0087	0.004	**0.035**	0.993
	Body Mass Index	0.0008	0.008	0.920	-
	L Ventricular Mass	-0.0033	0.002	0.117	-
R Postcentral	Age	0.0019	0.004	0.647	-
	VO_{2max}	0.0083	0.004	**0.039**	0.998
	Body Mass Index	-0.0097	0.008	0.230	-
	L Ventricular Mass	-0.0013	0.002	0.528	-
L Postcentral	Age	0.0041	0.005	0.374	-
	VO_{2max}	0.0064	0.004	0.140	-
	Body Mass Index	-0.0069	0.009	0.434	-
	L Ventricular Mass	0.0009	0.002	0.687	-

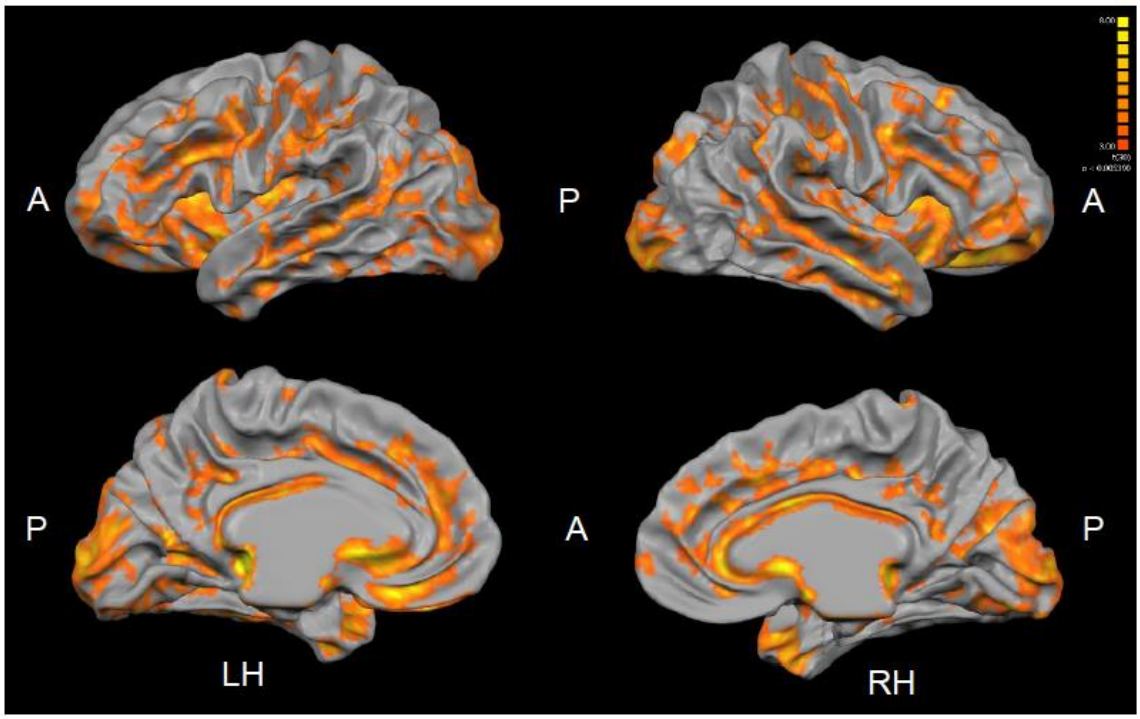
Note: all individuals were entered into regression, irrespective of group. L ventricular mass indexed to body surface area. Std: Standardized; L: left; R: right.

Figure 3.1 Regression analysis of region-of-interest subcortical volumes with age.



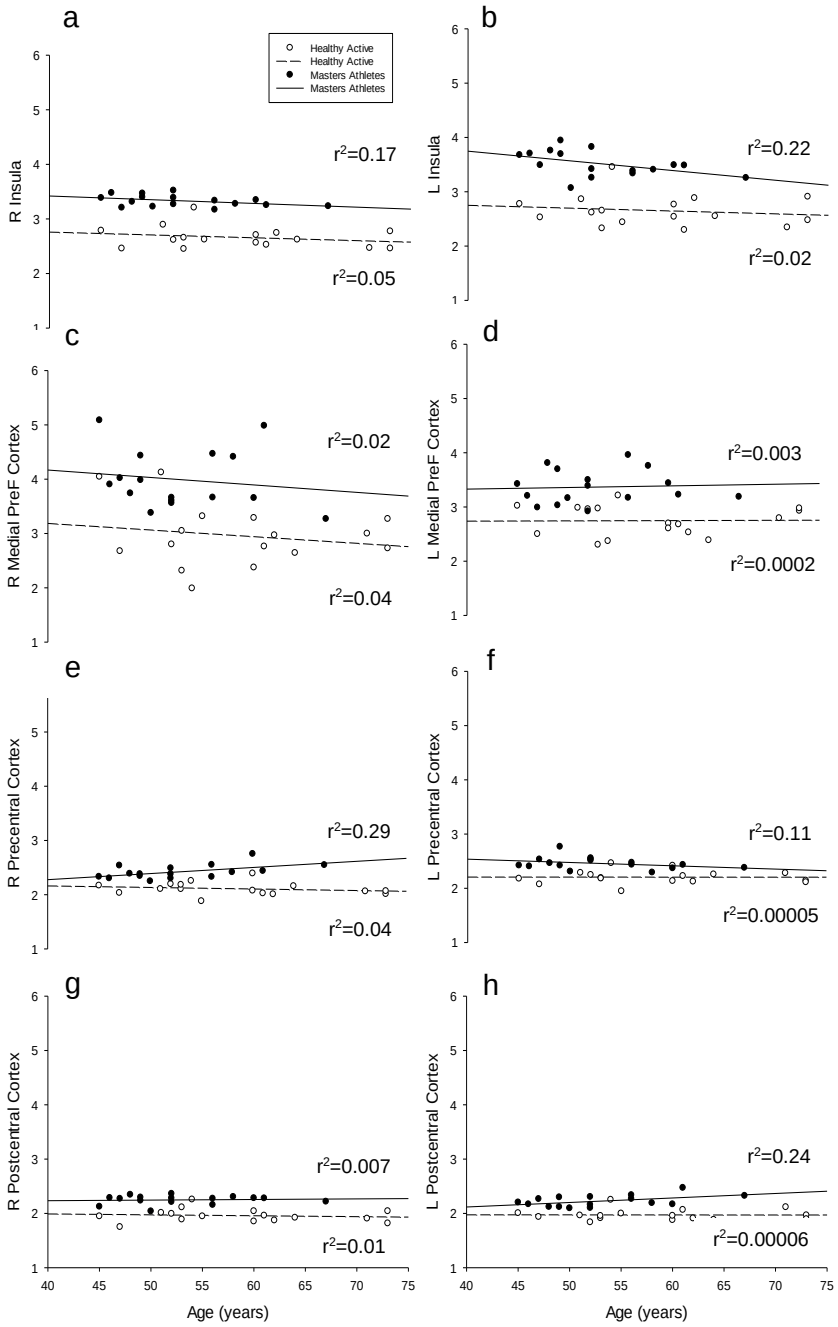
Slope of regression was not different between groups for (a) lateral ventricle volume (probability=0.75); (b) anterior cingulate cortex (probability=0.31); (c) right hippocampus (probability=0.57); (d) left hippocampus (probability=0.25); (e) right amygdala (probability=0.44); and (f) left amygdala (probability=0.11). All volumes are indexed to total intracranial volume and measured in mm^3.

Figure 3.2 Group differences in cortical thickness (MA > HA).



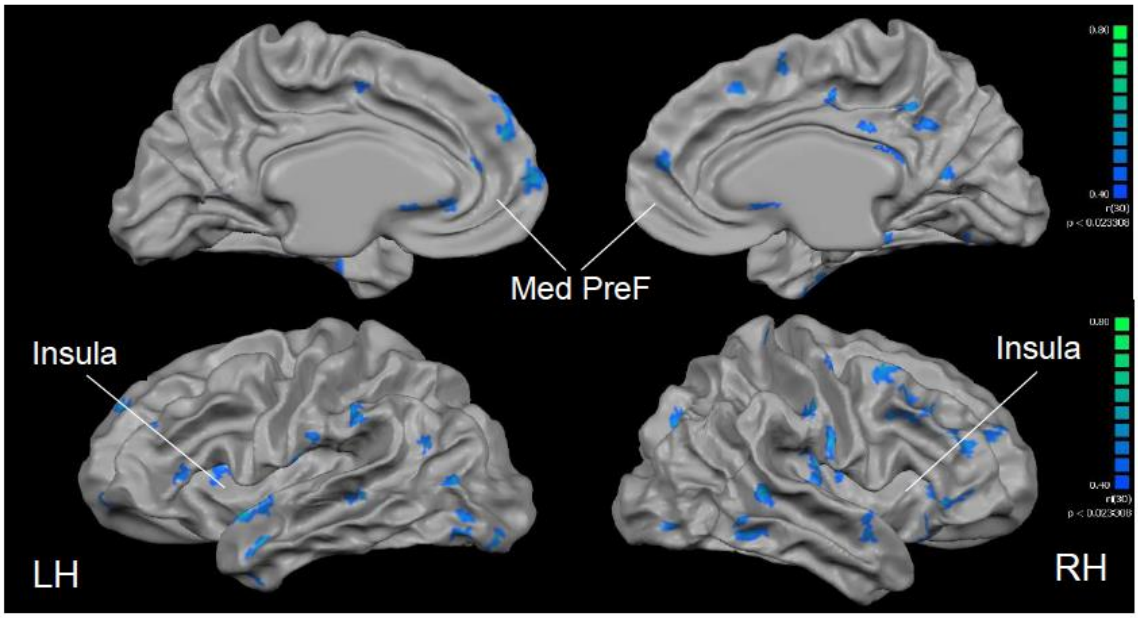
Results are shown at $p<0.005$, corrected by cluster size ($10mm^2$). Color scale at right denotes t values. LH: left hemisphere; RH: right hemisphere; A: anterior; P: posterior. Many regions in MA were significantly thicker than HA subjects (see Tables 3.4-3.5).

Figure 3.3 Regression analysis of region-of-interest cortical thickness with age.



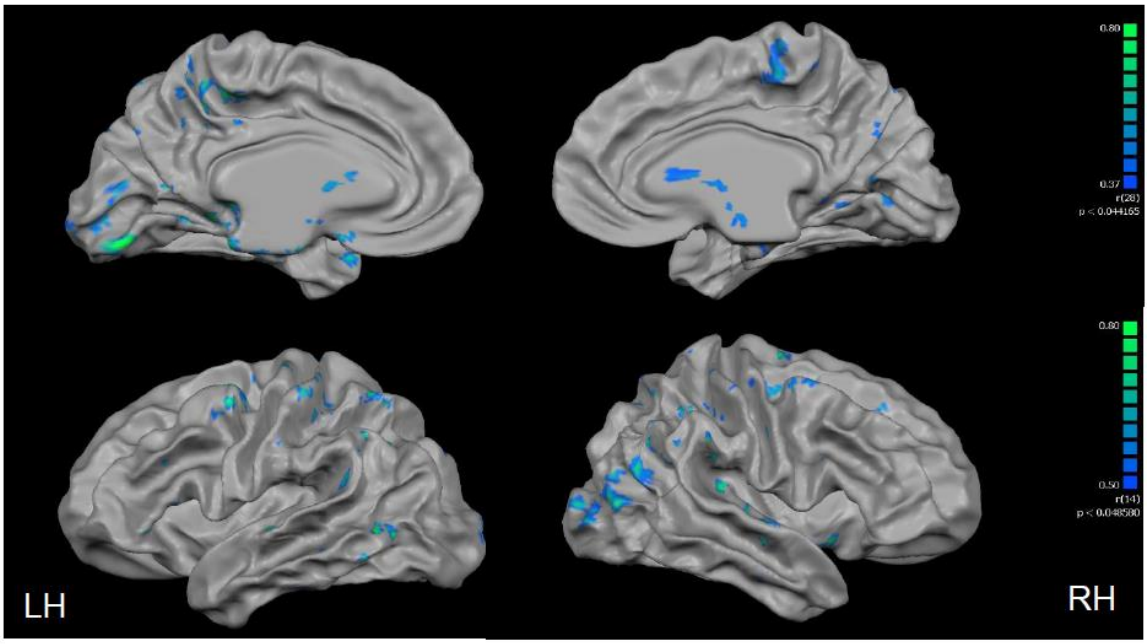
Slope of regression was not different between groups for (a) right insula (P =0.82); (b) left insula (P =0.32); (c) right medial prefrontal cortex (P =0.96); (d) left medial prefrontal cortex (P =0.88); (f) left precentral gyrus (P =0.33); (g) right postcentral gyrus (P =0.95); and (h) left postcentral gyrus (P =0.11). Masters athletes had a different trajectory with age at (e) right precentral gyrus (P =0.02). Cortical thickness measured in mm.

Figure 3.4 Full cortex correlation analysis with age covariate.



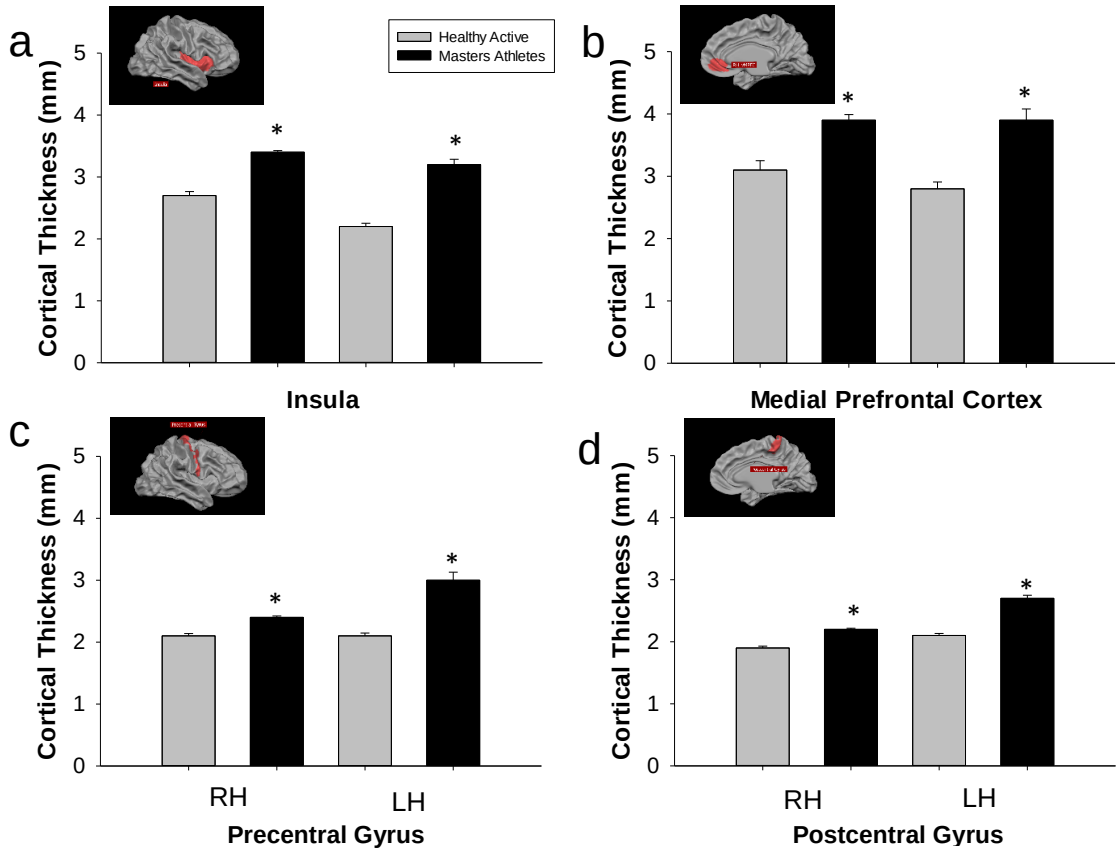
All subjects, irrespective of group. Results are shown at $p<0.05$, corrected by cluster size ($10mm^2$). Color scale at right denotes t values. LH: left hemisphere; RH: right hemisphere; Med: medial; PreF: prefrontal. Regions of interest are labeled.

Figure 3.5 Full cortex negative correlation analysis with VO_{2max} covariate.



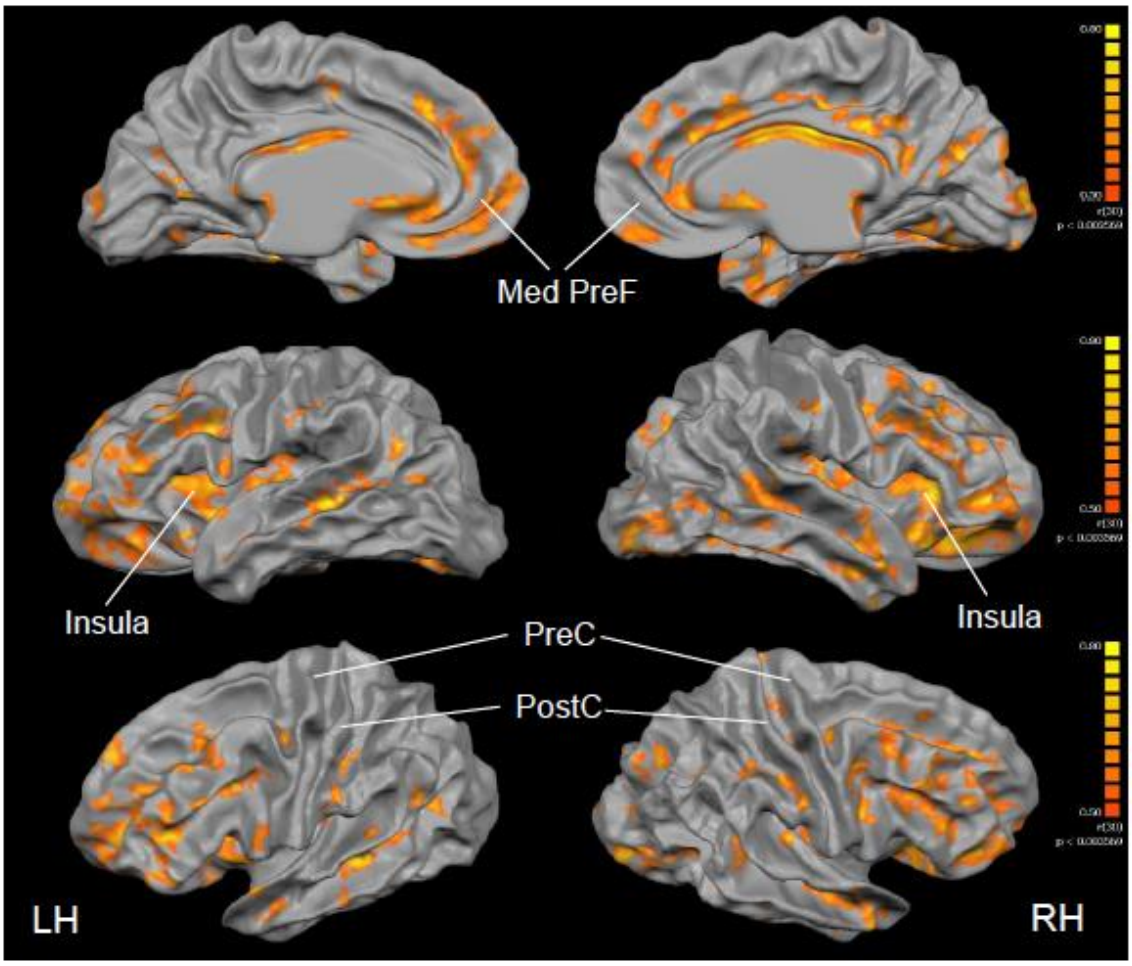
All subjects, irrespective of group. Results are shown at $p<0.005$, corrected by cluster size ($10mm^2$). Color scale at right denotes t values. LH: left hemisphere; RH: right hemisphere.

Figure 3.6



Cortical thickness at anatomically defined regions of interest in insula (a), medial prefrontal cortex (b), precentral gyrus (c), and postcentral gyrus (d). HA (gray bars), MA (black bars). RH: right hemisphere (left side pair of bars); LH: left hemisphere (right side pair of bars). * Different from HA, $p<0.0005$, corrected by cluster size ($10mm^2$).

Figure 3.7 Full cortex correlation analysis with VO_{2max} covariate.



All subjects, irrespective of group. Results are shown at $p<0.005$, corrected by cluster size ($10mm^2$). Color scale at right denotes t values. LH: left hemisphere; RH: right hemisphere; Med: medial; PreF: prefrontal; C: central. Regions of interest are labeled.

3.4 Discussion

This study examined the benefit that high levels of exercise training and cardiorespiratory fitness could have upon brain structure in a healthy human model. Overall, long-term training and high levels of cardiorespiratory fitness, elicited and sustained, greater cortical thickness over a large portion of the brain in MA than HA groups. However, life-long exercise training had little effect on the rate of age-related cortical atrophy and, had no effect on subcortical structures relative to that of modestly active adults. In addition, with the possible exception of focal regions of cortical thinning in the occipital and parietal lobes, life-long, high-intensity exercise training did not produce any apparent detrimental effect as indicated by greater total grey matter in MA, equivalent white matter hypointensities, and normal blood-borne inflammatory-markers, when compared to age-matched healthy active counterparts. Therefore, the current results illustrate the potent effect of this very high exercise stimulus to develop cortical (but not subcortical) reserve at younger ages, and sustain this reserve into the senior years. These data suggest that chronic endurance training provides a benefit in cortical neural reserve beyond that provided by guideline-based activity, but does not eliminate the effect of age on cortical grey matter atrophy.

The current study is the first to present evidence that life-long aerobic training, initiated early in life and sustained over decades, has a significant impact on total grey matter, and whole-brain cortical mass. Middle to older-aged MA were chosen for this study as they represent a dedicated athletic career lasting, in some cases, longer than 30 years, a proven athletic performance background, and a high to very high age-adjusted VO_{2max} (~125% of age-predicted maximum). Therefore, a reasonable conjecture is that these individuals represent the highest expected level of exercise training in aging adults and, therefore, provide an index of the potential to grow and preserve brain mass across the age span. Similarly, all HA participants were chosen based on their self-reported history of adherence to a healthy active lifestyle (>5 years) that met the age-adjusted physical activity guidelines (ACSM 1995). These guidelines emphasize 30 minutes of moderate intensity exercise on five or more days of the week, confirmed by an age-adjusted VO_{2max} of nearly 100%. In addition, our HA sample had normal weight and BMI scores, and

hemodynamic values statistically the same to those of MA and all within normal limits. Therefore, all participants in the current study were free of concerns related to sedentary living such as medications, brain pathology and cognitive dysfunction, and represent a model of active, successful aging (Thielke and Diehr 2012). Therefore, we propose that the current data represent a specific effect of exercise training.

3.4.1 Subcortical Gray Matter and Lateral Ventricular Volume

An interplay between subcortical mass and cerebral spinal fluid volume often results in the passive expansion of the lateral ventricles as neighboring grey matter mass declines (Weuve, Kang et al. 2004, Podewils, Guallar et al. 2005). Numerous cross-sectional imaging studies highlight the reduction in hippocampus (HC) volume with age, accompanied by a significant increase in ventricular volume (Gur, Mozley et al. 1991, Bhatia, Bookheimer et al. 1993, Blatter, Bigler et al. 1995, Forstl, Zerfass et al. 1995, Jack, Petersen et al. 1997, Mu, Xie et al. 1999, Jack, Knopman et al. 2013, Cash, Frost et al. 2015). However, contradictory data also exist regarding age (Mueller, Moore et al. 1998, Resnick, Goldszal et al. 2000) and HC volume (Jack, Twomey et al. 1989, Ohnishi, Matsuda et al. 2001). The current observations support the idea that age predicts lateral ventricular and HC volumes. However, Pearson correlation analysis revealed that ventricular volume also correlated weakly and inversely with VO_{2max} ($r = -0.28$, $p = 0.05$), due possibly to the expansion of the cerebral cortex space in the MA group, discussed below.

The HC is possibly the most commonly researched brain structure in the context of adaptations to exercise training. For example, Erickson et al. illustrate rapid expansion of the HC volume within 6 weeks of the onset of exercise training in older sedentary adults with a baseline state of cognitive impairment (Erickson, Voss et al. 2011). This conclusion contrasts with the current observations that indicate little association between cardiorespiratory fitness and HC volume. However, the current participants were all healthy, in the middle age range, and were at, or above, their age-predicted level of cardiorespiratory fitness for active individuals. Therefore, the potential capacity for HC volumetric changes in response to exercise are likely a function of the starting baseline

condition. In the current study, we suspect the lack of HC volumetric change between groups was due to a maximal effect achieved by guideline-based exercise training.

In contrast to the hippocampus, left amygdala volumes were greater in MA versus HA groups and were related to VO_{2max}. The specific benefit of this adaptive response to lifelong training could be multifactorial, given the role of this region in the processing of emotion, learning, memory, and autonomic cardiovascular adjustments.

3.4.2 Cortical Gray Matter

In contrast to the subcortical gray matter, we observed a widespread enhancement of cerebral cortex grey matter thickness in the MA group compared to the HA group. The impact of training in MA varied across the brain with some regions expressing no benefit while others indicated approximately 25% greater cortical thickness. These latter regions were evident in the frontal lobe, temporal lobe, insula cortex, parietal lobe and occipital regions. Thus, marked cortical "reserve" was evident in these regions across the entire age span including the middle age stages where the duration of training was less than those in the higher ages.

As mentioned, a notable outcome of the current study was the widespread cortical thickening in MA versus HA. Our whole brain analysis (Tables 3.4 and 3.5) reveals a number of cortical sites including the temporal and occipital lobes. Previously, Tseng et al. (Tseng, Uh et al. 2013) reported the confinement of cortical benefits of training in master's athletes to the posterior portion of the brain in the cuneus and precuneus. The current study differs from the previous Tseng (Tseng, Uh et al. 2013) study in the observation of widespread cortical thickening in MA. A wider age range and larger sample size may factor in these between-study differences. Nonetheless, the current results support those of Tseng et al. (Tseng, Uh et al. 2013) in the observation of greater cortical thickness in the cuneus and precuneus when compared to a sedentary elderly group. The possible implications of these observations are not clear but may relate to the functional role of the default mode network in neurocognitive and autonomic processing (Utevsky, Smith et al. 2014).

Further, the current data along with those of Tseng et al. (Tseng, Uh et al. 2013), give rise to a possible outcome that the posterior brain not only exhibits resistance to age-related atrophy but also demonstrates sensitivity to exercise stimuli across the age span studied here. Overall, the current observations support a speculation that the additional cortical reserve achieved early in life through high cardiorespiratory fitness will minimize the risk for neurological impairment in senescence and reduce the frailty period of life.

The mechanisms that operate to enhance cortical structure with training remain unknown and are likely multifactorial. In general, axonal, dendritic, and glial processes account for ~50% of grey matter volume whereas vascular (5%), other cell types (20%) and interstitial space (cerebrospinal fluid) account for the remainder (Thomas, Dennis et al. 2012). Recent evidence highlights the benefits of exercise training on pial vessel number, synaptogenesis (Cotman and Berchtold 2002), neurogenesis (van Praag, Christie et al. 1999) and astrocyte expansion (Saur, Baptista et al. 2014). To account for the regional ~25% greater cortical thickness in the MA group, some combination of expansion in any or all of these tissue types may be required.

3.4.3 Negative Association with High Fitness and Brain Structure

Chronic exposure to stress, whether it occurs during childhood, adolescence, adulthood or aging, has a negative impact on brain structure (Sapolsky 1999, Landfield, Blalock et al. 2007, Lupien, McEwen et al. 2009). Freund et al. (Freund, Faust et al. 2012) illustrated the significant, but reversible, reduction in brain volume following the TransEurope footrace that occurs over several weeks. The MA in our study were training >15 h/wk for decades which may amount to a significant level of physical stress. Therefore, concerns arise regarding the potential damage that intensive long-term competitive events may present to the brain, possibly due to persistent inflammatory mechanisms. However, no differences in white matter hypointensities, chronic inflammatory status (i.e., hsCRP levels), or altered lipidemic or glucogenic stress existed between groups. Therefore, little detrimental effect of chronic competitive training on brain structure occurred in the MA. In contrast, Tseng et al. (Tseng, Uh et al. 2013) observed an 83% reduction in white matter hypointensities in MA compared to sedentary adults. We attribute this discrepancy to the fact that our HA group was not sedentary and thus preserved white matter integrity

equally as well as our MA population. If so, then guideline-based training may be sufficient for maximal preservation of white matter integrity and subcortical structure volumes to the same degree as high-intensity long duration training. We did observe isolated regions of cortical thinning in MA relative to HA, located within the parietal and occipital regions. However, whether this observation reflects variations in regional adaptability or cortical damage remains unknown. It is noteworthy, however, that none of the MA group reported neurological cardiovascular problems such as fibrillation, and the regions of thinner cortex in these athletes have not been associated with autonomic cardiovascular function.

3.5 Limitations

Significant limitations of this study include lack of data on the proportional contributions of the exercise effect versus the environmental, genetic, or social situations on brain structure, that likely change over time.

We acknowledge the small sample size of the current groups and promote caution in interpreting the results. Nonetheless, using a random selection of 75% of our sample indicated that VO_{2max} remained the strongest predictor of cortical thickness and region-of-interest subcortical volume. We have also used a rigorous threshold protocol for the voxel-wise analysis ($p<0.005$, cluster size > $10mm^2$) to reduce the probability of false findings which could be associated with a small sample size.

Our study may also be limited by the age-range of our participants being 45-73 years in HA and 45-67 years in MA. This age range does not reflect the older age range in which cortical atrophy becomes more apparent.

Finally, we acknowledge the limitations of the cognitive testing protocol used in this study. We did not attempt to complete a comprehensive analysis of cognitive function. Rather, we collected MoCA and Trail Making questionnaires as a rapid but valid estimate of overall cognitive health.

3.6 Conclusion

We present novel findings that sustained high levels of cardiorespiratory fitness increase cortical thickness in many brain regions. However, the impact on subcortical grey matter appears to peak at levels of activity that meet the published guidelines for adults of middle to older age. Nonetheless, despite producing greater cerebral cortex thickness, the MA training load had no effect on the trajectory of age-related cortical atrophy. In fact, although based on small sample sizes, the rates of decline in the selected cortical regions of interest in the current study fall within the expected 0-0.5% range for this age group (Salat, Buckner et al. 2004, Raz, Lindenberger et al. 2005), in each of the MA and HA groups. Therefore, the benefits of life-long exercise training were limited to the expression and sustainment of cortical reserve, but not age-related decline. Therefore, in cognitively normal, healthy individuals, the neurological benefit of chronic training appears to include a sustained cortical reserve, producing greater cortical mass and possibly improved neurological function at every age. Furthermore, life-long, high-intensity exercise did not produce a notable detrimental impact on brain structure. The expectations of these observations are that the 25% reserve in cortical thickness that accompanied lifelong training will preserve neurologic function into the senescent years.

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Chapter 4

4 Cerebral Cortical Thickness Correlates with Autonomic Outflow

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4.1 Introduction

Chronic levels of sympathetic and parasympathetic outflow exert important implications for homeostasis and long-term health. Within the context of cardiovascular health, chronic elevations in sympathetic outflow, and/or reductions in parasympathetic cardiac control, contribute to the pathogenesis and progression of atherosclerosis, vascular wall thickening, cardiac damage (Amiya, Watanabe et al. 2014, Chistiakov, Ashwell et al. 2015) and disturbed cardiac rhythms (Pellman and Sheikh 2015), which combine to increase the risk of morbidity and mortality (Schmidt, Muller-Werdan et al. 2005). Advancing age often (Ebert, Morgan et al. 1992), but not always (Greaney, Schwartz et al. 2013), increases baseline muscle sympathetic nerve activity (MSNA). More consistently, advancing age decreases cardiovagal baroreflex sensitivity (Ebert, Morgan et al. 1992, Monahan, Dinenno et al. 2001). Combined, these outcomes suggest a chronic state of heightened peripheral sympathetic tone with parasympathetic withdrawal (Meredith, Eisenhofer et al. 1993, Monahan, Dinenno et al. 2001, Thayer and Lane 2007, Lambert, Dawood et al. 2008, Freeling and Li 2015). The mechanisms affecting this age-related autonomic dysregulation are not clear. Decreased baroreflex sensitivity accounts for some of the decrease in parasympathetic control of heart rate, as inferred by measures of cardiovagal baroreflex sensitivity (Ebert, Morgan et al. 1992, Monahan, Dinenno et al. 2001). However, age exerts little effect on baroreflex control of MSNA whether levels of sympathetic burst activity are elevated (Ebert, Morgan et al. 1992) or not (Greaney, Schwartz et al. 2013).

Alterations in cerebral cortex structure may contribute to these age-related autonomic changes. Certainly, experimental models in lower animals (Cechetto and Chen 1990, Dampney 1994, Verberne 1996), as well as clinical (Norris, Froggatt et al. 1978,

Critchley, Mathias et al. 2003, Woo, Macey et al. 2003, Soros and Hachinski 2012, Woo, Yadav et al. 2014), and observational studies in humans (Critchley, Corfield et al. 2000) (Cechetto and Shoemaker 2009, Thayer, Sollers et al. 2009, Shoemaker and Goswami 2015), indicate that several cortical sites modulate autonomic cardiovascular control. These regions include, but are not limited to, the IC, ACC, and MPFC (Barron and Chokroverty 1993, Soufer, Bremner et al. 1998, Critchley, Corfield et al. 2000, Gianaros, Van Der Veen et al. 2004, Gianaros, Derbyshire et al. 2005). Inasmuch as structure forms a basis of function, changes in cortical thickness in these regions may affect the neurologic outcomes.

Cerebral cortex atrophy begins in the third decade of life, with disproportionately high losses in the forebrain regions (Raz, Williamson et al. 2000). Average losses are estimated at roughly 15% of the cerebral cortex and 25% of the cerebral white matter between ages 30 and 90 (Jernigan, Archibald et al. 2001). It is noteworthy that those regions associated with autonomic cardiovascular control exist within regions that display high sensitivity towards age-related cortical atrophy. Nonetheless, age-related reductions in cortical tissue show considerable individual variation in their rate, extent, and location (Raz, Gunning et al. 1997, Raz, Rodrigue et al. 2007, Fjell and Walhovd 2010). This inter-individual variation offers an opportunity to explore potential relationships between cortical structure and autonomic changes in aging adults. Specifically, this study tested the hypothesis that inter-individual differences in cortical atrophy predict age-related changes in autonomic outflow. If so, then details related to cortical thickness would form a possible basis for explaining age-related autonomic dysregulation.

4.2 Methods

4.2.1 Participants

A total of 55 healthy, active individuals participated in this study across a range of fitness and age (26-81mL/kg/min; 21-73 years; 18 female). Table 4.1 provides group characteristics. All participants were right-handed, non-smokers, free of medications, and without diagnosed hypertension, diabetes, vascular or neurological impairments. The University of Western Ontario Health Sciences Ethics Review Board approved this study

and adhered to the Declaration of Helsinki. Each participant provided informed, written consent.

4.2.2 Assessment of Cardiorespiratory Fitness

A graded treadmill exercise test, conducted under standard clinical observation, provided information regarding each subject's peak oxygen uptake (VO_{2max}). During this test, expired air samples were taken at 3-second intervals until the point of volitional exhaustion. Based on the American College of Sports Medicine guidelines (ACSM 1995), VO_{2max} was determined by meeting the following criteria: (1) VO_2 ceased to increase with increasing workloads (plateau); (2) heart rate reached the age-predicted maximum value (220-age); and (3) respiratory exchange ratio was > 1.0.

4.2.3 Physiological Data Acquisition

Participants reported to the laboratory following a 12h abstinence from nicotine, alcohol, caffeine and intense physical exertion. Heart rate was measured using a standard three-lead electrocardiogram. Continuous tracings of arterial blood pressure through finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands), and calibrated to manual sphygmomanometer blood pressure measurements, enabled calculation of the mathematical mean pressure throughout the protocol. The Modelflow algorithm provided an index of cardiac output from the finger blood pressure tracings (Wesseling, Jansen et al. 1993), incorporating participant's sex, age, height, and weight. A respiratory belt (Pneumotrace II, ADInstruments, Colorado Springs, Colo., USA) was secured around the thorax for collection of respiratory rate. All analog measures were sampled at 1000 Hz and stored for offline analysis (LabChart7 software; ADInstruments).

4.2.4 Cardiovagal Baroreflex Sensitivity

The sequence method, performed on approximately 240 consecutive cardiac cycles at rest, provided a measure of the spontaneous cardiovagal BRS (Blaber, Yamamoto et al. 1995, Parlow, Viale et al. 1995). On the basis of evidence that the R-R interval is generally modulated within the same cardiac cycle (Pickering and Davies 1973), data

were analyzed using either a *lag 0*, *lag 1* or a *lag 2* approach to achieve the greatest number of sequences (Blaber, Yamamoto et al. 1995). The mean slope of the identified sequences was taken to represent average cardiovagal baroreflex gain.

4.2.5 Heart Rate Variability

R–R intervals were collected during 10 min of quiet supine rest. Participants were instructed to remain awake and still.

Time domain HRV analyses included the standard deviation of normal-to-normal R-R intervals (SDNN), while frequency domain analysis included the high-frequency (0.15-0.4Hz) component of the power spectral band (expressed logarithmically, ln-hf), the standard deviation of the width of the Poincaré plot (SD1), and total spectral power.

4.2.6 Sympathetic Neural Recordings

Sympathetic neural recordings were obtained in the right peroneal nerve by microneurography, using standard procedures originally defined by Hagbarth (Hagbarth and Vallbo 1968), and reported frequently by our laboratory (Steinback, Salzer et al. 2009, Goswami, Frances et al. 2012) (662C-3; Bioengineering of University of Iowa, Iowa City, Iowa, USA). Integrated sympathetic nerve activity was expressed both as burst frequency (the number of bursts per minute) and burst incidence (the number of bursts per 100 heart beats). Total MSNA was quantified as the product of mean normalized burst amplitude and burst frequency.

4.2.7 Neuroimaging Data Acquisition

Participants completed a structural magnetic resonance neuroimaging session (Robarts Research Institute Centre for Functional and Metabolic Imaging) following a 12h abstinence from nicotine, alcohol, caffeine and intense physical exertion. All imaging data were collected using a whole body 3-Tesla imaging system (Magnetom Prisma, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil (Barberi, Gati et al. 2000). A high-resolution T_1-weighted structural volume was acquired with a 3D MPRAGE sequence at the beginning of the scanning session (sagittal, matrix 256x240mm, voxel resolution 1.0x1.0x1.0mm, 1mm slice thickness, no gap, flip angle

9°, TE = 2.98 ms, TI = 900 ms, TR = 2.3 ms). A head cradle packed with foam padding limited head movement. Each subject received instruction to avoid head movements during the imaging period.

4.2.8 Neuroimaging Data Analysis

Cortical thickness analysis was performed using Brain Voyager 2.8.4 (BVQX, Brain Innovation, Maastricht, Netherlands). The Laplace method provided measures of cortical thickness for each subject's right and left hemisphere (Jones, Buchbinder et al. 2000). Spatial intensity inhomogeneities in the original T_1-weighted scans were corrected and converted into volumes with 1.0mm isotropic voxel resolution using sinc-interpolation. These volumes were transformed spatially into standard Talairach space. Once normalized, an automatic segmentation of gray and white-matter boundaries was applied, and images were resampled to 0.5mm isovoxel resolution. Topological errors such as "bridges" and remaining fragments of dura mater or cerebellum were corrected manually. Additionally, manual correction of gray and white matter boundaries was performed on a slice-by-slice and individual basis. The reconstructed cortical hemispheres were morphed into a folded three-dimensional mesh. Cortex-based alignment reduced the effect of anatomical variability and improved the spatial correspondence of cortical areas between individual brains while producing a spherical representation of the folded cortex. Finally, curvature information of individual brains was used for inter-subject alignment (Goebel, Esposito et al. 2006), and resulted in average curvature maps for each hemisphere (n=55).

4.2.9 Statistical Analysis

To assess the relationship between autonomic function and regional brain tissue thickness, we performed a full cortex correlation analysis in Brain Voyager using each individual's autonomic and cortical thickness variables. Simply stated, a linear regression model assessed the relationship between each participant's BRS, HRV and MSNA values, and the estimated peak cortical thickness value at 42 cortical sites bilaterally. The final correlation maps were adapted by increasing the minimum threshold value ($p<0.05$) and adding a cluster threshold ($10mm^2$). Subsequently, an initial series of linear regressions examined how cortical thickness at the predefined regions of interest

predicted the autonomic variables. Significant associations were followed by multiple linear regression to assess independence of the relationships after adjusting for age and cardiorespiratory fitness (VO_{2max}). A threshold for significance was set at $p<0.05$. All regression analyses passed the normality test, as assessed by the Shapiro-Wilk test for normal distribution.

4.3 Results

4.3.1 Whole-Brain Regression Results

Significant positive (BRS, HRV: SD1, SDNN, ln-hf, total spectral power) and negative (muscle sympathetic nerve activity: burst frequency, burst incidence, total MSNA) correlations were widespread and bilateral when whole-brain cortical thickness was regressed with values of autonomic outflow. These peak-vertex correlation results are given in Appendix 1 for each variable across all 42 cortical sites. Selected whole-brain results including baroreflex sensitivity, ln-high frequency, and MSNA burst frequency, are illustrated in Figure 4.1.

Table 4.2 illustrates significant relationships from the preliminary whole-brain linear regression analysis. BRS correlated with the average right hemisphere cortical thickness ($p<0.05$), and the left insula ($p=0.04$). Cortical thickness was correlated with SDNN over the right hemisphere ($p=0.03$), as well as the right ($p=0.01$) and left MPFC ($p<0.001$). Total spectral power (HRV) had a significant relationship with cortical thickness over the right hemisphere ($p=0.02$), as well as the right ($p=0.02$) and left MPFC ($p<0.001$). SD1 was correlated with cortical thickness at the left MPFC ($p=0.01$). Burst frequency, burst incidence, total MSNA, and ln-high frequency of HRV were correlated with the average cortical thickness in the right and left hemisphere, as well as the regions of interest, namely the bilateral MPFC and bilateral insula.

Subsequent multiple regression analysis of significant associations from the linear regression analysis (Table 4.3) included age and fitness as possible covariates. Fitness (VO_{2max}) did not alter the relationship between cortical thickness and any of our autonomic variables, with the exception of SD1 ($p<0.05$). Age influenced the relationship between cortical thickness and HRV (total power ($p=0.03$), ln-high frequency power

(p=0.02), SD1 (p<0.005)), as well as burst incidence (p=0.02). The thickness of the left MPFC was a dominant predictor of SDNN (p=0.01), HRV total power (p=0.01), ln-high frequency power (p=0.05), burst frequency (p=0.01), burst incidence (p=0.02), and total MSNA (p=0.05). Figure 4.2 illustrates the consistent presence of the left MPFC in these regressions. In addition, burst frequency and burst incidence can be predicted by the average cortical thickness of the right hemisphere (p=0.03 and 0.02, respectively), while burst incidence had further significant relationships with the average cortical thickness of the left hemisphere (p=0.01) and left insula (p=0.05).

Table 4.1 Anthropometric and baseline autonomic data (mean ± SD).

	Average Group Data	Value Range
Age (years)	45±15	21-73
Resting MAP (mmHg)	87±9	69-107
Resting Heart Rate (bpm)	57±9	41-90
BMI (kg/m^2)	24±3	17-36
VO_{2max} (mL/kg/min)	51±12	26-81
Cardiac Output (L/min)	2.8±0.7	1-4
Baroreflex Sensitivity (ms/mmHg)	23±17	2-84
SDNN (ms)	59±27	14-116
Total Power (ms^2)	3743±3306	143-12206
lnHF (ln ms^2)	6.1±1.5	2-9
SD1 (ms)	28±27	4-112
Burst Frequency (bursts/min)	28±11	9-50
Burst Incidence (bursts/100 heart beats)	48±19	14-83
Total MSNA (V/min)	5±3	1-15

MAP, mean arterial pressure; BMI, body mass index; VO_{2max}, maximal oxygen consumption; SDNN, standard deviation of normal to normal intervals; HF, high frequency-power; SD1, Poincare plot width; MSNA, muscle sympathetic nerve activity. Burst frequency, burst incidence and total MSNA: n=25. Cardiac output indexed to body surface area.

Table 4.2 Linear regression of autonomic variables on cortical thickness.

		RH	LH	RH MPFC	LH MPFC	R Insula	L Insula
BRS (ms/mmHg)	P	**0.046**	0.120	0.095	0.054	0.088	**0.041**
	R	0.270	0.212	0.227	0.261	0.232	0.276
Heart Rate Variability							
SDNN (ms)	P	**0.032**	0.142	**0.014**	**<0.001**	0.194	0.100
	R	0.295	0.204	0.336	0.467	0.181	0.229
Total Power (ms^2)	P	**0.015**	0.100	**0.015**	**<0.001**	0.125	0.070
	R	0.333	0.229	0.331	0.486	0.213	0.251
lnHF (ln ms^2)	P	**0.009**	**0.038**	**0.009**	**0.003**	**0.011**	**0.006**
	R	0.355	0.286	0.357	0.400	0.347	0.370
SD1 (ms)	P	0.082	0.579	0.051	**0.009**	0.187	0.335[ł]
	R	0.241	0.078	0.270	0.354	0.184	0.135
Sympathetic Nerve Activity							
BF (bursts/min)	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	R	0.742	0.731	0.664	0.809	0.708	0.717
BI (bursts/100 heart beats)	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	R	0.768	0.779	0.670	0.784	0.738	0.742
Total MSNA (V/min)	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**0.002**
	R	0.648	0.632	0.613	0.750	0.593	0.598

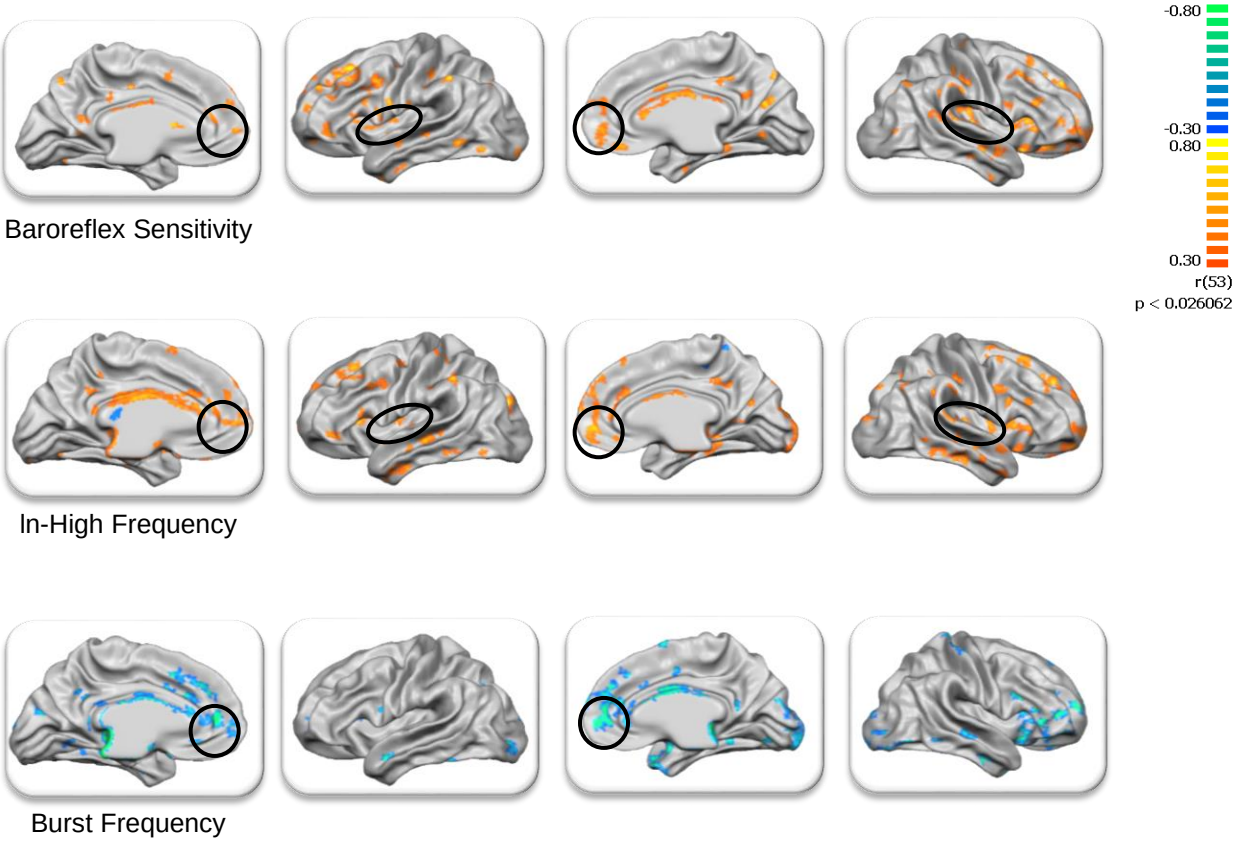
P value and correlation coefficient (R) determined from regression values. RH, right hemisphere; LH, left hemisphere; MPFC, medial prefrontal cortex; BRS, baroreflex sensitivity; SDNN, standard deviation of normal to normal intervals; HF, high frequency-power; SD1, Poincare plot width; BF, burst frequency; BI, burst incidence; MSNA, muscle sympathetic nerve activity. Bold type indicates significant relationships ($p<0.05$) carried into subsequent multiple regression.

Table 4.3 Results of multiple linear regression based on simple linear regression.

Dependent Variable	Independent Variable	Std. Coefficient	Std. Error	P value
Baroreflex Sensitivity	Age	-0.301	0.194	0.127
	VO_{2max}	-0.012	0.227	0.957
	Right Hemisphere	4.524	10.976	0.682
	L Insula	3.889	6.081	0.525
Heart Rate Variability				
SDNN	Age	-0.354	0.286	0.221
	VO_{2max}	-0.315	0.329	0.343
	Right Hemisphere	11.568	17.655	0.515
	R MPFC	6.425	6.009	0.290
	L MPFC	20.211	7.940	**0.014**
Total Power	Age	-80.752	36.362	**0.031**
	VO_{2max}	-52.573	39.320	0.188
	Right Hemisphere	1946.183	2117.005	0.363
	R MPFC	697.027	725.304	0.341
	L MPFC	2585.336	947.881	**0.009**
lnHF	Age	-0.0422	0.0176	**0.021**
	VO_{2max}	-0.0225	0.0183	0.226
	Right Hemisphere	1.013	0.954	0.294
	Left Hemisphere	0.345	0.902	0.704
	R MPFC	0.527	0.322	0.108
	L MPFC	0.895	0.442	**0.049**
	R Insula	0.651	0.648	0.320
	L Insula	0.712	0.530	0.185
SD1	Age	-0.831	0.274	**0.004**
	VO_{2max}	-0.670	0.315	**0.039**
	L MPFC	11.904	7.602	0.124
Sympathetic Nerve Activity				
Burst Frequency	Age	0.094	0.196	0.638
	VO_{2max}	-0.216	0.228	0.355
	Right Hemisphere	-29.940	12.893	**0.031**
	Left Hemisphere	-20.022	11.586	0.099
	R MPFC	-2.611	2.812	0.364
	L MPFC	-10.844	3.903	**0.012**
	R Insula	-11.708	7.259	0.122
	L Insula	-10.229	5.454	0.075
Burst Incidence	Age	0.710	0.272	**0.017**
	VO_{2max}	0.321	0.415	0.448
	Right Hemisphere	-56.569	21.581	**0.016**
	Left Hemisphere	-51.204	18.065	**0.010**
	R MPFC	-4.769	4.829	0.335
	L MPFC	-16.960	6.943	**0.024**
	R Insula	-20.574	12.465	0.114
	L Insula	-19.645	9.188	**0.045**
Total MSNA	Age	8.385	9.074	0.366
	VO_{2max}	-8.750	13.682	0.530
	Right Hemisphere	-946.417	868.848	0.289
	Left Hemisphere	-513.840	755.908	0.504
	L MPFC	-541.884	255.557	**0.047**
	L Insula	-219.490	360.751	0.550

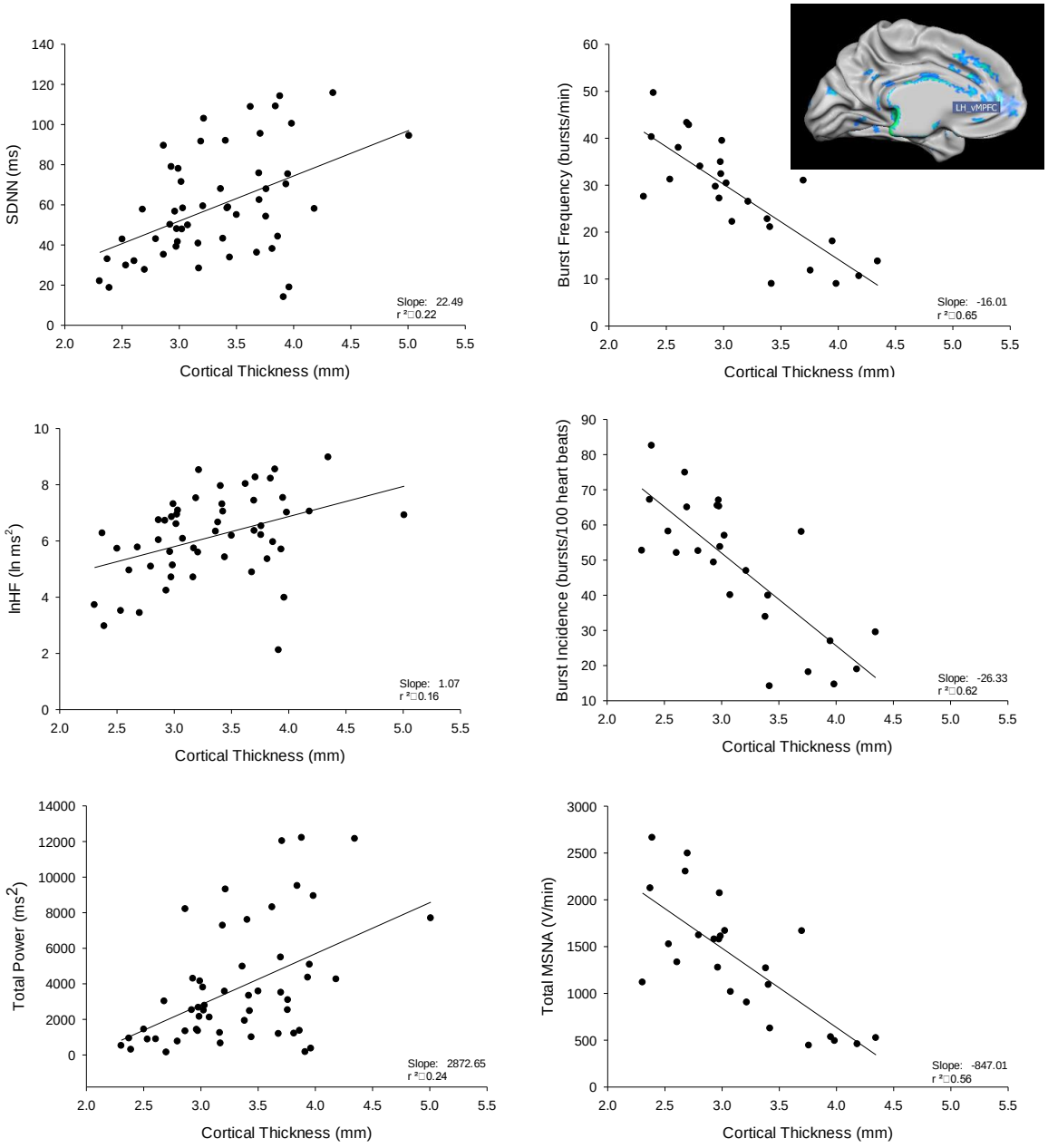
R, right hemisphere; L, left hemisphere; MPFC, medial prefrontal cortex; SDNN, standard deviation of normal to normal intervals; HF, high frequency-power; SD1, Poincare plot width; MSNA, muscle sympathetic nerve activity. Bold type indicates significant relationships ($p<0.05$).

Figure 4.1 Whole-brain correlation of selected autonomic variables and cortical thickness.



Top row: baroreflex sensitivity; middle row: ln high frequency (heart rate variability); bottom row: burst frequency (muscle sympathetic nerve activity). Pictures at right: right hemisphere; pictures at left: left hemisphere. Highlighted circles denote medial prefrontal and insula cortex regions of interest. Results are shown at $p<0.05$, corrected by cluster size ($10mm^2$). Color scale at right denotes t values.

Figure 4.2 Regression analysis of significant autonomic variables with cortical thickness at the left medial prefrontal cortex.



Talairach coordinates of peak vertex at region of interest (inset): -12, 42, 12. SDNN, standard deviation of normal to normal intervals; HF, high frequency power; MSNA, muscle sympathetic nerve activity. Sympathetic outflow (burst frequency, burst incidence, total MSNA) and cardiovagal indices (SDNN, lnHF, total power) can be predicted by thickness at the left medial prefrontal cortex ($p<0.05$, Table 4.3).

4.4 Discussion

The current study supports the hypothesis that cortical thickness predicts chronic levels of HRV and MSNA, particularly in the medial prefrontal cortex. Importantly, age was the primary determinant of the variations. The strength of the relationship between our autonomic variables and cortical thickness was not altered following adjustments for cardiorespiratory fitness.

The observation that the thickness of the MPFC predicts chronic levels of high HRV and low MSNA, forms a unique supportive concept towards understanding age-related autonomic dysregulation. However, the lack of relationship between cardiovagal baroreflex sensitivity and the MPFC was unexpected. Specifically, experimental studies in rodents indicate that this region exerts powerful modulatory effects over baroreflex function and has strong reciprocal connections with structures involved in autonomic function including the amygdala, hypothalamus, periaqueductal gray, the nucleus of the tractus solitarus and the caudal and rostral ventrolateral medulla (Neafsey 1990, Chiba and Semba 1991, Hurley, Herbert et al. 1991, Vertes 2004). Further, recent studies conducted in rodents and humans have revealed depressor sites within the ventral region of the MPFC that exhibit heightened levels of activity during periods of behavioral relaxation, including sleep and rest (Critchley, Mathias et al. 2001, Cechetto and Shoemaker 2009). The inverse relationship between MPFC activation and cardiovascular arousal in human neuroimaging studies suggests a direct effect of this region on cardiovascular function (Critchley, Corfield et al. 2000, Wong, Masse et al. 2007, Thayer, Sollers et al. 2009). Nevertheless, the current results do not suggest that cortical thickness at the MPFC is predictive of baroreflex function. One possible explanation for this is that the above results are based on functional associations between MPFC activation patterns and concurrent changes in heart rate, while the cardiovagal baroreflex is a complex signal involving both heart rate and arterial pressure. Other cortical areas such as the dorsal lateral prefrontal cortex may also modulate the baroreceptor reflex arc, since retrograde and anterograde transport studies indicate that this site may provide a quantitatively larger input to the nucleus of the solitary tract than the MPFC (van der Kooy, McGinty et al. 1982, van der Kooy, Koda et al. 1984). Indeed, the current results

support this connection as the dorsal lateral prefrontal cortex was correlated with baroreflex sensitivity in both the right and left hemispheres (see Appendix 1: superior frontal gyrus).

The current analysis examined the potential impact of cardiorespiratory fitness as a covariate in the age-cortical thickness relationship, and included the conceptual potential for underlying factors related to exercise training and increased fitness. Several factors contribute to this possibility. For example, cardiorespiratory fitness has been linked to improved hippocampal (Erickson, Voss et al. 2011, Varma, Chuang et al. 2014), MPFC (Colcombe, Erickson et al. 2003, Colcombe, Erickson et al. 2006, Kramer, Erickson et al. 2006), and anterior insula volumes (Peters, Dauvermann et al. 2009). In addition, high cardiorespiratory fitness has been associated with less structural brain atrophy (Colcombe, Erickson et al. 2003, Kramer, Erickson et al. 2006, Erickson, Voss et al. 2011) and higher estimates of white matter integrity (Marks, Katz et al. 2011, Johnson, Kim et al. 2012, Voss, Heo et al. 2013, Burzynska, Chaddock-Heyman et al. 2014). Recently, we reported preliminary results indicating a strong association between cardiorespiratory fitness and cortical "reserve" of gray matter at the MPFC (Norton, Heinecke et al. 2015). Furthermore, voxel-based morphometry measures indicate increased prefrontal cortex volume in cardiac patients undergoing cardiac rehabilitation exercise (Anazodo, Shoemaker et al. 2013). Nevertheless, the current results indicate that the strong relationships between cortical thickness and HRV and MSNA variables are not affected by inclusion of VO_{2max} in the regression model. This observation corresponds with the growing understanding that whereas exercise training can minimize the impact of age on autonomic function, the amount of exercise training or improved fitness needed to completely reverse age-related cardiovagal BRS in healthy humans are not known. Also, exercise training can reduce MSNA burst frequency in individuals with clinically or excessively elevated levels, such as those that occur with advanced age or pathology (e.g. hypertension) (Carter and Ray 2015). The current data support a speculation that the impact of exercise training on neural control of the circulation may include improvements to cortical thickness in pertinent brain regions. We acknowledge, however, that contributors to brain health extend well beyond exercise and can include sleep, diet,

lifestyle, social environment, cognitive stimulation, and genetics, among others. The relative impact of these contributors remains to be reported.

The insula cortex (IC) exhibits strong associations with cardiovascular control in functional imaging studies with humans (Cechetto and Saper 1990, Critchley, Corfield et al. 2000, Kimmerly, O'Leary et al. 2005, Cechetto and Shoemaker 2009, Macey, Wu et al. 2012), clinical studies (Critchley, Mathias et al. 2003, Woo, Macey et al. 2003, Soros and Hachinski 2012, Woo, Yadav et al. 2014), and experimental rodent models (Cechetto and Chen 1990, Verberne and Owens 1998). These associations are representative of strong sensory, as well as efferent top-down, sensitivities of this region. The topographical sensory organization of the insular cortex includes representation of baroreceptor afferent projections into the forebrain (Cechetto and Saper 1987). The relationship between cortical thickness in the insula and its sensitivity to sensory inputs was not studied in the current study. Indeed, the current autonomic indices are baseline values in healthy individuals with a rather narrow range of normal blood pressure values. Therefore, we do not think the current associations between insula cortical thickness and autonomic variables reflect a sensory mechanism. Rather, our approach was to assess the insula-autonomic outflow relationships. In this context, observations that direct stimulation of insular cortex regions produces cardiac damage through a sympathetic mechanism (Oppenheimer, Wilson et al. 1991) point to the potency of this region over autonomic function. The involvement of the IC has also been reported in situations of physical stress such as baroreceptor unloading (Kimmerly, O'Leary et al. 2005), and isometric exercise (Wong, Kimmerly et al. 2007, Goswami, Frances et al. 2012). Moreover, increased activity within the IC occurs during a variety of cognitive maneuvers that elevate autonomic arousal such as gambling (Critchley, Mathias et al. 2001), Stroop task (Gianaros, Van Der Veen et al. 2004), mental arithmetic (Critchley, Corfield et al. 2000) and many more. In the current study, MSNA burst incidence correlated with cortical thickness at the left insula. Thus, the structural approach in this study further supports the IC as an important region in generalized autonomic control. This study was not able to examine the gyri-specific associations across the various gyri in the IC, although such outcomes are apparent in functional MRI studies (King, Menon et al. 1999, Macey, Wu et al. 2012).

In addition to the regions of interest discussed above, our whole-brain approach exposed correlations between markers of autonomic outflow and cortical thickness of several other prefrontal brain regions – particularly the cingulate gyrus, and the superior frontal gyrus, or dorsal lateral prefrontal cortex. These regions are marked by their associations with functional autonomic outcomes. For example, peripheral changes in blood pressure are reflected in activity within the anterior cingulate cortex, a region that also may integrate peripheral cardiovascular changes with cognitive effort, motor and emotional states (Critchley, Corfield et al. 2000, Matthews, Paulus et al. 2004, Gianaros, Derbyshire et al. 2005). Woodward et al. (2008), and later Winkelmann et al (2016), reported associations between vagally-mediated heart rate variability and activation changes within the anterior mid-cingulate, or rostral dorsal anterior cingulate. In addition, functional magnetic resonance imaging methods exposed increased activity in the dorsal lateral prefrontal cortex during a maximal inspiratory apnea, a known maneuver of sympathoexcitation (Macefield, Gandevia et al. 2006).

4.4.1 Perspectives

The balance between sympathetic and parasympathetic regulation of the cardiovascular system is crucial to long-term health and to acute adjustments to stress. Chronic disturbances can contribute to the pathogenesis and progression of several cardiovascular disease states, thereby increasing the risk of morbidity and mortality (Schmidt, Muller-Werdan et al. 2005). Our study identifies a set of brain regions whose cortical thickness relates strongly to peripheral autonomic indices involved in cardiovascular control. These regions, specifically the MPFC and IC, along with the superior frontal gyrus, are highly susceptible to age-related cortical atrophy, as well as other pathologies. Moreover, these brain regions are also involved in cognitive and behavioural functions that also tend to decline in advancing age. Our findings are the first to report human data which suggest a link between cortical structure and autonomic function in these sites. Thus, strategies to sustain cortical thickness in these regions are expected to provide effective restraint of autonomic changes that occur with age and, thereby, improve overall health and mobility.

4.5 Limitations

The findings in the current study were limited to healthy participants to avoid comorbidity concerns associated with disease. Given the generalized sympathetic activation in various disorders such as cardiac disease, arthritis and neuropathologies (eg. Parkinson's Disease), it may be that the findings observed in this study are augmented in the disease state. Our region of interest analysis was based on previous literature and experience, both from our laboratory (Kimmerly, O'Leary et al. 2005, Wong, Masse et al. 2007, Cechetto and Shoemaker 2009, Goswami, Frances et al. 2012, Anazodo, Shoemaker et al. 2013, Norton, Luchyshyn et al. 2013) and that of others (Colcombe, Erickson et al. 2006, Macefield, Gandevia et al. 2006, Thayer and Lane 2007, Erickson, Voss et al. 2011). However, as suggested in the Appendices, there are many brain regions that appear to be correlated to autonomic function and may be studied in this context. For example, the dorsal lateral prefrontal cortex, the anterior cingulate cortex and the hippocampus are known to be associated with cardiovascular control (Mulder, Arts et al. 1997, Westerhaus and Loewy 2001, Castle, Comoli et al. 2005, Winkelmann, Thayer et al. 2016) and are affected by age (Raz, Gunning et al. 1997, Good, Johnsrude et al. 2001, Jernigan, Archibald et al. 2001, Allen, Bruss et al. 2005, Colcombe, Erickson et al. 2006, Thayer, Sollers et al. 2009, Anazodo, Shoemaker et al. 2013, Freeling and Li 2015).

4.6 Conclusion

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Chapter 5

5 High Cardiorespiratory Fitness in Middle-Age Preserves the Cortical Circuitry Associated with Brain-Heart Integration During Volitional Exercise

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5.1 Introduction

Rapid increases in HR represent a critical reactive response to physiologic stress, facilitating or sustaining blood pressure and organ perfusion. The tachycardia at the onset of exercise has been linked mechanistically to a rapid reduction in vagal chronotropic control (Hollander and Bouman 1975, Fagraeus and Linnarsson 1976, Mitchell, Reeves et al. 1989). However, although some inter-individual variation exists (Norton, Luchyshyn et al. 2013), advancing age impairs the magnitude of the HR and cardiac output responses at the exercise onset, resulting in a larger sympathetic drive to adjust blood pressure (Lalande, Sawicki et al. 2014). The mechanism(s) affecting the diminished cardiac response with exercise in aging adults remains unknown but may include either changes in autonomic neural adjustments and/or intrinsic cardiac events (Astrand 1960, Bruce, Blackmon et al. 1963, Lester, Sheffield et al. 1968, Dauchot and Gravenstein 1971, Kino, Lance et al. 1975, Seliger, Macek et al. 1978, Sheffield, Maloof et al. 1978, Yin, Spurgeon et al. 1979, Craft and Schwartz 1995, Lalande, Sawicki et al. 2014). Diminished HR variability (O'Brien, O'Hare et al. 1986, Schwartz, Gibb et al. 1991, Agelink, Malessa et al. 2001, Bonnemeier, Richardt et al. 2003, Russoniello, Zhirnov et al. 2013) and a reduced tachycardia following atropine administration (Dauchot and Gravenstein 1971) support the conclusion that age reduces chronic parasympathetic restraint of HR, suggesting that this effect may be a fundamental determinant of impaired cardiac acceleration with exercise in aging adults.

However, the neurological determinants of age-related impairment of cardiovagal function remain an attractive contributor, due to the important role of supramedullary sites on autonomic outflow. Specifically, neuroimaging techniques have enabled

investigation into a network of cortical regions associated with the autonomic nervous system and cardiovascular control in conscious humans (Critchley, Corfield et al. 2000, Gianaros, Van Der Veen et al. 2004, Williamson 2010, Basnayake, Green et al. 2012, Macey, Wu et al. 2012, Norton, Luchyshyn et al. 2013, Cechetto 2014, Shoemaker, Norton et al. 2014). These regions include the bilateral IC, ACC, PCC, MPFC, and HC. Importantly, experimental studies indicate that the IC, MPFC and HC are of particular relevance to HR control (Burns and Wyss 1985, Ruggiero, Mraovitch et al. 1987, Cechetto and Chen 1990, Yasui, Breder et al. 1991, Oppenheimer, Gelb et al. 1992, Verberne 1996, Fisk and Wyss 1997, Owens and Verberne 2001, Wong, Masse et al. 2007, Goswami, Frances et al. 2012, Norton, Luchyshyn et al. 2013, Shoemaker, Norton et al. 2014). Importantly, advancing age often associates with cortical atrophy (Raz, Lindenberger et al. 2005), changes in brain functional responses (Nyberg, Salami et al. 2010), and declines in cognitive performance (Ronnlund, Nyberg et al. 2005). Notably, the pattern of atrophy begins early in middle age (Raz, Gunning et al. 1997, Raz, Lindenberger et al. 2005, Raz and Rodrigue 2006, Kennedy, Erickson et al. 2009, Raz, Ghisletta et al. 2010) and preferentially affects frontal and parietal regions of the brain, including the MPFC and IC regions: therefore, diminished functional brain-heart associations may be expected in middle aged and older adults.

Nonetheless, substantial inter-individual differences exist in age-related cortical atrophy with some individuals showing resistance to major age-related brain pathology or neurological deficits (Nyberg, Lovden et al. 2012, Pudas, Persson et al. 2013). The determinants of this variability are not known. Of the many possible options, physical activity may be one of the factors affecting this heterogeneity as it demonstrates widespread benefits on brain health in aging individuals, including spared brain volume (Erickson, Prakash et al. 2009, Erickson, Voss et al. 2011, Niemann, Godde et al. 2014, Wood, Nikolov et al. 2016), improved task-related functional brain responses (Colcombe, Kramer et al. 2004, Voelcker-Rehage, Godde et al. 2010), increased white matter integrity (Johnson, Kim et al. 2012, Voss, Heo et al. 2013), and improved cognitive performance (Josefsson, de Luna et al. 2012). However, the functional consequences of this age-related heterogeneity on the forebrain circuitry associated with cardiovascular responses to physiological stress remain unknown.

The purpose of this study was to examine the impact of cardiorespiratory fitness on brain-heart associations in middle aged to older adults. The current study focused on healthy, middle-aged individuals to minimize the confounding variables of senescence and associated co-morbidities, as well as to engage the age-range where cortical vulnerability begins to be expressed and where the effects of advancing age on cardiorespiratory fitness remain weak. With this approach, we tested the hypothesis that high cardiorespiratory fitness improves the HR response to volitional handgrip in middle aged adults, and that such improvement is related to preservation of the entrainment of the MPFC, IC, and HC regions of the cortical autonomic network.

5.2 Methods

5.2.1 Participants

A total of 52 healthy, active individuals participated in this study across a range of fitness and age (26-66mL/kg/min; 45-73 years; 16 female). Table 5.1 provides group characteristics. All participants were non-smokers, free of medications, and without diagnosed hypertension, diabetes, vascular or neurological impairments. Pre-menopausal females were tested during days 1-14 of the menstrual cycle, with day one representing the first day of menstruation. None of the post-menopausal women were on hormone replacement therapy. The University of Western Ontario Health Sciences Ethics Review Board approved this study and adhered to the Declaration of Helsinki. Each participant provided informed, written consent.

5.2.2 Experimental Design

Participants completed two separate experimental sessions: 1) physiological recording (LAB session) and 2) functional magnetic resonance neuroimaging session (fMRI; Robarts Research Institute Centre for Functional and Metabolic Imaging). The sessions were performed at the same time of day and separated by a minimum period of 1 week. Participants were familiarized with the experimental procedures prior to their first test session. Participants were instructed to arrive following a 12h fast and to refrain from nicotine, alcohol, caffeine, and intense physical exertion for the same duration. Each

session began with a MVC handgrip calibration, in which the participant was instructed to squeeze a non-magnetic handgrip device connected in series to a pressure transducer (Edwards Lifesciences, PX272, Irvine CA) to their maximal ability while in the supine position. This was repeated twice with the larger value calibrated as 100%. All subjects were right handed and performed the isometric handgrip exercise (IHG) with their dominant hand. During each recording session, visual feedback was provided to the participant of their achieved force in real-time. Baseline data were collected over 5 min of quiet supine rest. Four repeated bouts each of 40% MVC force (LAB session), and seven repeated bouts of IHG at 40% MVC force (fMRI session) were performed, with each contraction lasting 20 sec and separated by 40 sec of rest. The number of trials was increased in the fMRI session to increase the signal-to-noise ratio. The level of perceived exertion produced by the exercise was monitored after each trial on a scale from 6-20 (Borg 1982). None of the participants reported feeling any significant degree of adverse emotional stress or forearm fatigue.

5.2.3 Assessment of Cardiorespiratory Fitness

A graded treadmill exercise test, conducted under standard clinical observation, provided information regarding each subject's peak oxygen uptake (VO_{2max}). During this test, expired air samples were taken at 3-second intervals until the point of volitional exhaustion. Based on the American College of Sports Medicine guidelines (ACSM 1995), VO_{2max} was determined by meeting the following criteria: (1) VO_2 ceased to increase with increasing workloads (plateau); (2) heart rate reached the age-predicted maximum value (220-age); and (3) respiratory exchange ratio was > 1.0.

5.2.4 Physiological Data Acquisition

During the LAB session, HR was monitored by standard 3-lead ECG techniques. Arterial BP was measured continuously from the finger of the non-exercising left hand, maintained at heart level, by photoplethysmography (Finometer; Finapres Medical Systems B.V. Amsterdam, NL). The BP readings recorded from the Finometer were corrected against sphygmomanometrically-obtained SBP and DBP pressures that were made intermittently during data collection.

5.2.5 Physiological Data Analysis

All measures were sampled at 1000 Hz, input into a data acquisition board (PowerLab ML795, ADInstruments) for analog-to-digital signal conversion with LabChart7 software (ADInstruments), and stored for offline analysis. HR was calculated from successive R-R intervals obtained from the ECG signal. Beat-by-beat HR data were averaged over 2.5s bins (the TR interval for functional scans) and time aligned to ensure a corresponding mean value for each functional scan obtained during the fMRI collection period. The HR response (ΔHR) to the IHG was determined by averaging the response over the last 20s of each rest, and last 10s of each IHG interval. Heart rate responses for each participant were averaged over the four repeated blocks. Blood pressure from the Finometer was converted to MAP using the formula MAP = 1/3 SBP + 2/3 DBP. The Modelflow algorithm provided an index of cardiac output from the finger blood pressure tracings (Wesseling, Jansen et al. 1993). Participant's sex, age, height, and weight were input manually into the Finometer to optimize estimation of cardiac output.

Linear regression analysis examined how cardiorespiratory fitness (VO_{2max}) predicted ΔHR to the IHG task. Although a box-plot analysis revealed several outliers with either a large positive or negative change in HR, removing these individual responses did not change the significance of our regression; therefore, we chose to include them for further analysis. A threshold for significance was set at $p<0.05$. All regression analyses passed the normality test, as assessed by the Shapiro-Wilk test for normal distribution.

5.2.6 Neuroimaging Recording Session

Imaging data were collected using a whole body 3-Tesla imaging system (Magnetom Prisma, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil (Barberi, Gati et al. 2000). A high-resolution T_1-weighted structural volume was acquired with a 3D MPRAGE sequence at the beginning of the scanning session (sagittal, matrix 256 X 240 mm, voxel resolution 1.0 X 1.0 X 1.0 mm, 1 mm slice thickness, no gap, flip angle 9°, TE = 2.98 ms, TI = 900 ms, TR = 2.3 ms). Transmission and detection of the BOLD contrast signal was acquired by T_2-weighted gradient echo-echo planar imaging

pulse sequence with the following parameters: TE = 30 ms; FOV = 240 x 240 mm, flip angle = 90°. Forty-five interleaved axial slices (3.0 X 3.0 mm in-plane voxel resolution, TR = 2.5 s) were acquired in each volume. Five volumes were acquired in the resting participant prior to actual data collection to allow for magnetization equilibrium; these were discarded prior to data analysis. Head movement was limited during the experimental session within a head cradle packed with foam padding, and each subject was instructed to avoid head movements during the scanning period. Beat-by-beat HR was calculated from the continuous signal derived from an MRI-compatible pulse Oximeter (Nonin Medical Inc, 8600FO MRI, Plymouth, MN) placed over the index finger of the non-exercising left hand. In each session, analog signals for pulse recordings and IHG contraction force were sampled at 1000 Hz with an on-line data acquisition and analysis system (PowerLab, ADInstruments, Mountain View, CA, USA). Respiratory frequency was monitored continuously to prevent Valsalva manoeuvres during the exercise period.

5.2.7 Neuroimaging Data Analysis

The ΔHR to IHG was determined by averaging the response over the last 20s of each rest, and last 10s of each IHG interval. Individual HR time courses were determined using 2.5s averages of the beat-by-beat HR measures to generate time-aligned data with the BOLD imaging acquisition. HR responses for each participant were averaged over the seven repeated blocks at 40% MVC. Figure 5.1 displays the average pattern of the HR response to the repeated IHG bouts.

All fMRI data were analyzed using Brain Voyager QX 2.8.4 (Brain Innovation, Maastricht, Netherlands) (Goebel, Esposito et al. 2006). At the first (individual) level, 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. Individual functional data were co-registered to their respective anatomical template, and subsequently transformed to Talairach space (Talairach and Tournoux 1988). The change in BOLD signal over the exercise period was modeled with a boxcar function

convolved with a canonical haemodynamic response function and regressed with the individual movement parameters generated during preprocessing. This resulted in subject-specific contrast images containing whole brain information related to sites of both increased and decreased BOLD signal, relative to baseline, during the IHG task as a function of the task itself and the individual HR correlation. The General Linear Model was used to calculate the parameter estimates for all brain voxels (Friston, Holmes et al. 1995). Corrections for multiple comparisons were made using the false discovery rate ($p<0.05$), as well as cluster level threshold estimation (Hagler, Saygin et al. 2006), with 1000 iterations of Monte Carlo simulation and a statistical threshold of $p < 0.05$ for the main task effects. Both corrections were performed sequentially, such that the final results represent only clusters > 10 voxels in size.

Subsequently, the individual BOLD data for all subjects were "collapsed" resulting in a mean effect estimate per condition. The estimated first level mean effects were analyzed across subjects using a RFX analysis which was performed both in response to the task and the HR regression to assess the consistency of effects between individuals based on the variability of the first-level estimates across subjects. Activation clusters with a minimum size of 300 voxels were converted into voxels of interest (VOI) resulting in a list of the most significant clusters at the whole-brain level. VOI details were transferred to Talairach Client (Research Imaging Institute, version 2.4.3) for the assignment of Talairach coordinates to the nearest grey matter voxel. Subsequently, a manual region of interest analysis was performed for relevant cortical autonomic network regions including the bilateral IC, ACC, PCC, MPFC, and HC, based on earlier data in young individuals performing the same IHG protocol (Wong, Masse et al. 2007, Norton, Luchyshyn et al. 2013). All fMRI data are represented in radiologic convention (i.e. subject's right appears on the left).

To assess the relationship between BOLD responses with our external covariate (VO_{2max}), we performed a full cortex subtraction (T-test) analysis on high-fit (equal to or > 90^{th} percentile of age-predicted VO_{2max}) versus low-fit (< 90^{th} percentile of age-predicted VO_{2max}) individuals. The 90^{th} percentile was the chosen threshold for fitness as it accurately depicts whether individuals were meeting the expected guidelines for their

age (ACSM 1995), and represents the median fitness level of our sample population. The final correlation maps were adapted by increasing the minimum threshold value ($p<0.05$) and adding a cluster threshold ($10mm^2$).

Task-dependent functional connectivity was assessed using psychophysiological interaction (PPI) analysis (Friston, Buechel et al. 1997). PPI analysis was applied to determine which voxels in the brain increase their relationship with the seed region of interest during the handgrip task, in reference to the HR time course. Based on previous BOLD responses to the handgrip task (Wong, Masse et al. 2007, Norton, Luchyshyn et al. 2013) and our a-priori hypothesis, the MPFC was selected as the seed region. Thus, a task-specific increase in the relationship between brain regions is suggestive of an interaction, or increase in the exchange of information, between the task (psychological factor) and the activation time course of the MPFC (physiological factor). Individual PPI maps were created and subsequently overlaid to obtain an RFX group PPI map.

5.3 Results

5.3.1 Physiological Results

Baseline anthropometric and cardiovascular characteristics are provided in Table 5.1. The ΔHR to the 40% MVC contraction was the same during the physiological and neuroimaging sessions.

Linear regression revealed a weak relationship between cardiorespiratory fitness ($p=0.09$) and the change in HR in response to the IHG task (Figure 5.2).

5.3.2 Functional (BOLD) Imaging Results: Handgrip Stimulus

A whole-brain group analysis of the cortical response to IHG revealed seven significant clusters of activation. An increase in BOLD signal relative to baseline was widespread and centered around the left fusiform gyrus, while decreases in activity were observed at the right superior and middle temporal gyrus, right insula, precuneus, left ACC gyrus, and middle temporal gyrus (Figure 5.3).

Further analysis of our *a-priori* regions-of-interest revealed increased activity in the bilateral IC and left HC, while reduced activity relative to baseline was observed in the left ACC and PCC, as well as the left MPFC and right HC.

5.3.3 Functional (BOLD) Imaging Results: Heart Rate

Whole-brain group analysis of the cortical response regressed with the HR time course revealed six significant clusters of activation. An increase in BOLD signal relative to baseline was observed in the right precentral gyrus and cerebellum, while decreases in activity were observed in the right superior temporal gyrus, as well as the left ACC, parahippocampal gyrus and superior temporal gyrus (Figure 5.4).

Region-of-interest analysis revealed similar results to the task, with increased activity in the bilateral anterior IC and left HC, and reduced activity relative to baseline observed in the left ACC and PCC, as well as the left MPFC and right HC.

5.3.4 Subtraction Analysis: Low VO_{2max}-High VO_{2max}

Subtraction analysis of BOLD activation patterns correlated to the HR response between the low-fit and high-fit groups revealed increased activation at the MPFC suggesting that those with a lower VO_{2max} had more activation/less deactivation than those with a high VO_{2max}. Age and the ΔHR response to the IHG task were the same between high vs low-fit individuals (Figure 5.5).

5.3.5 Psychophysiological Interaction Analysis: Heart Rate Time Course

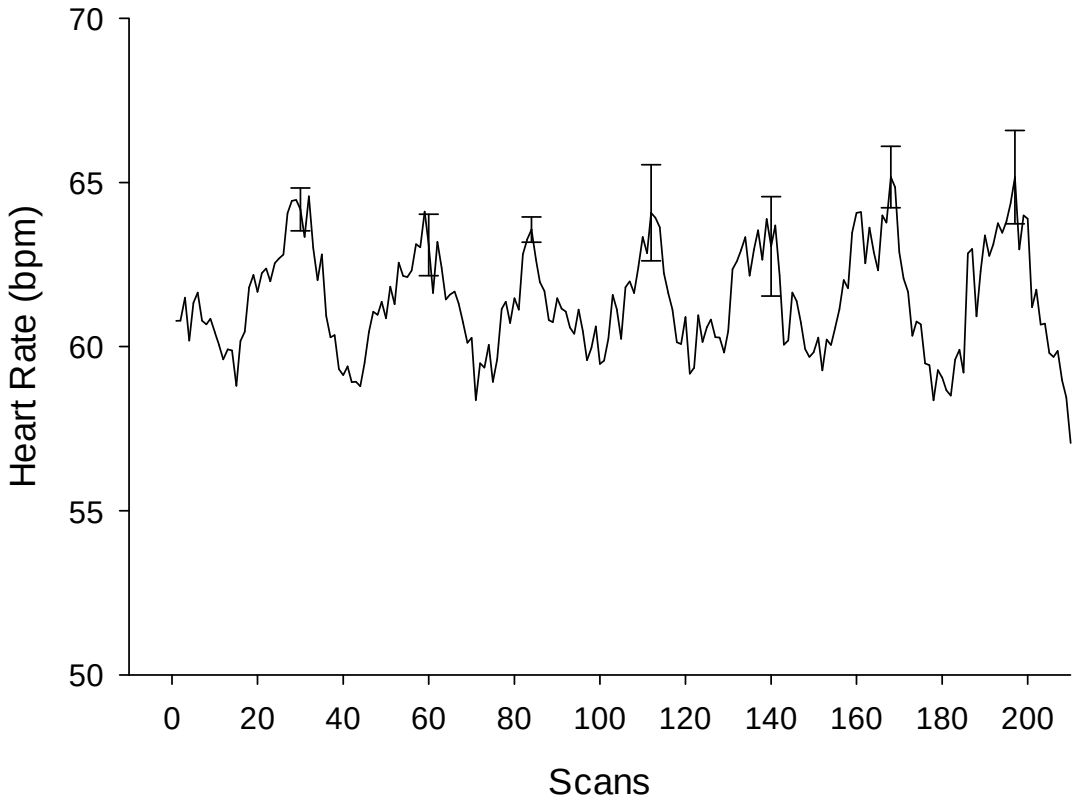
The PPI approach demonstrated that the MPFC (seed region) engaged in a task-specific increase in the exchange of information with nineteen activated clusters across the whole-brain, relative to the resting condition (Figure 5.6). Within the regions of interest, increased BOLD responses associated with HR connected to the MPFC seed region were observed in the bilateral IC. Decreased information exchange with the MPFC was observed in two clusters at the right middle temporal gyrus and right lingual gyrus (occipital lobe). No connections between the MPFC and HC were observed.

Table 5.1 Anthropometric and baseline cardiovascular data (mean ± SD).

	Average Group Data	Value Range
Age (years)	59±8	45-79
Resting MAP (mmHg)	87±9	73-114
Resting Heart Rate (bpm)	57±9	41-83
BMI (kg/m^2)	25±3	19-36
Cardiac Output (L/min)	6±2	3-11
ΔHR (LAB)	5±3	1-16
ΔHR (fMRI)	6±5	-12-22
VO_{2max} (mL/kg/min)	41±11	21-66
VO_{2max} Age Predicted Percentile	81±25	25-130

MAP, mean arterial pressure; BMI, body mass index; HR, heart rate; VO_{2max}, maximal oxygen consumption. Age-predicted percentile based on ACSM Guidelines. Cardiac output indexed to body surface area.

Figure 5.1 Average time course of the heart rate response for the isometric handgrip exercise trials of the neuroimaging session.



The error bars representing standard deviation are only shown at the end of the exercise for clarity.

Figure 5.2 Heart Rate (HR) response to isometric handgrip regressed with cardiorespiratory fitness (VO_{2max}).

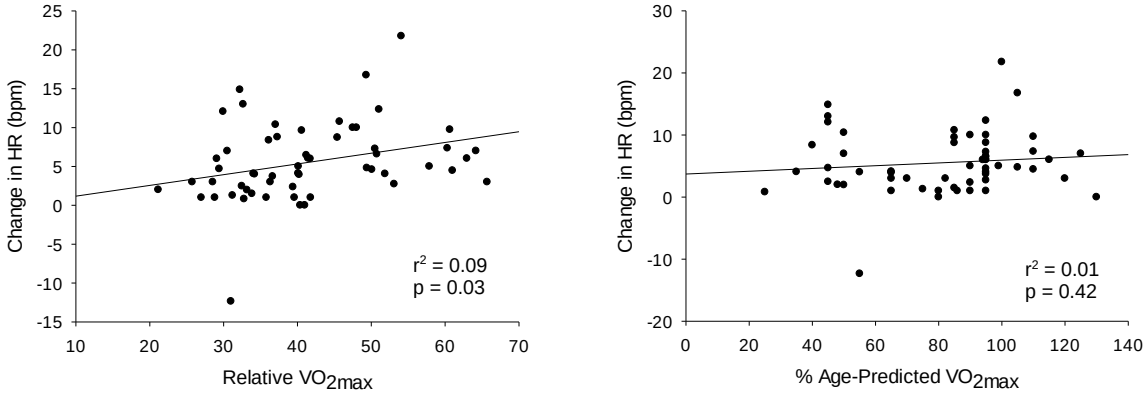
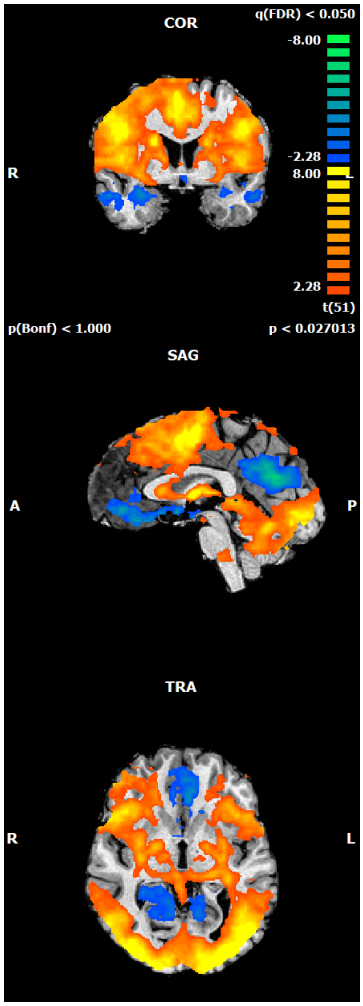
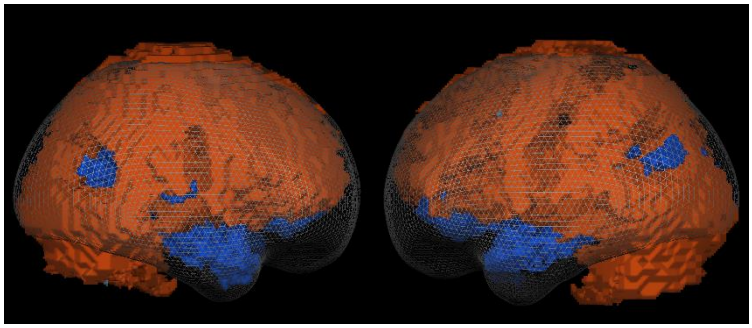


Figure 5.3 BOLD Imaging Results: Handgrip Stimulus.



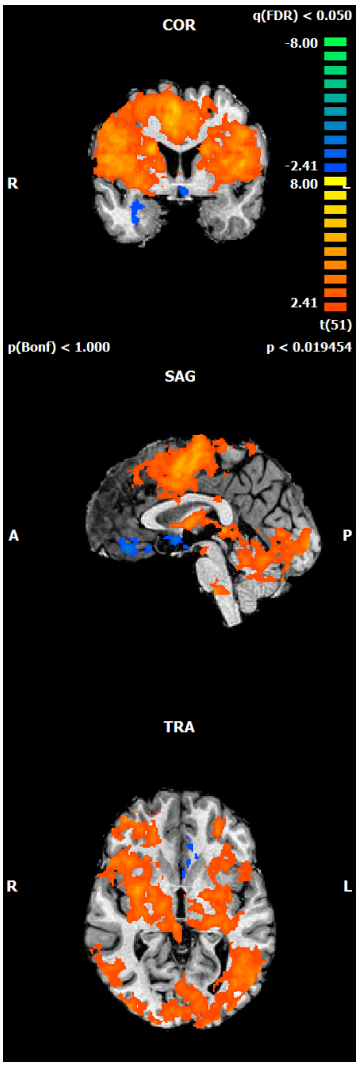


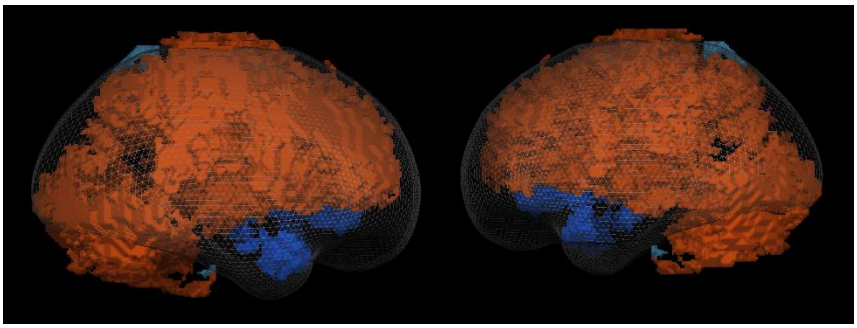
Cluster	X	Y	Z		Label	Voxels	r	T
1	36	8	-20	R	Sup Temporal	13097	-3.3	-7.3
2	45	-64	19	R	Mid Temporal	1184	-3.1	-5.1
3	33	-22	13	R	Insula	721	-2.9	-5.6
4	3	-61	19	R	Precuneus	45713	-3.5	-7.7
5	-9	29	-9	L	Ant Cingulate	10529	-3.4	-7.8
6	-42	-70	28	L	Mid Temporal	1343	-3.0	-5.5
7	-42	-67	-14	L	Fusiform	558931	4.8	19.6

ROI	X	Y	Z		Label	r	T
1	35	26	7	R	Insula	8.4	10.1
	-33	14	10	L	Insula	7.7	11.5
2	-3	32	6	L	Ant Cingulate	-2.3	-3.8
3	-5	-44	28	L	Post Cingulate	-3.9	-4.8
4	-3	38	-5	L	MPFC	-4.3	-6.4
5	27	-40	0	R	HC	-2.4	-4.2
	-33	-28	-4	L	HC	0.9	5.4

Whole-brain 2D (left panel) and 3D (glass brain, top right panel) representation of activated clusters. Peak cluster (top table) and region of interest details (bottom table) are given with Talairach coordinates. R, right; L, left; Sup, superior; Mid, middle; Ant, anterior; Post, posterior; MPFC, medial prefrontal cortex; HC, hippocampus. r, correlation value; T, T-score (beta value). Spherical region of interest represents nearest gray matter 257 voxels (same for all ROIs). FDR, $p<0.05$. Cluster threshold = 10 voxels.

Figure 5.4 BOLD Imaging Results: Heart Rate Regressor.



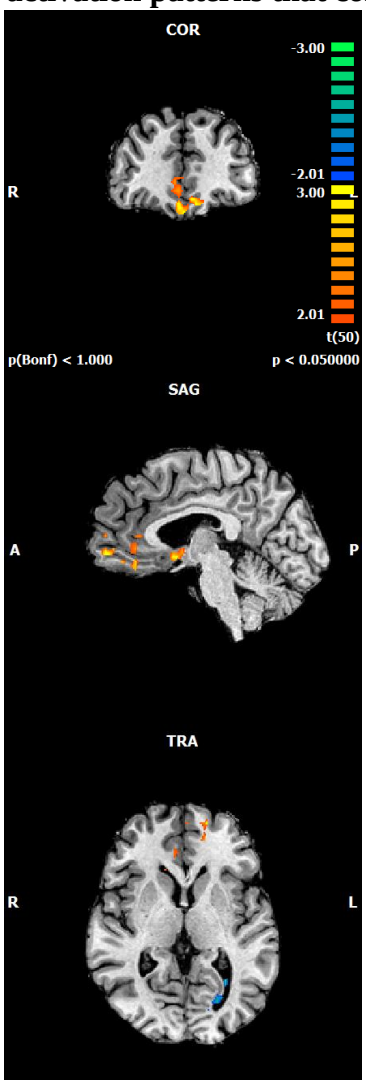


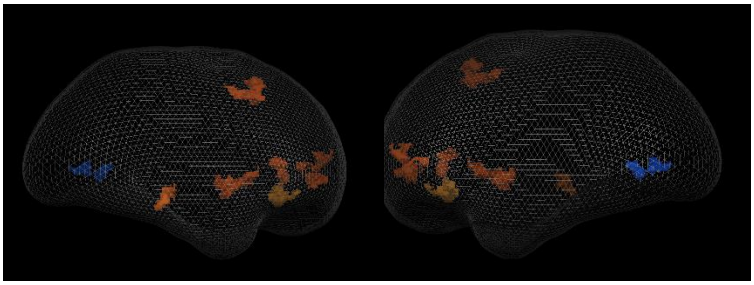
Cluster	X	Y	Z		Label	Voxels	r	T
1	30	-28	55	R	Precentral	445300	3.5	8.2
2	42	11	-20	R	Sup Temporal	4289	-3.1	-5.6
3	19	-85	-29	R	Cerebellum	414	2.8	3.9
4	-9	31	-11	L	Ant Cingulate	3785	-3.1	-5.6
5	-24	-10	-14	L	Parahippocampal	599	-2.9	-5.1
6	-39	20	-26	L	Sup Temporal	1842	-3.0	-4.6

ROI	X	Y	Z		Label	r	T
1	30	23	7	R	Insula	4.2	5.5
	-33	17	10	L	Insula	4.8	6.8
2	-3	32	6	L	Ant Cingulate	-0.2	-2.1
3	-6	-43	26	L	Post Cingulate	-1.5	-2.1
4	0	38	-8		MPFC	-2.9	-4.7
5	27	-39	1	R	HC	-1.1	-2.3
	-32	-28	-3	L	HC	0.7	3.9

Whole-brain 2D (left panel) and 3D (glass brain, top right panel) representation of activated clusters. Peak cluster (top table) and region of interest details (bottom table) are given with Talairach coordinates. R, right; L, left; Sup, superior; Ant, anterior; Post, posterior; MPFC, medial prefrontal cortex; HC, hippocampus. r, correlation value; T, T-score (beta value). Spherical region of interest represents nearest gray matter 257 voxels (same for all ROIs). FDR, $p<0.05$. Cluster threshold = 10 voxels.

Figure 5.5 Subtraction analysis of Low VO_2 – High VO_2 representing the difference in activation patterns that correlate with heart rate.



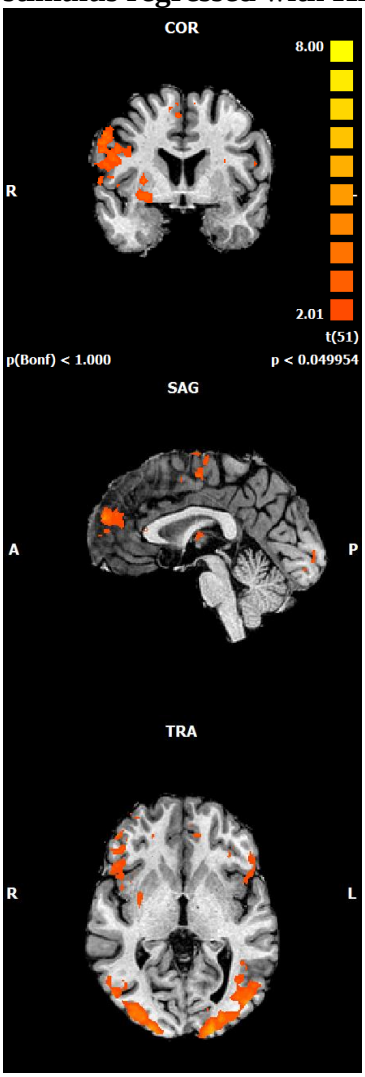


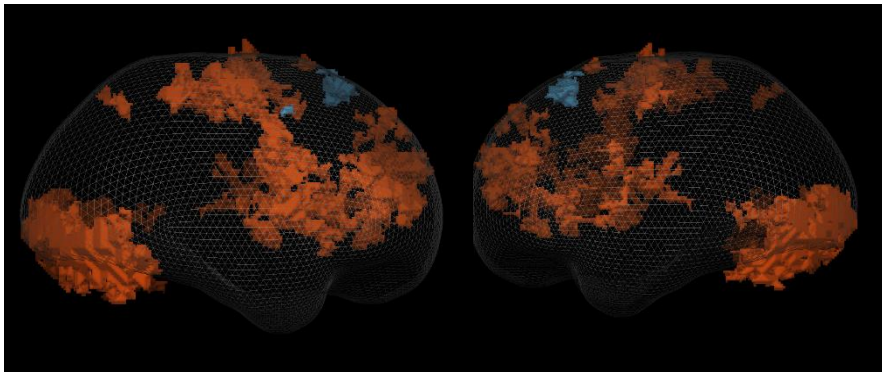
Cluster	X	Y	Z		Label	Voxels	T
1	42	-25	-8	R	Sup Temporal	388	2.3
2	30	20	52	R	Mid Frontal	582	2.3
3	9	5	-5	R	Caudate	1073	2.5
4	9	50	1	R	Frontal	472	2.5
5	6	35	7	R	MPFC	303	2.4
6	-18	56	6	L	Frontal	402	2.3
7	-9	32	-8	L	MPFC	624	2.5

	Age (years)	VO_2 Percentile	ΔHR
Low-fit (n=28)	61 ± 9	61 ± 18	4 ± 5
High-fit (n=29)	57 ± 8	101 ± 11*	6 ± 5

Whole-brain 2D (left panel) and 3D (glass brain, top right panel) representation of activated clusters are shown. Warm colors represent regions of the brain where the low VO_2 group has more activation/less deactivation than the high VO_2 group. Peak cluster details (top table) are given with Talairach coordinates. Group subject characteristics (bottom table) after assignment based on ACSM VO_{2max} age-predicted percentiles. R, right; L, left; Sup, superior; Mid, middle; MPFC, medial prefrontal cortex; T, beta value representing difference between groups; HR, heart rate. Cluster threshold = 10 voxels. * different from low-fit, $p<0.05$.

Figure 5.6 PPI Results: Functional connectivity of the MPFC during handgrip stimulus regressed with HR time course.





Cluster	X	Y	Z		Label	Voxels	r	T
1	54	2	34	R	Precentral	12854	2.5	5.2
2	42	-73	-8	R	Inf Occipital	11349	2.8	6.3
3	42	-61	25	R	Mid Temporal	956	-2.3	-4.2
4	36	-28	52	R	Postcentral	5377	2.5	4.6
5	27	59	9	R	Mid Frontal	1382	2.6	4.2
6	21	-70	52	R	Precuneus	361	2.3	3.3
7	9	-70	1	R	Lingual	25053	-2.6	-5.0
8	18	47	40	R	Sup Frontal	567	2.3	3.6
9	12	23	13	R	Caudate	372	2.6	4.4
10	3	2	52	R	Medial Frontal	2293	2.4	4.6
11	0	56	22	L	Sup Frontal	5618	2.5	4.9
12	-9	-19	10	L	Thalamus	388	2.3	3.4
13	-45	-76	-9	L	Mid Occipital	17636	2.8	6.4
14	-9	38	-5	L	Medial Frontal	476	2.6	4.3
15	-9	14	64	L	Sup Frontal	808	2.6	4.1
16	-27	-13	13	L	Putamen	1283	2.4	3.9
17	-36	47	26	L	Mid Frontal	473	2.4	4.0
18	-36	23	10	L	Inf Frontal	491	2.3	3.1
19	-42	-4	46	L	Precentral	325	2.3	3.4
20	-48	14	1	L	Inf Frontal	854	2.3	3.2
21	-54	8	31	L	Inf Frontal	312	2.3	3.3

ROI	X	Y	Z		Label	r	T
1	30	23	9	R	Insula	2.1	3.2
2	-35	17	13	L	Insula	0.8	2.1

Whole-brain 2D (left panel) and 3D (glass brain, top right panel) representation of activated clusters. Peak cluster (top table) and region of interest details (bottom table) are given with Talairach coordinates. R, right; L, left; Sup, superior; Mid, middle; Inf, inferior. r, correlation value; T, T-score (beta value). Spherical region of interest represents nearest gray matter 257 voxels. Cluster threshold = 10 voxels, $p<0.05$.

5.4 Discussion

To our knowledge, the present study represents the first exploration of the relationship between cardiorespiratory fitness and forebrain circuitry associated with cardiovascular control. Our first observation suggests that fitness does not predict the ΔHR to a volitional IHG task. Second, as a group, the BOLD response associated with the IHG task, and the with the HR time course, were very similar to those observed in young adults. Finally, in a group-wise contrast, cardiorespiratory fitness predicted the BOLD response associated with HR, but not the HR response itself. We interpret these findings to suggest that higher levels of fitness positively affect cortical neurocircuitry associated with cardiovascular arousal and that the effect of such neural activity is modulated in the middle to older age range due to factors related to brainstem pathways, and/or end organ responsiveness.

The preservation of deactivation patterns within the MPFC and HC in the highly fit group of the present study represents an important observation of the current study. These regions were specifically chosen for their known participation in cardiac adjustments to exercise and support the discrete patterns of activation consistently shown in young healthy subjects (Wong, Masse et al. 2007, Goswami, Frances et al. 2011, Norton, Luchyshyn et al. 2013). Further, high cardiorespiratory fitness has been linked to improved hippocampal (Erickson, Voss et al. 2011, Varma, Chuang et al. 2014), medial prefrontal (Colcombe, Erickson et al. 2003, Colcombe, Erickson et al. 2006, Kramer, Erickson et al. 2006), and anterior IC volumes (Peters, Dauvermann et al. 2009). In addition, cross-sectional studies show an association between fitness and brain function(Norton, Heinecke et al. 2015), particularly as it pertains to cognition (Dustman, Ruhling et al. 1984, Prakash, Voss et al. 2011, Voss, Heo et al. 2013, Dupuy, Gauthier et al. 2015, Gauthier, Lefort et al. 2015). The current results add additional novel outcomes that cardiorespiratory fitness positively affects MPFC and HC functional activity patterns to effortful tasks.

Nonetheless, the impact of fitness, while present in forebrain functional patterns to IHG, was not observed in HR responses. The paradoxical observation that fitness did not affect

the HR response to the IHG stimulus, but did preserve MPFC deactivation in this age group, may be due to challenges associated with the focus on middle-aged adults. Middle to late middle-age represents a period of physiological transition in many physiologic systems, including declines in fitness (Tanaka and Seals 2003) and brain mass (Raz, Ghisletta et al. 2010). Our recent study indicated that a reserve of cortical thickness is developed through higher levels of physical fitness in aging adults (Wood, Nikolov et al. 2016) although the trajectory of cortical atrophy with age persisted. The current results suggest that this cortical reserve induced by higher levels of physical activity may enhance cortical functional patterns as well. Nonetheless, advancing age often produces reductions in intrinsic HR (Craft and Schwartz 1995), and the parasympathomimetic effect of low-dose atropine (Lee, Picard et al. 2008), indicative of non-neural effects of age on cardiac function. Using independent group t-tests, and contrasting the current data with published data from previous research from our laboratory, we sought to further examine the age-dependent relationship between HR responses to IHG and the associated cortical activation patterns. In this retrospective analysis, we established that the current group of moderately-to-highly active individuals was somewhat younger (57±7 years) that a sedentary group reported previously (63±11 years, $p<0.05$) (Norton, Luchyshyn et al. 2013) who also generated a significantly lower HR response (2±2 bpm) to the same IHG intensity, compared with the current active participants (6±5 bpm, $p<0.05$). Importantly, the older sedentary group (Norton, Luchyshyn et al. 2013) failed to show MPFC or HC deactivation. Alternatively, young individuals (25±4 years; n=17) generated a much larger HR response (>10bpm) (Wong, Masse et al. 2007) with robust MPFC and HC deactivation, and IC activation. Therefore, the current data are consistent with a fitness-based preservation of forebrain activation patterns, but these neurologic benefits have little apparent benefit for HR responses to exercise due, likely, to a local age-related impairment of HR control.

In the current study, the MPFC engaged in significantly stronger connections with several brain regions when compared with baseline activity (Figure 5.6). Importantly, the bilateral IC appeared to correlate with MPFC activity. These results are consistent with known anatomical connections between the IC and MPFC, demonstrated in various

species (Augustine 1996, Ongur and Price 2000). In addition, our findings confirm the involvement of the IC and MPFC in HR control during effortful tasks previously reported in humans (Williamson, McColl et al. 2003, Wong, Masse et al. 2007), and rodents (Oppenheimer and Cechetto 1990). Age explains at least 10% of individual differences in functional connectivity (Boraxbekk, Salami et al. 2016) and the HC represents high sensitivity to age-related atrophy (Raz, Lindenberger et al. 2005, Cotman, Berchtold et al. 2007, Raz, Ghisletta et al. 2010). In this regard, the current group may be at the age where HC involvement in cardiovascular function declines.

5.5 Limitations

The current findings are based on cross-sectional brain imaging data. Additional prospective training studies in this age group are needed to see if elevations in cardiovagal function and cardiorespiratory function are linked with corresponding improvements in cortical neurocircuitry related to cardiovascular arousal.

In addition, there are determinants of cardiorespiratory fitness that account for variance beyond habitual physical activity such as genetics, which may account for about half of the variance in individual differences in fitness (Bouchard, An et al. 1999, Bouchard, Sarzynski et al. 2011). To our knowledge, no information exists regarding genetic linkages between brain function and cardiorespiratory function.

The current study focused solely on the HR outcomes during effortful IHG. However, measures of sympathetic nerve activity, also affected by the cortical autonomic network, may expose an additional outcome of interest. In particular, age elevates sympathetic outflow in many individuals (Seals, Taylor et al. 1994, Davy, DeSouza et al. 1998, Tanaka, Dinenno et al. 2000, Monahan, Dinenno et al. 2001, Monahan 2007) but this change can be mitigated by exercise training (Carter and Ray 2015). Moreover, IHG performed by older adults has been characterized by a greater increase in muscle sympathetic nerve activity, compensating for a smaller HR response. Therefore, it may be that the preservation of cortical activation patterns with high fitness in the current study exerted a positive influence over sympathetic outflow, rather than HR. Unfortunately, sympathetic nerve activity was not measured in the current study.

5.6 Conclusion

The current data indicate that high cardiorespiratory fitness sustained through middle-age contributes to the preservation of cortical circuitry associated with cardiovascular control. Overall, these findings support the hypothesis of a role for cardiorespiratory fitness to positively affect the cortical autonomic network, providing new insights into the mechanisms underlying preserved autonomic function and healthy brain aging in adults who engage in long-term regular exercise.

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Chapter 6

6 General Discussion

6.1 Perspectives

Given the economic, social, and personal burden associated with age-related neural deterioration, identifying cortical sites and patterns of the developing neurologic changes represents a necessary step from which strategies can be developed and tested to prevent declines in structural and functional brain health *before they begin*. The current observations support a role for sustained cardiorespiratory fitness in the maintenance and preservation of cortical tissue achieved early in life, which may minimize the risk for neurological impairment in senescence and reduce the frailty period of life.

6.2 Major Findings

This series of studies examined the potential benefit of sustained cardiorespiratory fitness on brain structure and autonomic function in a healthy human model. Overall, long-term training and high levels of cardiorespiratory fitness elicited, and sustained, greater cortical thickness over a large portion of the brain, with particular emphasis and relevance to the prefrontal cortex. In addition, physical activity preserved activation patterns within the medial prefrontal cortex and delayed the age-related decline in cortical circuitry associated with cardiovascular control. However, life-long exercise training had little effect on the rate of age-related cortical atrophy. In addition, the strength of the relationship between autonomic variables and cortical thickness was determined by age, and was not altered following adjustments for cardiorespiratory fitness. Therefore, the current results illustrate the potent effect of sustained cardiorespiratory fitness to develop cortical reserve beyond that provided by guideline-based activity, but does not eliminate the effect of age on cortical gray matter atrophy.

An initial important observation of this series of studies is the dominant effect of age on autonomic function and brain structure. In our second study, neither life-long exercise training nor guideline-based fitness had an impact on the rate of age-related cortical

atrophy. Our endurance-trained population showed a significant cortical "reserve" at every age beyond that of the healthy active group, yet the rate of decay with advancing age was still evident, and was not different from our healthy active group. Furthermore, in our third study, we observed that the thickness of the MPFC predicts chronic levels of high HRV and low MSNA; however, this correlation was not affected by inclusion of VO_{2max} in the regression model and was most strongly influenced by age alone.

If these observations reflect deterioration of autonomic cortical structure, the marked age-related decline and increased variability in the HR responsiveness to IHG becomes a further important observation. Taken together, this series of studies represents not only a continuum of risk for advancing age, but a continuum for fitness and its associated autonomic outcomes (Table 6.1). When compared with previously published data from our laboratory in young healthy, active individuals, the middle and older-aged trained adults in study four demonstrate a diminished HR response to a similar relative IHG tension ($p<0.005$). In addition, the older sedentary group from study one demonstrate a further diminished HR response when compared with young ($p<0.0001$). These differences are statistically significant, as determined by an independent group's t-test that contrasted the data from the current studies with those published earlier. This age-related difference, albeit significant, is not entirely unexpected given what we know about autonomic dysregulation with age. Mechanistically, this IHG protocol is designed to engage exercise-onset reflexive increases in HR that predominantly reflect reduced dominance of parasympathetic control. In this context, the larger the HR response, the greater evidence of efficient parasympathetic control. Therefore, the small HR responses in our older sedentary population, and smaller yet in the coronary artery disease patients, are likely a consequence of age-related impairment of parasympathetic outflow that is further negatively impacted by disease, but may be attenuated with physical fitness. Alternatively, age-dependent changes in the responsiveness of alpha- and beta-adrenergic, as well as cardiac muscarinic receptors may also explain this decrease in HR responsiveness and reflex bradycardia (Poller, Nedelka et al. 1997).

Nonetheless, the suppressed HR response in sedentary older adults appears to be consistent with the cortical patterns in response to the IHG task. In our first study, there

was an absence of deactivation associated with the (lack of) HR response to IHG in the MPFC in older subjects. However, we observed the preservation of expected activity patterns within the MPFC, HC, and bilateral insula in response to both the task, and the HR time course in our active (guideline and trained) middle and older aged groups from study four. These regions were specifically chosen for their known participation in cardiac adjustments to exercise and support the discrete patterns of activation consistently shown in young healthy subjects. Thus, these observations suggest that sustained cardiorespiratory fitness has the capacity to minimize the impact of age on the cortical autonomic circuitry, yet does not completely reverse the age-related neural decline in healthy humans.

Finally, evidence of a dose-response relationship with physical activity appears to exist in the maintenance of cortical mass and cardiovascular control across the middle to older aged period. This pattern raises questions regarding the maximal exercise-induced benefit possible across the adult age span, as well as the appropriate control group for such studies. In our first study, the older sedentary adults did not participate in any physical activity, which was associated with a lower HR response to the IHG task and a lack of cortical activation in expected autonomic regions. When compared to the trained group of the same age, despite a similar VO_{2max}, the improved fitness was associated with a larger HR response and preserved cortical response at the MPFC. Furthermore, when compared with the age-matched older sedentary group ($p<0.05$), our older trained group showed a preserved HR response equal to that of the middle-aged trained group ($p=0.4$) suggesting that when physical activity is continued through the early older years, cortical and autonomic function are preserved. Thus, sedentary activity is associated with a significant deterioration of cortical and autonomic function into the older years.

When comparing guideline-based fitness to long-term endurance training, our results from studies two and four indicate that long-term training and higher levels of cardiorespiratory fitness elicit and sustain, greater cortical thickness over a large portion of the brain than those following the age-appropriate guidelines for activity.

Interestingly, our population of middle and older aged adults, both those following the age appropriate guidelines for activity and those endurance-trained, show no differences in the HR response to the task and a preserved cortical response at the MPFC, despite a significant difference in age and fitness. This result appears to demonstrate a role for continued fitness through the crucial middle to older aged years with respect to preservation of HR control and cortical autonomic patterns, irrespective of activity level. A dose–response relationship between exercise duration/intensity and health-related quality of life has previously been reported, whereby the best outcomes are associated with moderate exercise (Larson, Wang et al. 2006).

However, despite high inter-individual variability, the MPFC factored importantly into the subtraction analyses when correlated with HR illustrating that those subjects with higher fitness (>50% of age-predicted maximum) had more deactivation than those subjects with lower fitness (<50%) in response to the exercise task. Therefore, higher cardiorespiratory fitness achieved through long-term training better preserved the age-related decline in cortical circuitry associated with cardiovascular control.

Overall, these data suggest that physical activity, beyond the age-appropriate parameters, may be better in terms of cortical structure, but it may only be necessary to follow the minimal recommended guidelines for benefits to be seen with autonomic function. These data engage the debate regarding the health benefits arising from physical fitness versus physical activity (Pogliaghi, Bellotti et al. 2014, Stathokostas, Dogra et al. 2015). In this context, it may be that cortical thickness is associated with the benefits rising from higher fitness whereas autonomic function is sensitive to a lower threshold level of activity.

6.3 Conclusion

Taken together, these results form a unique supportive concept towards understanding age-related autonomic impairment. These data illustrate the importance of limbic and forebrain structures in neural control of HR with specific new evidence supporting a link between structural cortical “reserve” and associated cortical function with long-term endurance training. Overall, these findings support the hypothesis of a role for cardiorespiratory fitness to positively alter these networks and brain regions, providing

new insights into the mechanisms underlying preserved autonomic function and healthy brain aging in adults who engage in long-term regular exercise.

6.4 References

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Table 6.1 Cortical and physiological results from all studies representing a continuum of risk for advancing age and reduced fitness with associated autonomic outcomes.

	YOUNG (GUIDELINE)	MIDDLE-AGED (GUIDELINE)	MIDDLE-AGED (TRAINED)	OLDER (TRAINED)	OLDER (SEDENTARY)	CAD (SEDENTARY)
N	17	12	41	25	23	17
AGE	25±4	58±9*+	53±6*	62±5*+	63±11*+	59±9*+
ΔHR	9±2	7±5	5±5*	6±4*	4±2*#	2±2*
VO_{2max}	49±8	38±7*+	55±10*#	39±10*+	38±7*+	26±5*+
MPFC (↓)	YES	YES	YES	YES	NO	NO
IC (↑)	YES	YES	YES	YES	YES	YES
HC (↓)	YES	YES	YES	YES	YES	NO

Values are mean ± SD. N = sample size; ΔHR = change in heart rate to 40% handgrip task; VO_{2max} = maximal aerobic capacity; MPFC = medial prefrontal cortex; IC = insula cortex; HC = hippocampus; (↓) = deactivation; (↑) = activation. * different from young ($p<0.005$); + different from middle-aged trained ($p<0.05$); # different from older trained ($p<0.05$).

Appendices

Appendix 1

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	-4	-75	15	-0.689	0.00014
Central Sulcus	-35	-26	47	-0.662	0.000311
Cingulate Gyrus	-9	-45	28	-0.761	0.00001
Cingulate Sulcus	-11	22	28	-0.742	0.000022
Collateral Sulcus	-28	-35	-10	-0.762	0.00001
Cuneus	-5	-77	17	-0.717	0.000056
Gyrus Rectus	-8	10	-11	-0.612	0.00116
Inferior Frontal Gyrus	-29	50	10	-0.723	0.000044
Inferior Frontal Sulcus	-43	24	30	-0.676	0.00021
Inferior Occipital Gyrus	-28	-85	-5	-0.758	0.000012
Inferior Parietal Lobe	-42	-56	39	-0.720	0.000049
Inferior Temporal Gyrus	-48	-56	-7	-0.707	0.000079
Inferior Temporal Sulcus	-49	-6	-14	-0.679	0.000188
Insula	-32	1	16	-0.790	0.000003
Intraparietal Sulcus	-26	-52	56	-0.714	0.000062
Lateral Occipitotemporal Gyrus	-40	-46	-17	-0.737	0.000027
Lateral Sulcus	-38	14	12	-0.761	0.00001
Medial Occipitotemporal Gyrus	-11	-56	-3	-0.708	0.000075
Medial Prefrontal Cortex	-12	42	12	-0.818	0.000001
Middle Frontal Gyrus	-27	49	10	-0.729	0.000036
Middle Occipital Gyrus	-27	-85	-4	-0.675	0.000216
Middle Temporal Gyrus	-46	-1	-15	-0.760	0.00001
Occipitotemporal Sulcus	-41	-27	-17	-0.672	0.000234
Olfactory Sulcus	-7	33	-13	-0.638	0.000608
Orbital Gyri	-18	9	-12	-0.757	0.000012
Orbital Sulci	-16	38	-11	-0.690	0.000133
Parahippocampal Gyrus	-24	-17	-21	-0.693	0.000124
Parieto-Occipital Sulcus	-5	-72	35	-0.632	0.0007
Postcentral Gyrus	-49	-20	45	-0.739	0.000024
Postcentral Sulcus	-55	-18	32	-0.755	0.000013
Precentral Gyrus	-37	-4	35	-0.767	0.000008
Precentral Sulcus	-19	-1	57	0.730	0.000034
Precuneus	-10	-46	27	-0.770	0.000007
Superior Frontal Gyrus	-12	42	12	-0.818	0.000001
Superior Frontal Sulcus	-21	14	43	-0.636	0.00063
Superior Occipital Gyrus	-14	-96	9	-0.747	0.000018
Superior Parietal Lobe	-9	-67	44	-0.662	0.000311
Superior Temporal Gyrus	-47	-9	-2	-0.682	0.000173
Superior Temporal Sulcus	-52	-21	-2	-0.685	0.000156
Supramarginal Gyrus	-52	-46	37	-0.716	0.000056
Transverse Occipital Sulcus	-29	-69	25	-0.628	0.000785
Uncus	-23	-17	-21	-0.697	0.000107

Left hemisphere full cortex correlation results on the basis of cortical thickness and burst frequency.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	-4	-75	15	-0.727	0.000038
Central Sulcus	-35	-26	47	-0.718	0.000053
Cingulate Gyrus	-9	-46	27	-0.794	0.000002
Cingulate Sulcus	-11	22	28	-0.773	0.000006
Collateral Sulcus	-28	-35	-10	-0.804	0.000001
Cuneus	-5	-77	17	-0.792	0.000002
Gyrus Rectus	-4	35	-13	-0.741	0.000022
Inferior Frontal Gyrus	-50	18	19	-0.761	0.00001
Inferior Frontal Sulcus	-39	25	24	-0.725	0.000042
Inferior Occipital Gyrus	-28	-85	-5	-0.783	0.000004
Inferior Parietal Lobe	-34	-46	41	-0.730	0.000034
Inferior Temporal Gyrus	-48	-56	-7	-0.767	0.000008
Inferior Temporal Sulcus	-54	-25	-16	-0.689	0.00014
Insula	-32	0	16	-0.815	0.000001
Intraparietal Sulcus	-19	-68	36	-0.754	0.000013
Lateral Occipitotemporal Gyrus	-40	-46	-17	-0.806	0.000001
Lateral Sulcus	-38	-11	18	-0.780	0.000004
Medial Occipitotemporal Gyrus	-11	-56	-3	-0.789	0.000003
Medial Prefrontal Cortex	-12	42	12	-0.820	0.000001
Middle Frontal Gyrus	-27	49	10	-0.742	0.000022
Middle Occipital Gyrus	-29	-84	-4	-0.716	0.000057
Middle Temporal Gyrus	-46	-1	-15	-0.757	0.000012
Occipitotemporal Sulcus	-39	-10	-24	-0.708	0.000075
Olfactory Sulcus	-7	33	-13	-0.695	0.000115
Orbital Gyri	-18	9	-12	-0.764	0.000009
Orbital Sulci	-16	38	-11	-0.729	0.000035
Parahippocampal Gyrus	-24	-17	-21	-0.717	0.000055
Parieto-Occipital Sulcus	-13	-63	48	-0.705	0.000084
Postcentral Gyrus	-5	-29	66	-0.775	0.000005
Postcentral Sulcus	-55	-18	32	-0.786	0.000003
Precentral Gyrus	-46	1	18	-0.767	0.000008
Precentral Sulcus	-19	-1	57	0.749	0.000016
Precuneus	-9	-46	27	-0.794	0.000002
Superior Frontal Gyrus	-12	42	12	-0.820	0.000001
Superior Frontal Sulcus	-22	8	44	-0.636	0.000631
Superior Occipital Gyrus	-14	-96	9	-0.791	0.000003
Superior Parietal Lobe	-21	-67	30	-0.704	0.000086
Superior Temporal Gyrus	-47	-9	-2	-0.749	0.000017
Superior Temporal Sulcus	-52	-20	-2	-0.685	0.000156
Supramarginal Gyrus	-46	-23	19	-0.723	0.000045
Transverse Occipital Sulcus	-27	-70	17	-0.710	0.000069
Uncus	-23	-17	-21	-0.719	0.00005

Left hemisphere full cortex correlation results on the basis of cortical thickness and burst incidence.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	-16	-55	6	0.458	0.000443
Central Sulcus	-34	-25	44	0.583	0.000003
Cingulate Gyrus	-9	-43	4	0.418	0.001496
Cingulate Sulcus	-14	-19	39	0.509	0.000073
Collateral Sulcus	-26	-67	-10	0.480	0.00021
Cuneus	-11	-94	10	0.417	0.001557
Gyrus Rectus	-5	12	-11	-0.371	0.005315
Inferior Frontal Gyrus	-44	37	-1	0.542	0.000019
Inferior Frontal Sulcus	-36	6	37	0.573	0.000005
Inferior Occipital Gyrus	-27	-90	-13	0.484	0.000179
Inferior Parietal Lobe	-44	-56	22	0.420	0.001416
Inferior Temporal Gyrus	-36	2	-31	0.455	0.000478
Inferior Temporal Sulcus	-55	-51	-6	0.625	0
Insula	-27	24	-2	0.491	0.000141
Intraparietal Sulcus	-29	-73	34	0.468	0.000316
Lateral Occipitotemporal Gyrus	-36	1	-32	0.524	0.00004
Lateral Sulcus	-36	-16	-3	0.528	0.000034
Medial Occipitotemporal Gyrus	-18	-37	-9	0.459	0.000429
Medial Prefrontal Cortex	-8	53	8	0.523	0.000042
Middle Frontal Gyrus	-32	24	38	0.547	0.000016
Middle Occipital Gyrus	-36	-80	10	0.393	0.002998
Middle Temporal Gyrus	-54	-50	-8	0.554	0.000011
Occipitotemporal Sulcus	-34	-48	-16	0.341	0.010876
Olfactory Sulcus	-5	48	-10	0.307	0.022668
Orbital Gyri	-27	37	-2	0.431	0.001015
Orbital Sulci	-27	40	-3	0.454	0.000504
Parahippocampal Gyrus	-27	-25	-17	-0.331	0.0137
Parieto-Occipital Sulcus	-19	-62	18	0.576	0.000004
Postcentral Gyrus	-35	-26	43	0.657	0
Postcentral Sulcus	-58	-17	21	0.501	0.000097
Precentral Gyrus	-51	-4	35	0.525	0.000039
Precentral Sulcus	-35	4	36	0.602	0.000001
Precuneus	-15	-55	7	0.508	0.000074
Superior Frontal Gyrus	-8	53	8	0.523	0.000042
Superior Frontal Sulcus	-19	25	45	0.636	0
Superior Occipital Gyrus	-12	-95	9	0.401	0.002434
Superior Parietal Lobe	-10	-68	44	0.517	0.000054
Superior Temporal Gyrus	-42	-27	7	0.459	0.000424
Superior Temporal Sulcus	-50	-10	-7	0.481	0.000203
Supramarginal Gyrus	-52	-45	39	0.678	0
Transverse Occipital Sulcus	-31	-77	19	0.391	0.003129
Uncus	-24	-14	-20	0.179	0.191934

Left hemisphere full cortex correlation results on the basis of cortical thickness and baroreflex sensitivity.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	-19	-58	6	0.418	0.00186
Central Sulcus	-11	-32	67	-0.388	0.004108
Cingulate Gyrus	-2	-8	28	0.481	0.000265
Cingulate Sulcus	-9	36	20	0.516	0.000076
Collateral Sulcus	-27	-68	-12	0.445	0.000833
Cuneus	-21	-85	24	0.474	0.000335
Gyrus Rectus	-7	11	-12	0.341	0.012594
Inferior Frontal Gyrus	-44	37	-1	0.484	0.000244
Inferior Frontal Sulcus	-39	13	32	0.458	0.000555
Inferior Occipital Gyrus	-14	-88	-17	0.400	0.002966
Inferior Parietal Lobe	-33	-46	40	0.429	0.001364
Inferior Temporal Gyrus	-50	-13	-23	0.449	0.000754
Inferior Temporal Sulcus	-55	-38	-11	0.349	0.010508
Insula	-30	11	16	0.499	0.000145
Intraparietal Sulcus	-42	-54	39	0.403	0.002762
Lateral Occipitotemporal Gyrus	-36	1	-32	0.387	0.004193
Lateral Sulcus	-37	-14	-5	0.482	0.00026
Medial Occipitotemporal Gyrus	-12	-87	-14	0.423	0.001615
Medial Prefrontal Cortex	-8	51	8	0.458	0.000565
Middle Frontal Gyrus	-26	43	23	0.486	0.000225
Middle Occipital Gyrus	-30	-83	-3	0.384	0.004544
Middle Temporal Gyrus	-49	-26	-1	0.484	0.000239
Occipitotemporal Sulcus	-46	-23	-20	0.382	0.004807
Olfactory Sulcus	-9	27	-12	0.410	0.002293
Orbital Gyri	-13	17	-14	0.390	0.003927
Orbital Sulci	-32	47	-3	0.403	0.002771
Parahippocampal Gyrus	-33	2	-33	0.321	0.018904
Parieto-Occipital Sulcus	-8	-59	48	0.460	0.000537
Postcentral Gyrus	-48	-17	31	0.474	0.00034
Postcentral Sulcus	-37	-34	40	0.485	0.000231
Precentral Gyrus	-37	-14	52	0.410	0.002274
Precentral Sulcus	-33	-9	46	0.442	0.000929
Precuneus	-12	-45	29	0.410	0.002286
Superior Frontal Gyrus	-7	50	38	0.493	0.000177
Superior Frontal Sulcus	-19	25	45	0.533	0.000041
Superior Occipital Gyrus	-22	-82	23	0.592	0.000003
Superior Parietal Lobe	-9	-45	63	-0.442	0.000919
Superior Temporal Gyrus	-48	2	-6	0.404	0.002703
Superior Temporal Sulcus	-48	-25	-2	0.514	0.000084
Supramarginal Gyrus	-52	-45	39	0.624	0.000001
Transverse Occipital Sulcus	-35	-81	24	0.418	0.001847
Uncus	-23	-15	-21	0.223	0.108889

Left hemisphere full cortex correlation results on the basis of cortical thickness and ln-high frequency.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	-14	-45	-1	0.279	0.043223
Central Sulcus	-34	-25	44	0.435	0.001145
Cingulate Gyrus	-4	-23	29	0.391	0.003826
Cingulate Sulcus	-14	-19	39	0.414	0.002056
Collateral Sulcus	-28	-38	-8	0.409	0.002336
Cuneus	-22	-84	23	0.456	0.000599
Gyrus Rectus	-5	12	-11	-0.356	0.008793
Inferior Frontal Gyrus	-45	35	-1	0.423	0.001619
Inferior Frontal Sulcus	-40	14	33	0.471	0.00037
Inferior Occipital Gyrus	-46	-63	-1	0.327	0.016955
Inferior Parietal Lobe	-41	-55	38	0.358	0.008551
Inferior Temporal Gyrus	-52	0	-24	0.471	0.000366
Inferior Temporal Sulcus	-55	-48	-9	0.393	0.003608
Insula	-34	-6	12	0.432	0.001246
Intraparietal Sulcus	-22	-62	53	0.392	0.003672
Lateral Occipitotemporal Gyrus	-36	2	-31	0.362	0.007818
Lateral Sulcus	-46	35	-1	0.438	0.001052
Medial Occipitotemporal Gyrus	-14	-51	-3	0.414	0.002055
Medial Prefrontal Cortex	-11	42	9	0.436	0.001093
Middle Frontal Gyrus	-26	43	23	0.493	0.000174
Middle Occipital Gyrus	-28	-84	-2	0.296	0.031384
Middle Temporal Gyrus	-49	-26	-1	0.432	0.001228
Occipitotemporal Sulcus	-44	-25	-18	0.288	0.036585
Olfactory Sulcus	-14	18	-12	0.304	0.026875
Orbital Gyri	-13	17	-14	0.397	0.003287
Orbital Sulci	-41	33	-7	0.320	0.019603
Parahippocampal Gyrus	-33	2	-33	0.312	0.023097
Parieto-Occipital Sulcus	-14	-73	44	0.413	0.002105
Postcentral Gyrus	-36	-26	42	0.476	0.000317
Postcentral Sulcus	-37	-34	40	0.462	0.000493
Precentral Gyrus	-9	-27	70	-0.366	0.00704
Precentral Sulcus	-33	-9	46	0.476	0.000313
Precuneus	-6	-52	10	0.347	0.010837
Superior Frontal Gyrus	-18	24	48	0.479	0.000288
Superior Frontal Sulcus	-19	35	43	0.506	0.00011
Superior Occipital Gyrus	-22	-82	23	0.521	0.000063
Superior Parietal Lobe	-14	-72	45	0.440	0.000978
Superior Temporal Gyrus	-50	-35	11	0.483	0.000248
Superior Temporal Sulcus	-48	-25	-2	0.446	0.000817
Supramarginal Gyrus	-52	-43	39	0.619	0.000001
Transverse Occipital Sulcus	-35	-80	25	0.342	0.01227
Uncus	-24	-14	-20	0.158	0.258131

Left hemisphere full cortex correlation results on the basis of cortical thickness and SD1.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	-8	-73	8	-0.427	0.001411
Central Sulcus	-43	-13	32	0.463	0.000484
Cingulate Gyrus	-6	36	7	0.554	0.000017
Cingulate Sulcus	-7	37	14	0.440	0.000989
Collateral Sulcus	-27	-68	-12	0.409	0.00234
Cuneus	-22	-84	23	0.550	0.00002
Gyrus Rectus	-5	12	-11	-0.336	0.01396
Inferior Frontal Gyrus	-45	35	-1	0.411	0.002253
Inferior Frontal Sulcus	-40	14	33	0.434	0.001151
Inferior Occipital Gyrus	-40	-65	-11	0.382	0.004725
Inferior Parietal Lobe	-42	-56	39	0.446	0.000806
Inferior Temporal Gyrus	-50	-13	-23	0.462	0.00049
Inferior Temporal Sulcus	-55	-39	-11	0.390	0.003897
Insula	-33	-5	12	0.420	0.001746
Intraparietal Sulcus	-30	-46	53	0.454	0.000644
Lateral Occipitotemporal Gyrus	-47	-24	-20	0.472	0.000362
Lateral Sulcus	-37	-14	-5	0.447	0.000801
Medial Occipitotemporal Gyrus	-10	-87	-15	0.371	0.006177
Medial Prefrontal Cortex	-6	36	7	0.554	0.000017
Middle Frontal Gyrus	-26	43	23	0.525	0.000054
Middle Occipital Gyrus	-29	-83	-2	0.348	0.010631
Middle Temporal Gyrus	-48	-36	3	0.474	0.000333
Occipitotemporal Sulcus	-46	-23	-20	0.474	0.000335
Olfactory Sulcus	-9	32	-12	0.293	0.03327
Orbital Gyri	-21	46	-4	0.440	0.000964
Orbital Sulci	-19	48	-4	0.434	0.001175
Parahippocampal Gyrus	-33	2	-33	0.356	0.008943
Parieto-Occipital Sulcus	-8	-59	48	0.350	0.010235
Postcentral Gyrus	-42	-13	31	0.467	0.000429
Postcentral Sulcus	-37	-34	40	0.505	0.000116
Precentral Gyrus	-30	-11	46	0.361	0.007959
Precentral Sulcus	-33	-9	46	0.441	0.000962
Precuneus	-8	-56	28	0.470	0.000389
Superior Frontal Gyrus	-10	63	5	0.546	0.000024
Superior Frontal Sulcus	-19	25	45	0.495	0.000162
Superior Occipital Gyrus	-22	-82	23	0.639	0
Superior Parietal Lobe	-27	-46	53	0.476	0.000311
Superior Temporal Gyrus	-51	-36	11	0.407	0.002517
Superior Temporal Sulcus	-48	-25	-4	0.478	0.000294
Supramarginal Gyrus	-52	-43	39	0.578	0.000006
Transverse Occipital Sulcus	-36	-80	24	0.331	0.015412
Uncus	-23	-16	-21	0.217	0.119327

Left hemisphere full cortex correlation results on the basis of cortical thickness and SDNN.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	-7	-71	8	-0.443	0.00089
Central Sulcus	-43	-13	32	0.496	0.000162
Cingulate Gyrus	-6	36	7	0.561	0.000012
Cingulate Sulcus	-17	-39	40	0.420	0.001737
Collateral Sulcus	-21	-84	-15	0.433	0.001198
Cuneus	-22	-84	23	0.592	0.000003
Gyrus Rectus	-5	12	-11	-0.342	0.012231
Inferior Frontal Gyrus	-45	35	-1	0.446	0.000815
Inferior Frontal Sulcus	-40	14	33	0.437	0.001069
Inferior Occipital Gyrus	-41	-59	-14	0.383	0.0047
Inferior Parietal Lobe	-41	-55	38	0.500	0.000137
Inferior Temporal Gyrus	-36	2	-31	0.499	0.000141
Inferior Temporal Sulcus	-55	-38	-11	0.421	0.001673
Insula	-35	13	14	0.524	0.000056
Intraparietal Sulcus	-27	-51	38	0.500	0.000137
Lateral Occipitotemporal Gyrus	-36	2	-31	0.499	0.000141
Lateral Sulcus	-40	-35	19	0.466	0.000436
Medial Occipitotemporal Gyrus	-12	-87	-14	0.398	0.003203
Medial Prefrontal Cortex	-6	36	7	0.561	0.000012
Middle Frontal Gyrus	-26	43	23	0.535	0.000037
Middle Occipital Gyrus	-29	-85	1	0.392	0.003706
Middle Temporal Gyrus	-48	-26	-3	0.453	0.000661
Occipitotemporal Sulcus	-46	-23	-20	0.501	0.000132
Olfactory Sulcus	-9	32	-12	0.308	0.024758
Orbital Gyri	-21	46	-4	0.475	0.000321
Orbital Sulci	-19	48	-4	0.466	0.000436
Parahippocampal Gyrus	-33	2	-33	0.346	0.01104
Parieto-Occipital Sulcus	-12	-71	47	0.416	0.001934
Postcentral Gyrus	-43	-14	29	0.521	0.000063
Postcentral Sulcus	-25	-39	53	0.561	0.000013
Precentral Gyrus	-31	-11	46	0.399	0.003081
Precentral Sulcus	-33	-9	46	0.592	0.000003
Precuneus	-8	-57	29	0.435	0.001149
Superior Frontal Gyrus	-10	63	5	0.546	0.000023
Superior Frontal Sulcus	-19	6	54	0.535	0.000037
Superior Occipital Gyrus	-22	-82	23	0.675	0
Superior Parietal Lobe	-29	-41	49	0.529	0.000047
Superior Temporal Gyrus	-51	-36	11	0.496	0.000157
Superior Temporal Sulcus	-48	-25	-2	0.513	0.000085
Supramarginal Gyrus	-52	-43	39	0.623	0.000001
Transverse Occipital Sulcus	-23	-77	22	0.429	0.001333
Uncus	-23	-16	-21	0.209	0.132587

Left hemisphere full cortex correlation results on the basis of cortical thickness and total power.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	-4	-75	15	-0.632	0.000708
Central Sulcus	-34	-27	46	-0.600	0.001525
Cingulate Gyrus	-9	-45	28	-0.714	0.00006
Cingulate Sulcus	-11	22	28	-0.736	0.000027
Collateral Sulcus	-30	-34	-10	-0.696	0.00011
Cuneus	-5	-77	17	-0.662	0.00031
Gyrus Rectus	-6	55	-1	-0.621	0.000921
Inferior Frontal Gyrus	-50	18	19	-0.630	0.000737
Inferior Frontal Sulcus	-39	24	25	-0.633	0.000691
Inferior Occipital Gyrus	-28	-85	-5	-0.663	0.000308
Inferior Parietal Lobe	-42	-56	39	-0.685	0.000156
Inferior Temporal Gyrus	-39	0	-32	-0.617	0.001029
Inferior Temporal Sulcus	-61	-27	-8	-0.633	0.000678
Insula	-32	0	16	-0.708	0.000075
Intraparietal Sulcus	-26	-52	56	-0.683	0.00017
Lateral Occipitotemporal Gyrus	-40	-46	-17	-0.665	0.00029
Lateral Sulcus	-38	14	12	-0.668	0.00026
Medial Occipitotemporal Gyrus	-11	-56	-3	-0.681	0.00018
Medial Prefrontal Cortex	-11	42	11	-0.750	0.000016
Middle Frontal Gyrus	-43	24	30	-0.618	0.001004
Middle Occipital Gyrus	-39	-66	14	-0.636	0.00064
Middle Temporal Gyrus	-47	1	-15	-0.669	0.000256
Occipitotemporal Sulcus	-41	-27	-17	-0.610	0.001203
Olfactory Sulcus	-7	33	-13	-0.563	0.003379
Orbital Gyri	-18	9	-12	-0.669	0.000253
Orbital Sulci	-16	38	-11	-0.663	0.000304
Parahippocampal Gyrus	-24	-17	-21	-0.575	0.002659
Parieto-Occipital Sulcus	-20	-59	13	-0.601	0.001502
Postcentral Gyrus	-49	-20	45	-0.688	0.000145
Postcentral Sulcus	-25	-43	51	-0.638	0.000601
Precentral Gyrus	-7	-26	48	-0.663	0.000302
Precentral Sulcus	-19	-1	57	0.707	0.000078
Precuneus	-11	-52	31	-0.697	0.000107
Superior Frontal Gyrus	-6	37	48	-0.753	0.000014
Superior Frontal Sulcus	-13	54	28	-0.605	0.001341
Superior Occipital Gyrus	-14	-96	9	-0.645	0.0005
Superior Parietal Lobe	-28	-64	43	0.641	0.000559
Superior Temporal Gyrus	-47	-9	-2	-0.671	0.000239
Superior Temporal Sulcus	-52	-21	-2	-0.724	0.000044
Supramarginal Gyrus	-52	-46	37	-0.625	0.000838
Transverse Occipital Sulcus	-30	-72	22	-0.553	0.004167
Uncus	-24	-17	-21	-0.575	0.002659

Left hemisphere full cortex correlation results on the basis of cortical thickness and total MSNA.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	15	-91	-6	-0.699	0.000101
Central Sulcus	29	-28	46	-0.740	0.000023
Cingulate Gyrus	13	35	18	-0.759	0.000011
Cingulate Sulcus	12	40	20	-0.783	0.000004
Collateral Sulcus	17	-87	-15	-0.841	0
Cuneus	10	-87	-1	-0.743	0.000021
Gyrus Rectus	5	24	-15	-0.672	0.000233
Inferior Frontal Gyrus	47	32	6	-0.839	0
Inferior Frontal Sulcus	40	39	9	-0.681	0.000176
Inferior Occipital Gyrus	15	-88	-15	-0.816	0.000001
Inferior Parietal Lobe	46	-51	22	-0.701	0.000094
Inferior Temporal Gyrus	50	-11	-23	-0.767	0.000008
Inferior Temporal Sulcus	54	-14	-19	-0.709	0.000072
Insula	29	17	14	-0.822	0
Intraparietal Sulcus	25	-62	34	-0.705	0.000084
Lateral Occipitotemporal Gyrus	40	-46	-15	-0.747	0.000018
Lateral Sulcus	25	13	-7	-0.775	0.000005
Medial Occipitotemporal Gyrus	14	-67	-10	-0.781	0.000004
Medial Prefrontal Cortex	11	50	6	-0.807	0.000001
Middle Frontal Gyrus	37	43	12	-0.766	0.000008
Middle Occipital Gyrus	30	-81	-3	-0.707	0.000078
Middle Temporal Gyrus	51	-51	4	-0.735	0.000029
Occipitotemporal Sulcus	32	-46	-14	-0.727	0.000039
Olfactory Sulcus	14	17	-9	-0.696	0.000111
Orbital Gyri	26	19	-5	-0.748	0.000017
Orbital Sulci	32	31	-2	-0.757	0.000012
Parahippocampal Gyrus	28	2	-34	-0.733	0.000031
Parieto-Occipital Sulcus	10	-61	52	-0.798	0.000002
Postcentral Gyrus	18	-36	67	-0.759	0.000011
Postcentral Sulcus	51	-19	35	-0.705	0.000082
Precentral Gyrus	48	-10	38	0.653	0.000398
Precentral Sulcus	45	8	26	-0.705	0.000082
Precuneus	9	-53	29	-0.760	0.000011
Superior Frontal Gyrus	11	50	6	-0.807	0.000001
Superior Frontal Sulcus	23	54	15	-0.749	0.000017
Superior Occipital Gyrus	15	-87	15	-0.763	0.000009
Superior Parietal Lobe	23	-64	33	-0.723	0.000044
Superior Temporal Gyrus	51	-18	6	-0.742	0.000022
Superior Temporal Sulcus	43	-39	5	-0.726	0.00004
Supramarginal Gyrus	50	-44	38	-0.700	0.000098
Transverse Occipital Sulcus	31	-74	21	-0.762	0.00001
Uncus	22	-13	-18	-0.520	0.00773

Right hemisphere full cortex correlation results on the basis of cortical thickness and burst frequency.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	15	-91	-6	-0.720	0.00005
Central Sulcus	29	-28	46	-0.802	0.000001
Cingulate Gyrus	10	-5	42	-0.788	0.000003
Cingulate Sulcus	9	43	22	-0.786	0.000003
Collateral Sulcus	17	-87	-15	-0.874	0
Cuneus	10	-87	-1	-0.778	0.000005
Gyrus Rectus	5	24	-15	-0.728	0.000036
Inferior Frontal Gyrus	47	32	6	-0.828	0
Inferior Frontal Sulcus	40	39	9	-0.699	0.000101
Inferior Occipital Gyrus	15	-88	-15	-0.847	0
Inferior Parietal Lobe	46	-51	21	-0.674	0.000222
Inferior Temporal Gyrus	50	-12	-21	-0.770	0.000007
Inferior Temporal Sulcus	56	-33	-10	-0.733	0.00003
Insula	29	17	14	-0.816	0.000001
Intraparietal Sulcus	25	-62	34	-0.767	0.000008
Lateral Occipitotemporal Gyrus	40	-46	-13	-0.783	0.000004
Lateral Sulcus	25	18	-7	-0.761	0.00001
Medial Occipitotemporal Gyrus	10	-87	-1	-0.778	0.000005
Medial Prefrontal Cortex	11	50	6	-0.787	0.000003
Middle Frontal Gyrus	37	47	11	-0.783	0.000004
Middle Occipital Gyrus	30	-81	-3	-0.714	0.000062
Middle Temporal Gyrus	61	-37	-2	-0.751	0.000015
Occipitotemporal Sulcus	41	-39	-17	-0.760	0.00001
Olfactory Sulcus	14	17	-9	-0.723	0.000044
Orbital Gyri	23	35	-5	-0.763	0.000009
Orbital Sulci	32	31	-2	-0.786	0.000003
Parahippocampal Gyrus	28	2	-34	-0.715	0.000059
Parieto-Occipital Sulcus	10	-61	52	-0.856	0
Postcentral Gyrus	20	-35	66	-0.813	0.000001
Postcentral Sulcus	40	-31	58	-0.723	0.000044
Precentral Gyrus	30	-24	49	-0.642	0.000542
Precentral Sulcus	26	-8	48	-0.742	0.000022
Precuneus	4	-64	29	-0.717	0.000056
Superior Frontal Gyrus	10	53	5	-0.819	0.000001
Superior Frontal Sulcus	23	54	15	-0.726	0.00004
Superior Occipital Gyrus	15	-87	15	-0.763	0.000009
Superior Parietal Lobe	24	-63	33	-0.751	0.000015
Superior Temporal Gyrus	51	-16	6	-0.767	0.000008
Superior Temporal Sulcus	43	-38	7	-0.768	0.000007
Supramarginal Gyrus	40	-42	38	-0.761	0.00001
Transverse Occipital Sulcus	31	-74	21	-0.780	0.000004
Uncus	22	-13	-18	-0.541	0.005272

Right hemisphere full cortex correlation results on the basis of cortical thickness and burst incidence.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	16	-68	6	0.420	0.001404
Central Sulcus	24	-26	61	0.459	0.000425
Cingulate Gyrus	4	9	31	0.454	0.000496
Cingulate Sulcus	10	47	19	0.423	0.001279
Collateral Sulcus	35	-13	-25	0.457	0.000453
Cuneus	9	-89	10	0.441	0.000757
Gyrus Rectus	6	33	-10	0.444	0.000684
Inferior Frontal Gyrus	38	40	9	0.508	0.000074
Inferior Frontal Sulcus	39	24	22	0.492	0.000137
Inferior Occipital Gyrus	20	-95	-5	0.384	0.003785
Inferior Parietal Lobe	43	-63	34	0.496	0.000116
Inferior Temporal Gyrus	46	-5	-29	0.510	0.000069
Inferior Temporal Sulcus	56	-18	-15	0.445	0.000674
Insula	31	-21	17	0.550	0.000013
Intraparietal Sulcus	23	-61	54	0.415	0.001641
Lateral Occipitotemporal Gyrus	36	-10	-26	0.539	0.000022
Lateral Sulcus	23	15	-10	0.597	0.000002
Medial Occipitotemporal Gyrus	8	-62	-2	0.487	0.000164
Medial Prefrontal Cortex	10	49	3	0.442	0.000733
Middle Frontal Gyrus	25	49	6	0.566	0.000007
Middle Occipital Gyrus	44	-71	3	0.456	0.000463
Middle Temporal Gyrus	52	-7	-16	0.446	0.000645
Occipitotemporal Sulcus	38	-9	-25	0.432	0.000999
Olfactory Sulcus	12	28	-10	0.430	0.001044
Orbital Gyri	20	34	-8	0.511	0.000068
Orbital Sulci	29	36	-1	0.550	0.000014
Parahippocampal Gyrus	29	-9	-23	0.486	0.000171
Parieto-Occipital Sulcus	20	-63	20	0.688	0
Postcentral Gyrus	36	-33	46	0.551	0.000013
Postcentral Sulcus	48	-23	35	0.515	0.000058
Precentral Gyrus	25	-26	62	0.471	0.00028
Precentral Sulcus	35	4	29	0.450	0.000563
Precuneus	6	-70	39	0.479	0.000214
Superior Frontal Gyrus	19	42	35	0.602	0.000001
Superior Frontal Sulcus	21	28	39	0.492	0.000137
Superior Occipital Gyrus	8	-95	3	0.373	0.005075
Superior Parietal Lobe	30	-48	51	0.447	0.000626
Superior Temporal Gyrus	31	-21	17	0.550	0.000013
Superior Temporal Sulcus	45	-13	-11	0.502	0.000095
Supramarginal Gyrus	57	-15	18	0.527	0.000036
Transverse Occipital Sulcus	25	-70	29	0.486	0.000168
Uncus	23	-12	-20	0.350	0.008742

Right hemisphere full cortex correlation results on the basis of cortical thickness and baroreflex sensitivity.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	17	-52	8	0.461	0.000518
Central Sulcus	54	-6	36	0.406	0.002546
Cingulate Gyrus	10	36	12	0.488	0.000211
Cingulate Sulcus	9	-46	60	-0.459	0.000553
Collateral Sulcus	17	-86	-14	0.447	0.000795
Cuneus	9	-89	10	0.428	0.001406
Gyrus Rectus	6	51	-7	0.372	0.006054
Inferior Frontal Gyrus	47	9	23	0.515	0.000079
Inferior Frontal Sulcus	39	23	23	0.423	0.001602
Inferior Occipital Gyrus	15	-85	-12	0.442	0.000933
Inferior Parietal Lobe	38	-59	36	0.455	0.000625
Inferior Temporal Gyrus	53	-20	-19	0.412	0.002149
Inferior Temporal Sulcus	58	-26	-12	0.444	0.000855
Insula	31	13	7	0.560	0.000013
Intraparietal Sulcus	35	-56	36	0.518	0.000071
Lateral Occipitotemporal Gyrus	36	-9	-26	0.521	0.000064
Lateral Sulcus	39	-23	20	0.489	0.000202
Medial Occipitotemporal Gyrus	20	-65	-8	0.427	0.001442
Medial Prefrontal Cortex	10	48	1	0.562	0.000012
Middle Frontal Gyrus	26	49	5	0.515	0.00008
Middle Occipital Gyrus	44	-71	3	0.372	0.006074
Middle Temporal Gyrus	54	-47	6	0.443	0.000884
Occipitotemporal Sulcus	37	-9	-26	0.516	0.000076
Olfactory Sulcus	13	28	-10	0.486	0.000224
Orbital Gyri	14	28	-12	0.548	0.000022
Orbital Sulci	14	52	-8	0.470	0.000379
Parahippocampal Gyrus	30	-1	-34	0.314	0.021909
Parieto-Occipital Sulcus	20	-63	20	0.514	0.000081
Postcentral Gyrus	42	-27	38	0.551	0.000019
Postcentral Sulcus	41	-36	39	0.509	0.000099
Precentral Gyrus	8	-25	70	0.385	0.004462
Precentral Sulcus	20	-5	59	0.459	0.000542
Precuneus	12	-49	33	0.449	0.000743
Superior Frontal Gyrus	10	48	1	0.562	0.000012
Superior Frontal Sulcus	25	50	27	0.463	0.00048
Superior Occipital Gyrus	12	-89	11	0.511	0.000092
Superior Parietal Lobe	26	-71	21	0.404	0.002723
Superior Temporal Gyrus	61	-31	12	0.472	0.000365
Superior Temporal Sulcus	45	-14	-9	0.439	0.000999
Supramarginal Gyrus	55	-42	29	0.526	0.000053
Transverse Occipital Sulcus	29	-70	19	0.442	0.000917
Uncus	24	-19	-19	0.333	0.014862

Right hemisphere full cortex correlation results on the basis of cortical thickness and ln-high frequency.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	12	-98	-3	0.440	0.000983
Central Sulcus	54	-6	36	0.416	0.001933
Cingulate Gyrus	8	35	14	0.512	0.000088
Cingulate Sulcus	8	50	28	0.423	0.001613
Collateral Sulcus	34	-9	-27	0.523	0.000058
Cuneus	21	-82	33	0.445	0.000841
Gyrus Rectus	6	39	-15	0.387	0.004247
Inferior Frontal Gyrus	27	53	1	0.415	0.001994
Inferior Frontal Sulcus	45	35	6	0.388	0.004115
Inferior Occipital Gyrus	45	-59	-7	0.354	0.009221
Inferior Parietal Lobe	42	-64	32	0.548	0.000022
Inferior Temporal Gyrus	31	2	-33	0.404	0.002731
Inferior Temporal Sulcus	57	-22	-13	0.444	0.000876
Insula	37	16	14	0.582	0.000005
Intraparietal Sulcus	47	-53	33	0.479	0.000286
Lateral Occipitotemporal Gyrus	35	-9	-27	0.507	0.000107
Lateral Sulcus	38	14	13	0.643	0
Medial Occipitotemporal Gyrus	21	-67	-8	0.458	0.000566
Medial Prefrontal Cortex	10	48	1	0.549	0.000021
Middle Frontal Gyrus	32	1	55	0.494	0.000171
Middle Occipital Gyrus	29	-83	-2	0.290	0.034953
Middle Temporal Gyrus	54	-47	6	0.474	0.000337
Occipitotemporal Sulcus	37	-9	-26	0.492	0.000184
Olfactory Sulcus	13	29	-11	0.405	0.002636
Orbital Gyri	14	27	-12	0.459	0.000551
Orbital Sulci	29	36	-1	0.393	0.00363
Parahippocampal Gyrus	31	-9	-27	0.371	0.006203
Parieto-Occipital Sulcus	11	-60	53	0.455	0.000613
Postcentral Gyrus	42	-28	38	0.512	0.00009
Postcentral Sulcus	41	-38	39	0.526	0.000053
Precentral Gyrus	53	-5	37	0.504	0.000121
Precentral Sulcus	44	2	23	0.454	0.000645
Precuneus	8	-60	20	0.348	0.010699
Superior Frontal Gyrus	15	9	57	0.581	0.000005
Superior Frontal Sulcus	31	1	55	0.496	0.000158
Superior Occipital Gyrus	24	-85	12	0.412	0.002169
Superior Parietal Lobe	9	-58	54	0.479	0.000289
Superior Temporal Gyrus	61	-30	11	0.588	0.000004
Superior Temporal Sulcus	49	2	-12	0.420	0.001751
Supramarginal Gyrus	55	-42	29	0.631	0
Transverse Occipital Sulcus	29	-70	19	0.348	0.010561
Uncus	22	-13	-18	0.307	0.025303

Right hemisphere full cortex correlation results on the basis of cortical thickness and SD1.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	12	-98	-3	0.453	0.000665
Central Sulcus	36	-22	41	0.408	0.002393
Cingulate Gyrus	10	36	12	0.502	0.000126
Cingulate Sulcus	11	-39	49	0.456	0.000596
Collateral Sulcus	29	-32	-18	0.435	0.001145
Cuneus	23	-78	33	0.469	0.000401
Gyrus Rectus	6	51	-7	0.471	0.000369
Inferior Frontal Gyrus	45	11	11	0.464	0.000468
Inferior Frontal Sulcus	39	23	23	0.387	0.00422
Inferior Occipital Gyrus	15	-85	-12	0.420	0.001723
Inferior Parietal Lobe	42	-62	35	0.506	0.000109
Inferior Temporal Gyrus	44	-40	-14	0.460	0.000527
Inferior Temporal Sulcus	53	-21	-16	0.473	0.000342
Insula	34	-17	21	0.542	0.000028
Intraparietal Sulcus	29	-55	48	0.448	0.000763
Lateral Occipitotemporal Gyrus	41	-41	-14	0.500	0.000135
Lateral Sulcus	44	11	10	0.488	0.000208
Medial Occipitotemporal Gyrus	18	-66	-9	0.451	0.000704
Medial Prefrontal Cortex	10	49	3	0.607	0.000001
Middle Frontal Gyrus	24	50	7	0.493	0.000178
Middle Occipital Gyrus	20	-95	-5	0.364	0.007345
Middle Temporal Gyrus	45	-15	-10	0.462	0.0005
Occipitotemporal Sulcus	34	-54	-14	0.450	0.000729
Olfactory Sulcus	13	29	-11	0.467	0.000422
Orbital Gyri	14	28	-12	0.535	0.000037
Orbital Sulci	14	50	-9	0.449	0.000745
Parahippocampal Gyrus	29	-10	-23	0.396	0.003348
Parieto-Occipital Sulcus	12	-60	53	0.518	0.000072
Postcentral Gyrus	42	-26	39	0.542	0.000028
Postcentral Sulcus	41	-36	39	0.485	0.000232
Precentral Gyrus	8	-25	70	0.449	0.000754
Precentral Sulcus	46	8	24	0.431	0.001269
Precuneus	12	-48	33	0.441	0.00096
Superior Frontal Gyrus	10	49	3	0.607	0.000001
Superior Frontal Sulcus	22	28	38	0.413	0.002098
Superior Occipital Gyrus	26	-87	9	0.467	0.000416
Superior Parietal Lobe	26	-71	21	0.452	0.00068
Superior Temporal Gyrus	48	4	-7	0.458	0.000564
Superior Temporal Sulcus	45	-14	-9	0.474	0.000332
Supramarginal Gyrus	56	-27	30	0.522	0.00006
Transverse Occipital Sulcus	27	-70	20	0.491	0.000192
Uncus	23	-12	-20	0.304	0.027101

Right hemisphere full cortex correlation results on the basis of cortical thickness and SDNN.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	12	-98	-3	0.490	0.000194
Central Sulcus	35	-21	39	0.462	0.000502
Cingulate Gyrus	7	-38	38	0.473	0.000351
Cingulate Sulcus	11	-39	49	0.481	0.000267
Collateral Sulcus	29	-32	-18	0.469	0.000401
Cuneus	23	-77	34	0.466	0.000438
Gyrus Rectus	6	51	-7	0.472	0.000357
Inferior Frontal Gyrus	43	22	20	0.445	0.00084
Inferior Frontal Sulcus	39	25	23	0.456	0.000608
Inferior Occipital Gyrus	15	-85	-12	0.450	0.000722
Inferior Parietal Lobe	43	-63	34	0.597	0.000002
Inferior Temporal Gyrus	44	-40	-14	0.489	0.000202
Inferior Temporal Sulcus	54	-21	-14	0.559	0.000013
Insula	34	-17	21	0.607	0.000001
Intraparietal Sulcus	47	-53	33	0.468	0.000406
Lateral Occipitotemporal Gyrus	41	-40	-15	0.552	0.000018
Lateral Sulcus	38	14	13	0.561	0.000012
Medial Occipitotemporal Gyrus	18	-66	-9	0.503	0.000123
Medial Prefrontal Cortex	10	49	3	0.591	0.000003
Middle Frontal Gyrus	24	50	7	0.527	0.00005
Middle Occipital Gyrus	30	-82	-1	0.383	0.004669
Middle Temporal Gyrus	45	-15	-10	0.528	0.000049
Occipitotemporal Sulcus	37	-9	-26	0.472	0.000365
Olfactory Sulcus	13	29	-11	0.521	0.000064
Orbital Gyri	14	28	-12	0.568	0.000009
Orbital Sulci	38	37	-4	0.477	0.000301
Parahippocampal Gyrus	29	-9	-23	0.396	0.003361
Parieto-Occipital Sulcus	12	-60	53	0.538	0.000033
Postcentral Gyrus	42	-28	38	0.540	0.00003
Postcentral Sulcus	42	-35	39	0.484	0.000244
Precentral Gyrus	8	-25	70	0.451	0.000711
Precentral Sulcus	24	-4	50	0.424	0.001538
Precuneus	11	-48	35	0.499	0.000143
Superior Frontal Gyrus	10	49	3	0.591	0.000003
Superior Frontal Sulcus	22	28	38	0.490	0.000195
Superior Occipital Gyrus	24	-89	11	0.474	0.000339
Superior Parietal Lobe	17	-65	43	0.506	0.000112
Superior Temporal Gyrus	53	-18	7	0.465	0.000448
Superior Temporal Sulcus	45	-14	-9	0.527	0.000051
Supramarginal Gyrus	55	-42	29	0.576	0.000006
Transverse Occipital Sulcus	23	-77	34	0.466	0.000438
Uncus	23	-12	-20	0.304	0.026957

Right hemisphere full cortex correlation results on the basis of cortical thickness and total power.

	TAL X	TAL Y	TAL Z	r	p
Calcarine Sulcus	11	-98	0	-0.649	0.000454
Central Sulcus	30	-28	46	-0.689	0.000141
Cingulate Gyrus	5	-45	10	-0.709	0.000072
Cingulate Sulcus	9	43	30	-0.768	0.000007
Collateral Sulcus	30	-44	-9	-0.760	0.000011
Cuneus	23	-79	32	-0.728	0.000038
Gyrus Rectus	6	26	-16	-0.650	0.00044
Inferior Frontal Gyrus	49	30	4	-0.792	0.000002
Inferior Frontal Sulcus	46	34	4	-0.674	0.000221
Inferior Occipital Gyrus	15	-88	-15	-0.729	0.000036
Inferior Parietal Lobe	42	-65	19	0.651	0.00042
Inferior Temporal Gyrus	49	-8	-22	-0.664	0.000294
Inferior Temporal Sulcus	54	-14	-19	-0.627	0.000798
Insula	25	17	-6	-0.733	0.000031
Intraparietal Sulcus	25	-62	34	-0.615	0.001073
Lateral Occipitotemporal Gyrus	28	2	-34	-0.749	0.000017
Lateral Sulcus	25	17	-7	-0.750	0.000016
Medial Occipitotemporal Gyrus	14	-67	-10	-0.685	0.000158
Medial Prefrontal Cortex	11	50	6	-0.743	0.000021
Middle Frontal Gyrus	37	47	11	-0.750	0.000016
Middle Occipital Gyrus	30	-81	-3	-0.610	0.001199
Middle Temporal Gyrus	51	-51	4	-0.636	0.000634
Occipitotemporal Sulcus	33	-47	-14	-0.657	0.000362
Olfactory Sulcus	13	26	-9	-0.622	0.0009
Orbital Gyri	25	17	-6	-0.733	0.000031
Orbital Sulci	37	44	-4	-0.688	0.000145
Parahippocampal Gyrus	28	2	-34	-0.749	0.000017
Parieto-Occipital Sulcus	10	-61	52	-0.726	0.00004
Postcentral Gyrus	29	-34	62	-0.767	0.000008
Postcentral Sulcus	40	-31	58	-0.628	0.00077
Precentral Gyrus	5	-22	52	-0.582	0.002293
Precentral Sulcus	18	-13	58	-0.618	0.000997
Precuneus	8	-55	30	-0.710	0.000069
Superior Frontal Gyrus	10	52	6	-0.768	0.000008
Superior Frontal Sulcus	31	9	52	-0.741	0.000023
Superior Occipital Gyrus	24	-79	30	-0.692	0.000128
Superior Parietal Lobe	23	-64	33	-0.649	0.000443
Superior Temporal Gyrus	51	-18	6	-0.674	0.000223
Superior Temporal Sulcus	48	-43	6	-0.690	0.000137
Supramarginal Gyrus	40	-42	38	-0.717	0.000054
Transverse Occipital Sulcus	31	-74	21	-0.671	0.000242
Uncus	22	-13	-18	-0.529	0.006551

Right hemisphere full cortex correlation results on the basis of cortical thickness and total MSNA.

Appendix 2 Ethics Approval

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Kevin Shoemaker
Review Number: 17810
Review Level: Delegated
Approved Local Adult Participants: 200
Approved Local Minor Participants: 0
Protocol Title: Architecture of Cortical Somatosensory and Autonomic Neural Networks Supporting Muscular Movement: Impacts of Vascular Disease and Exercise Interventions.
Department & Institution: Kinesiology, University of Western Ontario
Sponsor: Canadian Institutes of Health Research

Ethics Approval Date: August 12, 2011 **Expiry Date:** December 31, 2016
Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Revised Letter of Information & Consent		
Other	Revised recruitment letter	

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (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 revision(s) or amendment(s) on the approval date noted above. The membership of this REB 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.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion 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

___ Janice Sutherland (jsuther@uwo.ca)	___ Grace Kelly (grace.kelly@uwo.ca)	✓ Shantel Walcott (swalcott@uwo.ca)

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Curriculum Vitae

EDUCATION AND TRAINING:

Jan 2013 – Present	**PhD Candidate** Department of Kinesiology, Integrative Physiology, Neurovascular Research Laboratory The University of Western Ontario, London, ON *Thesis: Neuroprotective Effect of Exercise* *Supervisor: Dr. J Kevin Shoemaker*
Sept 2008 – 08/2010	**Masters of Science** Department of Kinesiology, Integrative Physiology, Neurovascular Research Laboratory The University of Western Ontario, London, ON *Thesis: Forebrain Cortical Alterations with Hypertension* *Supervisor: Dr. J Kevin Shoemaker*
September 2009	**Invited fMRI Visiting Fellowship** Martinos Center for Biomedical Imaging; Boston, Massachusetts
Sept 2004 – 05/2008	**Bachelor of Arts; Honors Specialization** Department of Kinesiology, Faculty of Health Sciences The University of Western Ontario, London, ON

ACADEMIC AWARDS AND ACCOLADES:

		Value
2016	Recognition of Achievement: Outstanding Contribution to Teaching	Certificate
2015	CSEP Oral Presentation Graduate Award Finalist	$500.00
2015	American Autonomic Society Travel Fellowship	$2,000.00
2015	Advanced Teaching Program	Certificate
2015	Certificate in University Teaching and Learning	Certificate
2014	3-Minute-Thesis Competition Finalist	University-level
2009	NSERC Alexander Graham Bell Scholarship – CGS M	$17,500.00
2008	NSERC Undergraduate Student Research Award	$4,500.00
2008	The University of Western Ontario Dean's Honor List	Average >85%
2004	The University of Western Ontario Scholarship of Distinction	$2,500.00

EXPERIENCE:

Research Experience

09/2010 – 12/2012	**Neuroimaging Research Assistant** Neurovascular Research Laboratory, Department of Kinesiology The University of Western Ontario, London, ON
01/2008 – 09/2008	**Research Assistant** Neurovascular Research Laboratory, Department of Kinesiology The University of Western Ontario, London, ON
09/2006 - 04/2008	**Research Assistant** Exercise Nutrition Laboratory, Department of Kinesiology The University of Western Ontario, London, ON

Teaching Experience

09/2009 – Present	**Wellness Instructor** IVEY Spencer Leadership Centre, London, ON
02/2016 – 04/2016	**Part-Time Faculty, Yoga Instructor**

	Department of Kinesiology, Western University, London ON
09/2013 – 04/2015	**Graduate Bioscience Coordinator**
	Kinesiology Graduate Students
	The University of Western Ontario, London, ON
04/2014 – 04/2014	**Invited Guest Lecture, Exercise Physiology**
	Fanshawe College, London, ON
01/2013 – 05/2013	**Teachers Assistant: Clinical Anatomy**
	The University of Western Ontario, London, ON
09/2009 – 06/2010	**Anatomy Instruction for Yoga Teacher Training**
	Osoyoga Inc, London, ON
09/2009 – 05/2010	**Teachers Assistant: Clinical Anatomy**
	The University of Western Ontario, London, ON
05/2008 – 08/2009	**Senior Fitness Instructor**
	Retired Researchers Association, London, ON

Academic and Administrative Experience

May 2014	GradCast – CHRW Graduate Student Research Radio; *UWO, London, ON*
April 2014	Participation on a hiring committee as a graduate student; *UWO, London, ON*
09/2009 – 05/2010	Graduate Students Health Plan Chairperson; *UWO, London, ON*
09/2009 – 05/2010	Levy Funds Masters Representative; *Dept. of Kinesiology, UWO, London, ON*
10/2008 – 08/2009	Graduate Student Health Plan Committee Member; *UWO, London, ON*
01/2006 – 05/2007	Undergraduate Teachers Assistant; *Dept. of Anatomy, UWO, London, ON*
09/2005 – 05/2006	Physiotherapist Assistant; *Fowler Kennedy Sports Medicine Clinic; London, ON*

RESEARCH PUBLICATIONS AND ACTIVITIES:

Articles Published or Accepted in Refereed Journals

1. **KN Wood**, R Nikolov, JK Shoemaker. (2016) Impact of Long-Term Endurance Training Versus Guideline-Based Physical Activity on Brain Structure in Healthy Aging. Frontiers in Aging Neuroscience; 8.
2. **KN Norton**, MB Badrov, CC Barron, N Suskin, A Heinecke, JK Shoemaker. (2015) Coronary Artery Disease Affects Cortical Circuitry Associated with Brain-Heart Integration during Volitional Exercise. J Neurophysiol. 114(2):835-45.
3. JK Shoemaker, **KN Norton**, J Baker, T Luchyshyn. (2015) Forebrain organization for autonomic cardiovascular control. Invited Review: Autonomic Neuroscience, Basic and Clinical;188:5-9.
4. **KN Norton**, TA Luchyshyn, JK Shoemaker. (2013) Evidence for a medial prefrontal cortex - hippocampal axis associated with heart rate control in conscious humans. Brain Research;1538:104-15.
5. AM Kiviniemi, S Tiinanen, AJ Hautala, T Seppänen, **KN Norton**, MF Frances, RP Nolan, HV Huikuri, MP Tulppo, JK Shoemaker. (2010) Low-frequency oscillations in R-R interval and blood pressure across the continuum of cardiovascular risk. Auton Neurosci.; 158(1-2):92-9.
6. M Zamir, **KN Norton**, A Fleischhauer, MF Frances, R Goswami, CW Usselman, RP Nolan, JK Shoemaker. (2009) Dynamic responsiveness of the vascular bed as a regulatory mechanism in vasomotor control. J Gen Physiol;134(1):69-75.

Papers in Submission

1. **KN Wood**, MB Badrov, MR Speechley, JK Shoemaker. (2016) Regional cerebral cortical thickness correlates with autonomic outflow. *Submitted to Brain Structure Function July 18, 2016: BSAF-D-16-00378.*

2. **KN Wood,** TA Luchyshyn, JK Shoemaker. (2016) High cardiorespiratory fitness in middle-age preserves decline in cortical circuitry associated with brain-heart integration during volitional exercise. *Submitted to J Neurophysiol July 23, 2016: JN-00592-2016.*

Reports

1. **KN Norton**. Analysis of Microvasculature Modeling Parameters. Masters Independent Study; April 2008.

Published Abstracts

1. **KN Norton**, A Heinecke, JK Shoemaker (2015) The Neuroprotective Effects of Endurance Training on the Aging Brain. Autonomic Neuroscience: Basic and Clinical, Vol. 192, p110.
2. M Daley, **KN Norton**, JS Gati, JK Shoemaker. (2015) Single-subject functional parcellation of the human brainstem. Autonomic Neuroscience: Basic and Clinical, Vol. 192, p11–12.
3. **KN Norton**, JK Shoemaker. (2011) The hippocampal connection to the autonomic network. Autonomic Neuroscience: Basic and Clinical, Vol. 163, Issues 1-2, p50
4. R Goswami, **KN Norton**, MF Frances, HA Sharma, JK Shoemaker. (2011) Aging is associated with reduced white matter connectivity and fractional anisotropy in cortical autonomic regions**.** Autonomic Neuroscience: Basic and Clinical, Vol. 163, Issues 1-2, p50–51.
5. **KN Norton**, M Zamir, MF Frances, CW Usselman, A Fleischauer, JK Shoemaker. (2009) Modeling Forearm Vascular Mechanics in Hypertension. *FASEB J.* 23:1017.41
6. GJ Hodges, **KN Norton**, J Chia, KA Zuj, JK Shoemaker. (2009) Distensibility of the common carotid and carotid sinus during lower body negative pressure. *FASEB J.* 23:957.5.
7. AM Kiviniemi, MP Tulppo, T Seppänen, **KN Norton**, MF Frances, RP Nolan, HV Huikuri, JK Shoemaker. (2009) Effects of aging, hypertension, and diabetes on low-frequency arterial pressure oscillations. *FASEB J* 23:1019.21.

Conference Presentations

1. **KN Norton**, A Heinecke, JK Shoemaker. Impact of Long-Term High Level Endurance Training on Brain Structure. Presented at The Saltin International Graduate Course in Clinical and Exercise Physiology; October 2015.
2. **KN Norton**, A Heinecke, R Nikolov, JK Shoemaker. Impact of Long-term High-level Endurance Training on Brain Structure in Healthy Aging. Presented at CSEP; October 2015.
3. **KN Norton**, TA Luchyshyn, J Drozd, R Bartha, JK Shoemaker. Impact of Long-Term Marathon Training on Age-Related Neuroanatomical Decline. Presented at ACSM; May 2015.

Conference Posters

1. KN Norton*, JK Shoemaker. Impact of age and hypertension on forebrain organization associated with heart rate regulation during exercise. Presented at the International Society for Autonomic Neuroscience; September 2011.
2. KN Norton*, TA Luchyshyn, J Drozd, R Bartha, JK Shoemaker. Impact of Long-Term Marathon Training on Age-Related Neuroanatomical Decline. Presented at CSEP; October 2014.
3. KN Norton*, R Goswami, RP Nolan, JK Shoemaker. Cortical Autonomic Alterations with Hypertension. Presented at the 21st International Symposium on the Autonomic Nervous System; Nov 5, 2010.
4. KN Norton*, M Zamir, M Frances, CW Usselman, A Fleischhauer, JK Shoemaker. Mechanical Properties of the Forearm Vasculature in Hypertension. Presented at the Federation for American Societies of Experimental Biology; May 2009.

5. KN Norton*, M Zamir, MF Flamengo, CW Usselman, A Fleischauer, JK Shoemaker. Mechanical Properties of the Forearm Vasculature in Hypertension. Presented at the Aging and Rehabilitation Geriatric Conference; January 2009.