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CO-AUTHORSHIP STATEMENT

Dr. Kevin Shoemaker: Dr. Shoemaker assisted in the design of this study, guided the data analysis, and supervised the writing of this document.

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ABSTRACT

Objective: This study tested whether the medial prefrontal cortex (MPFC) is differentially involved in human cardiovagal control in normotensive (NT) versus hypertensive (HT) subjects.

Design: Functional magnetic resonance imaging was combined with measures of heart rate (HR) and baroreflex sensitivity (BRS) during a 30-sec static handgrip (HG; 30% maximal strength) task.

Results: Baseline HR was higher in HT (68±3 bpm) versus NT (59±2 bpm). Cardiovagal baroreflex sensitivity was lower in HT (6.8±1.7 msec/mmHg) versus NT (16.4±2.2 msec/mmHg). During HG, HR increased similarly in HT (2±1 bpm) and NT (4±1 bpm). In NT, the HR response was associated with deactivation in the MPFC. MPFC activity did not change in HT. In 11 of the total 23 subjects, HR increased ≥ 3 bpm and MPFC deactivation was correlated with the HR time course.

Conclusions: Overall, hypertension appears to be equivalent to normotension in terms of the HR response to HG and the MPFC-HR association.

Keywords: Vagal withdrawal, Heart Rate, Cortical Autonomic Network, Hypertension

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AC	Anterior Commissure
ACC	Anterior Cingulate Cortex
ACE	Angiotensin Converting Enzyme
ACh	Acetylcholine
AChE	Acetylcholinesterase
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
AP	Arterial Pressure (mmHg)
ARB	Angiotensin Receptor Blocker
AV	Atrioventricular Node
BOLD	Blood-Oxygen-Level-Dependent
BP	Blood Pressure (mmHg)
bpm	Beats per minute
BRS	Baroreflex Sensitivity
CAN	Cortical Autonomic Network
CBF	Cerebral Blood flow
CV	Cardiovascular
DBP	Diastolic Blood Pressure (mmHg)
dHb	Deoxyhemoglobin
EPI	Echo-planar Imaging
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging

FOV	Field-of-view
FWE	Family Wise Error
FWHM	Full-width half maximum
Hb	Haemoglobin
HDR	Hemodynamic Response
HG	Handgrip
HR	Heart Rate (bpm)
HT	Hypertension
IC	Insular Cortex
K^+	Potassium
mmHg	Millimetres of mercury
MPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
MVC	Maximum Voluntary Contraction
NR	Non-Responder
NT	Normotension
NTS	Nucleus Tractus Solitarius
PC	Posterior Commissure
PCC	Posterior Cingulate Cortex
PFC	Prefrontal Cortex
PNS	Parasympathetic Nervous System
R	Responder
RF	Radiofrequency

ROI	Region of Interest
RVLM	Rostral Ventrolateral Medulla
SA	Sinoatrial node
SBP	Systolic Blood Pressure (mmHg)
SNA	Sympathetic Nerve Activity
SNR	Signal to Noise Ratio
SNS	Sympathetic Nervous System
SPM	Statistical Parametric Mapping
TE	Time to Echo
TI	Inversion Time
TR	Time to Repetition

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

In humans, the sensitive balance between sympathetic and parasympathetic regulation of the cardiovascular (CV) system is crucial to blood pressure stability and long-term health. The sympathetic nervous system elevates heart rate (HR) and blood pressure (BP) by stimulating adrenergic receptors on heart vasculature; whereas, the parasympathetic nervous system slows the heart through cholinergic activity focused at the sinoatrial node. Chronic disturbances of this dynamic equilibrium, in which sympatho-excitation is excessive and parasympathetic activation is simultaneously suppressed and unable to restrain the cardiac sympathetic drive, can contribute to the pathogenesis and progression of several CV disease states, thereby, considerably increasing the risk of morbidity and mortality (117).

There is substantial evidence to indicate that essential hypertension (HT) may be related to centrally mediated autonomic dysfunction as illustrated by elevated levels of sympathetic nervous activity and depressed sensitivity of the baroreflex-mediated control of heart rate (38). An understanding of the mechanisms that regulate arterial pressure under physiological conditions, and in the context of pathophysiological situations such as HT, represents a major challenge. In particular, the roles of normal and disordered parasympathetic innervation in patients with HT are not understood comprehensively and have not been elucidated in relation to cortical function (14, 98, 115, 118).

Both HT and the normal aging process are accompanied by a complex series of changes in autonomic control of the CV system favoring heightened cardiac sympathetic tone with parasympathetic withdrawal and blunted cardiovagal baroreflex sensitivity (BRS) (79, 116). This age-related dominance of sympathetic activity may contribute to the increased risk of hypertension with aging. Indeed, in persons older than 40 years, a strong association exists between resting levels of sympathetic nerve activity and resting arterial BP (90). This association does not exist in younger persons (50), suggesting that the mechanisms that buffer or balance the hypertensive influences of higher sympathetic activity are less active or missing in older persons (19, 128).

The autonomic dysfunction that occurs with aging and hypertension has attracted recent research focus and initiated many clinical and experimental studies to explore at the unknown role cortical regions may play in mediating the impact of hypertension on parasympathetic control of heart rate. A network of forebrain regions, termed the 'cortical autonomic network' (CAN), with influence over autonomic outflow and CV control have been identified and are now providing researchers with a means to study the physiological responses to different stressors at the central level. Given that the CAN likely acts as a modulator of the autonomic nervous system, we hypothesize that altered activation patterns within this network may be involved in the observed hyperadrenergic activity found in hypertensive patients, as well as the substantial decline in parasympathetic regulation of cardiac function observed with age.

In healthy subjects, moderate intensity handgrip (HG) exercise of short duration produces a rapid and large tachycardia that precedes sympathetic activation and can be severely attenuated by atropine administration causing vagal blockade (52, 83, 134). Therefore, using a short-term volitional isometric handgrip contraction model we were able to isolate the cortical modulation of vagal outflow specifically, and differentiate its actions from those of the sympathetic nervous system. By combining handgrip exercise with the capabilities of functional magnetic resonance imaging, we were able to determine whether cortical activation patterns are related to these peripheral changes in autonomic outflow emphasizing, in this study, the role of the parasympathetic nervous system (PNS).

1.2 PURPOSE

The purpose of this study was to evaluate the impact of hypertension on the cortical autonomic network (CAN) and its associated alterations in cardiovagal control. The temporal relationship between activation patterns in the *a priori* autonomic regions of interest, namely the medial prefrontal cortex (MPFC) and the subgenual cingulate cortex, were compared in age-matched hypertensive, and normotensive populations.

1.3 HYPOTHESIS

The decrements in cardiovagal control in hypertension are associated with modified activation patterns within the CAN during short-term exercise requiring PNS withdrawal. This hypothesis predicts that hypertensive subjects, as compared to age-matched normotensive (NT) controls, would produce smaller changes in HR during an

isometric handgrip task. This lack of a response in HR would be associated with a lack of change in MPFC activity.

1.4 STUDY APPROACH

We will apply a 30% maximum voluntary contraction (MVC) handgrip task, which is known to elicit a withdrawal of PNS activity and an increase in HR in young individuals, to a group of hypertensive and normotensive older adults. We will use functional magnetic imaging to examine activation patterns in the CAN with *a priori* emphasis on the MPFC.

CHAPTER 2: LITERATURE REVIEW

2.1 THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system (ANS) provides control of 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. Anatomically and functionally, the ANS is comprised of two divisions: the sympathetic and parasympathetic nervous systems. It is through these two centrally orchestrated neural systems that the body achieves regulation over the cardiovascular system. The sympathetic and parasympathetic nervous systems are not opposites; rather, their interactions are complementary. A dynamic balance occurs between these two effectors such that the development and control of cardiovascular regulation at any given level depends on the balance between their respective activities.

2.1.1 SYMPATHETIC NERVOUS SYSTEM

The sympathetic nervous system (SNS) generally works to mobilize the body's resources for action under stress and, therefore, may be thought to counteract the parasympathetic system, which generally works to promote maintenance of the body at rest.

There are two groups of neurons involved in the transmission of signals through both effector arms of the ANS: these are the pre- and post-ganglionic axons. 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 (121). 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, 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 (121).

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 (NT) storage and release. There are several NTs that are 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 (111).

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 heart rate. 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 heart rate to rise and is particularly important during periods of excitement and stress.

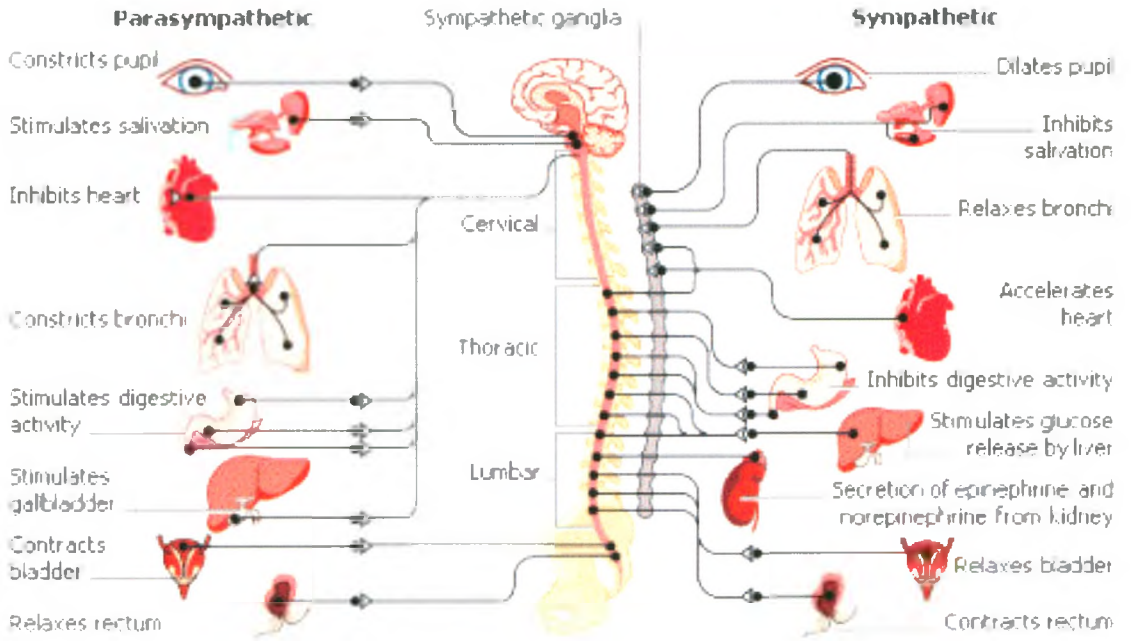

Figure 2.1.1 Actions of the Autonomic Nervous System

2.1.2 PARASYMPATHETIC NERVOUS SYSTEM

Similar to the SNS, the 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 (98). In the medulla, the cranial nerves III (occulomotor nerve), VII (facial nerve), IX (glossopharyngeal nerve), and X (vagus nerve) form the preganglionic parasympathetic fibers (48). The most important cranial nerve responsible for cardiovascular control is the vagus nerve, containing nearly 90% of the preganglionic parasympathetic fibers in the body (73).

The dorsal motor nucleus gives rise to preganglionic fibers, which project to ganglia very close to their visceral targets. As indicated in the SNS, preganglionic innervation in the PNS is mainly cholinergic with these terminals releasing acetylcholine (ACh) at the ganglion synapses onto nicotinic receptors. The major PNS postganglionic NT is also ACh, which binds to muscarinic ACh receptors (4).

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 (4).

It is important to note that the kinetics of the two autonomic divisions differ 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 (111). Once stimulation has ended, the return back to control level 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 (135). As a result, PNS activation is associated with immediate reductions in arterial pressure and heart rate 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 (89). 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.

2.1.3 REGULATION OF HEART RATE

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 (5). Resting and exercise HR are controlled by the sympathetic and parasympathetic nervous systems. Once exercise begins, the sympathetic nervous system is activated and the HR rises quickly. The SA node is the pacemaker of the heart, responsible for setting rate and rhythm. The SA node is usually under the tonic influence of both the sympathetic and parasympathetic nervous systems, such that changes in HR usually 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) (57). In 1969, through constant, simultaneous vagal and sympathetic stimulation in the anesthetized dog, Levy and colleagues further proved that parasympathetic influences prevail at the SA node (64). 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 nerves were 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 (62).

2.1.4 THE BAROREFLEX

Arterial blood pressure (AP), 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 AP is rapidly and reflexively modulated is called the baroreflex. The baroreflex is a neurocardiovascular reflex that operates in a negative feedback loop in an attempt to maintain AP homeostasis in the short-term (87). The purpose of the baroreflex is to regulate mean AP, preventing large transient changes that may arise from sudden stressors (24).

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 (58). Functionally, the baroreflex is a negative feedback reflex. In response to an increase in systemic AP, 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 ambiguus 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 AP (5). When AP decreases, "disinhibition" (less inhibition, hence activation) 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. 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 environmental perturbations that arise during everyday living (34).

The study of the relationship between changes in AP and HR is used as a measure of the sensitivity of the arterial baroreflex (86). Acute changes in AP 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 (58).

2.1.5 SUMMARY

The sympathetic and parasympathetic systems work in tandem to create a synergistic stimulation that is not merely on or off, but acts on a continuum depending

upon how vigorously each division is attempting to carry out its actions. Through the two branches of the autonomic nervous system, adjustments are made to vascular smooth muscle and cardiac pacemaker cells which in turn bring about changes in arterial pressure and heart rate. Under normal circumstances, stimulation of sympathetic efferents elicits increases in vascular resistance and heart rate, raising arterial pressure. On the other hand, vagal efferents target the heart to reduce cardiac output and heart rate when stimulated, decreasing arterial pressure. These pathways do not exist in isolation. Rather, they overlap through reflexes initiated by changes in arterial pressure. The baroreflex is one such reflex that relies on inputs from cells within the vascular walls to relay information about AP. Parasympathetic and sympathetic outflow are then adjusted to restore AP.

2.2 ROLE OF THE ANS DURING ISOMETRIC EXERCISE

In young healthy subjects, sustained isometric muscular contractions produce rapid and large increases in heart rate, cardiac output, and systemic arterial pressure (65, 67, 84, 94). The body's response to this exercise task is owing to actions of both the sympathetic and parasympathetic nervous systems and is dependent on the intensity, duration and type of exercise performed. Because the two autonomic divisions are tonically active and often work together, differentiating the systemic reactions and cortical influences of the PNS and SNS is challenging. Therefore, a 30s handgrip (HG) exercise task is used, which has been shown to isolate the PNS component in young individuals. In the current study, this 30s HG task will be applied to a hypertensive and normotensive population, isolating the PNS component of the HR response to exercise.

Short-term volitional isometric handgrip contractions offer a unique opportunity to separate the cortical modulation of PNS and SNS outflow. The observed elevation in heart rate is manifested very rapidly at the beginning of the isometric contraction (within the first 500ms of exertion) (9, 106). Furthermore, the magnitude of this tachycardia seems to depend directly on the relative degree of muscle force developed (32, 66). The autonomic regulation of the heart rate response is based on a biphasic mechanism, with parasympathetic withdrawal initiating the immediate rise in heart rate that occurs in response to the isometric exercise (35, 70, 77, 94, 106). Sympathetic activation makes a larger contribution to the chronotropic response after 30 seconds of isometric exercise at 30% of maximum voluntary capacity (MVC) (76, 77). Contractions of higher intensities (50-95% MVC) still emphasize PNS withdrawal initially, but the SNS contribution begins earlier in the contraction time course (70) and the associated responses do not stabilize, rather they progressively increase as the contraction is maintained (32, 66). During a 30% MVC HG contraction, the increase in forearm blood flow, blood pressure and heart rate all rise to a steady state, which is maintained throughout the contraction (66).

Importantly, the tachycardic response to isometric handgrip can be attenuated or eliminated by vagal blockade. In studies by Freyschuss and colleagues, atropine (muscarinic receptor antagonist which acts to block parasympathetic outflow) inhibited the heart rate response when contractions of varying intensities (50-90% of MVC) were maintained for short periods of time (5-10s) (40). Furthermore, Mitchell and colleagues showed that this response is not affected by beta adrenoceptor blockade (82). These

studies indicate that the initial tachycardia observed at the onset of handgrip exercise is predominately mediated by the withdrawal of PNS activity (40, 52, 83, 134).

2.3 ANS REGULATION BY CORTICAL INFLUENCE

Although it has been well established that the medulla oblongata is the primary CV control site (48), recent studies in rodent models have highlighted the importance of certain forebrain structures involved with the modulation of efferent autonomic outflow (17, 30, 132). In addition, clinical neuroimaging research has revealed that the basal levels of autonomic tone may be disrupted in patients with stroke or epileptic seizures in the higher regions of the brain such as the prefrontal and insular cortices, suggesting that cortical regions are involved in cardiovascular regulation (20, 23, 99). A network of forebrain regions, termed the 'cortical autonomic network' (CAN), has been shown to exert influence over autonomic outflow and CV control. These regions include the insular cortex (IC) (Brodmann areas 13, 14), and an area in front of the corpus callosum called the prefrontal cortex (PFC) which encompasses the anterior cingulate cortex (ACC) (Brodmann areas 24, 32, 33), and medial prefrontal cortex (MPFC) (Brodmann areas 10 and 11) (Figure 2.3.1) (25, 43, 44, 59, 125, 136).

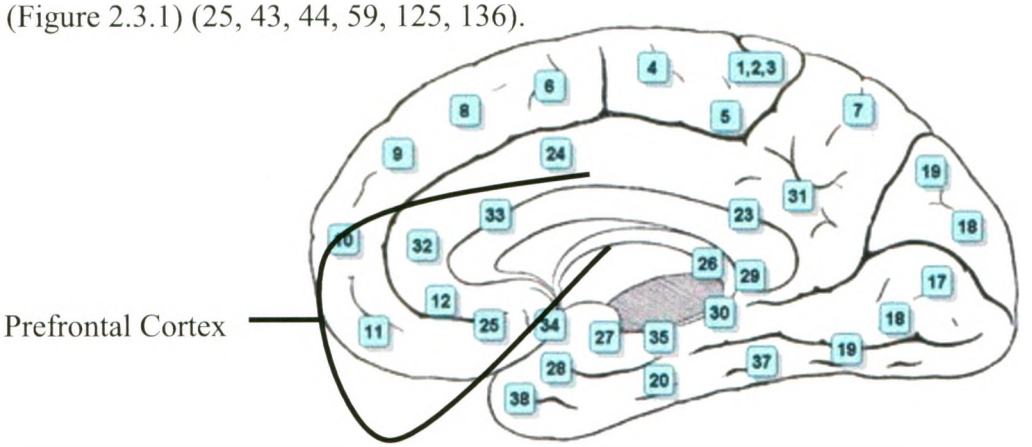

Figure 2.3.1 Brodmann area distribution on right medial hemisphere.

The above diagram highlights the regions involved with the CAN. However, the IC is not identified due to its embedded location beneath the temporal lobes on either side of the brain (Figure 2.3.2).

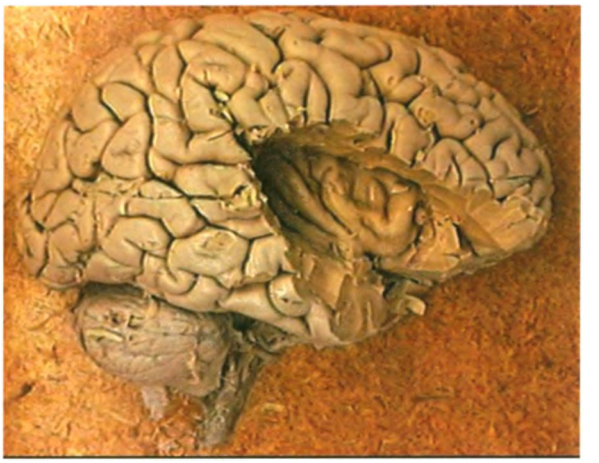

Figure 2.3.2 Histological view of insular cortex hidden beneath the temporal lobe.

The IC and ACC are activated during a variety of cognitive maneuvers that elevate autonomic stimulation such as gambling (26), Stroop task (43), mental arithmetic (25) and many more. The involvement of the ACC and IC in influencing SNA are not only observed during cognitive tasks that induce mental arousal, but are also reported in situations of physical stress such as baroreceptor unloading (59), and isometric exercise (136). Also, neuronal responses have been recorded in the IC when HR and BP changes were elicited by stimulation of the vagus nerve (3) and baroreflex afferents (17).

Functional magnetic resonance imaging (fMRI) techniques have enabled the non-invasive assessment of cortical autonomic correlates in healthy, young conscious humans (25, 49, 51, 60). Most of these studies, however, have focused solely on the cortical network modulating sympathoexcitatory responses. There is little data examining cortical

involvement in vagal responses, even though mounting evidence suggests that vagal activity is an important predictor of CV prognosis in humans (28). There is strong evidence associating the ventral portion of the MPFC with cardiovascular modulation (91, 132), and it has been shown to have strong efferent 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 (21, 55, 91, 132, 133). 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 (18, 26). As noted above, the rapid HR response at the onset of handgrip exercise is predominantly mediated by the withdrawal of PNS activity (76) and has shown to be associated with deactivation in the MPFC (136). These observations suggest that the MPFC is involved in mediating behavioural procedures that reduce cardiovascular response to psychological or physical stress, while augmenting vagal efferent control of HR (27), thus supporting a direct relation between MPFC activity and cardiovagal control in young, healthy adults.

2.4 ANS DYSFUNCTION WITH AGE

The normal aging process is accompanied by a complex series of changes in the autonomic control of the CV system, favoring heightened cardiac sympathetic tone with parasympathetic withdrawal and blunted cardiovagal BRS. One key feature also includes progressive arterial stiffening, which is accompanied by increases in systolic blood pressure, pulse pressure, and as a result left ventricular mass (58). Although the effect of aging on the human sympathetic nervous system has been studied extensively, it remains

a subject of considerable mystery. Continued research on this topic relates to the fact that a close interaction between cardiovascular disease and sympathetic nervous overactivity exists (58, 68, 79). Notably, essential hypertension, cardiac failure, and ventricular arrhythmias are all accompanied by elevated SNA (79, 116).

In contrast to the increased SNA, vagal control of the aging cardiovascular system is diminished, which is manifested by decreases in cardiac output responses to parasympathetic withdrawal (70, 74, 76, 127). This is due primarily to a reduction in baseline vagal tone, thus attenuating cardiac responses to both sympathetic stimulation and to vagal withdrawal with normal aging (127).

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 its evaluation by mathematical analyses, most notably power spectral analyses (1) and the sequence method (6). 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 (107, 124). High-frequency portions of the power spectrum (0.15-0.5Hz) are under the sole influence of parasympathetic control. The spontaneous cardiovagal baroreflex can also be calculated using the sequence method, which scans blood pressure

recordings for sequences of SBP that are characterized by either increases (or decreases) in SBP of at least 1 mmHg during each of three or more blood pressure 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 coefficent of determination (r^2) is taken as the measure of gain or sensitivity of the changes in heart rate induced by the blood pressure changes, as it is done by the baroreceptor reflex (6). Such analysis techniques have confirmed that healthy aging is associated with reductions in BRS and parasympathetic modulation of HR, with a greater loss of the high-frequency parasympathetic component (58, 85, 111). This decline in BRS is associated with functional changes in BP control, and an impaired ability to reflexively engage the cardiovascular system. That is, the degree of heart rate slowing that occurs with increasing blood pressure is blunted with aging (Figure 2.4.1).

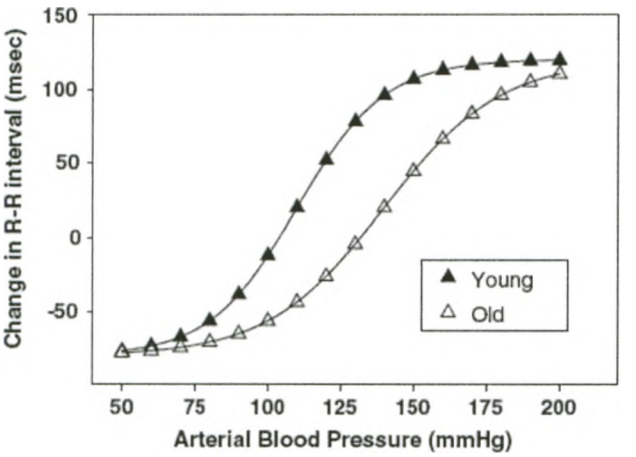

Figure 2.4.1 Baroreflex curves depicting blunting of the HR response to changes in BP in older individuals compared to younger subjects (58).

Age is the dominant factor associated with reduced BRS, although increasing BP is associated with further blunting of BRS in older populations (38). The mechanisms responsible for this age-associated change remain unclear. Traditional thinking has been

that increased blood vessel stiffening impairs the function of the afferent baroreceptors in the carotids and aortic arch, through either structural (atherosclerosis) or functional (reduced nitric oxide activity) changes (111). However, Robinson and colleagues found that BRS can be altered acutely by stroke, which may lead to altered central processing as the explanatory variable (112). The role of the brain, and in particular the forebrain, in cardiovagal baroreflex control and its change with age, is not known.

2.5 THE EFFECTS OF AGE ON THE BRAIN

As previously discussed, there is growing evidence in humans to suggest age-related changes in systemic cardiovascular properties. However, there are relativity few studies to date that examine potential age-related differences in CAN activity. It is known that aging is associated with ANS dysfunction and that the cortex modulates ANS outflow (11, 18, 50). However, much of the minimal research available is directed at structural age-related differences rather than functional changes. The adult human brain shrinks slowly with age but the regional, and tissue specificity of this loss is unclear. Major findings include widespread ventricular enlargement (10, 37) and whole brain atrophy (47), as well as a loss of brain volume in both gray and white matter (109). 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 (109) with degradation of fiber myelination (93), and a loss of structural connectivity (95). The presence of these structural differences in regions specific to autonomic control, suggests that aging may be linked with cortical functional differences and control of the ANS.

2.6 ANS DYSFUNCTION WITH HYPERTENSION

Essential hypertension is a chronic, age-related condition associated with multiple changes in the vascular system (75), and in the vast majority of cases it has no singular identifiable pathological cause. The definition of hypertension varies, although most authorities agree that sustained diastolic pressure greater than 90 mm of mercury (mmHg), and systolic pressure greater than 140 mmHg constitutes a cause for diagnosis (22). In spite of a notable decline in prevalence, still over 20% of adults (and over 55% of those are older than 70) suffer from hypertension (13). There is substantial evidence to indicate that essential hypertension may be related to elevated levels of sympathetic nervous activity, which originates within the central nervous system (98). An understanding of the mechanisms that regulate AP under physiological conditions and in the context of pathophysiological situations such as hypertension represents a major challenge. In particular, the roles of normal and disordered parasympathetic innervation in patients with HT are not understood comprehensively, and scientists are only beginning to consider how activation of the PNS may have important therapeutic implications for this population (98).

2.7 THE EFFECTS OF HYPERTENSION ON THE BRAIN

The effects mild vascular conditions, such as hypertension, have on the brain have been well examined in terms of structural change; however, they have not been examined in the context of autonomic function (14, 115, 118). In 2003, Raz and colleagues confirmed that otherwise asymptomatic older adults who have chronic elevation in blood pressure have increased white matter deterioration and reduced volume of brain tissue

(110). However, those differences are not global and appear to be confined to the PFC and underlying white matter. Very little is known about what effects, if any, these structural changes may have on functional responses within the aged brain and more research is needed to examine this potential more closely.

2.8 BLOOD-OXYGEN-LEVEL-DEPENDENT (BOLD) FMRI

Modern *in vivo* imaging is one of medicine's most powerful and exciting success stories. It has optimized diagnostics and enabled us to monitor treatment, providing not only clinically essential information but also insight into the basic mechanisms of brain function and malfunction. Although the brain comprises only 2% of the total body mass, it receives 12-15% of the cardiac output and consumes about 20% of the oxygen entering the body (122). At rest, the brain consumes oxygen at an average rate of 3.5mL of oxygen per 100g of brain tissue per minute (122). Approximately 50-60% of the energy produced by this oxygen consumption supports electrophysiological function. Therefore, the brain's substantial demand for substrates requires the adequate delivery of oxygen and glucose via the cerebral blood flow (CBF). There are elaborate mechanisms regulating the CBF and that these mechanisms are closely coupled with regional neural activity (54).

The physical principles on which magnetic resonance imaging (MRI) is based are complex and beyond the scope of this thesis. I will only be scratching the surface with a brief overview of the basic concepts involved in a much larger, more intricate process.

However, there are several excellent works on the details of MR physics (Abragam 1961, Callaghan 1991, Haacke et al. 1999, Stark & Bradley 1999).

As its name implies, MRI uses strong magnetic fields to create images of biological tissue. The strength of the static magnetic field (strength at the center of the magnet which does not change over time) created by an MRI scanner is expressed in units of Tesla, and is created by passing a current through multiple tight coils of wire – a process called electromagnetism. This static field is necessary for MRI, but does not produce an MR signal itself. The MR signal is produced by different electromagnetic coils, which generate and receive electromagnetic fields at the resonant frequencies of the atomic nuclei within that static field (54). When a human body is placed into the magnetic field, the hydrogen atoms align along and an equilibrium is reached. The application of an excitation pulse to a spin system disturbs this equilibrium state and causes some of the spins to change from a low-energy state to a high-energy state. When atomic nuclei are excited, they absorb the energy of the radiofrequency (RF) pulse. When this pulse ends, the atomic nuclei return to the equilibrium state and release the energy that was absorbed during excitation. This release of energy can be detected by the RF coils and is known as reception, which defines the raw MR signal.

One of the remarkable developments in recent work on MRI is the recognition that changes in the metabolic state of the brain affect the local MR signal and provide an intrinsic mechanism for detecting brain activation (63, 96). The origin of this effect is that hemoglobin (Hb) is diamagnetic when oxygenated; that is, it has no unpaired electrons

and zero magnetic moment. When Hb is deoxygenated it can be termed paramagnetic, meaning that it has both unpaired electrons and a significant magnetic moment (104). The presence of deoxyhemoglobin (dHb), which has a magnetic moment about 20% greater than fully oxygenated blood, alters the local magnetic signal and creates distortions within and around the blood vessels, producing a slight alteration in the local MR signal. This change in the MR signal, which is triggered by neural activity, summarizes the blood-oxygen-level dependent (BOLD) effect and is known as the hemodynamic response (HDR). The BOLD response measures changes in the total amount of dHb in a specific region of the brain over time. However, this quantity depends not only on the extraction of oxygen by active neurons, but more importantly on changes in blood flow and blood volume which together shape the BOLD HDR (Figure 2.8.1).

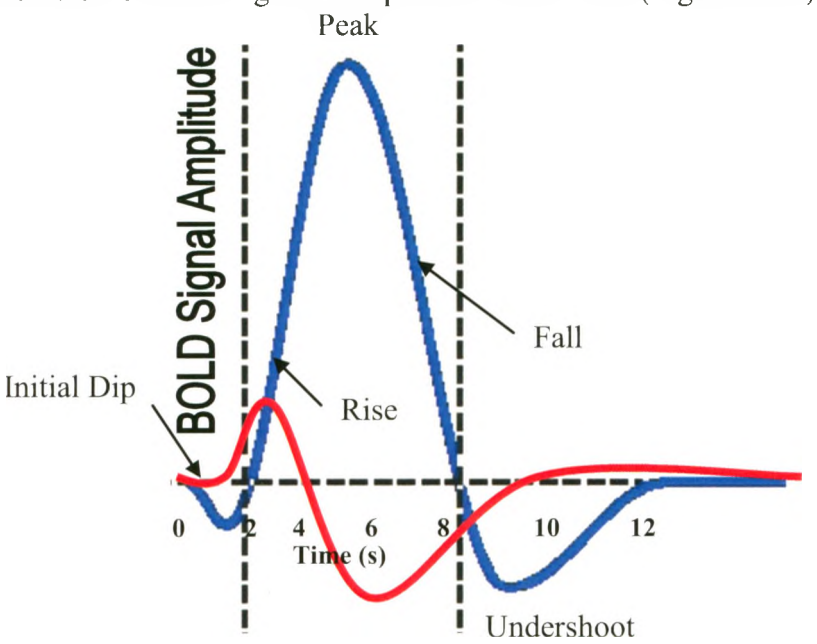

Figure 2.8.1 Schematic representation of the BOLD hemodynamic response (blue) and deoxygenated hemoglobin (red) following neuronal stimulation (0s).

The shape of the HDR (blue line on Fig 2.8.1) is relatively well explained by the interplay between oxygen consumption and blood flow and can be divided into phases.

At the onset of stimulus presentation, some studies have reported an initial dip of 1-2s duration, which has been attributed to the initial oxygen extraction from blood currently in the active tissues (137). The next phase follows the increased neural activity in the brain, with the local blood flow increasing much more than the metabolic rate of oxygen consumption, and as a result, the amount of extracted oxygen decreases (39). Because the local blood is more oxygenated, there is less deoxyhemoglobin present and less magnetic field distortion, which increases the MR signal reaching its peak at about 5-6s (72). Once the neural activity has stopped, the BOLD signal decreases to a below-baseline level. This decrease below baseline is known as the post-stimulus undershoot, and is due to a combination of reduced blood flow and increased blood volume in the area of interest. This process by which neural activity influences the haemodynamic properties of the surrounding vasculature is referred to as neurovascular coupling (54). Although the exact mechanisms that underlie neurovascular coupling are not completely understood, there is empirical evidence that these mechanisms might be altered in normal aging and disease. As most fMRI studies are performed on healthy, young individuals, little attention has focused on the effects of changes in the cerebrovascular system on the BOLD signal. However, direct comparisons of the BOLD signal made between groups of individuals rely heavily on comparable neurovascular coupling, and any alterations to these dynamics could affect this relationship.

2.8.1 THE EFFECTS OF AGE ON THE BOLD SIGNAL

As outlined in previous sections, anatomical, physiological, and metabolic changes in the human brain have been reported to be associated with aging. However,

these changes may not be concomitant with changes in neuronal density over the course of normal aging, and may or may not affect the BOLD signal. In early studies, it was thought that neuron loss accompanied the aging process even in the absence of disease (11). However, more recent studies have failed to reproduce this finding and support the idea that the cognitive functional decline observed with age is not due to decreased neuron density (88). The effects of aging on cerebral blood flow and metabolism are less well established and more controversial, with some studies reporting a decline with age, while others do not (45). Given this uncertainty, we would presume that any age-related change in brain anatomy, neuronal density, vasculature or metabolism would influence the fMRI-measured hemodynamic response. Taoka and colleagues found that there were age-related increases in the rise-time of the fMRI BOLD signal, but not in the fall (129). This slowing of signal rise was attributed to vascular effects, including stiffening of the artery wall. In contrast, D'Esposito and colleagues (29), as well as Huettel and colleagues (53), found no age-related differences in the fMRI HDR in the motor or visual cortices, respectively.

2.8.2 THE EFFECTS OF HYPERTENSION ON BOLD

As stated in previous sections, hypertension is a chronic, age-related condition associated with multiple changes in the vascular system (75), and negative affects on brain morphology (14, 115, 118). Although the effect of hypertension on the brain has been studied extensively through structural and cognitive research, the effect of these changes on autonomic function has not been examined in the context of its affects on the BOLD response.

CHAPTER 3: METHODOLOGY

3.1 SUBJECTS

A total of twelve normotensive (NT) older adults (6 male, 6 female; 56 ± 5 years; SBP: 123 ± 9 mmHg, DBP: 75 ± 10 mmHg) (mean ± SD) and eleven medicated, hypertensive (HT) age-matched adults (6 male, 5 female; 63 ± 6 years; SBP: 141 ± 12 mmHg, DBP: 92 ± 6 mmHg) provided informed written consent before participating in the present study, which was approved by The University of Western Ontario Health Sciences Ethics Review Board. All NT subjects were recreationally active and participated in regular physical activity. All female participants were post-menopausal, with two women currently taking hormone replacement therapy (Table 3.1.1).

All participants who had been diagnosed with hypertension were undergoing treatment with various antihypertensive medications that were prescribed and monitored by their physicians. Their full medication profile is presented in Table 3.1.1. The most frequently administered type of antihypertensive drug in this sample was Angiotensin-II Antagonists.

Medical screening and magnetic resonance imaging guidelines questionnaires were given to each subject to ensure safe compatibility within a high magnetic field environment. Subjects had no prior history of cardiovascular disease or neurological disorders. Subjects were instructed to consume a light meal approximately 3 hours prior to the experiment and to abstain from nicotine, alcohol, caffeine, and intense physical exertion for 12 hours prior to the experiment.

	Sex		Age	BMI	Resting HR	Resting BP	BRS	Medication Profile						
	M	F	(years)		(bpm)	(mmHg)	(slope)	DR	BB	ACE	ARB	CCB	Statins	HRT
HT Group	*6*	*5*	*63 ± 2*	*27 ± 1*	*68 ± 3*	*141/92 ± 4*	*6.8 ± 1.7*	*3*	*2*	*2*	*9*	*1*	*5*	*1*
Participant 1	x		66	24	67	132/90	3.2				x		x	
Participant 2	x		60	26	70	132/90	3.4				x	x	x	
Participant 3	x		73	25	71	130/90	2.9				x			
Participant 4	x		61	27	67	160/100	3.4				x		x	
Participant 5	x		51	23	65	N/A	18.4	x		x			x	
Participant 6	x		68	30	54	160/98	10.5				x			
Participant 7		x	66	28	64	126/84	2.6		x	x			x	
Participant 8		x	61	26	86	142/94	3.1		x		x			x
Participant 9		x	61	29	78	152/92	12.7	x			x			
Participant 10		x	60	27	54	134/84	11.3				x			
Participant 11		x	68	32	76	140/100	3.2	x			x			
NT Group	*6*	*6*	*56 ± 1*	*25 ± 2*	*59 ± 2*	*119/70 ± 3*	*16.4 ± 2.2*	*0*	*0*	*0*	*0*	*0*	*0*	*1*
Participant 1	x		55	24	46	125/75	14.9							
Participant 2	x		56	24	55	130/85	15.3							
Participant 3	x		59	28	66	135/85	6.3							
Participant 4	x		56	30	64	124/84	10.9							
Participant 5	x		62	23	55	135/85	28.0							
Participant 6	x		53	27	72	115/70	18.5							
Participant 7		x	59	21	59	118/70	11.6							
Participant 8		x	50	37	54	115/55	16.5							
Participant 9		x	52	25	58	135/85	14.3							
Participant 10		x	62	26	65	110/70	7.7							x
Participant 11		x	58	22	56	110/60	32.5							
Participant 12		x	53	22	60	125/80	20.2							

Table 3.1.1. Summary Subject Profile. The first row of both groups (italics) denotes group average data, presented as means ± SEM. HT=hypertensive; NT=normotensive; BMI=basal metabolic rate (weight/height^2); HR=heart rate; BP=blood pressure; BRS=baroreflex sensitivity; DR=diuretic; BB=beta-blocker; ACE=ACE inhibitor; ARB=angiotensin II antagonist/blocker; CCB=calcium channel blocker; HRT=hormone replacement therapy.

3.2 NEUROIMAGING DATA ACQUISITION

A mercury sphygmomanometer with a brachial cuff was used to obtain baseline systolic and diastolic blood pressure before each neuroimaging session began. Measures were obtained from subjects who were seated in a comfortable chair in a climate-controlled room. Participants were familiarized with the experimental procedures prior to completion of a functional magnetic resonance imaging (MRI) session, which was performed at the Robarts Research Institute at The University of Western Ontario. All imaging data were collected using a whole body 3-Tesla imaging system (Magnetom TRIO TIM, Siemens Medical Solutions, Erlangen, Germany) with a maximum gradient strength of 45 mT/m and a slew rate of 200 T/m/sec. A transmit-receive cylindrical hybrid birdcage radio frequency (RF) 32-channel head coil (2) was used for transmission and detection of the blood oxygen level dependent (BOLD) contrast signal. Prior to imaging, a global shimming procedure (RASTAMAP) using first- and second-order shims was performed to optimize the magnetic field over the imaging volume of interest (61). Functional data were collected using a T_2* echo planar imaging (EPI) pulse sequence (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, TE = 30ms) were acquired in each volume. Our initial protocol involved a 30s baseline period before the first HG task, but this was increased to 1min after the first 4 subjects to maximize scanning efficiency. Thus, these subjects had a total of 134 volumes collected per session, and the remaining 19 participants had 147 volumes. Regardless of volume total, 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. A corresponding high-resolution

T_1-weighted structural volume was acquired at the beginning of the same scanning session using 3D Turbo FLASH (TE = 2.98 ms, TI = 900 ms, TR = 2.3 ms) with a voxel resolution of 1.0 X 1.0 X 1.0 mm. T_1-weighting refers to images based on a tissue-dependent recovery of the longitudinal component of net magnetization over time, and T_2-weighting refers to the relaxation time of the transverse component. 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.

3.3 EXPERIMENTAL DESIGN

A non-magnetic handgrip device, which consisted of an inflated rubber bladder, was connected in series to a disposable pressure transducer (Edwards Lifesciences, PX272, Irvine CA) and a bridge amplifier located outside of the MRI suite. Each session began with a maximal voluntary contraction (MVC) handgrip calibration, in which the participant was instructed to squeeze the handgrip device (using their right hand) to their maximal ability while in the supine position. This MVC was calibrated as 100%. During each recording session, visual feedback was provided to the participant on their achieved force in real-time.

To establish a stable baseline level, participants remained in the supine position for a minimum of 10 minutes before recording started. The protocol consisted of an initial 30 s or 1 min of baseline recording (review of initial protocol necessitated an increase in duration of the first resting baseline), followed by three 30 s blocks of

handgrip exercise at 30% of their MVC, separated by 1 min of rest (Figure 3.3.1). To monitor the level of perceived stress of the exercise, participants were asked to verbally rate their level of perceived exertion after each trial (3 HG intervals = 1 trial) using the BORG scale rating system from 6-20 (8). None of the participants reported feeling any significant degree of aversive emotional stress or forearm fatigue during the fMRI experiments (all subjects reported values < 8 RPE). Imaging and HR data time points were taken from the last 30 s of each baseline period and the last 10 s of each exercise task. These time frames reflect those at which PNS activity is at its minimum (during HG), and therefore HR is at its maximum.

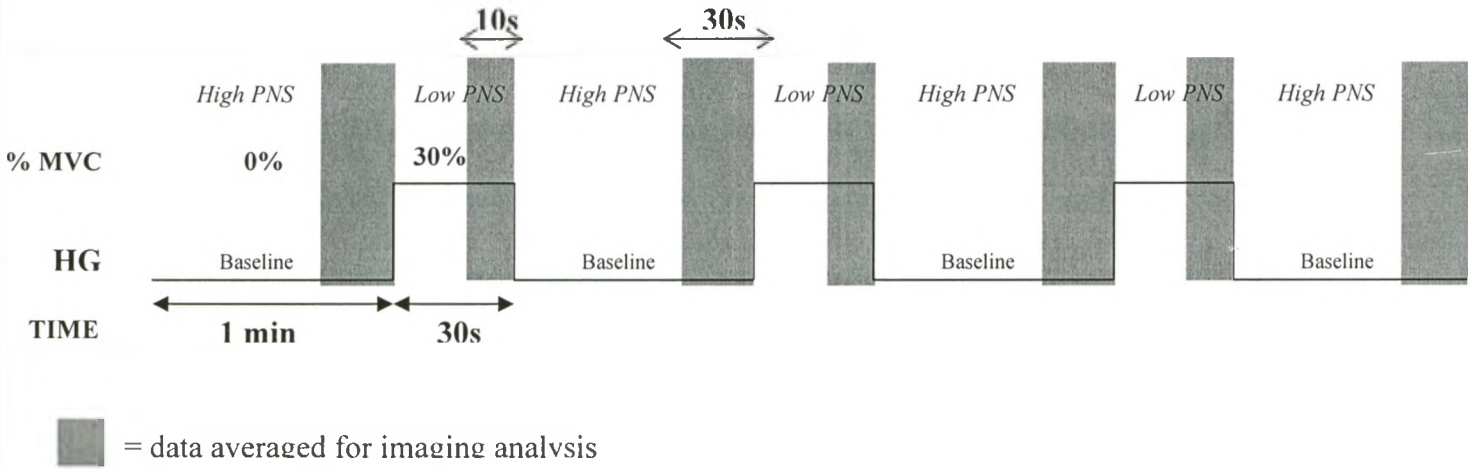

Figure 3.3.1 Boxcar design matrix for neuroimaging protocol.

3.4 PHYSIOLOGICAL DATA

During data acquisition, HR was calculated from pulse-by-pulse recordings on an MRI-compatible Oximeter (Nonin Medical Inc, 8600FO MRI, Plymouth, MN) placed over the index finger of the non-exercising left hand. Analog signals for pulse recordings and handgrip contraction force were sampled at 200-Hz with an on-line data acquisition

and analysis system (PowerLab, ADInstruments, Mountain View, CA, USA). Handgrip 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 (i.e. 147 time points). HR data values were determined by averaging the beat-by-beat response over the last 30s of the collection period for baseline, and the last 10s of HG intervals. Subsequently, the HR response to exercise was calculated as the average HR during the three exercise periods.

In a second set of analyses done on a separate day, the spontaneous cardiovagal baroreflex gain was determined using the sequence method as a measure of individual parasympathetic control (7, 103, 124). Briefly, regression analyses were performed on sequences of three or more consecutive cardiac cycles (pulse oximeter) exhibiting concurrent changes in systolic blood pressure and R-R interval (both rising and falling). The analysis was performed on about 240 cardiac cycles during 5 minutes of rest. The mean slope of identified sequences was taken to represent cardiovagal baroreflex sensitivity (BRS). Regression analyses were subsequently performed with HR responses of all individuals regressed against both age and level of BRS.

3.4.1 STATISTICAL ANALYSIS

The effect of group (normotensive/hypertensive) and the exercise task were assessed using a mixed one-way analysis of variance (ANOVA) with an alpha level of $p < 0.05$. Significant values were adjusted using the Tukey-Kramer correction. The impact of the exercise on HR from baseline was tested using the Student's T-Test, $p < 0.05$.

Statistical analyses were performed using SAS (version 9.1.3, 2002). All data are presented as mean ± standard error of the mean (S.E.M.).

3.5 NEUROIMAGING DATA

In this section the standard method for BOLD brain mapping will be described, which was used in the initial analysis stage to identify regions of activation. Subsequently, the need for an optimized method is highlighted and the various steps of the creation of a subject-matched customized template for spatial normalization will be described.

3.5.1 STANDARD ANALYSIS PROTOCOL

All fMRI data were analyzed with statistical parametric mapping software (SPM2, Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks, Sherborn, MA, USA). A two-level statistical paradigm was used for all functional imaging data. First, individual design matrices were constructed to analyze the participant-session interactions. The change in blood oxygen level-dependent (BOLD) signal over the exercise period was modeled with a boxcar function convolved with a canonical haemodynamic response function (HRF). This resulted in subject-specific contrast images containing whole brain information related to sites of both increased and decreased BOLD signal during the isometric HG task. The General Linear Model was used to calculate the parameter estimates for all brain voxels (41). The functional EPI images were realigned to the first scan of that session using a 4^{th} degree B-spline reslice interpolation method to correct for head motion. A mean functional image was created and coregistered with the participants' T_1-weighted anatomical image. The origin (e.g. $x,y,z = 0,0,0$ mm) was set at the anterior commissure (AC) parallel to the AC-posterior commissure (PC) line.

After coregistration, the anatomical image was transformed into a canonical stereotactic space (International Consortium for Brain Mapping, National Institutes of Health P-20 project; template image, avg152T1.mnc) using a trilinear interpolation method with a bounding box of dimensions: *x*, -78:78cm, *y*, -112:76cm and *z*, -50:85cm and a resampled voxel size of 2mm x 2mm x 2mm. For optimal coregistration with this structural template, the anatomical image was normalized with 16 non-linear iterations, a 25mm cut-off, and heavy regularization. These parameters were determined by Rosario et al. (113) to be optimal for the spatial normalization of mid-life/elderly imaging data for better matching of deformations and to limit distortions that might occur when warping to a young-adult template. The same normalization parameters were applied to all the functional images. To correct for resultant errors from fixed deterministic drifts (low-frequency noise) a high-pass filter at 250 seconds was applied to all functional images. The scans were smoothed using a Gaussian kernel set at 6 mm full-width at half-maximum (FWHM).

3.5.2 MOTIVATION FOR OPTIMIZED BRAIN TEMPLATE

An essential task in group comparison studies is the normalization of data from all subjects to a pre-determined standard template (as described above), which provides the reference coordinate system for statistical analysis. However, as noted by Good et al. (46), it is typically advantageous to use a customized template generated from the population specific to the study itself, rather than a generic template shared by many different studies when the images to register are very different in terms of morphology.

There is compelling evidence from post mortem and *in vivo* studies that there is ventricular enlargement and loss of brain volume in both gray and white matter with age (92, 109, 123) (Figure 3.5.2A). Due to these drastic structural and demographic differences between this subject population and that of the MNI template (young adult brains), the analysis of segmented images from the standard normalization algorithms described above often showed several small areas of mis-segmented nongrey matter voxels and produced inaccurate coordinate correspondences. For example, voxels from the dural venous sinuses and surrounding scalp/cerebrospinal fluid regions were often misclassified as grey matter and thus led to inaccurate cluster identification (Figure 3.5.2B).

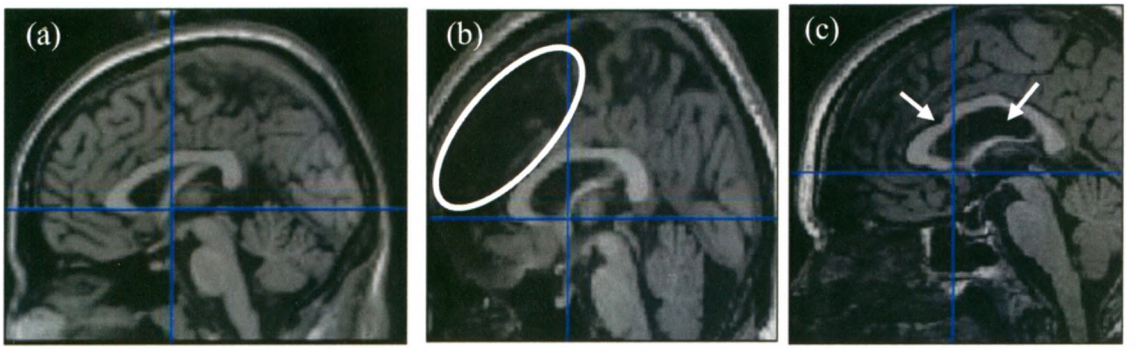

Figure 3.5.2A (a) Typical young brain. (b, c) Evidence of structural changes in an aged brain including decreased cortical thickness, prefrontal white matter atrophy (b), and ventricular enlargement (c).

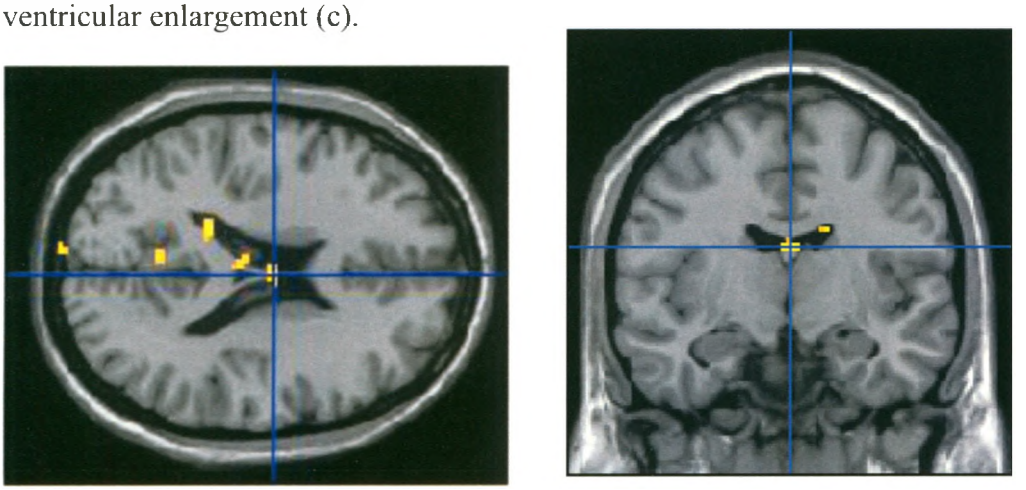

Figure 3.5.2B Misclassified activation clusters in non-cortical regions including ventricular and cerebrospinal fluid cavities when BOLD patterns of current participants were overlaid on the MNI template that was based on young individuals.

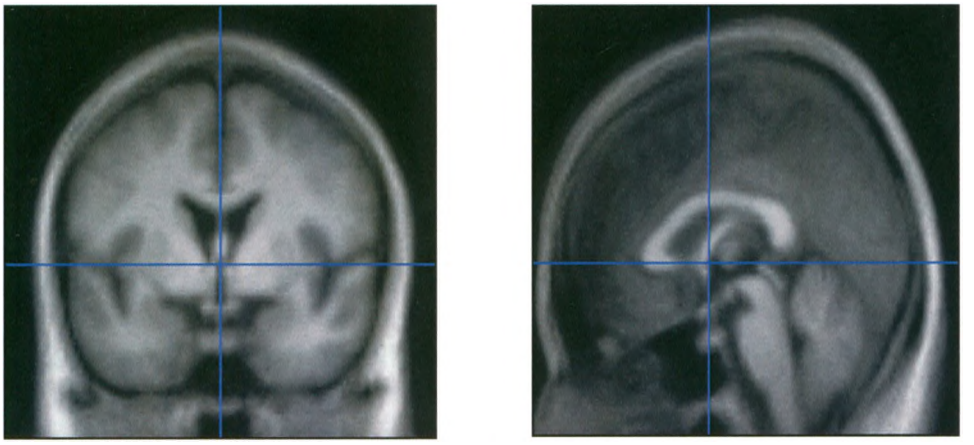

Figure 3.5.2C Customized study template created using average of all 23 aged subjects of the current study (hypertensive and normotensive).

3.5.3 OPTIMIZED FUNCTIONAL BRAIN TEMPLATE

The customized template (Figure 3.5.2C) was generated by spatially normalizing the individual T_1-weighted anatomical images of all 23 participants of the current study to a grey matter template released with SPM, which produced a group average. The construction of a template in such a fashion minimizes the deformation applied to all individual images when they are deformed to that template, thus increasing the average registration accuracy.

The standard protocol was followed as described above until the normalization step, when the anatomical image was then transformed into the stereotactic space of the customized template rather than that of the template image (avg152T1). A trilinear interpolation method with a bounding box of dimensions: *x*, -78:78cm, *y*, -112:76cm and *z*, -50:85cm and a resampled voxel size of 2mm x 2mm x 2mm was also used in this optimized image. As outlined above, the anatomical image was also normalized with 16 non-linear iterations, a 25mm cut-off, and heavy regularization. The same normalization parameters were then applied to all functional images, and the scans were spatially smoothed using a Gaussian kernel set at 8 mm FWHM. There is no optimal subject number for creation of a customized template, except to say that more subjects is always best. By changing the Gaussian kernel from 6 (standard) to 8 mm allows for the inclusion of fewer subjects in my template without compromising the model.

The resulting participant-specific contrast images representing the HG trial were initially analysed as individuals with the alpha level set at $p<0.005$ uncorrected for

multiple comparisons across the entire brain volume. Images were then entered into a repeated measures ANOVA model to estimate the main effect at the population level (HT vs. NT) (105). Based on observed variations in HR responses, an additional secondary analysis was conducted which re-grouped individuals as either a "responder" or a "non-responder". Responders were classified as having a change in HR of 3 or more beats per minute to the HG trial, and non-responders were those that did not elicit a significant change in heart rate, or had an observed decrease in HR. Three beats/min was determined to be the threshold between responder and non-responder subjects based on the median HR response of all individuals at 2.2 bpm and was below the 95% confidence interval of 4.9 bpm for HR responses in young individuals (n=8; average HR response =13.36bpm; Goswami R; PhD dissertation 2010).

In addition to a global analysis of forebrain responses in both ANOVA models, specific *a priori* regions of interest (ROI) were isolated based on previous animal and human data related to cortical structures implicated in central autonomic cardiovascular regulation (25, 59, 78). These anatomical regions included the insular cortex, anterior cingulate cortex (ACC), medial prefrontal cortex (MPFC), and the thalamus. All ROI masks were generated using the WFU_PickAtlas program (version 2.4) (71). Adjusted BOLD signal changes of these regions were extracted from the activation/deactivation peak of individual participants and were averaged across all subjects.

In a third set of analyses, the BOLD signal (both activation and deactivation) from the heart rate responder group (n=11) was regressed against the observed average HR

response during HG exercise. For each responder, the parameter estimates representing the magnitude of the activation associated with the HR response in the HG trial was entered into a repeated measures ANOVA model for the random effect analysis.

To reduce the risk of reporting false-positive results (type II error), a family-wise error correction for multiple comparisons across the entire brain volume is generally accepted as the 'gold standard' in young subjects (42). In this studied population of older adults, however, total brain activity was much lower than that of the young healthy and thus, any correction filter applied obliterated all clusters of activation. Thus, in an effort to preserve even minimal changes from baseline, all imaging data presented are uncorrected values, with an alpha level set at $p < 0.005$ unless otherwise stated. Clusters of significant activation/deactivation exceeding a minimum threshold of 10 voxels were color coded for T-score and overlaid onto the spatially normalized customized mean anatomical template. The effect size, representing the mean percent change in BOLD signal, was calculated for each significant cluster. All functional MRI data are represented in a neurological convention (i.e. subject's left appears on the left).

CHAPTER 4: RESULTS

4.1 PHYSIOLOGICAL DATA

Compared with NT (59 ± 2 bpm; $p < 0.05$), baseline heart rate was higher in HT (68 ± 3 bpm). Similarly, cardiovagal baroreflex sensitivity was lower in HT (6.8 ± 1.7 msec/mmHg) versus NT (16.4 ± 2.2 msec/mmHg; $p < 0.05$).

Heart rate increased during HG (main effect) ($p < 0.001$). However, the change in HR from baseline was not different between HT (2 ± 1 bpm; Figure 4.1.1) and NT (4 ± 1 bpm; Figure 4.1.2) ($p > 0.05$).

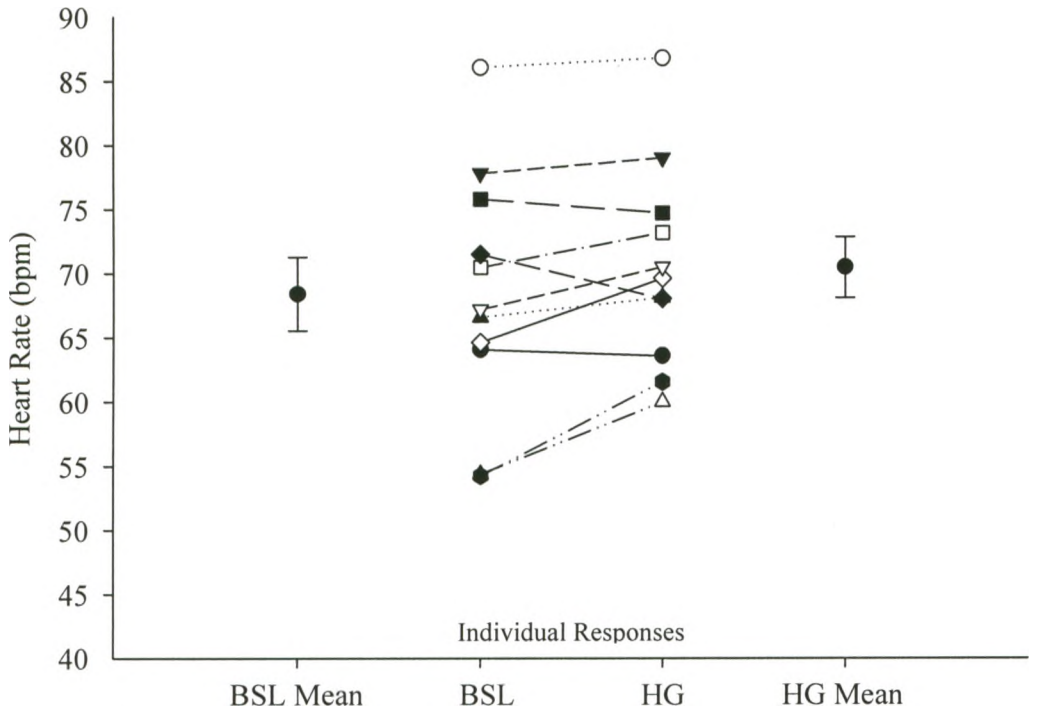

Figure 4.1.1 Heart rate response to HG exercise protocol performed by hypertensive individuals. BSL = Baseline; HG = Handgrip. Data are represented as mean ± S.E.M.

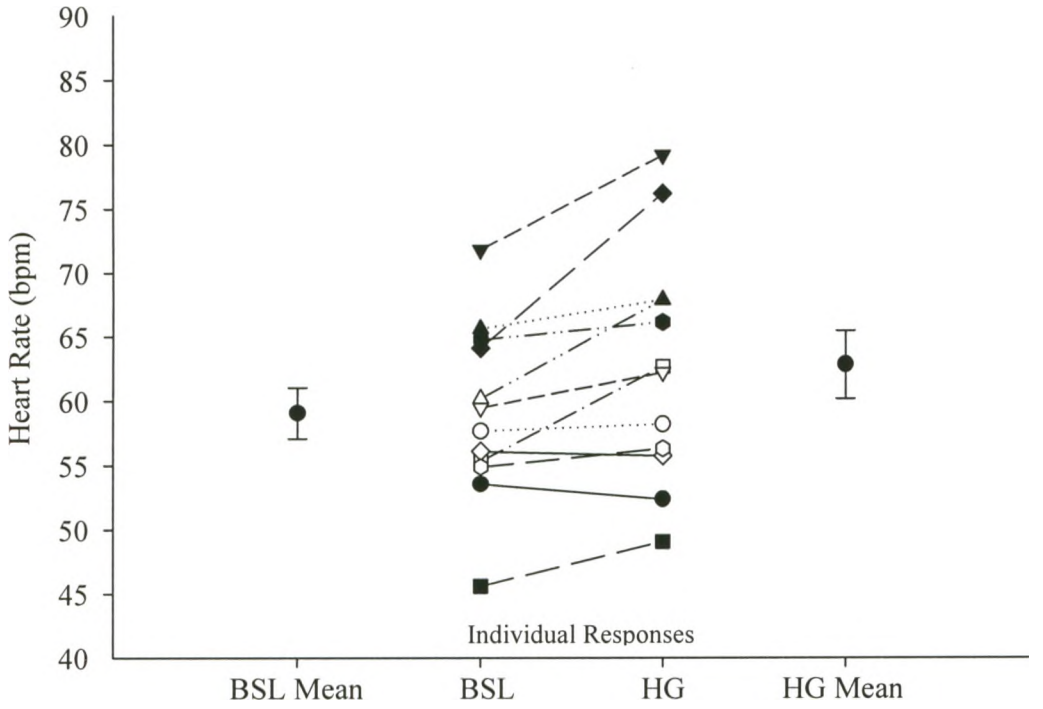

Figure 4.1.2 Heart rate response to HG exercise protocol performed by normotensive individuals.. BSL = Baseline; HG = Handgrip. Data are represented as mean ± S.E.M.

In an effort to investigate the lack of an observed HR response in both groups, individual HR responses were regressed against both age and cardiovagal baroreflex sensitivity (BRS) (shown in Figures 4.1.3 & 4.1.4, respectively). The correlation between changes in HR and age were minimal ($r^2 = 0.16$; p = not significant; Figure 4.1.3). The changes in HR to the HG task were not related to cardiovagal BRS ($r^2 = 0.03$; p = not significant; Figures 4.1.4) and did not explain our observed lack of a HR response when all individuals were considered. However, upon further analysis, two individuals were identified as having a 95% confidence interval (CI) markedly smaller than all other subjects and were confirmed to be outliers through box-plot analysis (Figure 4.1.5) and the observation that their HR responses were greater than two standard deviations away from the mean. Their subsequent removal of these two data points resulted in a correlation of significance ($r^2 = 0.25$; $p < 0.05$; Figures 4.1.4) and the conclusion that 25% of the variation in delta heart rate can be explained by BRS.

Of a total 23 subjects, eleven subjects were classified as R, and 12 were considered NR (Table 4.1.1). Among the responders, 5 were HT and 6 were NT, with male:female ratios being 4:1 and 4:2, respectively. Equal numbers of HT (n=6, 4F) and NT (n=6, 4F) subjects were included in the NR group.

Baseline HR was not different between R (61 ± 2 bpm) and NR (66 ± 3 bpm; p = 0.16). A significant tachycardic response to HG was observed in R, producing a change of 6 ± 1 bpm (p = 0.0001) from baseline, compared to NR in whom HR did not change (0.33 ± 0.41 bpm) from baseline (p = 0.43).

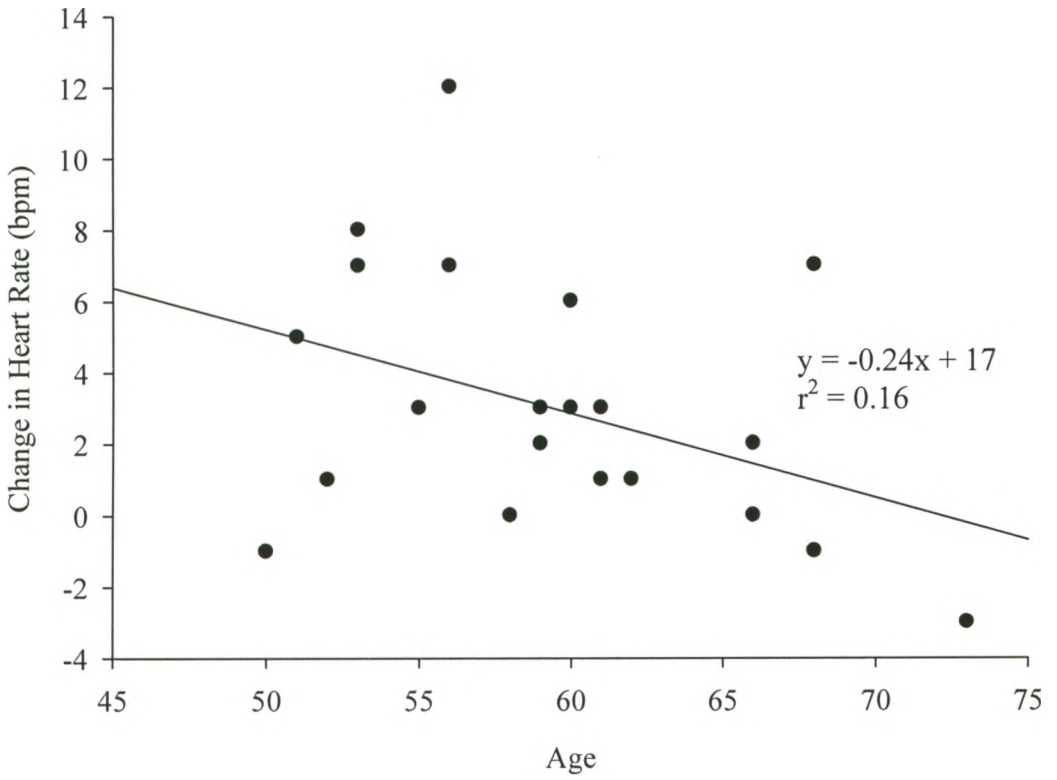

Figure 4.1.3 Individual HR responses for all subjects regressed against corresponding age.

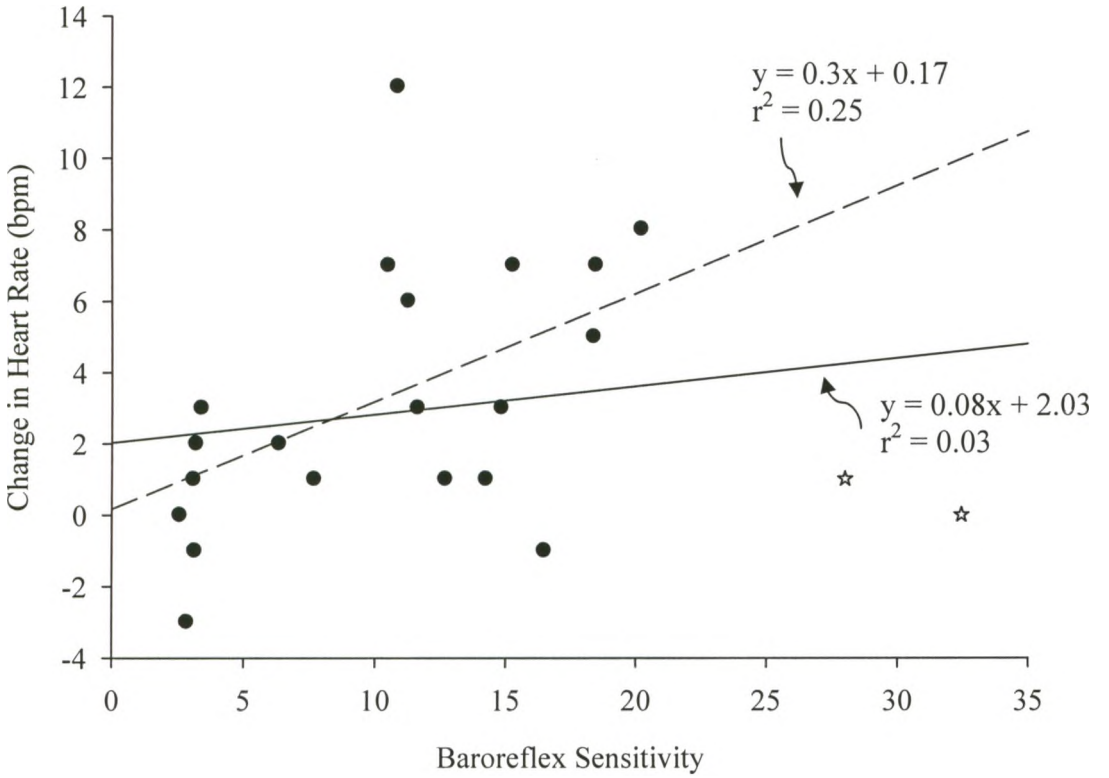

Figure 4.1.4 Individual HR responses for all subjects regressed against level of baroreflex sensitivity. Solid circles = all subjects; solid regression line ($r^2 = 0.03$). Open stars = outliers removed resulting in dashed regression line ($r^2 = 0.25$).

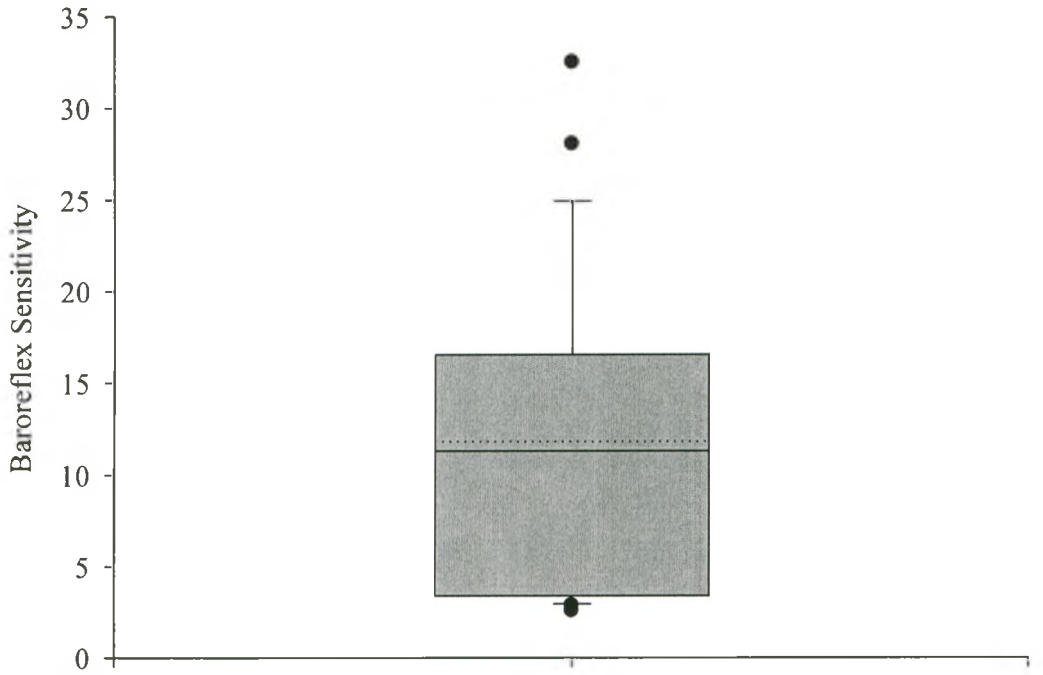

Figure 4.1.5 Box-plot analysis for baroreflex sensitivity (BRS) across all subjects. Box-plot analysis of BRS identified two outliers (i.e. greater than two standard deviations from the mean). Dashed line = mean BRS; solid line = median BRS; top and bottom of box = 25% and 75% for BRS respectively.

	Responder	Non-Responder	Baseline HR (bpm)	Z-Score	Handgrip HR (bpm)	Z-Score	ΔHG -BSL (bpm)	Z-Score
HT Group	***5***	***6***	***68 ± 3***		***70 ± 2***		***2 ± 1***	
Participant 1		x	67	-0.20	68	-0.30	2	-0.15
Participant 2	x		70	0.21	73	0.35	3	0.21
Participant 3		x	71	0.32	68	-0.31	-3	-1.73
Participant 4	x		67	-0.13	70	0.00	3	0.40
Participant 5	x		65	-0.40	70	-0.11	5	0.94
Participant 6	x		54	-1.49	62	-1.14	7	1.66
Participant 7		x	64	-0.46	64	-0.88	0	-0.79
Participant 8		x	86	1.85	87	2.08	1	-0.42
Participant 9		x	78	0.99	79	1.09	1	-0.26
Participant 10	x		54	-1.48	60	-1.34	6	1.13
Participant 11		x	76	0.78	75	0.54	-1	-0.99
NT Group	***6***	***6***	***59 ± 2***		***63 ± 3***		***4 ± 1***	
Participant 1	x		46	-1.97	49	-1.50	3	-0.06
Participant 2	x		55	-0.55	63	-0.02	7	0.90
Participant 3		x	66	0.95	68	0.55	2	-0.36
Participant 4	x		64	0.73	76	1.46	12	2.08
Participant 5		x	55	-0.61	56	-0.71	1	-0.57
Participant 6	x		72	1.85	79	1.79	7	0.90
Participant 7	x		59	0.06	62	-0.07	3	-0.25
Participant 8		x	54	-0.81	52	-1.14	-1	-1.23
Participant 9		x	58	-0.21	58	-0.50	1	-0.80
Participant 10		x	65	0.83	66	0.36	1	-0.59
Participant 11		x	56	-0.44	56	-0.77	0	-1.02
Participant 12	x		60	0.16	68	0.55	8	0.99

Table 4.1.1 Individual Heart Rate Data. The first row of each group (italics) denotes group averages, presented as means ± SEM. HT=hypertensive; NT=normotensive; HR=heart rate; bpm=beats per minute; HG=handgrip; BSL=baseline

4.2 NEUROIMAGING DATA

First-level, individual analysis of all 11 HT subjects revealed nonspecific activation in the bilateral posterior insula (left (L): n=5; right (R): n=9), dorsal (n=3) and mid-superior cingulate cortex (n=9). Deactivation was observed in the bilateral anterior insula (n=3), MPFC/subgenual cingulate cortex (n=5), and the dorsal (n=2), mid-superior (n=3) and posterior (n=6) cingulate cortex. In individual analysis of 12 NT subjects, activation was observed in the bilateral anterior insula (L: n=5, R: n=7), and the dorsal (n=4), mid-superior (n=7), and posterior cingulate cortex (n=3). Deactivation was observed in the MPFC (n=6), bilateral anterior (n=5) and posterior (n=4) insula, and throughout the cingulate cortex (n=6).

As indicated above, a second-level group analysis of the cortical response to HG was performed with both the HT and NT groups to focus on the *a priori* cortical regions associated with autonomic control. On average, deactivation was not observed in the MPFC in the HT group with HG (Figure 4.2.1A). However, deactivation was observed in the MPFC region (the subgenual ACC) and dorsal anterior cingulate cortex (ACC) of the NT group (Fig 4.2.1B). The posterior cingulate cortex (PCC) of HT subjects also showed deactivation (Fig 4.2.2).

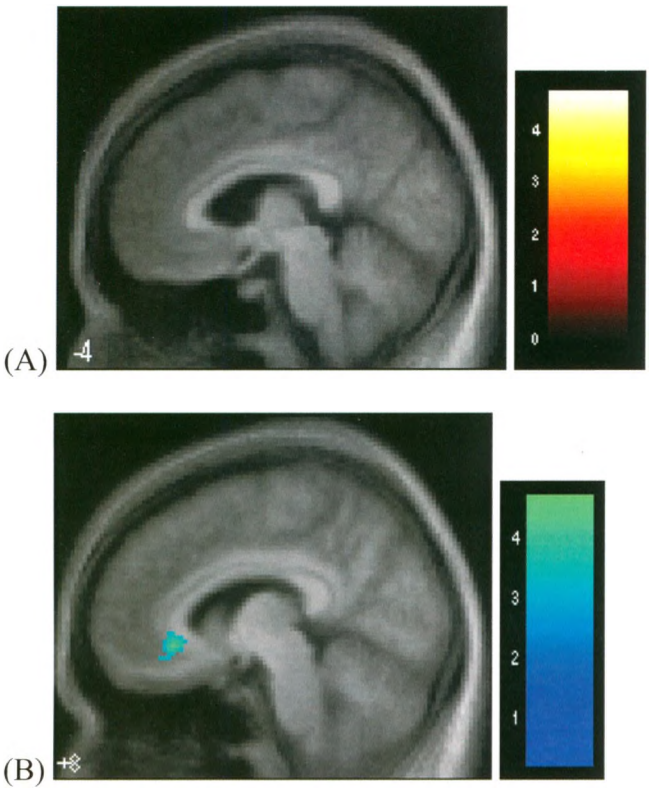

Figure 4.2.1 Cortical Response to 30% handgrip exercise protocol. (A) Deactivation was not found in the medial prefrontal cortex (MPFC) in HT subjects even at a threshold ($p < 0.005$, uncorrected). No HR change (Rest: 68 bpm, HG: 70 bpm; see Fig 4.1.1). (B) Deactivation in the subgenual and prefrontal cortex of NT subjects ($p < 0.005$, uncorrected). No HR change (Rest: 59 bpm, HG: 63 bpm; see Fig 4.1.2). The effect size (i.e. percentage of signal change) at the particular region is represented by the bar chart. The blue region denotes the deactivation cluster.

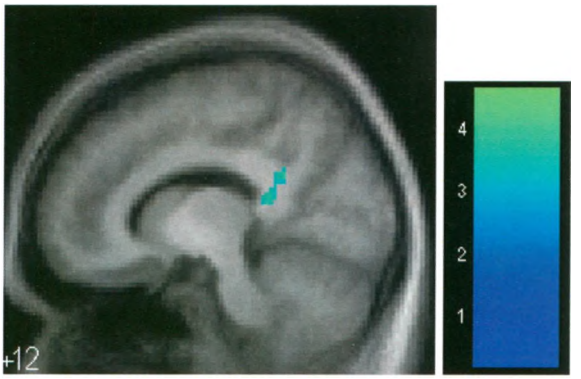

Figure 4.2.2 PCC Deactivation in HT subjects ($p < 0.005$, uncorrected). The blue region denotes the deactivation cluster as measured by the scale on the right.

In individual HR responders deactivation was observed in the prefrontal cortex during HG (Figure 4.2.3).

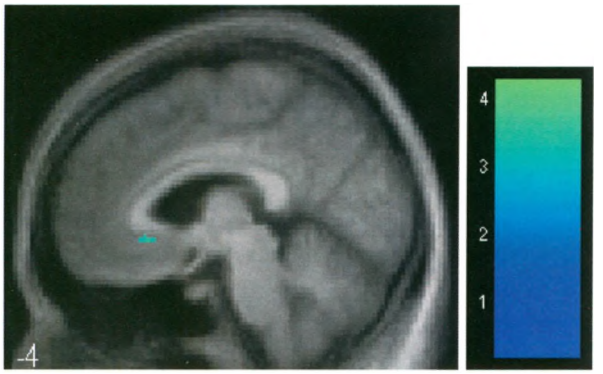

Figure 4.2.3 Subgenual cingulate cortex deactivation in R subjects ($p < 0.005$, uncorrected). HR increased with HG (Rest: 61 bpm, HG: 67 bpm). The blue region denotes the deactivation cluster as measured by the scale on the right.

In Figure 4.2.4, the time course of the BOLD change in the MPFC for the R group (n=11) was regressed with their average HR response. Consistently across all R participants, subgenual deactivation occurred during HG exercise and was correlated with an increase in HR. Heart rate increased progressively during the exercise period and peaked at the end of the exercise. Activity within the MPFC mirrored the HR response with progressive decreases in activity throughout the duration of the contraction period, reaching nadir at the end of the exercise. No such pattern was achieved in NR group, thus comparisons could not be made.

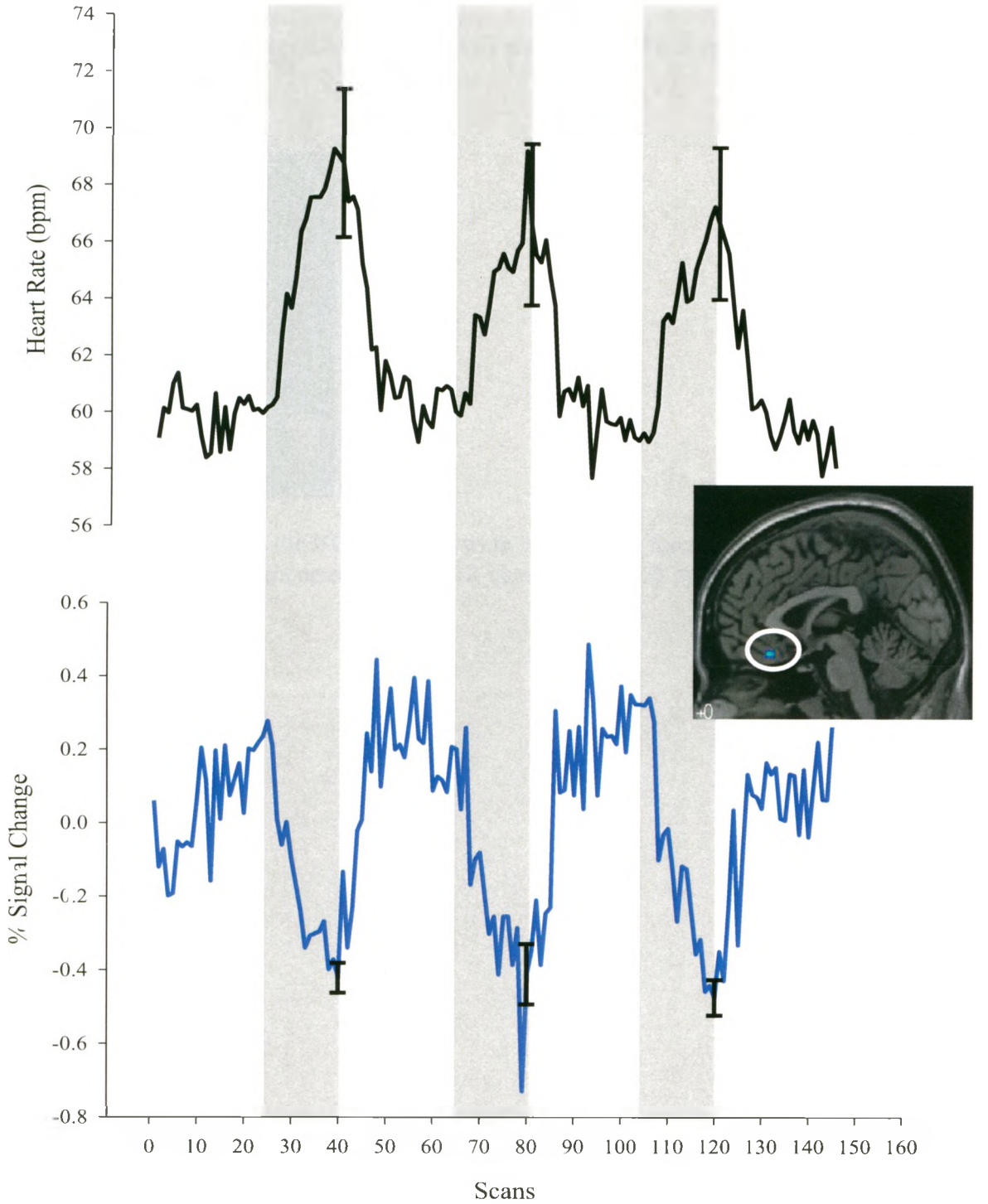

Figure 4.2.4. Average time course of the HR and BOLD response during HG in responder subjects. Top: average HR response (± SEM at end of exercise). Bottom: % signal change of MPFC. Gray vertical bars represent the HG period.

In addition to the MPFC, activation of the right insular cortex was observed in the NR group but not in the R, indicating a lack of association of this region with HR control. (Fig 4.2.5).

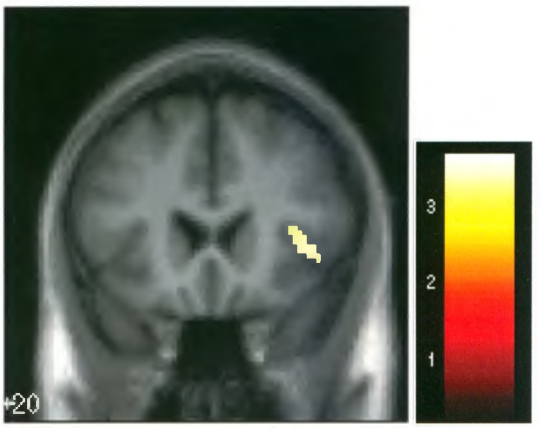

Figure 4.2.5 Increases in the BOLD signal were observed in the right anterior insula in NR subjects ($p < 0.005$, uncorrected). No HR change (Rest: 66 bpm, HG: 66 bpm).

Bilateral insular activation was also observed in the HT group (Fig 4.2.6) despite the lack of a significant increase in HR.

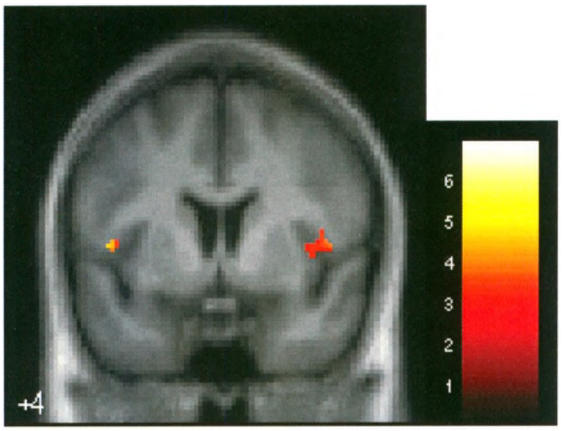

Figure 4.2.6 Bilateral insular activation in HT subjects ($p < 0.05$, corrected for multiple comparisons). Activation was constrained to the anterior region on both left (-48, 4, 4) and right sides (40, 20, 2). The effect size of the red activation patterns are represented by the bar chart.

Common cortical activities were also observed in the supplementary motor area (SMA), bilateral premotor area (precentral gyrus), bilateral somatosensory gyrus (postcentral gyrus), and cerebellum regardless of group designation (Table 4.2.1- 4.2.4). The level of motor control involved with the HG exercise allowed us to use the activity of the motor cortex as a control condition (i.e. common currency) to quantify a difference in BOLD reactivity between the two groups. The motor cortex activity was the same in both groups, but the activity of our region of interest (ROI) was different. Thus, the difference in our ROI is specific to group differences in activation patterns rather than an overall decrease in BOLD reactivity in aged subjects.

Table 4.2.1 BOLD signal changes to handgrip in hypertensive subjects.

Location		Side	Coordinates			T-score
			x	*y*	*z*	
Insula	(↑)	L	*-48*	*4*	*4*	4.74
		R	*36*	*18*	*8*	6.97
			40	*2*	*2*	3.48
	(↓)	L	*-24*	*10*	*-20*	5.13
PCC	(↓)	L	*-16*	*-44*	*6*	4.65
			14	*-48*	*26*	4.16
SMA	(↑)	L	*-4*	*2*	*52*	10.44
			-14	*-4*	*66*	7.33
		R	*6*	*12*	*46*	7.31
Precentral gyrus	(↑)	L	*-32*	*-4*	*54*	8.67
			-24	*-12*	*62*	8.21
		R	*48*	*10*	*36*	6.99
			38	*-8*	*56*	6.63
Postcentral gyrus	(↑)	L	*-42*	*-38*	*54*	10.02
			-36	*-26*	*52*	9.53
		R	*54*	*-24*	*40*	6.54
Cerebellum	(↑)	L	*-12*	*-74*	*-20*	9.31
			-34	*-80*	*-24*	8.84
		R	*42*	*-64*	*-22*	12.45
	(↓)	L	*-16*	*-40*	*-32*	4.2
		R	*22*	*-72*	*-36*	3.74
Precuneus	(↑)	L	*-12*	*-68*	*52*	4.79
		R	*10*	*-56*	*62*	7.45
	(↓)	L	*-18*	*-44*	*4*	5.42
		R	*20*	*-46*	*10*	5.82
Hippocampus	(↓)	L	*-32*	*-36*	*-8*	4.46
			-34	*-10*	*-20*	4.15
		R	*32*	*-16*	*-18*	3.66
Caudate	(↓)	L	*-10*	*12*	*-12*	4.38

SMA = supplementary motor area, PCC = posterior cingulate cortex. (↑) = activation; (↓) = deactivation. (*x* represents position in brain on horizontal axis, *y* represents position on vertical axis, *z* represents the depth position)

Table 4.2.2 BOLD signal changes to handgrip in normotensive subjects.

Location		Side	Coordinates			T-score
			x	*y*	*z*	
SMA	(↑)	L	*-4*	*-10*	*50*	3.43
		R	*6*	*4*	*52*	3.31
Subgenual ACC	(↓)	R	*10*	*32*	*-8*	4.65
			8	*38*	*-14*	3.21
Mid-superior CC	(↓)	R	*20*	*16*	*32*	3.74
		L	*-10*	*-16*	*30*	3.45
Precentral gyrus	(↑)	L	*-40*	*-24*	*58*	8.42
			-32	*-22*	*48*	5.42
		R	*50*	*8*	*48*	3.24
Postcentral gyrus	(↑)	L	*-34*	*-26*	*50*	9.17
Cerebellum	(↑)	L	*-34*	*-70*	*-24*	3.55

SMA = supplementary motor area, ACC = anterior cingulate cortex, CC = cingulate cortex. (↑) = activation; (↓) = deactivation. (*x* represents position in brain on horizontal axis, *y* represents position on vertical axis, *z* represents the depth position)

Table 4.2.3 BOLD signal changes to handgrip in heart rate responder subjects.

Location		Side	Coordinates			T-score
			x	*y*	*z*	
Subgenual ACC	(↓)	R	*6*	*34*	*2*	4.29
PCC	(↓)	R	*0*	*-54*	*28*	3.86
Mid-superior CC	(↑)	L	*-4*	*-2*	*48*	7.57
SMA	(↑)	L	*-4*	*0*	*48*	7.07
		R	*14*	*-6*	*62*	4.93
Precentral gyrus	(↑)	L	*-28*	*-22*	*68*	13.79
		R	*38*	*-10*	*58*	5.48
Postcentral gyrus	(↑)	L	*-38*	*-26*	*50*	14.47
Cerebellum	(↑)	L	*-18*	*-76*	*-20*	4.98
		R	*28*	*-70*	*-22*	4.58
Precuneus	(↓)	R	*2*	*-54*	*28*	3.8
Caudate	(↓)	L	*-10*	*16*	*-10*	5.53

SMA = supplementary motor area, ACC = anterior cingulate cortex, PCC = posterior cingulate cortex. (↑) = activation; (↓) = deactivation. (*x* represents position in brain on horizontal axis, *y* represents position on vertical axis, *z* represents the depth position)

Table 4.2.4 BOLD signal changes to handgrip in heart rate non-responder subjects.

Location		Side	Coordinates			T-score
			x	*y*	*z*	
Insula	(↑)	R	*40*	*20*	*4*	3.69
Subgenual ACC	(↓)	R	*6*	*34*	*2*	4.29
PCC	(↓)	R	*0*	*-54*	*28*	3.86
SMA	(↑)	L	*-6*	*-10*	*52*	3.57
		R	*12*	*-2*	*66*	3.82
Precentral gyrus	(↑)	L	*-36*	*-26*	*52*	7.38
		R	*50*	*8*	*38*	4.98
Postcentral gyrus	(↑)	L	*-36*	*-26*	*52*	7.38
		R	*58*	*-24*	*44*	4.69
Cerebellum	(↑)	L	*-30*	*-74*	*-26*	5.95
		R	*32*	*-72*	*-22*	4.67
Precuneus	(↓)	R	*2*	*-54*	*28*	3.8
Caudate	(↓)	L	*-10*	*16*	*-10*	5.53

SMA = supplementary motor area, ACC = anterior cingulate cortex, PCC = posterior cingulate cortex. (↑) = activation; (↓) = deactivation. (*x* represents position in brain on horizontal axis, *y* represents position on vertical axis, *z* represents the depth position)

CHAPTER 5: DISCUSSION

The major finding of this study was that hypertension is equivalent to normotension in terms of heart rate control and functional CAN responses to the HG task. Rather, the cardiovagal response to exercise and the observed correlation between medial prefrontal cortex (MPFC) activity was strongest in "responder" subjects, regardless of blood pressure status. Even though baroreflex sensitivity (BRS) was lower in HT versus NT group, these results suggest that HT itself does not directly predict a heart rate (HR) response to hand-grip (HG) in older individuals. Importantly, if there is a response in either HT or NT individuals, the association between MPFC deactivation and HR is retained.

5.1 PHYSIOLOGICAL RESULTS

Based on the fact that BRS is lower in HT than NT and the idea that BRS reflects cardiovagal control, the current study focused on the detrimental effect of hypertension (HT) on HR control and regulation of the parasympathetic nervous system (PNS). Considering that HR responses were similar between the two age-matched groups, this negative effect of HT was not supported by our physiological results. Similarly, there was no correlation when individual HR responses were regressed against BRS slope. Thus, these data failed to support the expected effect of hypertension on cardiac responsiveness to HG.

The range of BRS values were rather narrow in both subject groups, which may be limiting the current regression analysis. This becomes clear when comparing these

data to those obtained in young individuals. For example, Steinback and colleagues reported an average resting BRS slope of 23 ± 5 msec/mmHg in healthy, young subjects with a range of slope values from 15-25 (126). Normotensive subjects within this study thus lie at the low end of normal limits for a healthy, active population and exhibit an average BRS slope of 16.4 ± 2.2 msec/mmHg. Hypertensive subjects, however, show a marked decline in BRS with an average slope of only 6.8 ± 1.7 msec/mmHg. This is evidence that there is an effect of HT on BRS. To date, very limited work has been done looking at the effect of HT on BRS and could be further elucidated in a study involving more subjects.

This apparent separation between NT and HT subjects on the BRS curve may ultimately reveal an effect of HT on BRS and sympathetic regulation, or it may be attributable to a small effect of age. Both NT and HT groups are considered to be in older adulthood and fell within age-matched limits for study inclusion. However, the HT subjects were, on average, older than NT subjects (63 and 56 years, respectively) with all HT subjects being greater than 60 years of age. BRS has been shown to decline naturally with age (24, 34, 38, 58) and our results appear to support this idea of an age-dependent decline with the oldest HT subject (73y) having the lowest BRS slope (2.9 msec/mmHg) and the youngest HT subject (51y) having the highest BRS slope (18.4 msec/mmHg) respectively. This level of cardiovagal control is consistent with the idea that BRS affects the ability to increase their HR to the HG task as they consequently had the lowest, and one of the highest changes in HR (-3 and +5 bpm), respectively. Although regression

analysis determined this relationship to be weak, this is likely attributable to the small range of BRS values across the population, which is therefore limiting the correlation.

There were two outlying individuals who had very high BRS slopes (32.5 and 28; Fig 4.1.4) with a mismatched low heart rate response (0 and +1 bpm). Aside from the fact that they are both NT, they share no similarities that would explain this result. One was male and one was female; both are around 60 years of age with normal resting BP and HR values, and neither was on any form of medication. One possible explanation for their elevated level of BRS when compared to all other subjects is their level of physical activity. Both of these subjects lead chronically active lives including recreational running and fitness classes. Numerous studies have shown that long-term endurance training beneficially influences autonomic control of heart function (15). Endurance training increases parasympathetic activity and decreases sympathetic nerve activity, which causes a decreased resting HR. The decrease in parasympathetic control of the heart with aging can be minimized by regular endurance exercise and may explain why these two individuals show such a high level of BRS. Although this was not supported by a coordinated HR response to HG, both subjects had deactivation in the MPFC. Therefore, it appears as though the BRS is related to the regulation of the MPFC, but not necessarily HR. The MPFC is only one part of the CAN, and it may be that other manual elements of the heart simply do not respond normally in some individuals (ie. altered sinoatrial node conduction etc). Taking these two subjects out of the average calculation for BRS slope in the NT group nets an average slope of 13.6. Although this value is lower and more strongly supports the effect of age on BRS, it is still more than double

that of the HT group and just outside the limits of normal, young adults. All NT subjects were recreationally active and participated in regular physical activity. However, the activity levels of HT subjects were not monitored. This would be something to include in order to claim that regular exercise preserves PNS function. However, this can be difficult to measure in those individuals whose heart no longer responds.

Aging is associated with an increase in sympathetic nerve activity (SNA) and a decrease in PNS activity (38, 50, 58, 74, 119). As explained above, a tachycardic heart rate response to a handgrip (HG) exercise task is mediated solely by PNS withdrawal and independent of sympathetic activation (52, 76, 120). Thus, in an aged population with naturally lower PNS activity compared to young subjects, both HT and normotensive (NT) groups would be expected to have a decreased response to HG exercise. For example: young, healthy subjects exhibit HR responses of 10 ± 2 bpm (136), which, when compared to the observed average older adult response of 3 ± 1 bpm, illustrates the effect of advancing age on the control of HR. However, correlations were not impressive when individual HR responses were regressed against age. Thus, the narrow age range in the current study may explain the poor correlation between age and the lack of an observed HR response.

In addition, baseline HR may affect the HR response (114). Our baseline HR values were again, within a very small range, but illustrate a wide range of responses to the HG task (Table 4.1.1). The subject with the highest baseline heart rate of all individuals (86 bpm) was from the HT patient group and demonstrated a very low BRS

slope (3.1) with almost no HR response (+1 bpm) to the exercise task. We also found that the two HT individuals with the lowest resting HR (54 bpm) had the greatest increase from baseline with the exercise task (+6 and 7 bpm, respectively). While these individuals fit the expected relationship between baseline HR and HR responsiveness, the overall group did not fit this model likely because of the truncated range of HR responses.

The effect of medication within this population is also something to consider. As outlined in the methodology, the most frequently administered type of antihypertensive drug taken by 9 of the 11 HT subjects was an angiotensin-II antagonist. Angiotensin-II receptor antagonists (ARB) are vasodilators of the resistance vessels, and hence reduce peripheral vascular resistance. This class of drug blocks the activation of the angiotensin-II receptor by the circulating effector peptide of the renin-angiotensin system, angiotensin-II, which has several detrimental actions including vasoconstriction and a subsequent rise in blood pressure (31). Its suppression therefore directly causes vasodilation and a reduction in blood pressure (BP). The hemodynamic profile of the ARB is very similar to that of the angiotensin-converting-enzyme (ACE) inhibitors, which the remaining two participants were currently taking: heart rate remains unchanged and is not associated with any reflex tachycardia (131). Diuretics were combined with other medications in three individuals to help reduce the workload on the heart, but they act solely on the kidneys and have no known effect on the neural control of the heart.

Beta-blockers were prescribed to two individuals also in combination with another form of medication to combat their high BP. It is doubtful that these drugs affected the HR response of the current study specifically because pharmacological studies have concluded that the observed fluctuations in HR at the onset of light dynamic exercise can be explained by a rapid vagal withdrawal and are not altered by beta adrenoceptor blockade (35, 83, 134). Fagraeus and colleagues reported that β-blockers act solely on the SNS, and although these conclusions were established in studies performed in young individuals, they should not impact the PNS mediated responses the current study was examining (35). It was assumed that this response also applies to the older individuals of the current study. Despite being medicated by these different pharmacological interventions, the desired effect of these medications was not successful in 9 of the 11 HT persons who maintained uncontrollably high BP. In should be noted, however, that these resting BP values were taken 30 minutes before their fMRI test and may be confounded by increased anxiety.

5.2 NEUROIMAGING RESULTS

Evidence of BOLD changes were seen in both CAN and expected motor regions in both HT and NT groups, with HT subjects displaying more activation in motor control regions than NT counterparts. This finding was not preserved when subjects were re-analyzed as responders and non-responders; therefore, this was attributed to an effect of participant grouping but may be cause for further investigation in the future.

Despite the lack of a significant HR response to exercise in NT subjects, deactivation in the MPFC was observed in 11 out of 12 subjects in this group, and was preserved when analyzed at the group level. Although secondary analysis of HT subjects did not show MPFC deactivation, it was noted in individual analysis that 5 out of 11 HT subjects exhibited significant deactivation in this *a priori* region. Further analysis of all 23 subjects as individuals explained this inconsistency as subgenual deactivation was found in all 11 subjects who had been classified as a HR responder (R; $\Delta HR > 3bpm$). This pattern of deactivation follows previously established work in young, healthy controls relating to deactivation in the MPFC (136). Thus, the neuroimaging findings revealed that the observed heart rate response in select individuals occurred independent of blood pressure or pharmacologic status and was correlated with the deactivation of the MPFC (12, 132). It appears then that the connection between the MPFC and HR is still valid. It was interesting to observe however, that in contrast to marked consistency in the HR-MPFC linkage in young (136), only 50% of older individuals expressed this connection.

The observed deactivation in the posterior cingulate cortex (PCC) of HT subjects has previously been seen in healthy, young subjects performing the HG task (136), and was not found to correlate with the HR response as the MPFC/subgenual cortex did. Since HT subjects did not elicit a HR response, the observed deactivation in this region is likely attributed to its role in the integration of sensory information during the resting default state and its activities are hence attenuated during goal-directed behaviors, such as HG (108).

The insular cortex (IC) has received considerable attention along with the MPFC as being one of the dominant regions within the autonomic network. Autonomic responses, including changes in HR and BP, have been evoked by electrical stimulation within discrete regions of the MPFC (12, 101) and the IC (16, 99). Neuronal responses were also recorded in the IC when HR and BP changes were elicited by stimulation of the vagus nerve (3) and baroreflex afferents (17). It is therefore surprising that the two groups of subjects who did not have any fluctuations in HR and who, for the most part, have the lowest BRS values, would show activation in this region. The observed activation was predominantly on the right side of the brain and minimal, if any, activation was evident on the left side. The left IC has been shown to be predominantly responsible for parasympathetic cardiovascular effects, of which these two populations have very little (100). Left insular activation would suggest that there is some level of vagal control still present and thus being withdrawn from the demands of the task. Damage to this area could shift cardiovascular balance towards increased basal sympathetic tone (a pro-arrhythmic condition), and has been associated with a decrease in the randomness of heart rate variability (100). The observed insular activation could also be a somatosensory response as Zhang and colleagues suggested through its connections with the limbic system (138). Nevertheless, the insula appears to be involved in human heart rate regulation and damage to the left side specifically, may encourage a tendency towards reduced vagal outflow as observed in the HT and non-responder populations.

5.3 STUDY LIMITATIONS

All of the data presented thus far has been reported and analyzed as "uncorrected" for multiple comparisons. Generally, 14-16 subjects per group are required to obtain significant results after correcting for multiple comparisons (i.e. family-wise error; FWE, and FDR). Because our sample volume was smaller than this, application of a correction filter reduced the ability to observe borderline activation patterns. Although uncorrected results are not optimal, it is well known that aged and young subjects may show a difference in the reactivity of the BOLD response, which may explain why total brain activity was much lower in these older adults (53). Huettel and colleagues found a similar problem when analyzing aged brains, which they attributed to younger subjects having a higher signal-to-noise ratio (SNR) than older subjects (53). A 32-channel head coil was used in the current study to optimize the SNR across all subjects and we were able to definitively rule out head motion as a primary contributor given that overall movement distortions were less than 2mm and within acceptable limits. Nonetheless, a strong a priori hypothesis reduces the concern regarding statistical thresholds.

As previously explained in the results section, data were "unwarped" during the pre-processing stages of the raw data, which has been shown to be a correction of sufficient magnitude if head motion is less than 3mm (56). Even with such corrections, it is possible that the reduced spatial extent of activation is due to differences between the groups resulting from physiological changes accompanying aging, such as vascular and respiratory adaptations, which would necessarily contribute to the measurement of HDRs using fMRI.

Due to a lack of familiarization with the MRI environment, some subjects may have suffered mild anxiety or claustrophobia, potentially confounding the data. Future studies could involve the use of a "phantom" (0T) magnet, which would allow participants to better prepare for the confined space and feel more comfortable during the testing session. In an effort to control for this "fear bias" and to validate the observed neuroimaging responses, an identical protocol could be conducted in a controlled laboratory setting where HR and BP values can be monitored in a more neutral environment. We have used this approach in previous studies but it is uncertain that such measures provide additional benefit.

5.4 FUTURE DIRECTIONS

Future studies should make use of more direct measures of parasympathetic and/or sympathetic outflow, such as indwelling recordings of vagal outflow and SNA. This would provide further clarity regarding the pathways responsible for the observed HR changes and confirm that they were not elicited by peripheral sympathetic activation. In addition, more advanced imaging techniques could be administered such as arterial-spin labeling (ASL) and diffusion-tensor imaging (DTI). The addition of these two measurements would enable the assessment of potential age-related declines in cerebral blood flow and changes to axonal white-matter connection patterns, which may help elucidate more concrete conclusions regarding disease and age including whether diminished neurovascular coupling may explain the reduced BOLD response seen in HT and NR groups.

Further controlled studies are necessary to clarify the mechanisms through which blood pressure lowering might reduce the risk of autonomic dysfunction in older subjects and whether it is possible that antihypertensive medication may afford some degree of functional protection. The positive effects of antihypertensive medication on brain structure and cognitive function have been studied extensively, yet findings are still inconclusive. In 2003, Raz and colleagues found that brain structure and cognition were similar in medically treated hypertension when compared to untreated hypertension and concluded that antihypertensive medication did not afford a degree of functional brain protection (110). In contrast, Firbank and colleagues suggest that the increased progression of structural changes in the brain with age may be ameliorated by successful antihypertensive treatment (36). More research is needed to clarify the potential of drug therapy, but this may provide an avenue of exploration for autonomic rehabilitation.

Lastly, exercise-induced neuroplastic changes are thought to play critical roles in mediating important beneficial effects associated with physical activity, including improved memory, cognitive function and cardioprotection (33, 87, 130). In addition, exercise training has been shown to induce important adaptive and beneficial autonomic and cardiovascular adjustments, in order to ensure proper blood perfusion of peripheral tissues according to metabolic demands (69, 80, 81, 114). Additionally, the effect endurance training exerts on baroreflex function in older adults has been studied to provide insight into potential mechanisms underlying the cardioprotective effect of regular exercise (102). Cross-sectional studies suggest that endurance training is

associated with greater levels of cardiovagal BRS in older endurance-trained compared with older sedentary adults (87, 97) and age-associated declines in cardiovagal BRS are attenuated in endurance-trained compared with sedentary adults (86). Whether exercise-induced cardiovascular and autonomic adjustments involve neuroprotection as an underlying mechanism is at present not fully elucidated and further research is needed.

5.5 SIGNIFICANCE OF FINDINGS

The major finding of this study was that hypertension is equivalent to normotension in terms of the HR response to HG and the MPFC-HR association. Yet, both groups expressed responses that were less than observed in young individuals. Thus, aging appears to have an important, but as yet unexplained, effect on CAN function. Why and how this affects some, but not all aging individuals requires further study.

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APPENDIX I

Office of Research Ethics

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Western

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. J.K. Shoemaker
Review Number: 13395 **Review Level:** Full Board
Review Date: June 19, 2007
Protocol Title: Forebrain network involved in modulating autonomic and cardiovascular responses
Department and Institution: Kinesiology, University of Western Ontario
Sponsor: CIHR-CANADIAN INSTITUTE OF HEALTH RESEARCH
Ethics Approval Date: June 27, 2007 **Expiry Date:** December 31, 2012
Documents Reviewed and Approved: UWO Protocol and Letter of Information and Consent.
Documents Received for Information:

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 study 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.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

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.

Chair of HSREB: Dr. John W. McDonald
Deputy Chair: Susan Hoddinott

Ethics Officer to Contact for Further Information		
☑ Jennifer McEwen	☐ Denise Grafton	☐ Ethics Officer (ethics@uwo.ca)

cc: ORE File
LHRI

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