HUMAN CENTRAL AUTONOMIC CARDIOVASCULAR REGULATION DURING EXERCISE: BRAIN REGIONS INVOLVED WITH CENTRAL COMMAND AND THE EXERCISE PRESSOR REFLEX

by

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ABSTRACT

Background: Isometric handgrip (IHG) exercise increases heart rate (HR) and mean arterial pressure (MAP); MAP can be sustained after exercise via post-exercise ischemia (PEI). HR and MAP responses are mediated by feed-forward cortical signals (central command, CC) and neural feedback from active muscles (exercise pressor reflex, EPR). **Purpose:** Differentiate between cortical regions involved with CC versus the EPR via changes in alpha (8-12Hz) and beta (13-30Hz) power using magnetoencephalography (MEG). **Methods:** Participants (n=11, 22 ± 2 years) completed a repeated IHG and PEI protocol at 5% (control) and 40% maximum force. **Results:** HR and MAP increased ($p<0.04$) early during IHG (CC only), while MAP increased further ($p=0.03$) as IHG continued (CC & EPR). The MAP response persisted during PEI (EPR, $p=0.07$). During IHG, alpha and beta power decreased within the contralateral sensorimotor cortex. Power increased within MEG sensors associated with the ipsilateral (IHG-alpha) and contralateral (IHG-beta and PEI-beta) insular cortex.

LIST OF ABBREVIATIONS USED

ABP- arterial blood pressure

ACC- anterior cingulate cortex

ANOVA- analysis of variance

ANS- autonomic nervous system

BOLD- blood-oxygen level dependent

BP - blood pressure

CC- central command

CVLM- caudal ventrolateral medulla

DMNV- dorsal motor nucleus of the vagus

ECG- electrocardiograph

EMG- electromyography

EOG- electrooculogram

EPR- exercise pressor reflex

ERP- event related potential

fMRI- functional magnetic resonance imaging

HPI- head position indicator

HR- heart rate

IC- insular cortex

IHG- isometric handgrip exercise

MAP- mean arterial pressure

MEG- magnetoencephalography

MPFC- medial prefrontal cortex

MSNA- muscle sympathetic nerve activity

NTS- nucleus tractus solitarius

PAG- periaqueductal grey

PEI- post-exercise ischemia

PET- positron emission tomography

PNS- peripheral nervous system

PSP- post-synaptic potential

rCBF- regional cerebral blood flow

REB- research ethics board

RR- respiration rate

RVLM- rostral ventrolateral medulla

SA- sinoatrial

SNS-sympathetic nervous system

SQUID- super-conducting quantum interface device

STN- subthalamic nucleus

SV- stroke volume

TMS- transcranial magnetic stimulation

TPR- total peripheral resistance

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CHAPTER 1: INTRODUCTION

Cardiovascular (CV) disease is an umbrella term used to describe any disease that affects the structure or function of the heart or blood vessels. This class of diseases includes heart conditions such as myocardial infarction, atherosclerosis, stroke, atrial fibrillation, hypertension, and cardiac arrest (6). Diseases of the CV system are the second leading cause of death in Canada next to cancer, afflicting over 1.3 million Canadians (6). A fundamental determinant of CV health lies in the precise regulation of parasympathetic (heart) and sympathetic (heart and blood vessels) divisions of the autonomic nervous system (ANS). Decreased activity of the parasympathetic nervous system (PNS) and/or or elevated activity of the sympathetic nervous system (SNS) have been identified as significant risk factors for CV disease (7, 8)

During exercise, autonomic balance is shifted to meet the increased physiologic demands of the activity. Parasympathetic influence is immediately 'withdrawn', allowing for a rapid increase in heart rate (HR). As exercise intensity and/or duration increases, the SNS causes further increases in HR, as well as an increase in blood pressure (BP), which serves to redirect blood to the areas that are most metabolically active. Efferent neural signals from higher cortical brain regions, termed central command (CC), in combination with peripheral afferent feedback from the active skeletal muscles via the exercise pressor reflex (EPR), are two fundamental physiological control mechanisms. Both CC and the EPR are responsible for altering sympathetic and parasympathetic activity and coordinating the comprehensive CV response to exercise.

The objective of this magnetoencephalography (MEG) investigation was to uncover the cortical regions involved with CC and the EPR by examining changes in

cortical activation during an isometric handgrip exercise (IHG) and post-exercise ischemia (PEI) task in conscious humans. Specifically, we sought to uncover the brain regions involved with the immediate CV response to exercise (i.e. CC) separately from cortical areas that help mediate the sustained elevation in HR and BP that persists with prolonged isometric exercise (i.e. the EPR).

Participants completed ten bouts of IHG exercise (90 seconds each) that alternated between 5% and 40% of their maximal voluntary contraction (MVC) force output. Each bout of IHG exercise was immediately followed by a 90 second PEI period, which consisted of an inflated pressure cuff placed over their exercised arm that temporarily stopped the flow of blood to the previously exercised forearm muscles. The 5% IHG condition was included to serve as a sensory "control" that was not expected to elicit a significant CV response but would replicate some of the sensory experiences involved with performing the IHG and PEI protocols. The 40% IHG condition served to immediately activate CC, and as metabolic by-products accumulated (e.g., H^+, CO_2, etc.) within the forearm, initiate the delayed (~45 seconds) EPR response. The purpose of the PEI protocol was to isolate the EPR-mediated contribution of the CV response to exercise from CC. Specifically, at the end of the IHG period, CC signals cease while the post-exercise ischemic period serves to "trap" the metabolic by-products within the forearm to sustain the EPR. Continuous recordings of HR and BP were collected to represent the cardiovascular response.

Few studies have attempted to localize and differentiate between the brain regions associated with CC and the EPR (2). The current state of knowledge regarding cortical brain regions responsible for autonomic CV control is largely deduced from studies

conducted in anaesthetized animals (9-13). The few studies that have examined these relationships in conscious humans have employed technologies inadequate for accurately monitoring rapid neural transmission [e.g. blood-oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI)] or that have prevented concurrent recordings of both brain activity and cardiovascular variables, such as non-invasive continuous measures of BP (2-4, 14). This study will mark the first known attempt to simultaneously measure brain neural activity and non-invasive continuous BP and HR measurements in conscious humans during exercise.

1.1 CARDIOVASCULAR HEALTH

The Heart and Stroke Foundation's 2010 Annual Report on Canadians' Health did not project positively for the future CV health of Canadians (11). The report speculated that demographic changes in conjunction with increasing engagement in unhealthy heart behavior would lead to the "perfect storm". The risk for developing CV disease is now affecting both a younger and broader population, posing an unprecedented threat on the nation's already struggling health care system (11). The Canadian Community Heart Health Survey reported that 47% of Canadians between the ages of 20-34 are inactive, 41% are overweight or obese, and 29% consume tobacco products (11). Moreover, the heart healthy behaviors of the 13 Canadian provinces and territories were assessed according to the percent of the population that were smoke-free, physically active, maintained a healthy body weight, and consumed the recommended daily servings of fruits and vegetables; Nova Scotia ranked a disappointing third last (11). Furthermore, the percentage of Nova Scotians age 65 and older is expected to increase by approximately 55% between 2005 and 2021 (11). Provincial statistics regarding modifiable (e.g. diet and

exercise) and non-modifiable (e.g. age and genetics) risk factors suggest that an upward trend in CV disease rates can be expected in Nova Scotia within the coming years (11).

A basic physiological response associated with an elevated risk for CV disease is an increase in resting blood pressure. Healthy normotensive individuals' exhibit more pronounced vagal influence on the heart than their hypertensive counterparts (15). The natural rise in BP that accompanies exercise is also amplified in hypertensive individuals (16-18). An exaggerated BP response to exercise increases the likelihood that a hypertensive individual will suffer a cardiac event when engaging in physical activity. Despite this increased risk, CV treatment and prevention programs boast the heart healthy effects of physical activity; thus a deeper understanding of the mechanisms associated with the exercise response may aid in uncovering a safe method to get at-risk populations active. Second, an exploration into the brain regions implicated with CV control during rest and exercise is important to fully understand the development and mitigation of CV disease, and may lend to novel treatment and prevention strategies.

CHAPTER 2: REVIEW OF LITERATURE

In 1895, Johansson (19) noted that unlike voluntary exercise, passive stretching or electrical stimulation of rabbit muscles did not elicit a near-immediate increase in HR. Such observations led Johansson to postulate that signals altering HR must be generated centrally and transmitted neurally, as chemically mediated feedback could not account for the accelerated response noted during voluntary exercise (19). It was hypothesized that efferent signals originating in the motor cortex stimulate autonomic neural pathways that control CV function (20). Similar observations were made by Krogh and Lindhard in 1913 (21), which led them to coin the term "cortical irradiation" to describe the descending feed-forward propagation of neural activity from the cortex to both CV-related nuclei of the brainstem (medulla) and to active skeletal muscle. Cortical irradiation was initially viewed as a "top-down" diffusion of a neural signal as opposed to the propagation of signals through distinct neural networks dedicated to CV control (20). A feed-forward system serves to control specific physiological variables in the absence of system feedback in an effort to rectify disruptions that threaten homeostatic balance (22). The early concept of cortical irradiation was met with opposition; the notion of CV control as a feed-forward mechanism begged the question of how it was capable of such elegant CV responses in the absence of an internal error signal. Furthermore, criticism stemmed from the lack of quantifiable evidence to support the theory (20).

The early theories of Johansson and the work of Krogh and Lindhard were fundamental in understanding the basic structure of central CV control. However, with the emergence of new knowledge, the theory of cortical irradiation was replaced by the current theory of Central Command (23). The classic definition of CC posits that it is a

feed-forward mechanism involving parallel activation of both motor and CV centres within the cerebral cortex during exercise (4, 20, 24, 25). It is hypothesized that brain regions related to CC transmit signals to brainstem autonomic nuclei responsible for CV regulation. Within the brainstem, descending signals from the cerebral cortex integrate with ascending sensory afferent signals from exercising skeletal muscle (i.e. muscle mechanoreceptors and metaboreceptors) that constitute the EPR. Thus, the convergence of CC and the EPR within medullary integration sites are capable of generating a comprehensive CV response to exercise.

2.1 Basics of Autonomic Cardiovascular Control

The human nervous system is an intricate communication network subdivided in a hierarchical fashion. The term 'human nervous system' first branches into the central nervous system (including the brain and spinal cord), and the peripheral nervous system, which is comprised of all neurons lying outside of the central nervous system. The peripheral nervous system can be further divided into the somatic and autonomic nervous system (ANS). The somatic system functions through voluntary control of skeletal muscles, while the ANS acts to propagate involuntary neural signals. The ANS responds to deviations within the internal and external environment, and thus plays a paramount role in homeostatic maintenance. The autonomic division is comprised of central ganglia and peripheral nerves that innervate the viscera and vasculature (26, 27). Efferent peripheral neurons are integral to the control of smooth muscle and secretory cells, while afferent peripheral neurons are concerned with reflexes and the relay of sensory information to the brain and spinal cord (26). The autonomic nervous system further subdivides into two distinct branches with opposing physiological functions, commonly

referred to as the “rest and digest” (i.e. parasympathetic) and the “fight or flight” (i.e. sympathetic) systems.

The parasympathetic nervous system (PNS) is noted for promoting energy conservation and physiologic recovery. This system is the dominant regulator of visceral organ function during rest (26). The PNS is comprised of lengthy preganglionic neurons that originate within the brainstem and sacral region of the spinal cord, innervating the vagus nerve (cranial nerve X) amongst others (26). The vagus nerve governs the cardiac pacemaker or sinoatrial node (SA), thus playing a critical role in determining HR during rest. Increases in the magnitude of PNS innervation to the SA node results in a decrease in HR, and vice versa. As such, rapid increases in HR during exercise are mediated by a rapid withdrawal of PNS innervation to the SA node.

In contrast, the sympathetic nervous system (SNS) enables the body to respond to environmental and physiologic challenges (26). The preganglionic neurons of the SNS originate within the thoracic and lumbar regions of the spinal cord. The SNS is comprised of long postganglionic neurons that communicate with regions of the entire body (28). Among the end organ sites of the SNS are the heart and vasculature. At rest, the contribution of the SNS is minimal, however, in the presence of challenging conditions the activity of the SNS is amplified.

Elevated activity of the SNS leads to increased innervation of the SA node, ventricular cardiac muscle and the vasculature. The “fight or flight” response is therefore accompanied by more rapid and forceful cardiac contractions, effectively increasing HR and the volume of blood expelled with each beat [i.e. stroke volume, (SV)]. Changes in the rate and force of cardiac contractions are often quantified as cardiac output (CO), the

product of heart rate and stroke volume. Vasoconstriction, or the narrowing of blood vessels, to inactive regions of the body in conjunction with increased cardiac output, contributes to the rise in arterial blood pressure during exercise. These alterations are reflected by a measure known as total peripheral resistance (TPR), a quantification of the resistance to blood flow within the peripheral vasculature. This measure is defined as the sum of the resistance within the peripheral vasculature across the cardiovascular system, calculated mathematically as the quotient of MAP/CO. This value is often expressed in units of Pascal seconds per cubic meter ($Pa \bullet s/m^3$), which are often favored over the anticipated unit of millimeters of mercury seconds per liter (mmHg•s/L), as derived from the following equation:

$$= \frac{\text{(Mean Arterial Pressure [mmHg] – Mean Venous Pressure [mmHg])}}{\text{Cardiac Output (L/second)}}$$
$$= \text{Systemic Pressure Difference (mmHg)} \bullet 1/\text{Cardiac Output (second/Liter)}$$
$$= \text{mmHg} \bullet \text{second/L}$$

In 1920, Lindhard concluded that the increase in TPR observed during isometric exercise was significantly influenced by the mechanical nature of the contraction. The pressure increases induced by isometric exercise are sufficient to physically compress blood vessels, inhibiting blood flow to active muscles and causing TPR to increase (29). As such, TPR is expected to increase during the IHG utilized in this study, through vascular occlusion within the forearm during contraction, together with a corresponding increase in CO (30).

Finally, the cumulative sympathetic response to exercise is commonly expressed as an increase in mean arterial pressure (MAP), defined as an individual's average BP during a single cardiac cycle. The product of HR, SV, and TPR can be used to quantify MAP, such that the equation accounts for both the cardiac and vascular changes that

accompany the sympathetic response (Figure 1) (1). The value of MAP is telling in healthy and diseased populations, as it indicates how capable the ANS is in controlling the relative contribution of the PNS and SNS under various conditions. Although both branches of the ANS are integral to survival, changes in the SNS have garnered an infamous reputation in the realm of CV health. Chronic hyperactivity of the sympathetic system has been linked to destructive adaptations in of the heart and peripheral vasculature that speed the progression of CV disease (31, 32)

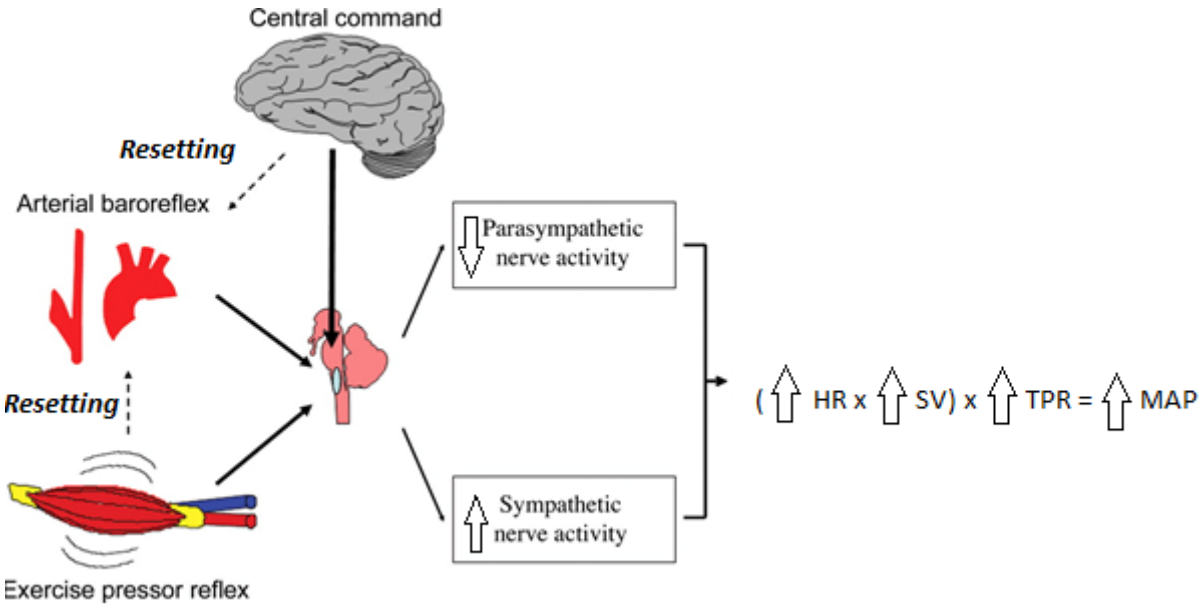

Figure 1: A schematic representation of the mechanisms contributing to neural CV control during exercise. Central command signals originating from higher brain centers, along with the exercise pressor reflex, and their combined influence on arterial baroreflex resetting (see below) act to modulate sympathetic and parasympathetic nerve activity during exercise. The alterations in autonomic activity mediate changes in the rate and force of cardiac contractions, as well as the diameter of blood vessels. Therefore, heart rate (HR), stroke volume (SV) and total peripheral resistance (TPR) enable mean arterial pressure (MAP) to increase directly with the intensity and duration of exercise (1).

2.2 Autonomic Cardiovascular Response to Exercise

The exercise state introduces a multitude of physiological challenges that are dependent upon exercise mode, duration, and intensity, as well as the fitness level and nutritional status of the individual (26). Strenuous physical activity requires the ANS and

CV systems to initiate an increase in the delivery of oxygen and nutrients to working skeletal muscle, and increase the removal of carbon dioxide and other metabolic waste products. The parasympathetic and sympathetic divisions of the ANS system have different temporal responses to exercise. Parasympathetic responses are astonishingly quick while the rate of sympathetic-mediated responses are delayed in comparison (20). At the onset of exercise, the parasympathetic nervous system immediately 'withdraws' innervations to the SA node, allowing for a rapid increase in HR, relating back to the initial observations by Johansson in 1895 (19,20). As the level of exercise increases and/or as exercise continues, the SNS causes further increases in HR, in addition to SV, which contributes to an increase in MAP (Figure 1). An increase in MAP is integral to the exercise response, as it is an influential driving force in increasing blood flow to active muscle, thus supplying oxygen and nutrients while removing metabolic by-products (33). Additionally, SNS-mediated vasoconstriction within less active regions of the body helps redirect this increased cardiac output towards the heart and active skeletal muscles. Therefore, coronary and active skeletal muscle blood flow are increased during exercise, while cerebral blood flow is maintained, and blood flow to less active regions such as inactive skeletal muscles, the kidney and splanchnic regions are decreased. The aforementioned hemodynamic alterations must be accompanied by increases in tidal volume (i.e. the volume of air per breath) and respiration rate (RR) (1). The cardiorespiratory responses that accompany physical activity along with the corresponding changes in PNS and SNS activation patterns provide a unique platform to examine the elegant interplay between the central and peripheral neural control mechanisms and the brain regions associated with them (1,26).

2.3 PORTAPRES®: CONTINUOUS, NON-INVASIVE MEASUREMENT OF BLOOD PRESSURE

The Portapres® Model-2 (Finapres Medical Systems, Amsterdam, Netherlands) is a portable battery-operated tool that obtains continuous non-invasive BP measurements within the arteries of the phalanges (34). The Portapres® is a novel addition to the current study, as this tool is somewhat MEG compatible and capable of tracking the BP waveform over time, monitoring beat-by-beat changes in BP throughout the IHG and PEI protocol. The Portapres® operates according to the volume-clamp method introduced by Peñaz in 1967, devised according to the dynamic unloading of arteries within the finger (34). The technique devised by of Peñaz, in hand with the ability of the Portapres® to recalibrate measurements, has been shown to non-invasively record reliable and continuous estimates of BP without the risks and ethical considerations inherent to invasive techniques (35).

The size of the artery within the finger is assessed using an infrared transmission plethysmograph within an inflatable finger cuff. The plethysmograph consists of a light source and detector embedded on opposite sides of the cuff. Infrared light emitted by the source is passed through the finger, the blood absorbs a fraction of the light and the remaining fraction reaches the detector on the opposite side. The detector receives the incoming light and indirectly determines BP using the changes in blood volume (and diameter) according to the difference in light emitted and light received. Therefore, the increase in arterial diameter associated with the systolic phase of the cardiac contraction, causes more light to be absorbed and less light to be detected than during the diastolic phase when the heart is relaxed (34).

In order for the Portapres® to obtain an accurate measurement of BP it must determine the unloaded arterial diameter of the finger, also referred to as the *set-point*. At the set-point, the arterial pressure and finger cuff pressure are equal, and the transmural pressure is equal to zero (34). When the set-point has been identified, the finger cuff inflates, clamping the artery, effectively maintaining the unloaded state. The light detector establishes a measured value halfway between the systolic and diastolic arterial diameter to compare with the set-point (35). The difference in amplitude between the measured value and set-point is used to control the pneumatic device of the Portapres® so that changes in cuff pressure mimic changes in arterial diameter (35). The changes in finger cuff pressure create a waveform of variable amplitude that can be assessed by a computer algorithm to calculate BP. Furthermore, the Portapres® incorporates a height correction feature that accounts for changes in arterial pressure that result from vertical movement of the hand/foot in relation to the heart level.(i.e. hydrostatic pressure changes).

The continuous BP recordings from the Portapres®, along with concurrent measurements of HR, will provide indirect insight into the contributions of the PNS and SNS on CV function. As previously mentioned, the PNS contributes to the rapid increase in HR during exercise, and the SNS is responsible for vascular constriction and contributes to the delayed increase in the rate and force of cardiac contractions. Therefore, the most prominent increases in BP will occur when PNS activity is withdrawn (elevating HR) and SNS activity is increased (elevation in HR, SV). Due to the delayed nature of the sympathetic response, we anticipate the greatest BP changes to occur in the latter half of the IHG exercise (both CC and EPR active). During the PEI

period following the IHG, we expect BP to remain elevated but anticipate a slight decrease in comparison to measures obtained during the handgrip exercise. At this time CC has been removed causing HR to decrease, leading to a subsequent decrease in BP (only the EPR active). The ability of the Portapres® to monitor BP (and HR) on a beat-by-beat basis will allow us to identify when the SNS began contributing to the exercise response and therefore when the EPR became active in response to the IHG (~45 seconds after initiation of the contraction), as well as to ensure that the EPR-mediated contribution to the increase in BP persisted during the post-exercise ischemic phase (see Methods below for details).

2.4 BRAINSTEM CARDIOVASCULAR CONTROL

The autonomic CV response to exercise is comprised of three primary components: the Exercise Pressor Reflex, the Arterial Baroreflex, and Central Command (22, 24, 36). A variety of experimental approaches have been employed in attempt to unveil the neuroanatomy involved with each component and to uncover the independent role of each, along with the relationships that exist among them.

Researchers confidently proclaim that the medulla of the brainstem is the site of integration between the descending efferent signals of the cerebral cortex and ascending afferent signals from baroreceptors within the aortic arch and carotid sinus (arterial baroreflex), as well as receptors located within skeletal muscle (i.e. EPR) (3,20,36-38). Afferent signals regarding blood volume and blood pressure are detected by arterial baroreceptors and transmitted to the medulla, while signals regarding metabolic by-product concentrations, pH, temperature, and the mechanical state of muscle cells are transmitted via mechanoreceptors and chemoreceptors and initiate the EPR. Specifically, the dorsal brainstem contains the Nucleus Tractus Solitarius (NTS), deemed the

"gatekeeper of the central nervous system" due to its chief role in autonomic regulation and its primary role in monitoring the aforementioned variables. The dynamic nature of the NTS ensures that all responses coincide with the global needs of the functioning body (38). Along with its extraordinary networking and relay capabilities, the NTS also acts as a powerful integration site capable of altering autonomic outflow. This is accomplished via reflex control using proximal brainstem circuits, such as the afferent signals originating from the EPR and the arterial baroreflex, or by receiving efferent signals from distant sites of autonomic regulation, such as the signals originating from the cortical regions involved with CC (20, 38).

2.5 Other Important Brainstem Regions

The periaqueductal grey (PAG) is a medullary structure noted for its ability to consolidate behavioral defense mechanisms. The PAG is recognized by its columnar structure and is divided into a dorsomedial, dorsolateral, lateral, and ventrolateral column, with each column playing a different role in autonomic regulation (24). Specifically, activation of the dorsomedial and dorsolateral columns of the PAG evokes a classic fight or flight response, including increased HR, SV, BP, and emotional correlates such as fear or anxiety. Activation of the lateral and ventrolateral columns elicits a passive "fight or flight" coping response that results in a decrease BP and a similar emotional reaction (38, 39). The PAG shows significant activity during exercise and is capable of eliciting increases in BP when directly stimulated (40). Furthermore, the level of activation in the PAG and rise in BP is proportional to the level of exercise intensity (24). Basnayake et al. (27) recorded local field potentials originating from 'deep' brain nuclei during exercise tasks constructed to prompt the exercise pressor response. The participants involved in this study were afflicted with movement disorders and thus had

electrodes implanted stereotaxically in the PAG, subthalamic nucleus (STN), or hypothalamus. Circulatory occlusion (i.e. isolation of the metaboreflex) of the contracting skeletal muscle following the exercise task resulted in significant PAG activation, while activation in the remaining aforementioned regions was negligible, with the exception of the STN, which was shown to increase alongside PAG (40). The STN is a medullary site involved with the parallel activation of CV and locomotor systems (24). An early study by Smith and colleagues (41) demonstrated that stimulating the STN in dogs triggers actions and responses that mimic the exercise state, including movement and increases in HR and BP. Overall, PAG exhibits activation patterns similar to regions of CC; despite similarities in activation and its prominent role in CV regulation, the PAG is more accurately viewed as an integration site closely linked to CC rather than a component of it (27).

The feed-forward actions of CC are not always able to adequately adjust to the metabolic demands of exercise. In addition to CC, resetting of the EPR and baroreflex allow for comprehensive correction of the metabolic error signal (i.e. mismatch between metabolic accumulation and muscle blood flow), with the composite providing a proficient CV response to exercise (22). The ability of the human body to utilize the strengths of both the feed-forward mechanisms of CC and feedback mechanisms of the EPR and arterial baroreflex gives the CV system a significant advantage over the use of a single mechanism in isolation (Figure 1) (22). Williamson et al. (22) notes the complexity of studying feedback mechanisms within the ANS of humans, for as one mechanism is attenuated others become more active to compensate.

2.6 THE EXERCISE PRESSOR REFLEX

The EPR is comprised of two receptor groups that respond to exercise-induced afferent signals arising from within the working muscles. The *muscle mechanoreflex* responds to mechanical stimuli such as stretch and pressure, as well as to the products of cyclooxygenase and lipoxygenase reactions, including arachidonic acid (33). In contrast, the *muscle metaboreflex* responds to hypoxia and chemical stimuli, primarily the by-products of muscle metabolism, including lactic acid, hydrogen ions, bradykinin, and potassium (33). Mechanoreceptive signals are transmitted to the CNS via type III afferent neurons, while metaboreceptive signals are transmitted to the CNS via type IV afferent neurons. Reflex-driven CV adjustments to exercise are initiated by transmitting afferent signals to the spinal cord, which are in turn passed to the brainstem, where sympathetic activity is increased. The NTS, caudal ventrolateral medulla (CVLM), rostral ventrolateral medulla (RVLM), nucleus ambiguus, and ventromedial region of the rostral PAG have all been implicated as important medullary sites of EPR integration (25,38,42)

Early CV control theories postulated by Zuntz and Geppert (43) were centered on examining the relationship between metabolism and muscle blood flow (MBF). They hypothesized that elevated levels of metabolites within the working muscle must be corrected for by an increase in MBF. Zuntz and Geppart (43) postulated that the SNS was responsible for increasing BP, which in turn increases MBF and decreases metabolite concentration (20). As previously mentioned, isometric exercise is an effective mode of stimulating the SNS, as it induces large increases in intramuscular pressure, temporarily inhibiting blood flow to the exercising muscle. Additionally, HR, SV, and BP are increased, assisting to elevate muscle perfusion with the release of the isometric contraction. The EPR plays a role in the aforementioned response, as it acts to detect

changes in intramuscluar pressure and concentration of metabolic by-products within the working muscle, transmitting this information to the CNS (42). Alam and Smirk (44) explored this hypothesis, discovering a persistent elevation in BP upon circulatory occlusion of a human limb following isometric exercise. Blood pressure remained elevated during circulatory occlusion following exercise, suggesting that as long as MBF was prevented from relieving metabolic build-up, BP would remain high (44). Furthermore, it was noted that the rise in BP was strongly correlated with the mass of ischemic muscle and the extent of hypoxia.

Paralytic agents have been administered to human subjects intravenously in order to examine the role of the EPR reflex-mediated CV responses to exercise versus those produced by CC (45-47). When subjects attempted to exercise a paralyzed skeletal muscle, feed-forward signals of CC were amplified despite the absence of muscular contraction. Because no contraction was elicited, no metabolic by-products were created and the EPR remained inactive. The results showed that HR increased as much during the attempted contraction as during the true contraction, illustrating that CC was equally active during the attempted exercise as it was during the static exercise (45). Furthermore, BP during attempted handgrip involving a paralyzed muscle was much lower than during an actual static handgrip but elevated in comparison to rest, underlining the importance of the EPR in generating the appropriate BP response to exercise. The results of this study illustrated that the sheer attempt to exercise is sufficient to activate CC and cause significant increases in HR and BP. However, an adequate HR and BP response is generated by the dual activation of CC and the EPR.

2.7 THE ARTERIAL BAROREFLEX

An integral BP monitoring system is rooted in the function of stretch-sensitive receptors lining the aortic arch and carotid sinus, termed arterial baroreceptors. Arterial baroreceptors detect BP on a beat-by-beat basis and convey the amount of stretch in the major vessels to the NTS via action potentials. When the vessels are distended, receptors fire action potentials more frequently, such that a greater amount of stretch (i.e. higher BP) results in more frequent action potentials (46). The NTS acts to weigh the frequency of the baroreceptor afferent signals in comparison to an "operating point" or "set point". If the frequency of action potentials deviates from the set point, a cascade of signals will act to return BP back to its initial level (20, 38). When second-order neurons within the NTS are stimulated by cardiac baroreceptors, they excite neurons that communicate with the parasympathetic preganglionic cell bodies within the dorsal motor nucleus of the vagus (DMNV) and nucleus ambiguus, as well as the γ–aminobutyric acid (GABAergic) transmitting neurons of the CVLM, an important CV medullary region. In the case of a transient increase in BP, the neurons of the CVLM act to inhibit neurons of the RVLM, the generator of pre-motor sympathetic outflow (38). According to this pathway, increased baroreceptor loading (i.e. an acute hypertensive stress) corresponds to a shift in autonomic balance that increases vagal influence and reduces sympathetic activity. This produces decreases in HR, SV, and TPR; the opposite actions are elicited during a sudden hypotensive stimulus (37). The arterial baroreflex is an acute negative-feedback mechanism set in place to counteract unwanted fluctuations in arterial pressure, thus maintaining a specific pressure range (20, 37).

Exercise is associated with a change in the homeostatic pressure range maintained by the arterial baroreflex. The increased metabolic demands of exercise require that blood

pressure rises higher than the resting "set-point". The actions of brain regions involved with CC, in combination with the actions of the EPR, act to hyperpolarize the NTS, altering its sensitivity to baroreceptor afferent input. This change in polarization effectively shifts the set-point of the baroreflex to a higher range (37, 46). Thus, until arterial blood pressure reaches this new set-point, afferent signals from the baroreceptors are interpreted as being "hypotensive". As such, the baroreflex will respond by acutely increasing SNS activity to the heart and blood vessels, which increases HR, SV, TPR, and ultimately arterial blood pressure (Figure 2). The magnitude of baroreflex resetting is dependent on the amount of active muscle mass used during exercise and the intensity of muscular exertion (i.e. factors associated with increased central command and EPR feedback, respectively). It is through the resetting of the baroreflex set-point by CC and the EPR that HR, SV, and TPR can all increase in unison, along with an increase in BP. Under resting conditions, the baroreflex would normally prevent this cascade of events from occurring.

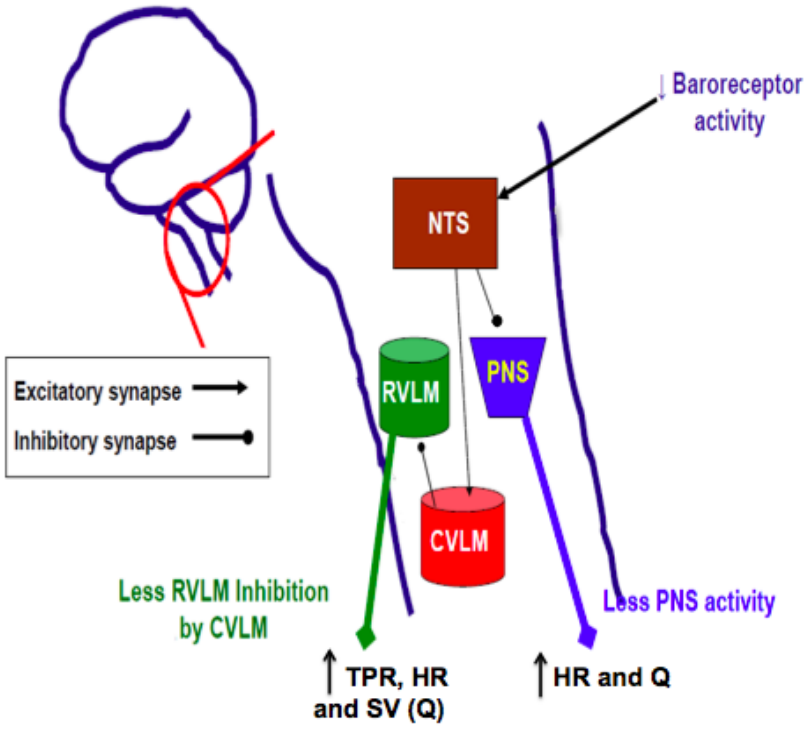

Figure 2: Activity of autonomic nuclei in the medulla in response to a physiologically perceived decrease in arterial blood pressure (ABP) during exercise as the result of baroreceptor resetting. Afferent signals from the baroreceptors are interpreted as being "hypotensive", and respond by acutely increasing SNS activity to the heart and blood vessels, which increases heart rate (HR), stroke volume (SV), total peripheral resistance (TPR), and arterial blood pressure (ABP).

Gallagher and colleagues (46) demonstrated the ability of CC and the EPR to independently reset the baroreflex; both CC and the EPR were manipulated separately and as a combination during static leg exercise at 20% MVC. The neuromuscular blocking agent Norcuron was administered to isolate CC activation, while in a separate trial, medical anti-shock trousers were used to isolate the EPR. In the study by Gallagher and colleagues (46), medical anti-shock trousers were positioned around the active leg and inflated to 100 mmHg following exercise, preventing muscle blood flow from removing the metabolic by-products of exercise. The final manipulation involved the simultaneous use of both neuromuscular blockade and the use of the anti-shock trousers in an effort to examine how changes in baroreflex resetting would differ from the

previous two conditions. Results of this study suggested that CC was the primary mechanism for altering HR during exercise, while both CC and the EPR were involved with increasing BP through baroreceptor manipulation. Baroreflex resetting was most pronounced when both mechanisms were active compared to isolated activation of either CC (Norcuron trial) or the EPR (anti-shock trouser trial without Norcuron) alone. This suggests that both mechanisms play a cooperative role in resetting of the baroreflex (46).

Neuromuscular blocking techniques such as the one described above are effective for providing insight into the role of CC versus the EPR. Despite the information afforded by neuromuscular blocking studies, this method is difficult to manage and often yields inconclusive results (48). Employing neuromuscular blocking techniques in the current study is unnecessary and not feasible as it requires dedicated medical personnel and equipment that is not compatible with the MEG scanning environment.

In opposition to this approach, we favored a repeated IHG exercise and PEI protocol. As previously mentioned, the IHG exercise serves to activate CC, causing near-immediate increases in HR. As exercise persists, the IHG causes type III and IV afferents to fire in response to muscle metaboreflex and mechanoreflex activation. When the arterial blood supply to the forearm is occluded just prior to cessation of the isometric exercise, the products of metabolism are trapped in the muscle, preserving the sympathoexcitation mediated by the EPR. The reflex response that accompanies PEI occurs in the absence of CC, as voluntary contraction has ceased, switching off activity within motor pathways. This protocol has been used many times with great success (2, 4, 49); the design is simple, yet reliable and effective.

2.8 Cortical Regions Implicated with Autonomic Cardiovascular Regulation

Uncovering the functional anatomy of CC has been of scientific interest since the work of Johnasson in 1895 (19). The medullary circuitry associated with modulating CV activity is well mapped, while much mystery still surrounds the boundaries of higher brain regions implicated in CV control. Lesion studies have revealed that in spite of a fully functioning medulla, electrolytic or stroke induced lesions within the cerebral cortex result in CV and autonomic dysfunction, including cardiac arrhythmias and irregular blood pressure (50, 51). Research has identified the insular cortex (IC), anterior cingulate cortex (ACC), medial prefrontal cortex (MPFC), and thalamus as key components of a neural network involved with the regulation of CV function (2,14,36,38,52-54).

The IC is an autonomic nucleus of the forebrain, located deep within the Sylvian fissure. The mammalian IC is involved in an incredible number of processes, including the perception of pain, production of speech, and processing of social emotions (55). Furthermore, direct stimulation of the IC leads to increases in HR, BP, and RR, similar to those elicited during voluntary exercise (56). Research suggests that the IC is important for augmenting BP by maintaining baroreflex sensitivity, and by regulating autonomic and limbic system function (4, 53). Research has confirmed baroreflex-governed neural connections between the IC and the ACC, ventral MPFC, and medial dorsal nucleus of the thalamus, as well as reciprocal innervation between the right and left insula (57-61).

Evidence suggests that in terms of CV regulation, the IC is lateralized; bradycardia (slowing of HR) is induced by stimulating the posterior region of the left insula, while right insular stimulation causes tachycardia (increasing HR) and an increase in BP (62). Electrophysiological studies during pharmacologic blood pressure challenges

have consistently reported a high percentage of sympatho-excitatory neurons within the right posterior insula (63-65). In 1999, Williamson and colleagues (66) discovered increases in regional cerebral blood flow in the right and left IC in response to increasing exercise intensity. However, only changes in the right IC showed a significant relationship with changes in BP and increases in the participants' perceived level of physical exertion. Additionally, a positive correlation between the magnitude of insular activation and the level of exercise intensity was found (66). Williamson and colleagues suggest that CV changes that accompany volitional exercise could be generated by the IC, with the left insular region serving as the cortical site for CV parasympathetic regulation during exercise and the right insular region serving as the cortical site for CV sympathetic regulation. Most importantly, an afferent pathway from the brainstem to the IC has not been identified, suggesting that the IC may be a paramount CC site integral for modulating BP during exercise (66).

Additional studies have confirmed the presence of interconnections among the aforementioned cortical regions fundamental to the autonomic CV response to exercise. Specifically, the ACC has been traced to the MPFC, and dorsal medial thalamus in addition to its connection with the IC. Furthermore, connections have been documented between the ACC and PAG, NTS, RVLM, DMV, and the nucleus ambiguus (67-69). Strong neuroanatomical connections have also been shown to link the MPFC to both the IC and subcortical autonomic regions, including the PAG, NTS, DMNV, nucleus ambiguous, CVLM, and RVLM (1, 22). These connections suggest that the MPFC may play a role in vagal control (14, 69, 70).

Furthermore, the successful execution of any goal-directed motor task, such as the one employed in this study, requires activation of the cortical regions of the motor pathway (71). The motor pathway involves direct and indirect communication among a number of brain regions in order to bring about the desired motor response (71). The posterior frontal lobe houses the motor cortex, the region most highly associated with the control of voluntary movement. In order to successfully complete goal-driven movements, the motor cortex must integrate signals from various regions of the brain to create a dynamic model of the task (71). The initial planning of a movement is carried out within the anterior portion of the frontal lobe from which signals extend to Area 6 of the motor cortex, the pre-motor area, which is responsible for directing movement through the integration of sensory information. The pre-motor area relays this information to Area 4, the primary motor cortex, which is capable of activating the muscles involved with motor execution (71). All the while, the cerebellum and basal ganglia process signals from sensory and motor cortices; the cerebellum also receives information from skeletal muscle proprioceptors, which are involved with the EPR (71). The processed information is then passed through the thalamus where it is relayed back to the motor cortex. The information afforded from the cerebellum and basal ganglia are used to adjust elements of movement necessary for successful execution.

Previous studies examining the CV response to exercise have implicated brain regions of the motor pathway as potential components of the CC network, particularly the thalamus (53, 60, 64, 72-74). The thalamus has been shown to link higher brain regions to areas of the midbrain, indirectly suggesting that this structure serves as a link to the medullary sites of CV control (72). A study conducted by Wong and colleagues (14)

discovered increased activation within the thalamus that was correlated with the ventral MPFC and HR response during graded IHG exercise. Additionally, changes in BP have been correlated with increased thalamic activation (60, 64) with direct stimulation of thalamic regions triggering increases in HR and BP (74). This research suggests that the thalamus likely plays a role in controlling baroreceptor activity, and modulating the cardio-vagal relationship (1, 14). In 2003, Williamson and colleagues (53) noted bilateral activation of the inferior thalamus throughout an IHG and PEI protocol, a region previously shown to interconnect with the IC, which has been highly implicated in CV control during exercise (73).

Previous findings that regions of the motor pathway, such as the thalamus, may play a role in the CV response to exercise introduces an important concept for the current investigation; changes in brain activity within regions of the motor pathway may represent more than sheer involvement with motor generation, and may be involved with the central- or reflex-mediated CV response to exercise

2.9 PAST RESEARCH

In 2010, Sander, Macefield, and Henderson (2) sought to investigate the time course associated with changes in cerebral cortex and cerebellum activation of conscious humans in response to an IHG and PEI protocol, and to differentiate between cortical sites associated with CC and those associated with the EPR input. The experimental design consisted of a two minute IHG exercise at 35% of the participants MVC followed by six minutes of PEI. Sander and colleagues used BOLD fMRI to quantify changes in signal intensity within the brain during IHG and PEI (2).

During the contraction period, parallel increases in neural activity were observed in the forearm region of the contralateral primary motor cortex, contralateral IC,

ipsilateral parietal association cortex and discrete cerebellar nuclei. The activation profile in these regions matched the exertion of the IHG exercise and decreased at the end of the contraction phase. Increasing signal intensity from within the contralateral insula, primary and secondary somatosensory cortices, along with decreasing BOLD signal intensity from within the perigenual anterior cingulate and midcingulate cortices, persisted throughout the PEI phase; leading to the conclusion that these areas were associated with the EPR. The increase in IC activity during the contraction phase and sustained elevation throughout PEI, along with a decrease in ACC activity across the same time periods suggests that these two regions are not part of the CC network. Furthermore, the IC has been shown to encode feelings of unpleasantness and pain (75), leading to the suggestion that the IC was more likely associated with the perception of pain and discomfort rather than dictating an autonomic response (2). This was further supported by the fact that neither direct nor indirect links between the IC and regions of the medulla were uncovered. It was also suggested that the IC might be stimulated by increases in baroreceptor signaling due to the increase in BP (2).Additionally, Sander et al. (2) also propose that the persistent drop in activation within the ACC is not attributable to parasympathetic withdrawal as previously thought (14, 52); it is believed that the activity of this region is also reflective of the muscle pain induced by the protocol (2, 76).

Research has confidently concluded that increases in HR at the onset of exercise are attributable to the actions of CC and vagal withdrawal (14, 20); the findings of Sander et al. (2) agree with this, as significant increases in HR and MAP were noted at the onset of the IHG exercise and subsided during PEI (Figure 3). It was also noted that MAP

elevated in a progressive fashion during the exercise protocol and began to plateau during PEI (Figure 3). Importantly, clusters of increased activity were observed within the dorsal and medial medulla, which are believed to correspond to the NTS and RVLM, respectively. Medullary activation mirrored changes in MAP, providing further support for the notion that the EPR is mediated by the medulla.

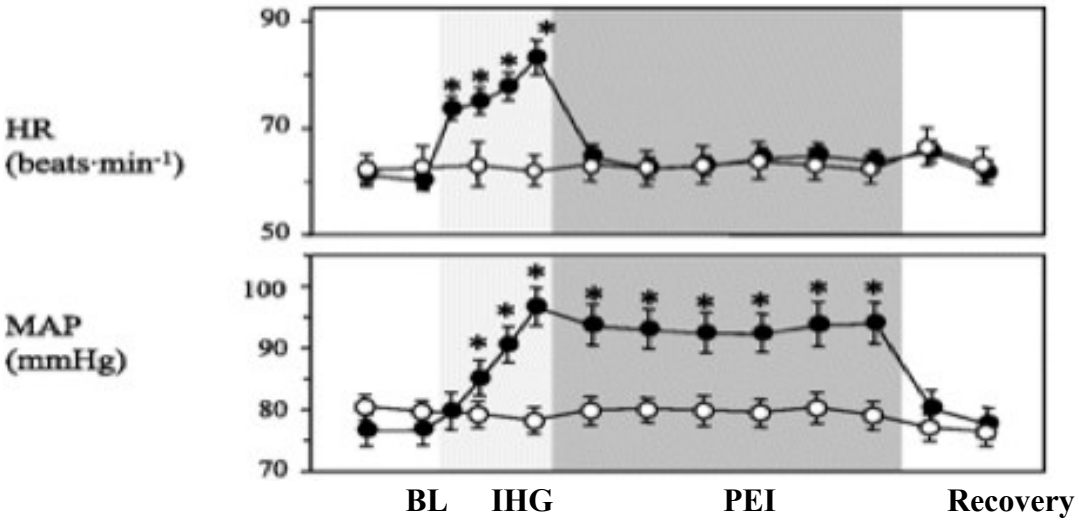

Figure 3: Summary data (means ± SEM) for heart rate (HR) and mean arterial pressure (MAP). The experimental condition (●) included a baseline (BL, 2 min), isometric handgrip (IHG, 2 min) exercise, post-exercise ischemia (PEI, 6 min) period, and recovery (2 min). The control condition (○) included a BL (2 min), PEI (6 min), and recovery (2 min), but no IHG exercise.*$p < 0.05$ versus BL (2).

2.10 Perception of Effort and Hypnosis

Williamson and colleagues (77) reported increased activity in the sensorimotor cortex, ACC, IC, MPFC, and thalamic regions of the brain in response to physical effort. Despite such findings, there was difficulty delineating the regions responsible for CV changes from those activated in response to concurrent cognitive and perceptual processes associated with the task. Such ambiguity led to the development of an experimental design capable of teasing apart the cortical regions involved with the task

perception versus the CV response. This experimental design served as the foundation of a body of research integral to our understanding of CV control mechanisms.

Perception is the process of bringing information into consciousness, be it from sensory afferent feedback or from memory (22); the senses associated with perception have led to the postulation that the mere awareness of physical exertion could elicit centrally mediated physiologic changes such as increases in HR and BP (22). The traditional definition that characterizes CC as a feed-forward system has been questioned by Williamson and colleagues (3). Although it is known that afferent feedback from working muscles can induce CV reactions, Williamson and colleagues (3) illustrated that one can trigger a CV response in the absence of afferent muscular feedback by augmenting the perception of effort.

Six healthy participants identified as highly hypnotizable were assessed at a constant workload under three hypnotic conditions that varied in their level of perceived exertion; this included the perception of cycling at a level grade, an incline, and a decline. The exercise protocol was 15 minutes for each condition. During the perceived incline and decline condition, the first ten minutes were spent at a perceived and true level grade and participants were hypnotically cued to the grade change in the last five-minute segment. Despite the constant workload throughout the study, the perception of cycling at an incline caused significant increases in the individuals' perceived level of exertion, HR, BP, right insular regional cerebral blood flow (rCBF) and right thalamus rCBF when compared across conditions (3). In contrast, the perception of cycling on a decline led to a decrease in their ratings of perceived exertion and decreases in rCBF within the left IC and ACC, but it did not significantly change HR or BP responses (Figure 4). During the

perceived decline condition, participants were actually cycling at a level grade; therefore it was postulated that afferent feedback from the exercising skeletal muscle maintained HR and BP at a level necessary to continue cycling on the level grade (Figure 4). In conclusion, Williamson and colleagues (3) discovered that the hypnotic manipulation of effort is capable of modifying patterns of neural activation and inducing CV adjustments. The results of this study suggest that the IC, thalamus, and ACC are critical players in the perception of effort, and also underline the importance of muscle afferent feedback in dictating a CV response to exercise (3). It has also been hypothesized that their summed actions elicit CV adaptations in the absence of afferent feedback by cortically resetting the baroreflex (3).

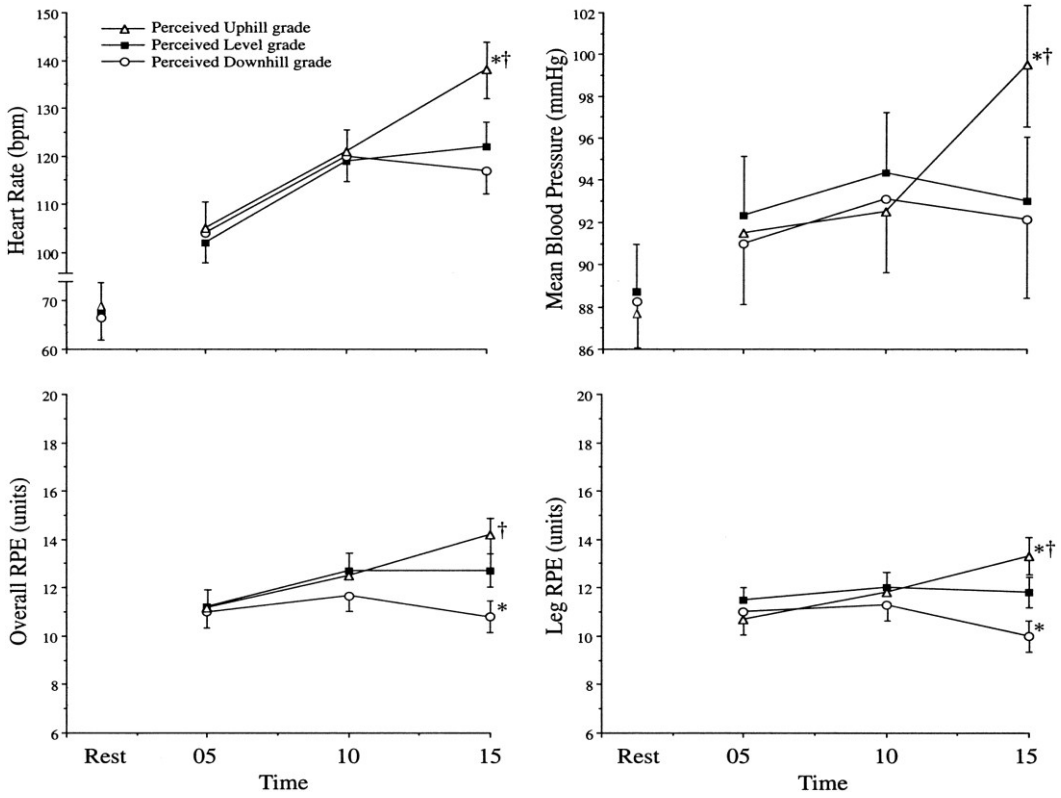

Figure 4: Means ± SEM for heart rate, mean blood pressure, overall rating of perceived exertion (RPE) and leg RPE for subjects cycling at a constant workload under the hypnotic impression that they were cycling at a level grade, uphill grade, and downhill grade. Subjects were given a hypnotic cue of grade change in the last five minute period of each exercise trial. *, $p < 0.05$ versus the control or level-grade condition. †, $p < 0.05$ between downhill and uphill conditions (3).

In a second study (4), patterns of brain activation in response to real and imagined handgrip exercise were examined in order to define the cerebral cortical brain regions involved with the CV response to exercise. Subjects were divided into two groups depending on the ease at which they could be hypnotized [high hypnotisability (HH) and low hypnotisability (LH)]. These two groups were tested under two experimental conditions; one in which they completed a real three minute isometric handgrip exercise at ~30% of their MVC and a second in which they completed an imagined three minute isometric handgrip exercise at ~30% MVC under hypnosis. Both groups elicited similar increases in RPE, HR and mean BP during the real handgrip exercise, while only the HH group demonstrated significant increases in RPE, HR, and mean BP during the imagined handgrip (Figure 5). In addition, the HH group presented significantly higher rCBF within the ACC and IC during the imagined exercise in comparison to their LH counterparts. It is important to note that muscle electromyographic recordings did not demonstrate any measurable increase in force during the imagined handgrip exercise. These findings provide further evidence that the anterior cingulate and insular cortices are two structures related to central command that can be activated in the absence of muscle afferent feedback (4).

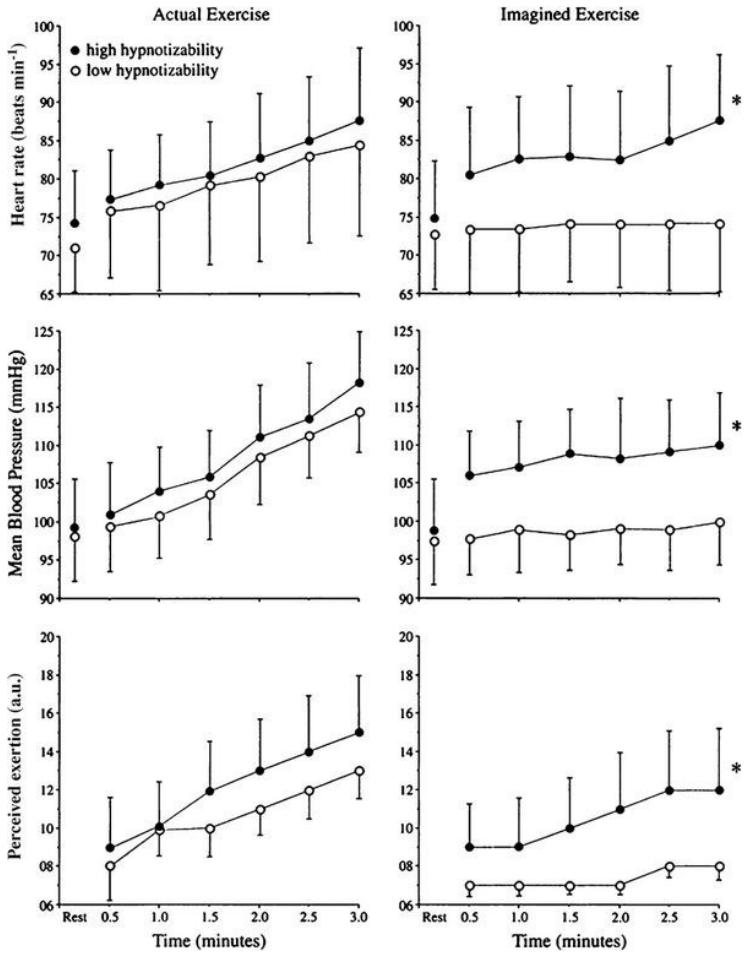

Figure 5: Heart rate, mean blood pressure and ratings of perceived exertion according to the Borg scale were recorded during actual and imagined handgrip exercise. The data are presented as Means ± SD. and significant differences between groups are shown as *$p < 0.05$ (4).

The traditional definition of CC as a feed-forward system that triggers the simultaneous activation of motor and CV centres during exercise is called into question by the results of the previously presented hypnosis studies. Although afferent feedback from exercising muscles has the potential to modulate CV activity, it is not required. Increasing the perception of effort is capable of causing an increase in the CV response through direct activation of the CC network. Thus, Williamson and colleagues have postulated that CV and motor pathways can be uncoupled and are capable of independent activation (22).

The hypnosis studies led by Williamson introduced a novel method of manipulating elements of the exercise experience and have created a body of knowledge integral to our current understanding of CC. However, this protocol is difficult to employ; a great deal of ambiguity surrounds evaluating if a participant is truly hypnotized and it would be difficult to find a competent hypnotist that could carry out hypnosis within the confines of the neuroimaging facility. Furthermore, in the current study activation of the motor cortex is a fundamental prerequisite for monitoring and qualifying CC activation, unlike the imagined exercise study conducted by Williamson et al. (4). As such, a hypnotic experimental protocol such as this would be incapable of effectively separating CC from the EPR, as the EPR could not be activated in the absence of a contraction.

2.11 Magnetoencephalography as a Tool to Measure Brain Activity

The brain is a complex structure with greater than 10^{10} neurons and 10^{14} synapses within the cerebral cortex alone (78). This structure forms a vast communication network that allows the brain to orchestrate a coordinated response according to input from multiple brain regions (79). The neural activity associated with perception, cognitive assimilation, and motor activation within the human brain occurs on the order of milliseconds (79). Therefore, examination of human brain neural activation patterns requires techniques capable of capturing the rapid electrical impulses characteristic of the human nervous system. The most common non-invasive electrophysiological measures currently capable of accurately capturing such swift changes are magnetoencephalography (MEG) and electroencephalography (EEG). Recordings of MEG and EEG both depend on synchronized neuronal activity within the brain. Both MEG and EEG boast a superior temporal resolution, three orders of magnitude smaller than BOLD fMRI (78). The electrical activity of a lone neuron lasts between

approximately one and tens of milliseconds, a temporal scale too fine for the likes of neuroimaging techniques such as BOLD fMRI (78). The millisecond temporal resolution of MEG and EEG is an invaluable characteristic, as it allows the rapid electrical changes in cortical activity to be followed throughout signal processing by the brain (78).

Robert Jaklevic, John J. Lambe, James Mercereau, and Arnold Silver of Ford Research Labs have been credited with inventing SQUID technology in 1964. SQUID is an acronym used to represent Superconducting QUantum Interference Device, an extraordinarily receptive set of amplifiers with wide-reaching application. These amplifiers are capable of detecting weak magnetic fields, as small as one-billionth the size of the earth's magnetic field (80). Upon the development of SQUIDs in the 1960s, D.S. Cohen employed the technology to measure the weak magnetic fields on the surface of the head that arise from the united action of tens of thousands of neurons, marking the first neuromagnetic MEG recording (81).

Synchronized currents, known as current dipoles, are reflective of brain activity and can be defined as a current (movement of charge per unit time) active over an infinitely short distance (82). Based on Maxwell's equations, each current dipole creates an orthogonal magnetic field (82). When there is united action of a large population of neurons within the brain, the SQUID sensors of MEG are capable of detecting these extremely small magnetic fields and can convert them into a measureable electric voltage.

The MEG scanner used in the current investigation includes a silicon chip sensor array composed of 510 coils that sample the magnetic field distribution at specific locations. These coils are organized into 102 triple sensor elements comprised of

gradiometers and magnetometers in a ratio of two to one. Magnetometers measure magnetic flux through a single coil and are more sensitive to deeper sources but also vulnerable to environmental noise. On the other hand, planar gradiometers are more sensitive to focal superficial sources. Planar gradiometers measure a difference in magnetic flux via two oppositely wound coils, under the assumption that interference is relatively uniform across sensors, resulting in a net flux of zero (82). Because magnetometers measure total flux and gradiometers measure a flux gradient, the units differ (T^2 and T^2/cm^4 respectively) and thus cannot be directly compared (83). The sensor array spans the top of the head, bordering the eyebrows, temporal lobes, and cerebellum (82). Mapping the magnetic field strength over the entire head across time allows for the interpretation of spatial and temporal characteristics of the magnetic field. The sampling rate of MEG can reach 2500 samples per second, with a temporal resolution of less than 1ms and spatial localization accuracy between 1 and 5mm (82).

The magnetic field of interest that results from brain activity can be defined as the signal and is ~10-100 $\times 10^{-15}$ Tesla (T) (84). Noise can be defined as all other magnetic fields outside of the signal that threaten to interfere with MEG's detection of the signal. Environmental noise, physiologic noise, brain noise, and intrinsic sensor (SQUID) noise are four prominent sources of signal interference. Environmental noise commonly stems from the magnetic fields created by power lines, elevators, and electronic devices in the vicinity of the MEG scanner and commonly lie on order of 10^{-9}T. Physiologically generated artifacts are approximately 10^{-12}T and are typically the product of movement (84), while extraneous brain signals and intrinsic sensor noise lie within the same range of magnitude as brain signals of interest (10-100 $\times 10^{-15}$T). Magnetic source imaging acts

to attenuate sources of interference and increase signal strength in order to maximize the signal-to-noise ratio (84).

A room structured from multiple layers of mu-metal, copper, and aluminum house the MEG scanner and act to attenuate high-frequency environmental noise. Low-frequency noise generated from moving objects in the vicinity of the MEG is minimized using a gradiometer that detects the spatial gradient of magnetic fields. Sources of magnetic fields lying at a distance from the MEG scanner introduce a small spatial gradient compared to the gradient generated within the brain, and thus can be attenuated from the signal (85). As long as noise occurs independently of the signal elicited by the experimental task, it can be extracted and eliminated. The most effective mode of attenuating unwanted artifacts is to average 20-300 responses, drawing the signal from the noise that surrounds it. A more problematic issue arises when the artifact is time-locked to the evoked response (79). The primary focus should always be on obtaining high quality raw data by minimizing sources of noise. Adequate shielding, high quality sensors, and effective gradiometers should be the first line of defence when trying to improve the signal-to-noise ratio.

2.12 Event-Related Synchronization and Desynchronization in the Alpha and Beta Bands

Adjunct to the invention of the EEG in the 1920s by Hans Berger, was the discovery of consistent neural oscillations in the frequency range of 8-12 Hz (86), which Berger referred as “alpha band rhythms”. Berger also observed that this activity was suppressed when a subject went from an eyes-closed state to an eyes-open state, leading him to term this phenomenon “alpha blockage” (86). In the 1970s a more inclusive term, event-related-desynchronization (ERD), was proposed by Gert Pfurtscheller and

colleagues to express suppression in oscillatory activity within a specific frequency band (86). The inverse of ERD, termed event-related synchronization (ERS), is also an important observable alteration in neural oscillatory activity and is commonly evaluated in conjunction with ERD. Since the early observations of Berger, changes in oscillatory activity have been noted in response to a multitude of physiologic and sensory experiences. Laboratories throughout the world employ the concepts of ERD and ERS to examine cognitive and motor processes within the brain (86).

Neural oscillations can be characterized according to frequency, amplitude and phase. The brain frequency spectrum is commonly divided into six frequency bands, including delta (2-4 Hz), theta (5-7 Hz), alpha (8-12 Hz), beta (13-30 Hz), gamma1 (31-60 Hz), and gamma2 (61-90 Hz) (87). The frequency components of brain neural networks are reliant upon the membrane characteristics at the level of the neuron, as well as the overall structure and connective pattern of the network as a whole. At the neuronal level, oscillatory activity is influenced by resting membrane potential, dynamics of synaptic exchange, the effect of neurotransmitters, and the strength and reach of neuronal connections as oscillatory synchrony within a neural network is largely maintained by feedback connections among neurons (15, 88). With respect to the neural network, oscillatory frequency tends to be inversely related to amplitude, and oscillatory amplitude within a neuronal network is proportional to the population of synchronous neurons (15). Therefore, high-amplitude, low-frequency oscillations are commonly associated with a greater number of active neural components than their low-amplitude, high-frequency counterparts (15).

Changes in neural synchronization are time-locked to an event but not phase-locked, meaning that they must be examined using a frequency analysis as opposed to the linear averaging techniques common to the extraction of event-related potentials (15). Changes in the synchronous firing of neuronal populations within a frequency band are most often assessed in relation to a baseline period and expressed as an increase or decrease in power with respect to the baseline measure (88). Therefore, the use of a true baseline is important, as the time-frequency results are dependent upon the "inactive" baseline period from which the brain activity of interest will be compared (88).

Of particular interest to the current study is the repeated demonstration of alpha and beta band desynchronization within the contralateral sensorimotor cortex during the execution of unilateral motor tasks (89-91). Alpha and beta ERD has been demonstrated during motor planning, execution, and even motor imagery, with beta ERD being more topographically discrete than alpha ERD (15, 89-91). Desynchronization within these bands is commonly viewed as an electrophysiological manifestation of cortical activation. In contrast, it has been proposed that the presence of oscillatory synchronization is characteristic of cortical "idling" or the absence/inhibition of activity (88).

Crone and colleagues (92) noted that alpha ERD was topographically widespread during both the early and late phases of an isometric contraction, with the desynchronization extending outside the area of the motor cortex associated with the moving body part. Crone et al. (92) suggest that the somatotopic representation of the motor cortex may be oversimplified and that there may be a significant amount of overlap between the networks that control movement of different body parts with respect to the

alpha band. This group of researchers also discovered a more somatotopically discrete beta ERD in comparison to alpha, in response to isometric motor tasks involving different body parts (92). Furthermore, the strength of alpha and beta ERD is positively correlated with both task complexity and the amount of muscle mass engaged in a motor task (93, 94)

Interestingly, it has been demonstrated that alpha and beta desynchronization during a motor task can been accompanied by an increase in neural synchrony within the surrounding brain regions (95). This pattern has been termed "focal ERD" or "surround ERS" and is believed to represent an inhibition of neural networks not associated with the execution of the task. It should also be noted that the regions of motor cortex that experience beta ERD undergo post-movement beta ERS, also referred to as beta-rebound (15). This rebound marks the reestablishment of beta oscillatory activity and tends to occur less than 1 second after movement termination (15).

The wealth of research examining changes in oscillatory activity within the alpha and beta frequency bands in response to sensorimotor tasks will be employed in the current study. As previously mentioned, changes in brain activity in response to such tasks most commonly manifest as changes in synchrony, and thus power, within these two frequency bands and have therefore been selected for closer examination. We will be examining the regions of the brain that exhibit changes in alpha and beta power in response to different periods of our experimental protocol. Specifically, we will be looking at the brain regions that undergo significant alterations in alpha and beta neural oscillation during activation of CC versus the EPR. These CV control mechanisms will be isolated by temporally parsing the data according to the temporal activation of CC

versus EPR and will be evaluated against a corresponding resting or baseline period, as well as a comparison of the experimental condition (i.e. 40% IHG) against the corresponding sensory control condition (i.e. 5% IHG). Please refer to the Methods section below for more detail.

2.13 PURPOSE AND HYPOTHESIS

Cardiovascular disease is a class of diseases that afflict the heart and blood vessels, and includes diseases such as hypertension and stroke. SNS over-activity has been correlated with CV disease and has been implicated with increased mortality and morbidity (96) Gaining a better understanding of the cortical structures responsible for initiating and sustaining the sympathetic response to exercise could assist in understanding the inner workings of the SNS and its relation to CV disease (96). Expanding our knowledge could aid in the development of novel prevention techniques, improved prognosis, and innovative treatment strategies. For example, transcranial magnetic stimulation (TMS) is a non-invasive tool capable of exciting or inhibiting brain regions using magnetic current flow (97). Research may soon uncover a method by which TMS can favorably modify the activity of specific brain regions involved in the autonomic response. The big picture of this approach would be to inhibit regions of the brain involved with sympathetic over-activity, and/or to excite regions of the brain that augment parasympathetic activity, under the assumption that restoring autonomic balance would result in a lower risk for developing CV disease.

The purpose of the current study was to examine the brain regions involved with two separate autonomic CV regulatory mechanisms (CC and the EPR) using an IHG exercise and PEI protocol. Specifically, we sought to uncover the cortical regions associated with initiating the rapid CV responses at the beginning of IHG (i.e. Central

Command) versus the brain regions involved with detecting and integrating sensory information from active skeletal muscles (i.e. the Exercise Pressor Reflex). On the most basic level, we anticipate that the contraction phase will be accompanied by alpha and beta desynchronization within the hand region of the contralateral primary motor cortex. Based on previous findings, it was hypothesized that alpha and beta desynchronization in the contralateral IC and ipsilateral parietal association cortex will parallel the decrease in alpha and beta power noted within the contralateral primary motor cortex and will be positively correlated with the exertion of the IHG exercise. Desynchronization from within the contralateral insula, primary and secondary somatosensory cortices, along with synchronization within the perigenual anterior cingulate and midcingulate cortices, is expected during the post-exercise ischemic phase. Based on the findings of Sander et al. (2), we expect to observe desynchronization within the contralateral IC that persists after exercise cessation, throughout PEI, while activity within the ACC is expected to demonstrate progressive synchronization.

This study is unique to its predecessors, as the design includes the simultaneous measure of brain neural activity using MEG, along with non-invasive continuous BP and HR measurements. The high-frequency radio signals emitted during fMRI have prevented the concurrent measurement of brain neural activity, and continuous recordings of BP in this environment. The inability to differentiate between the brain regions associated with the integration of afferent autonomic activity from those associated with efferent autonomic activity is a critical limitation of fMRI that can be bypassed using MEG. Additionally, the IHG/PEI protocol that will be used in the current study (see Methods) has been employed numerous times with great success throughout the years. Its

elegant simplicity and practicality make it an optimal fit for addressing these research questions when using a sample of conscious humans.

CHAPTER 3: METHODS

3.1 ANALYSIS STRATEGY

The purpose of this study was to compare changes associated with CC versus the EPR. This comparison was achieved by methodically parsing data into blocks according to the time at which each neural mechanism was most prominent, when they were both activated the same time or when neither was active. According to this theoretical framework, it was assumed that CC was immediately active upon IHG onset and deactivated with its offset. Additionally, activity of the EPR was assumed to emerge approximately 45 seconds into the IHG exercise period and remained active throughout the PEI period. Previous knowledge regarding the response times of CC and the EPR, together with the well-documented changes in HR and BP that accompany exercise (19,44), justified the analysis strategy outlined below and the comparisons between the CV and MEG data. This study marked the first known attempt to concurrently examine brain neural activity patterns and continuous recordings of CV data during exercise in humans. This study was granted ethical approval by the Research Ethics Board of the IWK Health Centre (Project #: 1011585, Appendix A).

The expertise of Dr. Derek Kimmerly and Dr. Tim Bardouille was imperative to the success of the study. Dr. Derek Kimmerly, an Assistant Professor within the School of Health and Human Performance at Dalhousie University was the primary investigator and expert in the field. Dr. Kimmerly led the acquisition of the physiologic data and directed the study. Dr. Tim Bardouille is a research scientist at the Laboratory for Clinical Magnetoencephalography (IWK Health Centre), as well as, an adjunct professor within the School of Physiotherapy and Department of Computer Sciences at Dalhousie University. Dr. Bardouille guided the acquisition, processing, and analysis of the MEG

data, and served as an invaluable source of MEG expertise. Ms. Van Gestel was a student at Dalhousie University pursuing a Master of Science degree in Kinesiology, and was responsible for participant recruitment and instrumentation, data collection, analysis and interpretation (MEG and physiologic data), as well as the dissemination of the results.

3.2 POPULATION SAMPLE

To determine sample size, we considered published mean values ± standard deviations (SD) of MAP responses to IHG and PEI in a population of normotensive individuals (49). Based on a resting MAP of 83 ± 8 mmHg and MAP increases of 105 ± 11 mmHg (during IHG) and 98 ± 11 mmHg (during PEI), we calculated values for Cohen's "d" (2.29 and 1.56, respectively) and effect size (0.75 and 0.62, respectively). These values were then entered into a power calculator (G Power 3.1.3, repeated measures ANOVA, within factors model) (98). An alpha value of 0.05 resulted in an estimated sample size of 8 and 11 (IHG vs. PEI MAP values, respectively). Participants were recruited through word of mouth and through poster advertisements (Appendix B).

3.3 FAMILIARIZATION SESSION

Individuals interested in participating in the study were emailed prior to the first session and asked to avoid intense physical activity and to abstain from consuming alcohol and caffeine 24 hours prior to all data collection sessions. An initial familiarization session was then scheduled with participants in which the protocol was reviewed, informed consent was obtained, and required questionnaires and documentation were completed; this included a medical history questionnaire, (Appendix C) physical activity readiness questionnaire (PAR-Q, Appendix D), and the Edinburgh handedness questionnaire to determine hand dominance (Appendix E), as well as a review of the pre-study instructions (Appendix F) and the inclusion/exclusion criteria,

(Appendix G). Furthermore, resting HR, BP, and measures of height and weight were recorded. Participants were also asked if they adhered to the pre-study guidelines and if they were experiencing any adverse physiologic symptoms as a result of partaking or failing to partake in the pre-study protocol (e.g. caffeine withdrawal or sustained elevations in HR due to prior intense physical activity). If a participant was experiencing symptoms, the symptoms were recorded so they could be referred to in the case of anomalous results.

Individuals were invited to partake in the study according to the information obtained from the previously mentioned questionnaires and forms. Young (age 18-64 years), healthy, normotensive (systolic ≤139 mmHg, diastolic ≤ 89 mmHg) men and women were eligible. Participants were deemed ineligible if they were smokers, obese (body mass index > 30 kg/m^2), pregnant, or if they were taking any medications for a CV, metabolic, pulmonary, or neural disease. Individuals with syndromes characterized by autonomic and/or CV effects (e.g. diabetes mellitus, Raynaud's disease) were also excluded from participation. Furthermore, because the MEG scanner is sensitive to subtle changes in magnetic fields and involves confinement within an enclosed environment, exclusion criteria included participants that had permanent or semi-permanent metallic objects in/on their body (e.g. braces, surgical screws) or were claustrophobic.

Once participants were deemed eligible to participate in the study they were instrumented with the two Portapres® pressure cuffs on the toes of their left foot and an automated blood pressure monitor on their dominant upper arm. After resting BP and HR data were recorded, participants performed three, 5 second maximal voluntary

isometric handgrip contractions with their non-dominant hand. They then completed the IHG and PEI protocols (see below) at both the 5% and 40% MVC force levels.

3.4 EXPERIMENTAL PROTOCOL

The experimental protocol was comprised of two conditions: 1) a 40% IHG exercise condition; and 2) an "effortless" 5% IHG exercise condition. The 5% IHG condition was included to serve as a sensory control that required the participants to carry out the same sensorimotor task at an intensity level below that necessary to elicit a CV response. Therefore, during the 5% IHG condition, brain regions associated with the performance of the task were activated, while brain regions responsible for orchestrating a CV response were not. Differences in brain activation between the two conditions were used to identify the brain regions involved with CV control during isometric exercise (40% IHG condition), versus those involved with mild motor activation and sensory perceptions involved with the task (5% IHG condition). As previously mentioned, both conditions were sub-divided into three periods: 1) three minutes of rest; 2) 90 seconds of IHG exercise; and 3) 90 seconds of PEI (Figure 6). The periods of interest for the analysis included the last 43 seconds of rest prior to IHG onset (Rest), the first 43 seconds of IHG (IHG-A), second 43 seconds of IHG (IHG-B), and the second 43 seconds of PEI (PEI-B). The 43 second window was chosen as opposed to 45 seconds (half of the 90 second period) to avoid any situation in which a period may have ended slightly prior to the 90 second mark. The 43 second segment thus ensured that the time frame was purely the period (or epoch) of interest and did not contain data from the neighboring epochs.

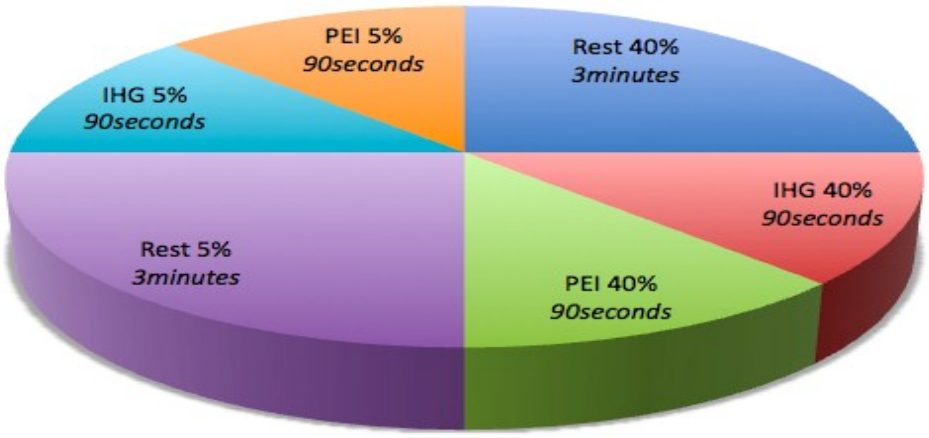

Figure 6: Illustration representing the time periods that comprised the experimental protocol. Each condition (i.e. IHG using 5% or 40% of MVC force) consisted of a 3 minute rest period, a 90 second isometric handgrip (IHG) period, and a 90 second post-exercise ischemia (PEI) period. The first condition performed was randomized and the above protocol was repeated a total of five times (five trials at 5% IHG and five trials at 40% IHG), alternating between conditions.

For the familiarization session, only single 40% IHG and 5% IHG conditions (Rest, IHG and PEI) were performed. During the IHG exercise, participants received real-time, continuous visual feedback on a visual feedback monitor (projection screen) regarding handgrip force production as a percentage of their MVC force output. With approximately five to ten seconds remaining in the IHG exercise period, the upper arm cuff was inflated to a supra-systolic pressure (~200 mmHg) to occlude blood flow to the exercising forearm for the 90 second PEI period. Once the cuff was inflated, the participant was notified to stop contracting. After 90 seconds of PEI, the cuff was deflated and a 3 minute recovery period ensued.

The inability to establish or maintain a stable Portapres® BP recording from a toe during the familiarization precluded further participation in the study. Participants were also required to be able to view the visual feedback monitor presenting the amount of force applied to the handgrip dynamometer. Furthermore, participants were expected to be able repeat the familiarization protocol four more times each during the MEG session,

totaling 10 IHG trials (five at 40% IHG and five at 5% IHG). Therefore, participants were required to have normal or corrected-to-normal vision and were not affected by any condition that prevented the full function of their non-dominant hand (e.g. arthritis, Dupuytren's contracture).

3.5 MEG SESSION

The MEG session was scheduled for each participant that successfully completed the familiarization session. Participants were instructed to arrive at the IWK Health Centre in a well-hydrated state ~3 hours after a light meal. Upon arrival, participants were directed to a washroom to change into a hospital gown or metal-free garments and asked to remove all external metal objects (e.g. underwire bra, eye make-up, hair accessories, upper body metal zippers/clasps/buttons, shoes, jewelry, and piercings). After changing, participants were asked to sit comfortably in the MEG chair within the shielded room while a short test scan was performed to confirm that they were free of objects that would interfere with the MEG scanner. Once completed, participants were removed from the scanner and outfitted with head position indicator (HPI) coils placed at four anatomical landmarks, two placed as far apart as possible on the forehead, and one behind each earlobe, as high up as possible so they could be tracked by the MEG sensor array. The head position was monitored throughout the scanning sessions by sending a continuous sinusoidal current through the HPI coils. A digitization device (Polhemus Incorporated, Vermont, USA) was used to digitize a 200-point head-shape and create a three-dimensional model of the participant's head in space. In addition, electrodes were placed just lateral to the right and left eye, as well as an electrode just superior and inferior to the left eye. Each pair of electrodes produces an electrooculogram (EOG) that measures the resting potential of the retina across time. Normal eye movements,

including blinks and saccades, bring about changes in resting potential and therefore changes in the magnetic field. The EOG records eye movement throughout the experimental protocol in order to detect and subsequently remove occular artifacts. Finally, non-dominant forearm electromyographic (EMG) signals of the wrist flexors served to document the duration of each contraction profile that was useful for accurate parsing of the data into the different time blocks (see below).

Participants were briefed once again on the experimental protocol and were asked to be cognizant of the level of exertion they experienced according to the Borg Scale, 6-20 (99) during the 5% and 40% IHG conditions, as well as, the level of pain or discomfort they experienced during the PEI periods via the McGill Pain Questionnaire (100) (Appendix H and Appendix I, respectively). The Borg Scale and McGill Pain Questionnaire ratings were obtained after the MEG scanning session was completed to avoid unnecessary thought processes that could confound the neuroimaging results.

After head digitization, participants were taken into the electromagnetically shielded MEG scanning room where they were outfitted with adhesive silver-silver chloride electrodes for recordings of an electrocardiogram (ECG, 3-lead, bipolar configuration). The Pneumotrace II, strain-gauge band (UFI, California, USA) was secured around the participants' thorax, underneath their xyphoid process to track respiratory movements. An inflatable pressure cuff (Hokanson) was secured around the participant's non-dominant arm, proximal to the elbow and the cuff of an automated BP monitor (Carescape v100, GE Healthcare Dynamap Technology) was secured in the same position around the dominant upper arm. Finally, a strain gauge-based handgrip dynamometer (ADInstruments) was placed within the participant's non-dominant hand.

All signals were recorded using dedicated data acquisition (PowerLab, ADInstruments) and analysis software (LabChart, ADInstruments).

A Portapres® (Finapres Medical Systems, B.V., The Netherlands) provided continuous non-invasive measurements of BP from the 1st and 2nd or 2nd and 3rd toes of the left foot, depending on cuff fit. The toe used by the Portapres® was switched during resting periods if stable BP recordings were not possible or if the participant requested that a different toe be used. The toes of the foot were used for the Portapres® BP recordings, as opposed to the fingers, in order to minimize the electromagnetic interference on the MEG sensors created by the front-end unit of the Portapres®, which controls the pump connected to the pressure cuffs. The height correction unit, stemming from the front-end unit was secured at the heart level, served to correct for orthostatic-induced changes in blood pressure between the levels of the heart and foot (i.e. corrected the pressures recorded in the toes to heart level). Due to the sensitivity of the MEG to electromagnetic noise, all electronic devices that could interfere with the MEG sensors were kept outside of the shielded room. Prior to data collection, the functional (or recording) end of each device was fed through a small passage in the wall of the shielded room, with the exception of the Portapres®, such that the functional end of the equipment entered the MEG room for recording while the electronic sources remained outside the MEG scanner.

The MEG chair was raised so the head of the participant came as close to the MEG sensors as comfortably possible. Once comfortable and secure, the participant was isolated within the shielded room. Resting MEG and physiologic measurements were collected with the eyes closed for a minimum of five minutes while the participant sat in

a relaxed position, breathing spontaneously. Members of the research team were able to communicate and visually observe participants throughout the experiment using a 2-way intercom system and video camera.

Following the collection of the resting HR and BP data, participants performed two to three 5 second isometric maximal voluntary contractions, each separated by 30 seconds of rest. The dynamometer force output was recorded and the greatest force generated from these trials represented 100% MVC and was used to calibrate the relative handgrip dynamometer force output. For each participant, the order of conditions always alternated between 5% and 40% during the experimental protocol. Conditions were alternated to avoid participant fatigue associated with repeated 40% IHG exercise periods. However, the order of which condition was performed first (5% versus 40% IHG) was randomized between participants.

Upon completion of the entire experimental protocol the participant was asked to provide Borg scale ratings (Appendix H) for the 40% IHG versus 5% IHG, to compare the 40% and 5% PEI period according to the McGill Pain Questionnaire (Appendix I), and asked to report on their experience in the MEG environment by completing the 'MEG Exit Questionnaire for Adults' (Appendix J).

3.6 CARDIOVASCULAR DATA COLLECTION

The ECG was sampled at 1000 Hz (band-pass filtered: 0.3-20 Hz). The Pneumotrace II was sampled at 40 Hz, the Portapres® waveform at 200 Hz, and the handgrip dynamometer at 20 Hz. The analogue signals for ECG, Pneumotrace, and Portapres® were "split", one directed to the MEG acquisition system and the other to the PowerLab data acquisition system.

3.7 MEG DATA COLLECTION

At the start of MEG scanning, the PowerLab (AD Instruments) and MEG data acquisition systems were time-aligned using a 5 volt transistor-transistor logic pulse signal. The MEG system acquired data from a single magnetometer and two orthogonal planar gradiometers at 102 locations across the entire head, sampling at a rate of 1500 Hz (low-pass filtered at 500 Hz). Throughout the protocol, HPI coils served to continuously monitor movement of the head. The experimental protocol consisted of 65 minutes of active MEG scanning. Due to the length of the scan and volume of data collected by the system, scanning was briefly stopped and saved during two intermittent rest periods as a precautionary measure. If a problem was encountered during the scanning session, this would minimize the amount of data lost.

3.8 CARDIOVASCULAR DATA ANALYSIS

Electrocardiogram-derived measurements of HR were quantified as the number of beats per minute. The toe arterial BP waveform generated by the Portapres® was calibrated to brachial artery BP using the automated blood pressure device (Carescape v100, GE Healthcare Dynamap Technology). The equation: ⅓ systolic pressure + ⅔ diastolic pressure, was used to calculate MAP. Average and peak data for HR and MAP were calculated during Rest, IHG-A, IHG-B, and PEI-B of each trial for both the 5% and 40% IHG conditions.

All CV data were for like time periods were averaged across trials for each participant (e.g. HR average during IHG-40A across five trials); the averaged values for each person was subsequently used to calculate group means and SD for each time period of the 40% and 5% condition. A statistical analysis of within-group differences were assessed by a two-way repeated measures (condition × time period) analysis of variance

(ANOVA). The level of significance was set at $p < 0.05$ and adjusted using the Bonferroni correction method as required.

3.9 MEG DATA ANALYSIS

Temporal signal-space separation (TSSS) was performed on the raw MEG data using Maxfilter™ and MaxST™ software (Elekta Neuromag Oy, Helsinki, Finland). This process served to attenuate environmental, physiologic, and intrinsic sensor noise by defining three independent subspaces within the MEG environment (Figure 7). One subspace included sources (*b*) emanating from inside of the helmet (b_{in}), the second included sources emanating from outside of the helmet (b_{out}), and the third represented those in very close proximity to the helmet's sensor array (*n*) (5). The signal-to-noise ratio was increased by suppressing sources of noise (b_{out} and *n*) that interfere with the brain signals that originate from within the sensor array (b_{in}). These sources of noise are identified by inordinately high spatial frequencies, uncharacteristic of true brain activity (5).

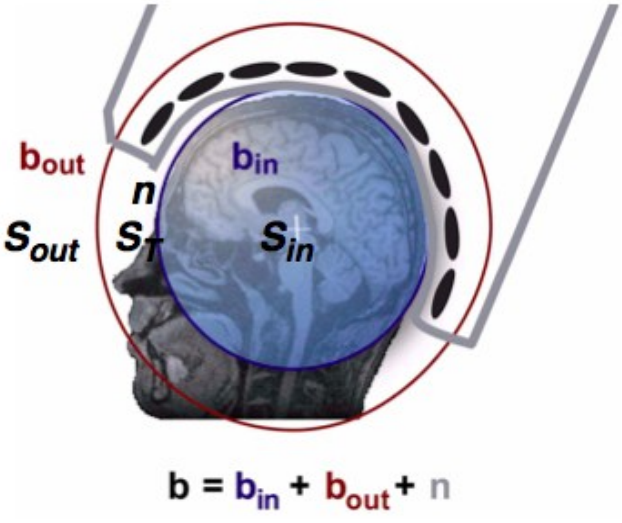

Figure 7: Illustration of the three independent subspaces created by Maxfilter™ and MaxST™. One subspace (Sin) includes the brain activity originating from within the helmet (bin). Another subspace (ST) encloses the sensor array of the helmet (n) and includes interference generated by the sensors or sources of noise located very close to the sensor array. The third (Sout) is comprised from all sources originating from outside of the previous two subspaces (bout) (5).

Head Position Indicator coil data were used to confirm that a stable head position was maintained throughout the experimental protocol. Position information afforded by the HPI coils was used to examine each participant's gradual drift in position throughout the scan, as well as their maximum movement during the experimental protocol. If the maximum movement exceeded 10 mm, plots illustrating translation and rotation in the x-, y-, and z-axis were generated. Rotation and translation thresholds were set at 10° and 6 mm, respectively (Figure 8). The experimental period corresponding to any movement exceeding the rotation or translation threshold was removed from the dataset and excluded from further analysis. Rotation and translation plots generated in response to maximum movement in excess of 10 mm are displayed in Appendix K.

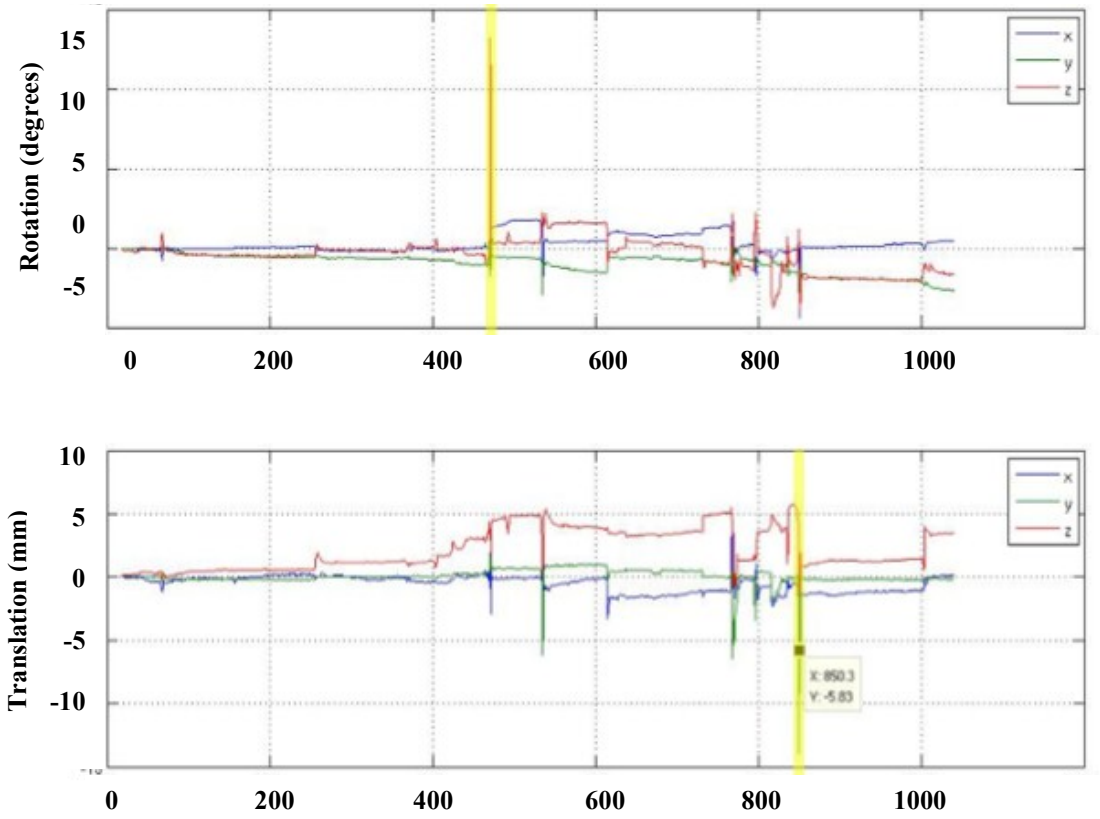

Figure 8: Sample rotation and translation plots. The time span of any rotation exceeding 10°, or any translation exceeding 6 mm, in the x-, y-, or z-axis (highlighted in yellow) were recorded and traced back to the corresponding experimental period. This period was then flagged for removal from further analysis.

Upon the head movement assessment, an EMG envelope was applied to the EMG data. This included full-wave rectification, low-pass filtering at 70 Hz, and down sampled to 250 Hz. The EMG profile was used to identify the start and ends of the IHG contraction periods. This information was used to epoch the processed MEG data into "active" data only; this included all data except the first 135 seconds of each rest period (i.e. only the last 43 seconds of rest data prior to IHG onset were maintained). Active MEG data were concatenated into one complete and orderly dataset. An independent component analysis was then applied to this file, serving to separate the multivariate signals into individual components. These components were then assessed separately under the assumption that they are statistically independent of one another. This statistical tool is effective for separating physiologic sources of noise (e.g. heartbeats and skeletal muscle contraction) from sources of brain activity (101). The final preprocessing step involved a visual check of each dataset to confirm that the data were free of major artifacts.

3.9.1 Parsing of MEG Data

Each participant's complete pre-processed dataset was imported into Brainstorm, a graphical user interface that is documented and freely available for download online under the GNU general public license (102). As previously mentioned, each trial was temporally parsed into events, including a 43 second rest period (rest segment immediately prior to the start of each IHG period), while the 90 second IHG and PEI period were divided into two consecutive 43 second segments (Figure 9). The rest period served as a baseline condition in which neither CC nor the EPR were active. The first segment of the 40% IHG (0-43 seconds, IHG-40A) represented predominantly CC activation, the second segment of the 40% IHG (43-86 seconds, IHG-40B) represented

the combined activation of CC and the EPR, and both the first and second segments of the 40% PEI period predominantly represented the EPR (PEI-40A and PEI-40B). Time segments of particular interest for this thesis included Rest, IHG-40A (i.e. CC), and PEI-40B (i.e. EPR). The latter segment of PEI was chosen as opposed to the first, due to the more pronounced physiologic response accompanying the latter half of this period (2, 22,103). It is important to reiterate the role of the 5% IHG condition as a sensory control that would not activate CV control mechanisms during exercise. The epochs of the 5% sensory control condition were compared to the corresponding epochs of the 40% IHG condition.

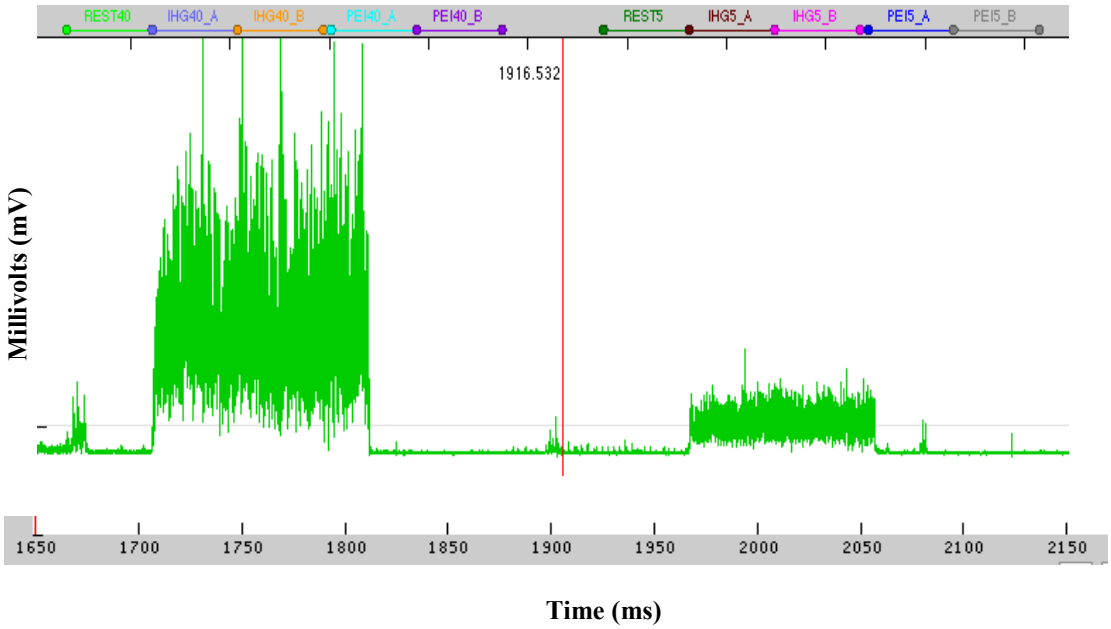

Figure 9: Sample EMG profile, displaying 40% and 5% isometric handgrip (IHG) exercise periods, respectively. Each 40% and 5% condition was parsed into five 43 second epochs, which included Rest, the first (IHG-A) and second (IHG-B) 43 second segments of exercise, and the first (PEI-A) and second (PEI-B) 43 second segments of post-exercise ischemia.

3.9.2 Time-Frequency Decomposition of MEG Data

Separate time-frequency (TF) decompositions were computed for each epoch using complex Morlet wavelets grouped in frequency bands (alpha, and beta). Morlet wavelets contained a central frequency of 1 Hz and a time resolution of 3 seconds. The

resulting TF decompositions were averaged across each of the time periods. This resulted in one file containing the average TF changes during each experimental period for each participant. Group averaged difference plots were then generated based on the comparisons of interest, the most important being the subtraction of the corresponding rest periods (i.e. IHG-40A – Rest-40, IHG-5A –Rest-5, PEI-40B – Rest-40, and PEI-5B – Rest-5) as well as the removal of the 5% sensory control condition from the 40% experimental condition (i.e. IHG-40A – IHG-5A and PEI-40B – PEI-5B) in the alpha and beta bands.

Paired t-tests were used to identify the presence of significant differences between group averages for the previously mentioned comparisons (α= 0.05, t-scores < -2.29 or > +2.29, based on 10 degrees of freedom according to a sample size of 11. A mask was then applied to the t-test data such that MEG sensors displaying a significant difference were multiplied by one, while sensors that did not display a significant difference were multiplied by zero. The results of this t-test mask were then applied to the difference plots, which revealed regions that displayed significant differences in power in the alpha and beta bands. For example, according to the theoretical framework, subtracting group averages of IHG-5A from IHG-40A would remove the sensory control and yield the changes in power within regions that correspond with CC. The t-test would then be used to discriminate between regions that exhibited a significant change in alpha and/or beta power from those that did not.

The results were plotted in the alpha and beta bands for each of the three sensor types, including the first set of planar gradiometers (Gradiometer 1), the second set of planar gradiometers (Gradiometer 2), and the magnetometers. All non-significant

changes in power were masked such that plots only depict regions that demonstrated significant increases (i.e. t-score > 2.29, $p < 0.05$) or decreases (t-score < -2.29, $p < 0.05$) in power within the alpha or beta bands. Again, increases in alpha and beta power are synonymous with increased neural synchrony and are indicative of inactive brain regions. In contrast, decreases in power are synonymous with neural desynchronization and are indicative of active brain regions.

CHAPTER 4: RESULTS

A total of 13 participants completed the experimental protocol; however, two datasets were removed from the study, one due to excessive head movement throughout the MEG scan and one due to a lost HPI coil signal. Furthermore, epochs from the remaining 11 participants were removed from analysis if they exceeded the rotation/translation threshold (10 mm and 6°, respectively). This resulted in the removal of 10 Rest-40 epochs, 11 Rest-5 epochs, 7 IHG-40A epochs, 8 IHG-5A, 5 PEI-40B, and 7 PEI-5B. Thus the total number of epochs used in the subsequent analysis were 45 Rest-40, 44 Rest-5, 48 IHG-40A, 47 IHG-5A, 50 PEI-40B, and 47 PEI-5B, out of a possible 55 epochs maximum per time period. Descriptive data from the remaining 11 healthy participants are included in Table 1.

Table 1: Descriptive statistics of the study participants

	Female (n=5)	**Male (n=6)**	**Total (n=11)**
Age (years)	21 ± 2	23 ± 2	22 ± 2
Height (cm)	165 ± 12	175 ± 7	170 ± 11
Weight (kg)	59 ± 4	81 ± 9	71 ± 13
BMI (kg/m^2)	22 ± 4	26 ± 2	24 ± 4
Resting HR (beats/minute)	66 ± 14	70 ± 14	68 ± 14
Resting MAP (mmHg)	79 ± 8	90 ± 9	85 ± 9
Handedness	Right=5, Left =0	Right=6, Left=0	Right=11, Left=0

Data are presented as Means ± Standard Deviations. BMI, body mass index; HR, heart rate; MAP, mean arterial pressure

4.1 CARDIOVASCULAR RESULTS

4.1.1 MAIN EFFECTS

Heart rates and measures of MAP during the 40% IHG condition were found to be significantly higher than those of the 5% IHG condition ($p = 0.02$ and $p = 0.03$, respectively). Furthermore, an effect for period was found for HR ($p = 0.05$) with IHG-A significantly higher than Rest ($p = 0.001$) and PEI-B ($p = 0.006$). An effect for period was

also found for MAP ($p = 0.04$), with IHG-B significantly higher Rest ($p = 0.03$) and IHG A ($p = 0.05$). No significant interaction effect was uncovered ($p = 0.06$ for both).

4.1.2 WITHIN-CONDITION RESULTS

Group mean HR and MAP data are presented in Figures 10 and 11, respectively. No significant differences ($p > 0.28$, all comparisons) were noted for either HR (Figure 10) or MAP (Figure 11) across the four time periods during the 5% IHG condition. During the 40% IHG condition, HR was significantly greater during the first 43 second epoch of IHG (IHG-40A) than the preceding rest period ($p = 0.0004$) and the second segment of the PEI period (PEI-40B, $p = 0.003$). Mean arterial pressures during both IHG-40A and IHG-40B were significantly higher ($p = 0.04$ and $p = 0.03$, respectively) than the rest period (Figure 11). In addition, MAP during IHG-40B was greater ($p = 0.04$) than IHG-40A (Figure 11).

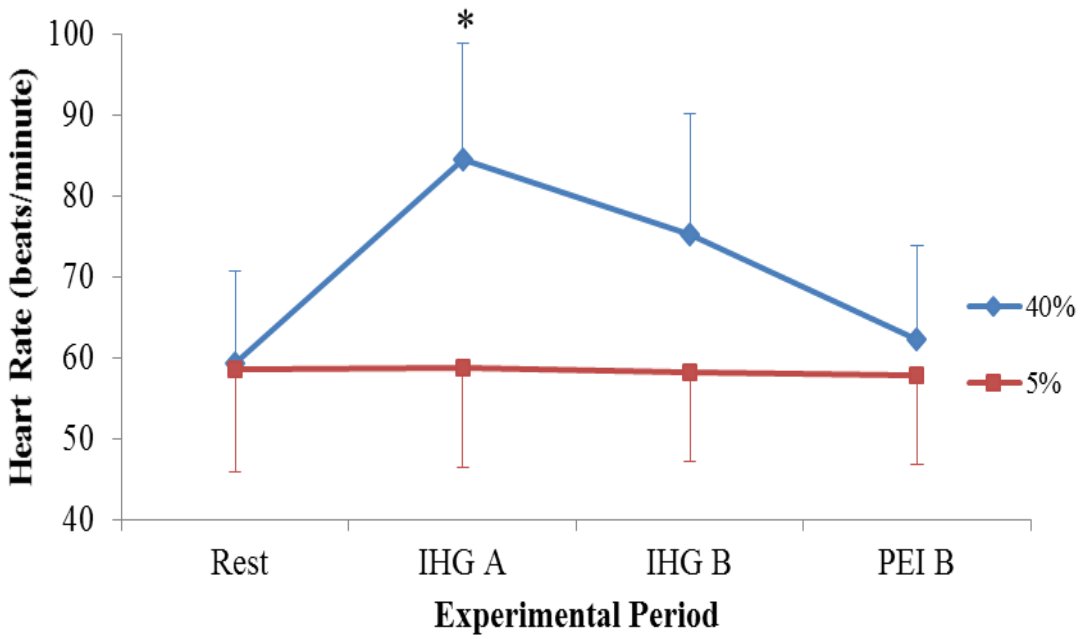

Figure 10: Heart rate responses during the 40% and 5% IHG conditions across time periods. Data are presented as Means ± SD. Rest, the rest periods preceding isometric handgrip (IHG) exercise; IHG-A, the first 43 seconds of handgrip exercise; IHG-B, the last 43 seconds of handgrip exercise; PEI-B, the last 43 seconds of post-exercise ischemia. *, $p < 0.004$ vs. Rest-40 and PEI-40B.

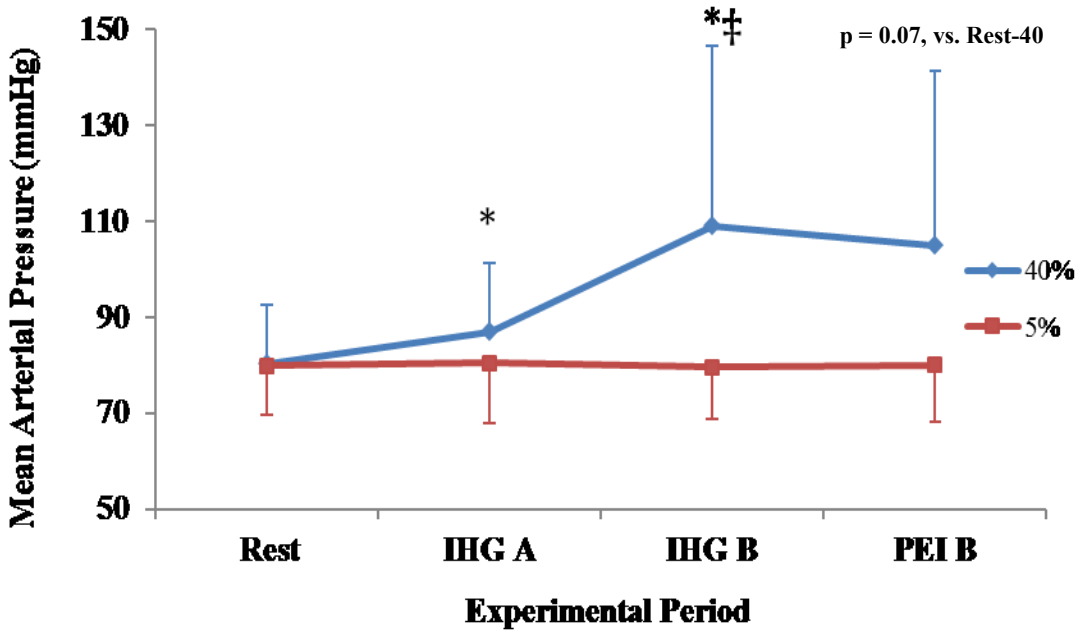

Figure 11: Mean arterial pressure responses during the 40% and 5% IHG conditions across time periods. Data are presented as Means ± SD. Rest, the rest periods preceding isometric handgrip (IHG) exercise; IHG-A, the first 43 seconds of handgrip exercise; IHG-B, the last 43 seconds of handgrip exercise; PEI-B, the last 43 seconds of post-exercise ischemia. *, $p < 0.04$ vs. Rest-40; ‡, $p < 0.04$ vs. IHG-40A; $p = 0.07$, PEI-40B vs. Rest-40.

No significant differences were found between the average isometric handgrip contraction percentages across trials of the 40% condition, expressed as a percent of maximal voluntary force output ($p > 0.97$, Figure 12). Furthermore, no significant differences ($p = 0.12$) were noted between the average isometric handgrip contraction percentages during the first versus the last 43 second segment of the 40% IHG (IHG-40A versus IHG-40B, Figure 13).

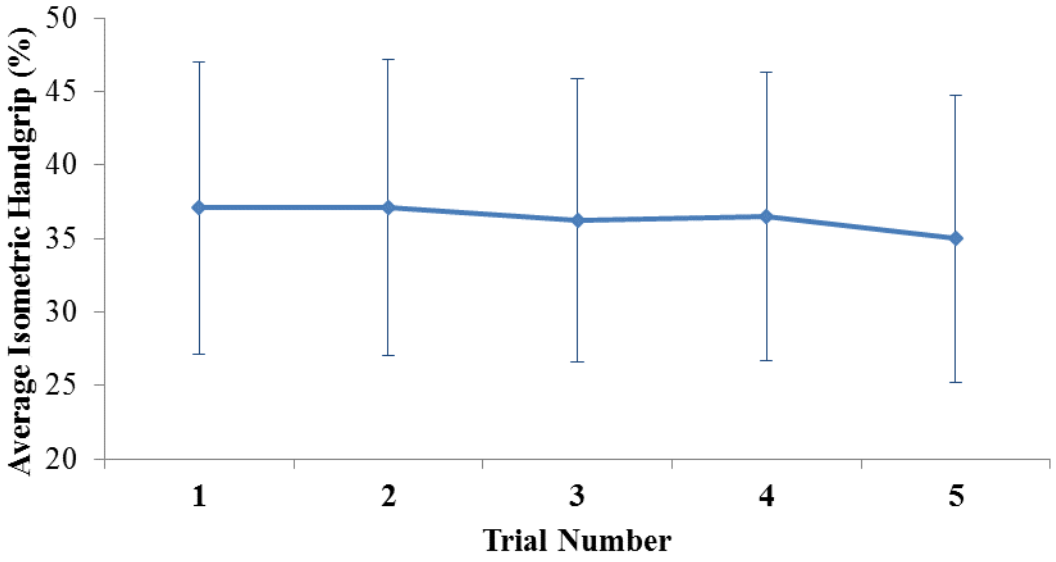

Figure 12: Average isometric handgrip (IHG) contraction during the 40% condition, as a percentage of maximum contraction force, across trials. Data are presented as Means ± SD. No significant differences were found across trials ($p > 0.97$).

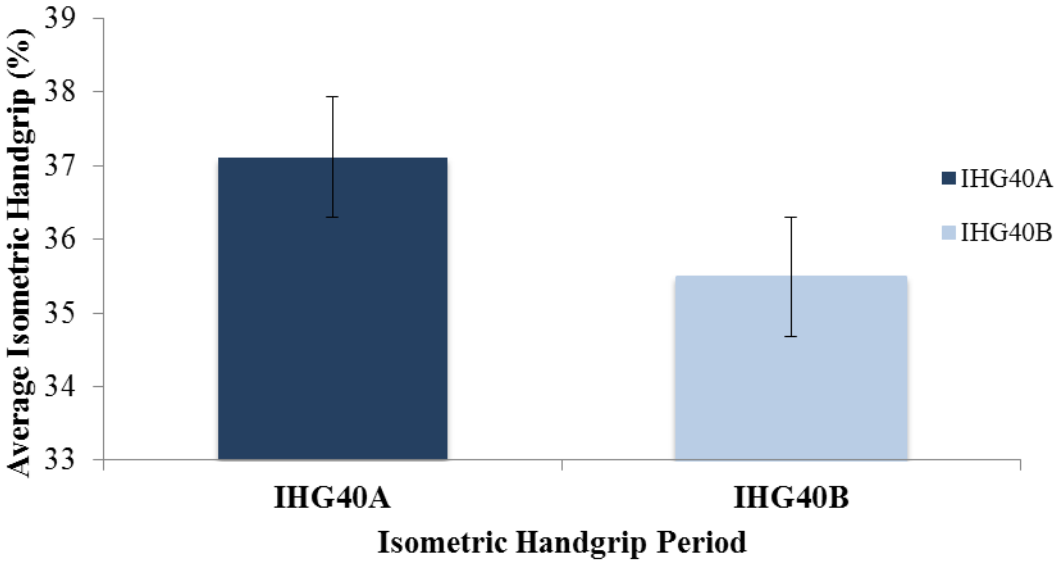

Figure 13: Average isometric handgrip (IHG) contraction during the first 43 seconds of the 40% IHG (IHG-40A) and second 43 seconds of the 40% IHG (IHG-40B). Data are presented as means ± SD. No significant differences were found between IHG-40A and IHG-40B ($p = 0.11$).

The mean rating of perceived exertion during the 40% IHG condition was 16 ± 2 according, which was significantly higher ($p = 0.0002$) than the rating during the 5% IHG condition (9 ± 2). Participants also reported that the pain associated with the 40% PEI period (10 ± 2) was significantly higher ($p = 0.0002$) than that noted during the 5% PEI period (3 ± 1) according to the Mcgill pain questionnaire. The sensations experienced during the 40% PEI period were commonly described as "hot-burning", "aching", and "throbbing". No participants reported any adverse effects related to the pre-study guidelines (e.g. caffeine withdrawal) or any lasting negative effects related to partaking in the experimental protocol.

4.2 MEG Results

4.2.1 Isometric Handgrip Comparisons, Changes in Alpha Power

Significant bilateral desynchronization in the alpha band for the comparison of IHG-5A versus Rest-5 were observed in all three sensor types (Figure 14). Both sets of gradiometers (Gradiometer 1 and Gradiometer 2) revealed extensive desynchronization

spanning from what appeared to be the posterior region of the frontal lobe to the occipital lobe. A pronounced desynchronization in alpha power, most evident in the first set of gradiometers (Gradiometer 1), was observed within the posterior region of the contralateral frontal lobe, potentially corresponding to the hand-region of the sensorimotor cortex. The magnetometers revealed more widespread decreases in alpha power for this the comparison of IHG-5A versus Rest-5. This decrease extended bilaterally throughout the posterior-frontal area, with a distinct region in the contralateral hemisphere expressing the greatest decrease in alpha power, again believed to be activation within the hand-region of the sensorimotor cortex (Figure 14). This region seems to be slightly more medial than the region highlighted by the gradiometers (Figure 14).

The comparison of IHG-40A versus Rest-40 displayed a similar pattern of alpha power change (Figure 14). Bilateral desynchronization was observable in both sets of gradiometers, again with a marked desynchronization displayed by Gradiometer 1 within the posterior region of the frontal lobe, particularly the hand-region of the sensorimotor cortex. Furthermore, the second set of gradiometers (Gradiometer 2) appears to illustrate a clustered bilateral desynchronization along the midline of the posterior parietal and/or occipital lobe, slightly more prominent on the ipsilateral hemisphere. The magnetometers also display a similar representation of power difference compared to the corresponding 5% comparison, with a more pronounced desynchronization observed along the midline of the contralateral posterior-frontal, and anterior-parietal regions (Figure 14).

The comparison of IHG-40A to IHG-5A revealed a significant ERD across sensor types within the contralateral posterior-frontal region, corresponding to the hand-region

of the sensorimotor cortex. The location of this decrease in alpha power was consistent across sensor types, but more widespread within the magnetometer representation and could potentially represent desynchronization within thalamic regions (Figure 14). Additionally, alpha ERS was noted in the first set of gradiometers and the magnetometers, just posterior and lateral to the aforementioned region of decreased power and may correspond to the contralateral insular cortex. Unlike the magnetometers, the region of ERS for Gradiometer 1 encompassed a larger area and decreased slightly in power as it extended anteriorly. Gradiometer 2 expressed a significant synchronization within the contralateral temporal region (Figure 14).

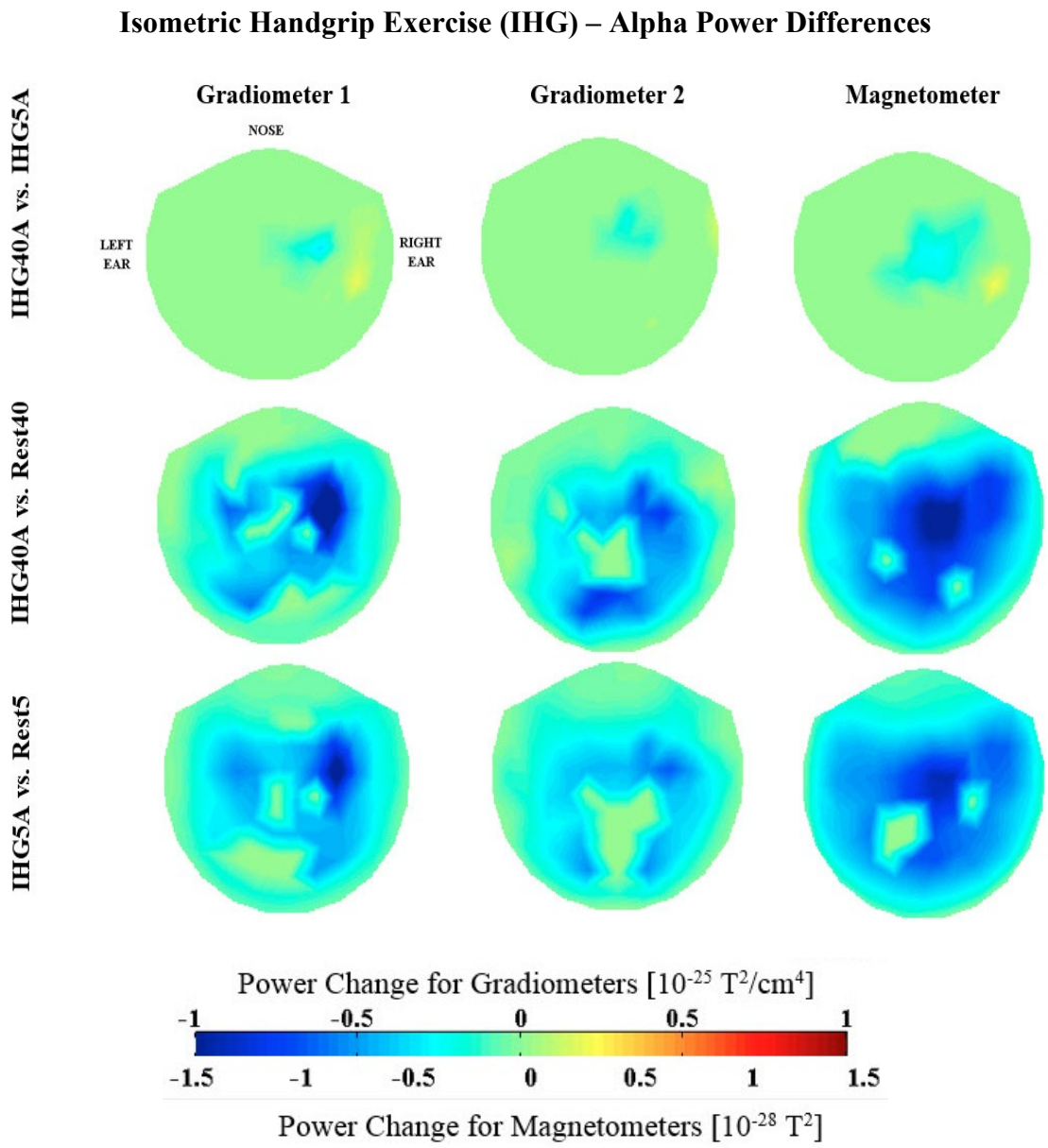

Figure 14: Group averaged power differences in the alpha band (8-12 Hz) displayed by both sets of planar gradiometers (Gradiometer 1, Gradiometer 2) and magnetometers**.** IHG-40A, first 43 seconds of the 40% isometric handgrip (IHG); IHG-5A, first 43 seconds of the 5% IHG; Rest-40, 43 second rest period preceding IHG-40A; Rest-5, 43 second rest period preceding IHG-5A. Non-significant power differences have been masked out, all displayed power differences are significant at $p < 0.05$.

4.2.2 Isometric Handgrip Comparisons, Changes in Beta Power

Figure 15 displays significant beta desynchronization across the three sensor types for the comparison of both IHG-40A and IHG-5A to rest. The differences are widespread but clustered medially. Furthermore, the comparison of IHG-5A versus Rest-5 and IHG-40A versus Rest-40 revealed a similar topographical pattern of beta ERD, especially within the first set of gradiometers. Both IHG-40A versus Rest-40 and IHG-5A versus Rest-5 illustrated a significant bilateral desynchronization within the posterior region of the frontal lobe. This desynchronization was more significant in the contralateral hemisphere during the 40% versus 5% IHG condition and is believed to correspond with the hand-region of the sensorimotor cortex. Furthermore, both conditions demonstrated a bilateral desynchronization in the region of the occipital lobe. Again, this desynchronization was more significant and had more distinct boundaries within the 40% IHG condition.

Within the second set of gradiometers, the IHG-5A versus Rest-5 comparison showed a diffuse decrease in beta power, spanning from the posterior-frontal region to the occipital lobe. For the IHG-40A versus Rest-40 comparison, this pattern was also apparent but contained more focal regions of desynchronization. Gradiometer 2 results for both conditions highlighted a region of beta desynchronization within the contralateral posterior-frontal region. Again, the IHG-40A versus Rest-40 comparison presented a more noticeable desynchronization within the posterior-parietal and/or anterior-occipital regions, extending bilaterally (Figure 15).

With respect to magnetometers, IHG-5A versus Rest-5 exposed significant beta desynchronization across the topographical representation of the cortex, with the exception of the ipsilateral frontal region. The most prevalent ERD was located within

the posterior-frontal region, slightly more medial than the localized regions expressed by the first and second set of gradiometers (Figure 15). Beta power increased outward from this region, emanating laterally and posteriorly. For IHG-40A versus Rest-40, the magnetometer results displayed a more somatotopically discrete ERD than the corresponding 5% comparison within what is believed to be the hand-region of the sensorimotor cortex. However, the most significant beta desynchronization was located within the same area (posterior-frontal, slightly toward the midline), with significant decreases in power stretching bilaterally from the contralateral posterior-frontal region and also posteriorly into the parietal and occipital lobes (Figure 15).

For the comparison of IHG-40A versus IHG-5A within the beta band, all sensor types identified a significant desynchronization in the posterior-frontal region, which corresponds to the hand region of the sensorimotor cortex (Figure 15). The beta power difference presented by the magnetometers encompassed a larger area than that revealed by the gradiometers. Furthermore, all three sensor types highlighted synchronization near the division of the ipsilateral temporal and parietal lobe, potentially corresponding to the ipsilateral insular cortex (Figure 15). Additionally, the first set of gradiometers displayed synchronization within contralateral temporal region for the comparison of IHG-40A to IHG-5A, while the magnetometers presented increased beta synchronization in the ipsilateral frontal region and posterior occipital region along the midline (Figure 15).

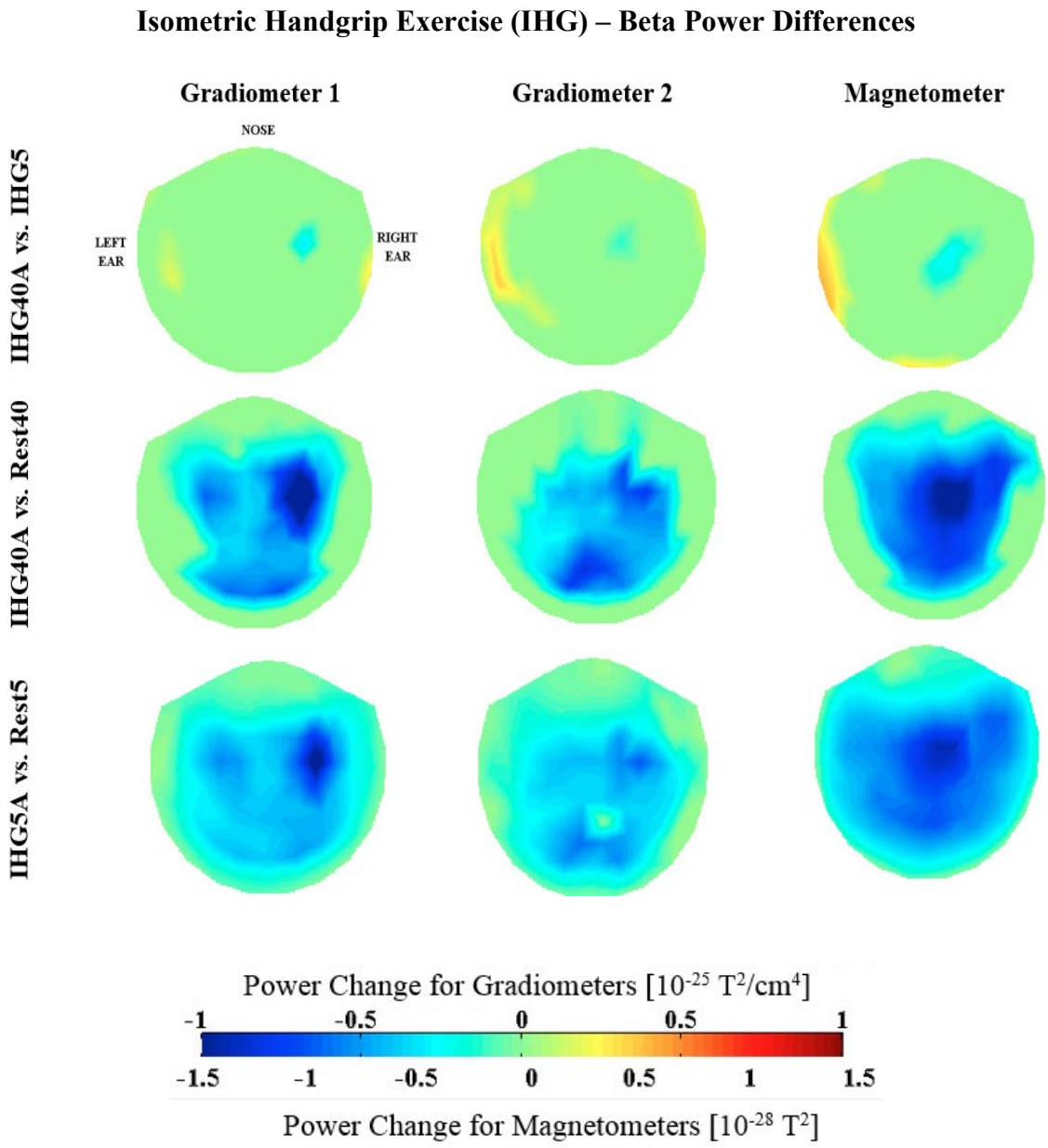

Figure 15: Group averaged power differences in the beta band (13-30 Hz) displayed by both sets of planar gradiometers (Gradiometer 1, Gradiometer 2) and magnetometers. IHG-40A, first 43 seconds of the 40% isometric handgrip (IHG); IHG-5A, first 43 seconds of the 5% IHG; Rest-40, 43 second rest period preceding IHG-40A; Rest-5, 43 second rest period preceding IHG-5A. Non-significant power differences have been masked, all deviations from zero power are significant at $p < 0.05$.

4.2.3 POST-EXERCISE ISCHEMIA COMPARISONS, CHANGES IN ALPHA AND BETA POWER

An examination of the last 43 seconds of the PEI period (PEI-B) in the alpha band, including PEI-5B versus Rest-5, PEI-40B versus Rest-40, and PEI-40B versus PEI-5B, did not produce any significant differences within any of the three sensor types (all t-scores between -2.29 and +2.29, $p > 0.05$).

In the beta band, the PEI-5B versus Rest-5 comparison highlighted an increase in synchrony along the midline over the occipital/cerebellar region. This increase in synchrony extended through the occipital lobe into the contralateral hemisphere, as observed in the first set of gradiometers (Figure 16). The second set of gradiometers for the comparison of PEI-5B versus Rest-5 also exposed a larger region of increased synchronization located in the contralateral parietal and occipital regions (Figure 16). With respect to the magnetometers, a focal region of increased beta synchronization was identified along the midline of the posterior-parietal and/or anterior-occipital regions (Figure 16). In addition, there was an increase in beta synchrony around the posterior-temporal or occipital region (Figure 16).

An examination of PEI-40B versus Rest-40 showcased four distinct regions of increased beta synchronization within the first set of gradiometers (Figure 16). Two were located within the contralateral parietal region, another in the ipsilateral parietal region, and the fourth stretched bilaterally into the occipital region (Figure 16). Both the second set of gradiometers (Gradiometer 2) and the magnetometers demonstrated bilateral increases in beta synchrony in the occipital and parietal regions of the cortex (Figure 16). For the comparison of PEI-40B versus Rest-40, magnetometers also displayed an increase in beta synchrony that was focused along the midline over the occipital lobe.

This region of increased beta synchrony was most pronounced along the midline but extended into the contralateral hemisphere.

Finally, no significant differences were noted within the first set of gradiometers or the magnetometers for the comparison of PEI-40B versus PEI-5B. The only significant power difference that resulted was an increase in synchrony observed in the second set of gradiometers, within the ipsilateral posterior-temporal region and may correspond to the ipsilateral insular cortex (Figure 16).

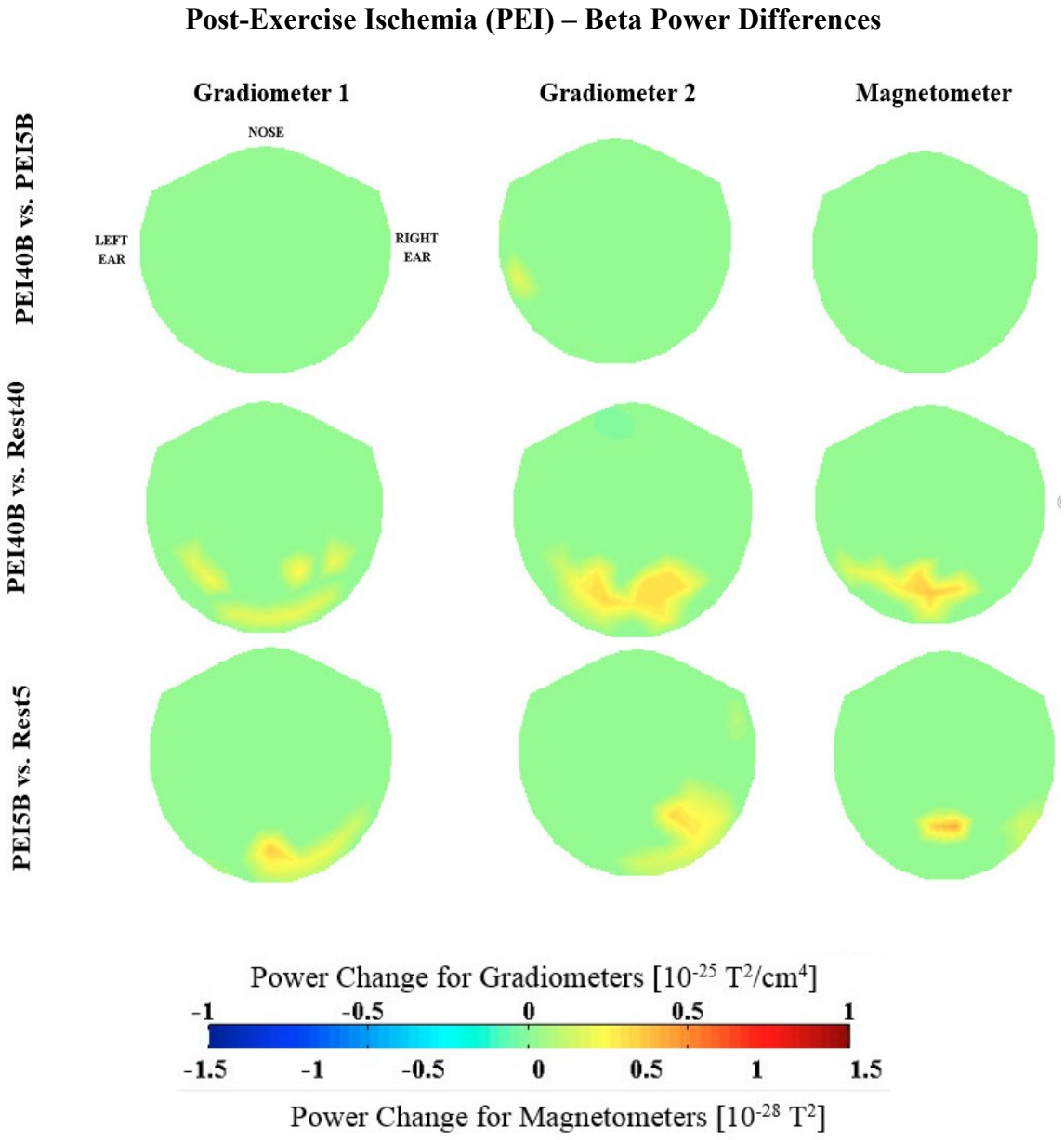

Figure 16: Group averaged power differences in the beta band (13-30 Hz) displayed by both sets of planar gradiometers (Gradiometer 1, Gradiometer 2) and magnetometers**.** PEI-40B, last 43 seconds of the 40% post-exercise ischemia (PEI); PEI-5B, last 43 seconds of the 5% PEI; Rest-40, 43 second rest period preceding the 40% isometric handgrip (IHG); Rest-5, 43 second rest period preceding the 5% IHG. Non-significant power differences have been masked, all deviations from zero power are significant at $p < 0.05$.

CHAPTER 5: DISCUSSION

The specific purpose of this study was to examine the brain regions involved with two separate CV control mechanisms during exercise, Central Command and the Exercise Pressor Reflex. Continuous, non-invasive measures of HR and MAP were used as the main cardiovascular-related dependent variables, while changes in brain activity were determined via magnetoencephalography and assessed as group-averaged power changes in the alpha and beta frequency bands.

During the first half of the 40% handgrip exercise (IHG-40A), HR and MAP increased significantly in comparison to rest, a change mediated predominantly by CC. During the second half of the IHG (IHG-40B) MAP underwent a further increase (significantly greater than rest and IHG-40A, a change mediated by both CC and the EPR. The MAP response decreased during PEI but there was a trend ($p = 0.07$) that suggested it was close to being higher than the rest period. With respect to MEG data, alpha and beta desynchronization were noted within the hand-region of the sensorimotor cortex during the IHG exercise. Furthermore, increases in synchronization during the IHG were noted within sensors that may correspond to both the ipsilateral and contralateral IC for the comparison of IHG-40A versus IHG-5A. More specifically, increases in alpha synchronization within what may be the IC were noted in the contralateral hemisphere, while increases in beta synchrony within this region were noted within the contralateral hemisphere. An increase in beta synchrony during PEI was also noted in what may be the ipsilateral IC, while no significant differences were noted within the alpha band during this time period.

This study is novel compared to previous research conducted in the field for a number of reasons. First, this study employed a sample of conscious humans while the majority of previous research has drawn conclusions derived from animal models (9-13). Second, the use of MEG rather than other neuroimaging techniques (i.e. functional MRI, EEG) afforded important benefits. Specifically, the spatial resolution of MEG is superior to that of EEG and is less affected by variations in tissue conductivity within head (78); MEG has superior temporal resolution (i.e. milliseconds versus seconds) and is a more direct measure of neural activity in comparison to BOLD fMRI (104). Third, the MEG environment is much more conducive to the simultaneous recordings of brain neural activity and continuous non-invasive recordings of HR and MAP. The strong magnetic field and emission of high-frequency radio waves inherent with the fMRI environment prevents this opportunity, making it necessary to record task-related changes in physiological variables and brain data on separate days (2, 14, 52). Efforts can be made to replicate conditions between these two days, but it is impossible to ensure that the cardiovascular responses to any stressor (i.e. handgrip exercise) are reproducible during repeated experimental sessions (14).

5.1 CARDIOVASCULAR DATA

Continuous recordings of HR and MAP were used to confirm that the handgrip exercise paradigm (i.e. 5% versus 40% IHG and PEI periods) conformed to the expected pattern of response (Figure 10 and 11) (2). Both heart rate (Figure 10) and mean arterial pressure (Figure 11) reactions to IHG and PEI followed in the anticipated manner according to their temporal response patterns mediated by CC (i.e. early rise in HR during IHG that returns near resting levels during PEI) and the EPR (delayed MAP response to IHG and sustained elevation during PEI).

The 5% IHG condition displayed an unchanging HR and MAP profile that did not differ between rest, the IHG, and PEI time periods (Figure 10). These findings confirm that the 5% IHG condition did serve as an effective sensory control condition, such that the physiologic demands of the 5% IHG contraction were insufficient to elicit CC or EPR-mediated increases in HR or MAP.

The resting heart rate values were similar between the 5% and 40% IHG conditions (Figure 10). During the transition from rest to the end of IHG-40A, the 40% IHG condition elicited a significant increase in HR (Figure 10). This early increase in HR confirmed that the feed-forward CC mechanism was activated within the first 43 seconds of IHG, and capable of triggering a significant cardiac response to the handgrip exercise at 40% of the participant's maximum voluntary contraction force. This initial CC-mediated increase in HR is similar to previous findings, as displayed in the results of Sander et al. in Figure 3 (2). One unforeseen result was that the HR during the second segment of the IHG (i.e. IHG-40B) underwent a slight depression from IHG-40A and was not found to be significantly different from rest or from PEI-40A (Figure 10). It was expected that HR during the 40% IHG condition would demonstrate a further increase toward the latter stages of the IHG period, as CC increased alongside increases in exercise duration. It was expected that CC would increase during the latter stages of the IHG to combat the effects of fatigue, recruiting larger motor units in order to maintain the desired force output. In an effort to explain this phenomenon the average IHG-40 contraction force output (as a percentage of maximum) was examined during the first and second segments of the handgrip exercise (Figure 13). It was speculated that a significant decrease in percent handgrip contraction force during IHG-40B (versus IHG-40A) could

account for the slight decrease in HR and lack of significance noted during this time period. That is, if participants failed to maintain 40% of their MVC contraction force throughout the latter half of the 90 second exercise period, the decreased contraction force may have been insufficient to elicit the anticipated HR response. However, no significant differences were noted between IHG-40A and IHG-40B, nor were there any significant differences between the average percent handgrip contractions across trials of the 40% IHG condition (Figure 12). The ECG and Portapres® signal for one participant contained several trials with large amounts of movement-related noise during IHG-40B. Values of HR during noisy trials could not obtained during these trials and were thus excluded from the analysis. It is possible that the removal of these trials had an effect on statistical power.

The MAP profile across periods (Figure 11) nicely depicts the time course of EPR activation in response to exercise and is similar to findings of previous studies that have examined the MAP response to an IHG and PEI protocol (2,53,103). Again, the results of the Sander et al. study (2) displayed a similar MAP profile, as illustrated in Figure 3. Average resting MAP for both conditions was ~80 mmHg, confirming a stable and similar baseline period (Figure 11). With respect to the 40% IHG condition, IHG-40A resulted in a significant increase in MAP from rest, suggesting that the CC-mediated elevation in HR was capable of causing significant increases in MAP within the first 43 seconds of the IHG. As metabolic by-products accumulated within the active forearm muscles during the second half of the 40% handgrip exercise, type III and type IV afferent signals from muscle mechanoreceptors and chemoreceptors transmitted these afferent signals to the brainstem (i.e. NTS and RVLM) leading to an increase in efferent

sympathetic outflow to the heart and blood vessels. The rise in MAP during the latter half of the IHG (i.e. IHG-40B) is attributed to the dual activation of CC and the EPR. Upon cessation of the 40% IHG, MAP was expected to remain significantly higher than rest during PEI. At this time CC has ceased while the ischemic period serves to "trap" the metabolic by-products in the forearm, sustaining the EPR. As expected, the PEI period for the 40% IHG condition did display a sustained increase in MAP; however, the MAP value during PEI-40B underwent a slight decrease from IHG-40B and was not significantly different from rest (Figure 11). Although not statistically significant, it is important to note that a trend towards significance occurred in comparison to resting values ($p = 0.07$). It is expected that this lack of significance was due to greater than expected variability in these responses and insufficient statistical power. More specifically, one participant expressed surprisingly low MAP values during PEI40-B across trials despite inflation of the occlusion cuff to supra-systolic levels. This lack of significance in the MAP response during PEI-40B could have been affected by the data from this participant.

5.2 MAGNETOENCEPHALOGRAPHY DATA

Medullary regions responsible for integrating descending efferent signals from the cerebral cortex and ascending afferent signals from the arterial baroreceptors, muscle mechanoreceptors and chemoreceptors have been clearly identified (2,3,20,36-38,46). The medullary NTS, CVLM, RVLM, nucleus ambiguus, and ventromedial region of the rostral PAG are integral components of the CV response to exercise. In contrast, relatively little is known about the human cortical regions involved with initiating the cardiovascular component of CC, and regulating the EPR, which represents the focus of

the current investigation. Specifically, cortical changes in alpha and beta power associated with each of these neural mechanisms were investigated.

A number of studies have attempted to address similar questions regarding the cortical networks of CC and the EPR (2-4,21,23,36,40,53,66,77,105,106). Despite the similarities in purpose across investigations, a variety of experimental modalities have been employed, including BOLD fMRI (2,105), positron emission tomography (PET) (106), single-photon emission computed tomography (3,4,53,66,77), and measures of rCBF (36). While there are similarities among the aforementioned techniques, they are also very different from one another, primarily in relation to the manner in which they assess changes in brain neural activation. Increased neural activation illustrated using one technique would not necessarily translate into the same activation pattern using a different technique.

For example, the impressive spatial resolution of BOLD fMRI has made it the leading tool for examining the location and function of neural networks in response to various tasks (104). As previously mentioned, the BOLD signal is not a direct representation of neural activity but rather utilizes correlated changes in cerebral blood volume, blood flow, and the metabolic rate of oxygen consumption to draw indirect conclusions regarding neuronal activation (104). The way in which the hemodynamic responses measured by BOLD fMRI relates to changes in neural synchrony as measured by MEG and EEG is not fully understood (107,108). Simultaneous BOLD fMRI and EEG studies have discovered a positive correlation between the BOLD signal and changes in oscillatory power in high frequency bands (30 -150 Hz), while a negative correlation has been revealed between the BOLD signal and low frequency bands (i.e. the

alpha and beta bands, 8-30 Hz) (107,108). Yaun and colleagues (87) examined changes in the BOLD signal and neural synchrony within the alpha and beta bands in response to a motor task. This study identified a negative co-variation between the two measures, and has demonstrated that reciprocal changes (i.e. an increase in BOLD signal/decrease in power, and vice versa) could be elicited in the region of the sensorimotor cortex responsible for controlling the motor task (87) These researchers support the notion that neural desynchronization is indicative of increased neural activity, similar to an increase in the BOLD signal (87). Zumer and colleagues (107) warn that the relationship between the BOLD signal and neural activity is not linear, but rather is dependent on the frequency band and cortical location (18). Additionally, support has been provided for comparing changes in neural synchrony as measured by MEG with changes noted by other neuroimaging modalities such as EEG and PET (109). This support justifies the evaluation of changes in alpha and beta power noted in this study in comparison to previous studies that have used other neuroimaging modalities to explore similar questions.

No significant differences in alpha or beta power were found between the rest periods of the 40% or 5% IHG conditions. This finding was important, as the resting period of each condition (Rest-40 and Rest-5) were used as a baseline to 'subtract' the brain activity associated with rest from those active during both the IHG and PEI periods. In order to examine these comparisons in relation to each other (IHG-A vs. Rest and PEI-B vs. Rest, for both conditions), we needed to confirm that resting alpha and beta power between conditions were not significantly different.

5.3 Power Differences and the Sensorimotor Cortex

In line with past research (89-91), significant alpha and beta desynchronization were uncovered in what appears to correspond to the hand-region of the sensorimotor cortex during the handgrip exercise (Figures 14 and 15). The most prominent decreases in power for both bands were located when comparing each IHG period to the corresponding rest period (IHG-40A vs. Rest-40 and IHG-5A versus Rest-5) and when comparing the experimental condition to the sensory control (IHG-40A vs. IHG-5A). Decreases in power were larger during the 40% IHG-A versus 5% IHG-A condition in comparison to rest within both frequency bands (Figures 14 and 15). As more motor units are recruited to maintain the target 40% of MVC force output, the neural network experiences a stronger and broader level of desynchronization (93, 94).

Crone and colleagues (92) demonstrated that movement-related ERD often involved a topographical area that extended outside the region of the motor cortex dedicated to the moving body part and suggested that this pattern was more prevalent within the alpha band, and more topographically constrained in the beta band (Figure 14 versus Figure 15). Furthermore, Pfurtscheller and colleagues (15) noted that alpha desynchronization results from the execution of almost any task and is typically widespread with the amount of desynchronization reflective of task performance and attention. The findings of Crone et al. (92) and Pfurtscheller et al. (15) can be observed in the results of the current study, as the regions expressing significant changes in power were similar for both the alpha and beta bands but appear to be slightly more somatotopically discrete in the beta band (Figures 14 and 15). For example, beta desynchronization in the sensorimotor cortex, resulting from the comparison of IHG-40A versus IHG-5A, was more focused and localized to the hand-region of the sensorimotor

cortex in comparison to the alpha desynchronization. Changes in power observed by the magnetometers demonstrated a more diffuse decrease in power believed to extend outside the hand-region of the sensorimotor cortex, consistent with Crone et al. (92). It is possible that this diffuse desynchronization is more related to the sensitivity and decreased precision of the magnetometers in comparison to the gradiometers as opposed to extended regions of significant desynchronization (82).

As previously mentioned, it is possible that the desynchronization noted within the sensorimotor cortex could represent more than just activation of the hand-region. Figure 14 and 15 consistently showed a more medial desynchronization in the magnetometers, most obvious in the comparison of IHG-40A versus IHG-5A. It is possible that this extended desynchronization represents the activation of other brain regions of the pathway. The thalamus is located in a region that may correspond to this extended desynchronization (Figure 14 and 15). Alpha and beta desynchronization of the thalamus during IHG-A would support previous findings that have implicated the thalamus in cardio-vagal control (14, 74).

5.4 Experimental versus Sensory Control Conditions

The most important comparisons in this study were between IHG-40A versus IHG-5A (i.e. CC-mediated cardiovascular control) and PEI-40B versus PEI-5B (i.e. Exercise Pressor Reflex-mediated cardiovascular control). In theory, the subtraction of the 5% IHG time periods should minimize brain regions involved with sensation during the handgrip exercise and ischemia periods, such as the neural processes associated with adjusting contraction force to attain the desired value on the projection monitor during the IHG, or the feeling of the cuff inflating around the upper arm during the onset of the PEI period. It is important to mention that the one sensation likely not accounted for by

the 5% condition is the discomfort experienced during the latter stages of the 40% IHG and PEI periods. Thus the 5% condition served to remove the commonly activated areas of the 40% and 5% IHG conditions that were not involved with the CV response to exercise, while brain regions involved with the pain pathway would not be accounted for by the 5% condition

Neural synchrony within the alpha and beta bands is largely associated with resting or "idling" states (15, 86, 89,110,111). This premise is important to our findings, as the aforementioned comparisons of experimental and sensory conditions only revealed decreases in power within what is believed to be the hand-region of the sensorimotor cortex. All other regions that displayed significant changes in power expressed an increase in alpha and beta synchrony during both the IHG and PEI periods. This finding suggests that the 40% IHG condition "turns-off" or inhibits brain regions in comparison to the 5% IHG sensory control condition.

Many studies have implicated the IC as an integral component of the neural network involved with the regulation of CV function (36, 38, 53, 54). These studies have all documented increases in the activity of the IC during comparable investigations, yet our results illustrate a dissimilar trend. As previously mentioned, decreases in activity (synchronization) were noted in what may correspond to the IC during both the IHG (alpha and beta) and PEI (beta) periods for the comparisons of IHG-40A versus IHG-5A and PEI-40B versus PEI-5B. Significant increases in beta power were noted within the area of the contralateral IC during IHG. Additionally, increases in alpha power were uncovered within the ipsilateral IC during IHG and beta band during PEI.

Sander and colleagues (2) reported an increase in contralateral IC activity during the IHG that remained elevated into the PEI period. Due to this activation profile, they proposed that activity of the IC may be related to the unpleasantness that participants experienced (2). The increases in synchronization noted above are fundamentally different from the patterns noted in the Sander study (2); our results illustrate deactivation in regions that are believed to correspond to the regions where Sander et al (2) noted increases in activation. Moreover, our results suggest that both the contralateral and ipsilateral (dependent upon frequency band and period) IC undergo a significant increase in synchronization as opposed to just the contralateral IC (2). It is possible that these changes represent some manifestation of the brains response to a sensory element of the protocol, such as discomfort. As suggested in the Sander study (2), it is possible that regions such as the ACC and IC are implicated in the pain pathway as opposed to being regions of CC. Thus the activity of the ACC and IC may be associated with a frequency band more highly associated with the pain pathway, such as the gamma band (117), as opposed to the bands most highly associated with the motor pathway (alpha and beta).

Although the 5% IHG condition served as an appropriate sensory control (from a CV response to IHG and PEI perspective), it is possible that the changes in oscillatory power within the alpha and beta bands elicited by the 5% IHG condition (versus Rest) were significant enough that any further changes could not be realized when comparing the 40% IHG to the 5% IHG time periods. Figures 14 and 15 illustrate that mild isometric activity of the forearm is sufficient to cause widespread and significant desynchronization in comparison to rest. Moreover, participants reported significantly higher ratings of perceived exertion and expressed greater levels of discomfort during the

40% IHG versus the 5% IHG condition. This suggests that the similar changes in alpha and beta power were not likely attributable to sensations related to muscle fatigue and pain. Aside from this, comparisons for HR and MAP still support the activation of CC and the EPR in the 40% IHG versus 5% IHG conditions (Figure 10 and 11). Therefore, we expected changes in alpha and beta power to reflect these differences in the CV response to IHG and PEI, respectively. The scope of this study was restricted to examining changes in power within the alpha and beta bands only due to their strong relationship with the motor network (15, 86, 89-91). As previously mentioned, it is plausible that cortical regions important for regulating the CV response to exercise operate within oscillatory frequencies outside of these ranges (i.e. delta and gamma). As such, future studies should also examine power changes within these other frequency bands.

5.5 Assumptions and Limitations

The experimental design of this study improved upon many limiting features common to previous investigations examining the brain regions implicated in the autonomic CV response to exercise. Major improvements included the recruitment of conscious humans (versus anesthetized animals), the use of MEG neuroimaging techniques, along with the ability to gather continuous non-invasive recordings of HR, MAP, and brain data. Aside from the benefits afforded by this paradigm, we were faced with a significant number of challenges, particularly during data collection and analysis.

A fundamental issue rooted in the study design was encountered during data collection and transcended into data analysis. On the neuroimaging day it took approximately one hour for instrumentation, over one hour to complete the experimental protocol, and approximately 30 minutes to have the equipment removed and complete the

exit questionnaires. Aside from this significant time commitment, the participants were connected to a multitude of different recording devices, had their head partially restrained within the rigid MEG sensor helmet, and were asked to remain as still as possible throughout a lengthy and challenging protocol. Despite providing participants with blankets to keep them warm, inserting cushioning for a better fit in the MEG chair, and periodically checking on their comfort level, all participants expressed a level of discomfort associated with the protocol. The discomfort was most likely reflected by the large amount of head movement present in the data (Figure 8, Appendix K). Minimizing movement is critical in MEG, as shifts in head position result in different sensors recording the activity of the same brain region over time. An unstable head position over the course of the MEG scan limits a researcher's ability to confidently localize brain regions that exhibit significant changes in activity. Future researchers employing this experimental design may consider using an IHG protocol that involves many brief intermittent contractions at a higher force level. In order to obtain enough MEG data to have sufficient statistical power, this revised protocol would require a greater number of these shorter intermittent contractions in comparison to a lesser number of longer repetitions, as used in the current study. As long as this design is capable of eliciting a significant CV response that can be sustained throughout the ischemic period, it could ameliorate some of the discomfort and resultant head movement.

Another possible modification could be the incorporation of a rest block part way through the experiment that would allow the participant to take their head out of the sensor array to stretch their neck and shoulders. This would also give participants a mental break from the repetition of the study. Although a mid-scan resting block would

be beneficial for participant comfort, it may be implausible for two reasons. First, the recording of certain variables, namely muscle sympathetic nerve activity (MSNA) may prevent this amount of movement. This is a direct measure of sympathetic neural traffic within peripheral nerves and involves the insertion of recording and reference electrodes into a peripheral nerve site, with the common fibular nerve being a conventional choice. The slightest of movements can shift the electrodes, causing the signal to be lost. If this situation occurs, or if many recording devices need to be disconnected in order for the participant to take advantage of the mid-scan rest block, it could add a significant amount of time on to the study and defeat its purpose. This measure was recorded during the study but lies outside the scope of the analysis. Second, having a participant remove their head from the sensor array would mean that they would have two different original head positions in relation to the sensor array, which would affect the consistency between the two data collection segments, affecting the reliability of the comparisons made between them.

The Portapres® was an important feature in this study. Manual or automated measurements of BP take a significant amount of time to obtain and provide a single value of BP over that period. Furthermore, it would be implausible to conduct manual measurements of BP during the current study due to the enclosure of the participant within the shielded room, and conducting repeated automated measures of BP throughout the protocol would provide an unsatisfactory profile of how BP changes over time. In contrast, the Portapres® provides continuous non-invasive measures of BP that allowed us to closely monitor how BP changed over time and how it changed in response to periods of the protocol. The Portapres® allowed us to monitor BP online and allowed us to assess

BP changes in relation to other cardiovascular variables, namely HR. Finally, the sensor array of the MEG is highly sensitive to electronic devices, which interfere with their ability to accurately measure changes in brain activity (5, 84). Employing the Portapres® allowed us to collect BP from the toe (112), distancing the electronic front-end unit of the Portapres® from the MEG sensors. Electronic sources of magnetic fields lying at a distance from the MEG scanner introduce a small spatial gradient compared to the gradient generated within the brain, and thus can be removed from the signal using techniques such as TSSS (85)

Despite the many benefits afforded by the Portapres® device, we experienced difficulty establishing and maintaining accurate measurements of BP from the toes. In order to keep the level of impedance low enough for the MEG sensors to detect the extremely small magnetic fields that exist outside of the head, the sensors are bathed in liquid helium at a temperature of approximately -269°C (80). This functional property of the MEG caused the shielded room to be at a temperature below room temperature, resulting in decreased circulation to the extremities of participants in the MEG for an extended period of time. A space-heater was placed near the left foot during instrumentation and a heated gel pack was placed on the foot during the experimental protocol in order to encourage blood flow in hopes of maintaining the blood pressure signal. This practice was successful for some participants, but not others. The cool environment caused vasoconstriction within the arteries of the recording phalange and thus resulted in dubious Portapres® recordings. Recordings from an automated blood pressure cuff positioned over the brachial artery of the dominant arm were collected at each time period during the familiarization session (i.e. Familiarization Day) and at the

beginning of each rest period during the MEG session in order to check the validity of the measurement and to recalibrate the Portapres®. Future studies employing Portapres® technology in the MEG environment may consider doing further pilot testing using the phalanges of the hand to determine if there is a more optimal strategy for minimizing or removing the electromagnetic interference it introduces on the MEG sensors. Other strategies may focus on other methods of keeping the foot warm or encouraging circulation. Reheating the gel-pack during a scheduled resting session may be beneficial. A more reliable solution to the issues mentioned above may be to use a BP device designed for the MRI environment as opposed to the Portapres®. Our laboratory is in the process of ordering such a device (BIOPAC Systems, Inc.), which we hope will provide accurate measures of BP from the finger in the MEG scanning environment.

A major roadblock to this study was the use of Brainstorm software in the analysis of MEG data. Brainstorm is a remarkable analysis tool that is well designed, intuitively constructed, and can easily conduct basic and popular tasks such as pre-processing techniques and TF decompositions. However, the Brainstorm project was started in the late 1990's and its capability is still rudimentary for more intricate or less common processes (102). With respect to the current study, the t-test results were generated by Brainstorm but needed to be exported to Matlab in order for the figures to be displayed. More specifically, the Brainstorm software is currently incapable of running a t-test on TF decompositions of MEG data and displaying sources on a cortical representation. The barriers imposed by Brainstorm were a large factor in our choice to conclude the study with analysis performed on sensor-level data only. Analyzing changes

in brain activity in the absence of a cortical representation has inhibited the ability to localize brain regions exhibiting significant changes in power with anatomical precision.

Lastly, this experimental design assumed that the cardiovascular responses noted during the first half of the 40% IHG exercise period (i.e. IHG-A) was attributed to the sole actions of Central Command. However, there is evidence to suggest that stretch and pressure sensitive mechanoreceptors, associated with the Exercise Pressor Reflex, transmit afferent signals from the active muscles to the brainstem at the initiation of exercise. Cui and colleagues (113) noted that the isolated stimulation of mechanoreceptors can induce changes in muscle sympathetic nerve activity in young healthy individuals, including a transient but significant increase in HR and BP. It is important to reiterate the transient nature of this effect, with the significant rise in HR occurring between the first and third heartbeat after isolated mechanoreflex activation, followed by a significant increase in mean BP between the third and seventh heartbeat. Furthermore, Herr and colleagues (114) used repeated quadriceps contractions in humans to examine the effects of mechanoreflex activation. Their findings challenge the long-held assumption that the sympathetic response to exercise in humans is always associated with a lengthy onset latency in comparison to the parasympathetic system (114). As such, previous research does suggest that afferent signals from the mechanoreflex do contribute to the CV changes noted during the early stages of the IHG. However, the HR and BP changes noted in the current study during the early stages of exercise (IHG-40A) remained consistently elevated throughout the IHG period. This suggests that CC was the dominant mechanism active and that the transient effects of mechanoreflex activation was unlikely significantly contribute to the CV response.

5.6 Future Directions and Conclusion

This sensor-level analysis will serve as a stepping-stone, as a great deal of information is left to extract from the MEG data. In the near future, the data will be carried forward from the sensor-level to the source-level estimation, by co-registering MEG data with the individual's anatomical MRIs. Brainstorm is capable of this co-registration, however because of its inability to display t-test results comparing TF decompositions, we will likely employ another software program to carry out the co-registration. Anatomical representations of each participant's brain will afford a much more accurate localization of cortical activation for the networks of CC and the EPR. The same time period comparisons will be used, but the information garnered will be much more specific and localized.

Furthermore, the successful execution of a complex task requires a cooperative response from brain regions (115). An analysis of functional connectivity examines neuronal synchrony between regions under the assumption that distant brain regions expressing a consistent phase relationship in the frequency domain across time are evidence of neuronal communication (115). Utilizing functional connectivity would provide the opportunity to map neural communication patterns amongst the brain regions of CC versus that of the EPR. The ability to not only map the neuroanatomical networks of each mechanism, but to uncover the time course and interconnections would be a great step forward in this field. Moreover, it is hoped that the source-level analysis previously mentioned will provide more clarity with respect to the results obtained at the sensor-level.

As previously mentioned, direct, microneurographic recordings of MSNA are the most effective method to examine efferent sympathetic drive to the blood vessels.

Obtaining direct measures of MSNA throughout the protocol on a sample of 8 to 11 participants would provide considerable insight into the changes occurring in the vasculature in response to sympathetic vasoconstrictor activation mediated by the EPR (2, 49, 105, 116). Future studies should attempt to obtain continuous recordings of brain data, BP, HR, and MSNA for the most comprehensive understanding of the autonomic CV response to exercise. We were successful at collecting MSNA data during the 5 minute rest period prior to the onset of the exercise protocol in four participants. In the near future we will attempt to uncover the cortical regions that exhibit changes in neural activity each time a burst of MSNA is generated. A similar analysis will also be performed with resting Portapres® data to uncover cortical networks involved with sensing baroreceptor afferent information.

This preliminary investigation into the brain regions associated with CC and the EPR have made a significant contribution to the current body of work in this field. The study design proved effective for correcting some of the limitations associated with previous research, as we were able to conduct this investigation using a conscious human model, able to monitor changes in brain activity via MEG concurrently with continuous measures of HR and MAP. The results of this study also uncovered interesting patterns of increased power within the alpha and beta band that were not previously reported in studies that employed other techniques, such as BOLD fMRI (2,105), positron emission tomography (106), single-photon emission computed tomography (3,4,53,66,77), and measures of regional cerebral blood flow (36). Furthermore, translating the results from the sensor domain to the source-level and will greatly elaborate on the presented findings.

5.7 PERSPECTIVE

The growing prevalence of disease states afflicting the heart and blood vessels has placed CV research at the forefront (6). The Canadian demographic threatened by CV disease is younger and broader than ever before (11). Inadequate nutrition, and an increase in inactivity, obesity, and smoking, in-hand with an aging demographic, only continue to make matters worse (11).

Cardiovascular treatment and prevention programs are keen to promote the heart-healthy effects of physical activity. However, an exaggerated BP increase is elicited by exercise in hypertensive individuals in comparison to their normotensive counterparts, increasing the likelihood that they will suffer a cardiac event while engaging in physical activity (16-18). Therefore, a better understanding of the mechanisms associated with the exercise response may reveal methods to safely increase the activity level of at-risk populations.

Additionally, it is hoped that the findings of this study will be a positive contribution to the growing body of literature dedicated to refining the neuroanatomy responsible for initiating and sustaining the sympathetic response during exercise and/or the development of CV disease, as well as providing a more thorough understanding of mechanistic action of these brain regions. Greater clarity surrounding the networks that control sympathetic outflow could uncover innovative treatment strategies to target brain regions that exhibit dysfunctional activity.

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APPENDIX A: IWK REB APPROVAL

IWK Health Centre
Research

5850/5980 University Avenue
PO Box 9700, Halifax
Nova Scotia B3K 6R8
Canada
tel: 902.470.8888
www.iwk.nshealth.ca

Approval – Delegated Review
October 16, 2012

Principal Investigator: Dr Timothy Bardouille
Co-Principal Investigator: Derek Kimmerly
Title: Functional Connective Brain Networks Involved with Cardiovascular Regulation During Exercise in Humans
Project #:1011585

On behalf of the IWK Research Ethics Board (IWK-REB) I have reviewed the documents included in this study. I am pleased to confirm the Board's full approval for this research study, effective today. This includes approval for the following study documents:

Document Name	Version Date
Research Summary	2012/06/25
Protocol #2	2012/06/25
Information and Consent Form	2012/09/15

The Board's approval for this study will expire one year from the date of this letter (October 16, 2013). To ensure continuing approval, submit a Request for Continuing Review to the Board 2 - 4 weeks prior to the renewal date. If approval is not renewed prior to the anniversary date, the Board will close your file and you must cease all study activities immediately. To reactivate a study, you must submit a new Initial Submission (together with the usual fee, if applicable) to the IWK-REB and await notice of re-approval.

Please be sure to notify the Board of any of the following:
§ Proposed changes to the initial submission (i.e. new or amended study documents)
§ Additional information to be provided to study participants
§ Material designed for advertisement or publication with a view to attracting participants
§ Serious adverse events experience by local participants
§ Unanticipated problems involving risks to participants or others
§ Sponsor-provided safety information
§ Additional Compensation available to participants
§ Upcoming audits/inspections by a sponsor or regulatory authority
§ Closure of the study (within 90 days of the event)

Approved studies may be subject to internal audit. Should your research be selected for audit, the Board will advise you and indicate any other requests at that time.

Important Instructions and Reminders

Submit all correspondence to Ethics Manager Bev White or Ethics Assistant, Joanne Leonard at the address listed at the top of this letter (do not send your response to the IWK-REB Chair or Co-Chair)

Be sure to reference the Board's assigned file number, 1011585 on all communications.

Highlight all changes on revised documents and remember to update version numbers and version dates, include a clean copy of all revised documents.

Best wishes for a successful study.

Research Ethics Board Committee Members		
Robert	Bortolussi	Pediatrics (Clinical Researcher)
Jill	Chorney	Psychology (Clinical Researcher)
Elaine	Cumming	Legal Representative
Eleanor	Fitzpatrick	Nursing (Clinical Researcher)
Margo	Fulmer	Lay Representative
Jane	Gillis	Medical Genetics (Clinical Researcher)
Linda	Hamilton	Obstetrics and Gynecology, Co-Chair
Adam	Huber	Rheumatology (Clinical Researcher), Co-Chair
Faye	Jacobson	Nursing (Research Coordinator)
Sarah	Matheson	Lay Representative
Susan	McKinney	Legal Representative
James	Morrison	Anaesthesia, Executive Chair
Victoria	Price	Hematology/Oncology (Clinical Researcher)
Pierre	Schmit	Diagnostic Imaging (Clinical Researcher)
Valerie	Shaffner	Privacy
Marilyn	Tiller	Pharmacy

* REB members are not in attendance during review of their own proposed research involving human subjects or where there is conflict of interest with the proposed research

This statement is in lieu of Health Canada's Research Ethics Board Attestation: *The Research Ethics Board for the IWK Health Centre operates in accordance with:*
- Food and Drug Regulations, Division 5 "Drugs for Clinical Trials involving Human Subjects"
- The Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans - TCPS(2)
- International Conference on Harmonization - Good Clinical Practice Guidelines - ICH-GCP

APPENDIX B: STUDY RECRUITMENT POSTER

Do you want to find out more about how your brain is involved with the control of blood pressure during exercise?

Researchers at the Exercise and Cardiovascular Physiology Laboratory (Dalhousie University) and the *Laboratory for Clinical Magnetoencephalography* (IWK Health Centre) are currently recruiting healthy individuals between the ages of 18 – 64 years to participate in a study looking at the brain regions involved with the control of blood pressure during handgrip exercise

We invite people who:

- ♥ ***have no history of disease or illness (e.g., high blood pressure, diabetes, etc.)***
- ♥ ***are not obese, pregnant or have any permanent metal objects on their body.***
- ♥ ***can come to Dalplex (one, 1 hour visit) and the IWK for two separate visits (one, 2-hour and one, 30-minute visit)***

IF YOU ARE INTERESTED AND WOULD LIKE MORE INFORMATION PLEASE CONTACT:

Derek Kimmerly (dskimmerly@dal.ca) or Holly Van Gestel (H.VanGstel@Dal.ca)

APPENDIX C: HEALTH HISTORY QUESTIONNAIRE

NAME:

ADDRESS:

CITY:

POSTAL CODE:

PHONE: (home) **(work)**

DATE OF BIRTH:

E-mail address:

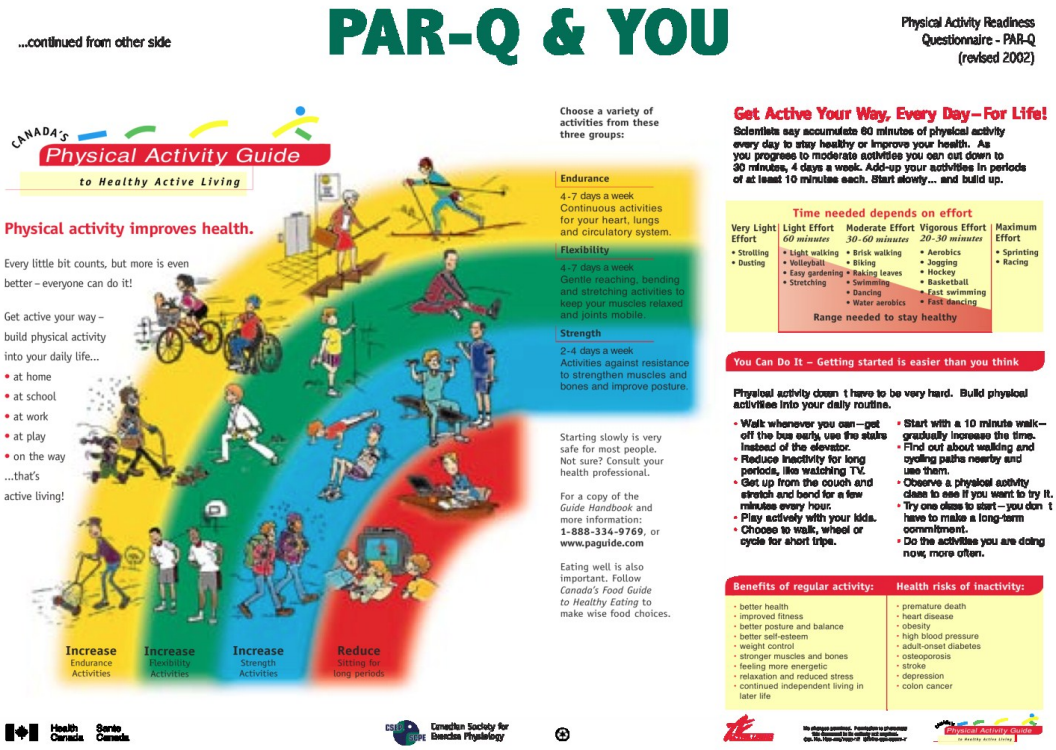
Name:

1.	Do you consider yourself to be in good health?	Yes	No
2.	Do you exercise on a regular basis?	Yes	No
	If yes, what type of exercise:		
	How often:		
3.	Have you ever had high blood pressure?	Yes	No
4.	Have you ever had migraines?	Yes	No
5.	Have you ever had diabetes?	Yes	No
6.	Have you ever had chest pain, heart disease or a heart murmur?	Yes	No
7.	Do you have any bleeding or clotting problems?	Yes	No
8.	Have you ever had kidney disease?	Yes	No
9.	Have you ever had liver disease such as hepatitis?	Yes	No
10.	Do you have an ulcer or any stomach problems?	Yes	No
11.	Have you ever had any thyroid disease?	Yes	No
12.	Do your hands or feet blanche or become painful in cold weather?	Yes	No
13.	Have you ever had asthma or lung disease?	Yes	No
14.	Are you aware of any allergies to “freezing medication”?		
	i.e. Lidocaine, novacaine, procaine	Yes	No
15.	Are you aware of any drug, food or other types of allergies?	Yes	No
16.	Are you taking any medications?	Yes	No
17.	Have you taken any Aspirin recently? If yes, please specify:	Yes	No
18.	Do you drink alcohol more than twice a week?	Yes	No

Name:

19.	Do you take coffee or any other stimulants?	Yes	No
	If yes, approximately how many cups/day _____ ?		
20.	Do you smoke?	Yes	No
21.	Have you taken any medication that might stimulate or depress your nervous system?	Yes	No
22.	Have you ever fainted?	Yes	No
23.	Is there any possibility that you may be pregnant?	Yes	No

APPENDIX D: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE



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PAR-Q & YOU

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

CANADA'S Physical Activity Guide to Healthy Active Living

Physical activity improves health.

Every little bit counts, but more is even better – everyone can do it!

Get active your way – build physical activity into your daily life...

- at home
- at school
- at work
- at play
- on the way

...that's active living!

Increase Endurance Activities | Increase Flexibility Activities | Increase Strength Activities | Reduce Sitting for long periods

Choose a variety of activities from these three groups:

Endurance
4-7 days a week
Continuous activities for your heart, lungs and circulatory system.

Flexibility
4-7 days a week
Gentle reaching, bending and stretching activities to keep your muscles relaxed and joints mobile.

Strength
2-4 days a week
Activities against resistance to strengthen muscles and bones and improve posture.

Starting slowly is very safe for most people. Not sure? Consult your health professional.

For a copy of the *Guide Handbook* and more information: **1-888-334-9769**, or **www.paguide.com**

Eating well is also important. Follow *Canada's Food Guide to Healthy Eating* to make wise food choices.

Get Active Your Way, Every Day – For Life!

Scientists say accumulate 60 minutes of physical activity every day to stay healthy or improve your health. As you progress to moderate activities you can cut down to 30 minutes, 4 days a week. Add-up your activities in periods of at least 10 minutes each. Start slowly... and build up.

Time needed depends on effort

Very Light Effort	Light Effort *60 minutes*	Moderate Effort *30-60 minutes*	Vigorous Effort *20-30 minutes*	Maximum Effort
• Strolling • Dusting	• Light walking • Volleyball • Easy gardening • Stretching	• Brisk walking • Biking • Raking leaves • Swimming • Dancing • Water aerobics	• Aerobics • Jogging • Hockey • Basketball • Fast swimming • Fast dancing	• Sprinting • Racing

Range needed to stay healthy

You Can Do It – Getting started is easier than you think

Physical activity does t have to be very hard. Build physical activities into your daily routine.

- Walk whenever you can – get off the bus early, use the stairs instead of the elevator.
- Reduce inactivity for long periods, like watching TV.
- Get up from the couch and stretch and bend for a few minutes every hour.
- Play actively with your kids.
- Choose to walk, wheel or cycle for short trips.
- Start with a 10 minute walk – gradually increase the time.
- Find out about walking and cycling paths nearby and use them.
- Observe a physical activity class to see if you want to try it.
- Try one class to start – you don t have to make a long-term commitment.
- Do the activities you are doing now, more often.

Benefits of regular activity:	Health risks of inactivity:
• better health • improved fitness • better posture and balance • better self-esteem • weight control • stronger muscles and bones • feeling more energetic • relaxation and reduced stress • continued independent living in later life	• premature death • heart disease • obesity • high blood pressure • adult-onset diabetes • osteoporosis • stroke • depression • colon cancer

Health Canada Santé Canada | Canadian Society for Exercise Physiology

Source: Canada's Physical Activity Guide to Healthy Active Living, Health Canada, 1998 http://www.hc-sc.gc.ca/hppb/paguide/pdf/guideEng.pdf

FITNESS AND HEALTH PROFESSIONALS MAY BE INTERESTED IN THE INFORMATION BELOW:

The following companion forms are available for doctors' use by contacting the Canadian Society for Exercise Physiology (address below):

The **Physical Activity Readiness Medical Examination (PARmed-X)** – to be used by doctors with people who answer YES to one or more questions on the PAR-Q.

The **Physical Activity Readiness Medical Examination for Pregnancy (PARmed-X for Pregnancy)** – to be used by doctors with pregnant patients who wish to become more active.

References:

Arraix, G.A., Wigle, D.T., Mao, Y. (1992). Risk Assessment of Physical Activity and Physical Fitness in the Canada Health Survey Follow-Up Study. **J. Clin. Epidemiol.** 45:4 419-428.

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PAR-Q Validation Report, British Columbia Ministry of Health, 1978.

Thomas, S., Reading, J., Shephard, R.J. (1992). Revision of the Physical Activity Readiness Questionnaire (PAR-Q). **Can. J. Spt. Sci.** 17:4 338-345.

For more information, please contact the:

Canadian Society for Exercise Physiology
202-185 Somerset Street West
Ottawa, ON K2P 0J2
Tel. 1-877-651-3755 • FAX (613) 234-3565
Online: www.csep.ca

The original PAR-Q was developed by the British Columbia Ministry of Health. It has been revised by an Expert Advisory Committee of the Canadian Society for Exercise Physiology chaired by Dr. N. Gledhill (2002).

Disponible en français sous le titre «Questionnaire sur l'aptitude à l'activité physique - Q-AAP (revisé 2002)».

Supported by: Health Canada Santé Canada

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

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APPENDIX E: EDINBURGH HANDEDNESS INVENTORY

Please indicate your preferences in the use of hands in the following activities *by putting a check in the appropriate column*. Where the preference is so strong that you would never try to use the other hand, unless absolutely forced to, *put 2 checks*. If in any case you are really indifferent, *put a check in both columns*.

Some of the activities listed below require the use of both hands. In these cases, the part of the task, or object, for which hand preference is wanted is indicated in parentheses.

Please try and answer all of the questions, and only leave a blank if you have no experience at all with the object or task.

	Left	Right
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking Match (match)		
10. Opening box (lid)		
TOTAL (count checks in both columns)		

Difference	Cumulative TOTAL	Result

Scoring:
Add up the number of checks in the "Left" and "Right" columns and enter in the "TOTAL" row for each column. Add the left total and the right total and enter in the "Cumulative TOTAL" cell. Subtract the left total from the right total and enter in the "Difference" cell. Divide the "Difference" cell by the "Cumulative TOTAL" cell (round to 2 digits if necessary) and multiply by 100; enter the result in the "Result" cell.

Interpretation (based on Result):

- below -40 = left-handed
- between -40 and +40 = ambidextrous
- above +40 = right-handed

APPENDIX F: PRE-STUDY INSTRUCTIONS

STUDY INFORMATION

Human central autonomic cardiovascular regulation during exercise:
Functional connective brain networks involved with central command and the exercise pressor reflex

Derek S. Kimmerly, PhD, Dr. Timothy Bardouille, PhD, and Holly Van Gestel, BSc.

Laboratory for Clinical Magnetoencephalography, IWK Health Center, 5850/5980 University Avenue, PO Box 9700, Halifax, NS B3K 6R8.

hollyvangestel@hotmail.com

PRE– STUDY REMINDERS

Please avoid the following 24 hours before each study session:

- Intense physical activity (running, bicycling, weight training, etc.)
- Alcoholic beverages
- Caffeinated products (coffee, tea, chocolate, etc.)
- Nicotine containing products (cigarettes, Nicorette gum, etc.)

Please bring along a comfortable T-shirt/tank top and pair of shorts to both the familiarization session and MEG session. Females should also bring a sports bra or bra without a metal underwire.

Eat a light meal ~3 hours before each study and record the meal contents.
Please bring the contents list with you to the study
Drink plenty the night before (~5 hours before bed)

APPENDIX G: INCLUSION/EXCLUSION CRITERIA

YOU MAY NOT PARTICIPATE IN THE STUDY IF:
You may not participate in the study if, you have metal objects inside your body. The following list is not necessarily complete. Please discuss with your physician and/or the study personnel if you have or may have any object in your body that was not there when you were born. ▪ Surgery involving metal, such as: clips, rods, screws, pins, or wires. ▪ Heart pacemaker ▪ Implanted electrodes, pumps or electrical devices ▪ Cochlear (inner ear) implants ▪ Intraocular lens (eye) implants (Cataract lenses are allowed if they are soft lenses) ▪ Any metallic foreign body, shrapnel or bullet (Please mention if you have ever been a grinder, metal worker, welder, wounded during military service, etc. ▪ Intrauterine contraceptive device (IUD) or contraceptive diaphragm ▪ Dental work held in place by magnets ▪ Non-removable dental braces and retainers ▪ Metal dental work, unless it is composed predominantly of precious or semiprecious alloy or amalgam (including multiple crowns or bridges) ▪ Tattooed eyeliner ▪ Some tattoos (if you do, please discuss with the Investigator) ▪ Non-removable metal jewellery (body piercing) ▪ Nicotine and/or contraceptive patches
You may not participate in the study if, in the opinion of the study personnel, you have a medical condition that could be made worse by any stress associated with participation in a research protocol. These conditions include: ▪ Heart and circulatory problems ▪ Metabolic disorders (e.g. diabetes) ▪ Neurological disorders (e.g. Raynaud's disease) ▪ Seizure disorders ▪ Anxiety disorders ▪ Mental disorders ▪ Chronic back pain
You may not participate in this study if you are a smoker or have a body mass index greater than 30 kg/m^2
You may not participate in the study if, you have claustrophobia, or if you are currently taking medication that could affect your performance (e.g. anti-depressants, anti-anxiety type drugs) or cardiovascular function (e.g. 'water pills', high blood pressure medicine).
You may not participate in the study if you are affected by a condition that affects the full function of your non-dominant hand.
You may not participate in the study if you are or may be pregnant.

APPENDIX H: BORG SCALE (RATING OF PERCEIVED EXERTION)

Table 5.2A The original Borg Scale Rating Perception of Effort (RPE)

Rating	Perception of effort
6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

From Borg (1973, p. 92). © by Lippincott, Williams & Wilkins. Adapted by permission.

APPENDIX I: THE MCGILL PAIN QUESTIONNAIRE

SHORT-FORM McGILL PAIN QUESTIONNAIRE

PATIENT'S NAME_____ DATE

Instructions: Since you have reported that one of your problems is **physical pain**, the purpose of this checklist is for you to give us an idea about what your **physical pain** feels like. Each of the words in the left column describes a quality or characteristic that pain can have. So, for each pain quality in the left column, check the number in that row that tells how much of that specific quality your pain has. Rate every pain quality.

PAIN QUALITY	NONE	MILD	MODERATE	SEVERE
1. Throbbing	(0)_____	(1)_____	(2)_____	(3)_____
2. Shooting	(0)_____	(1)_____	(2)_____	(3)_____
3. Stabbing	(0)_____	(1)_____	(2)_____	(3)_____
4. Sharp	(0)_____	(1)_____	(2)_____	(3)_____
5. Cramping	(0)_____	(1)_____	(2)_____	(3)_____
6. Gnawing	(0)_____	(1)_____	(2)_____	(3)_____
7. Hot-burning	(0)_____	(1)_____	(2)_____	(3)_____
8. Aching	(0)_____	(1)_____	(2)_____	(3)_____
9. Heavy	(0)_____	(1)_____	(2)_____	(3)_____
10. Tender	(0)_____	(1)_____	(2)_____	(3)_____
11. Splitting	(0)_____	(1)_____	(2)_____	(3)_____
12. Tiring-exhausting	(0)_____	(1)_____	(2)_____	(3)_____
13. Sickening	(0)_____	(1)_____	(2)_____	(3)_____
14. Fearful	(0)_____	(1)_____	(2)_____	(3)_____
15. Punishing-cruel	(0)_____	(1)_____	(2)_____	(3)_____

A. PLEASE MAKE AN "X" ON THE LINE BELOW TO SHOW HOW BAD YOUR PAIN IS RIGHT NOW.

NO PAIN |----------| WORST POSSIBLEPAIN

B. PLEASE CHECK THE ONE DESCRIPTOR BELOW THAT BEST DESCRIBES YOUR PRESENT PAIN.

0 NO PAIN _____

1 MILD _____

2 DISCOMFORTING _____

3 DISTRESSING _____

4 HORRIBLE _____

5 EXCRUCIATING _____

C. IS YOUR PAIN ?

(check one word)

_____ Brief

_____ Intermittent

_____ Continuous

Note: Adapted with permission from the "Short Form McGill Pain Questionnaire". Copyright 1987 Ronald Melzack.

S = ___/33 A/E = ___/12

APPENDIX J: MEG EXIT QUESTIONNAIRE FOR ADULTS

Protocol Number: _____ Date: _____

Research Subject Identifier: _____

Thank you for participating in our study. We would appreciate it if you would answer the following questions about your experience.

1. Did you experience any unusual sensations while in the MEG? Yes ☐ No ☐
   If yes, please describe it/them (what was it, how long did it last, when did it occur).

   _____

   _____

2. Did you experience any of the following:
   - nervousness Yes ☐ No ☐
   - sleepiness Yes ☐ No ☐
   - warmth Yes ☐ No ☐
   - cold Yes ☐ No ☐

   Comments: _____

   _____

3. Would you participate in an MEG study again? Yes ☐ No ☐

   If yes, may we contact you when you are eligible for another study? Yes ☐ No ☐

4. Please tell us how we could have made this experience more comfortable for you.

   _____

   _____

5. How did you feel about the way in which you were approached about participating in this study?

   _____

   _____

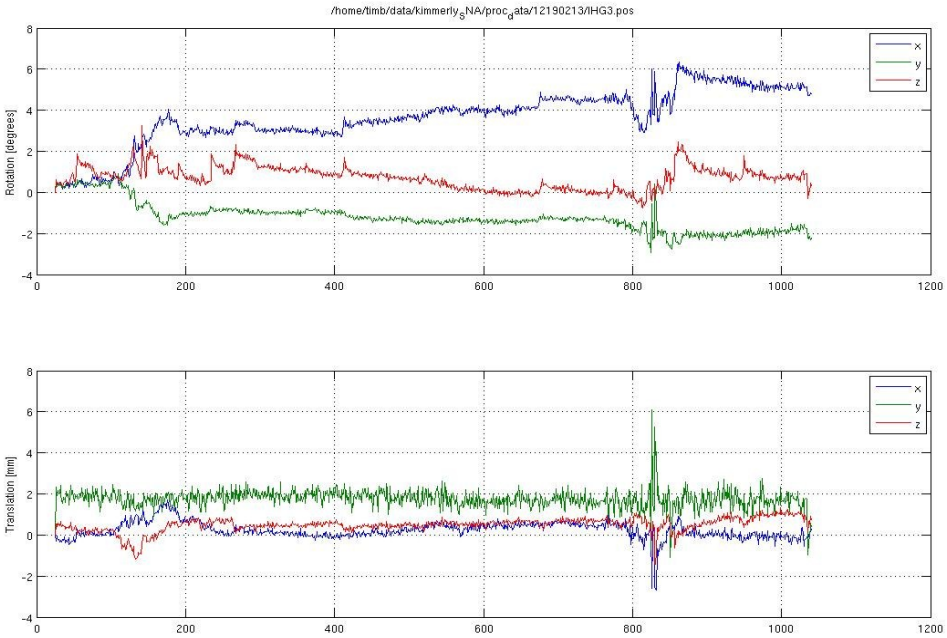
   Signature: _____ Date: _____

   Thank you ☺

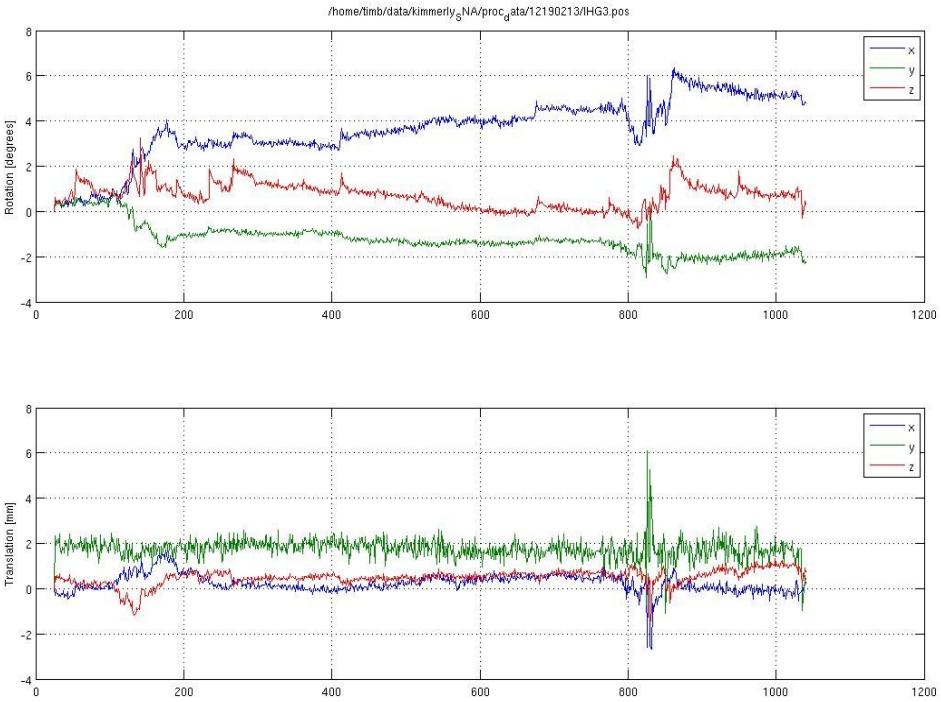
APPENDIX K: ROTATION AND TRANSLATION VERSUS TIME

Participant B001

IHG2



IHG3



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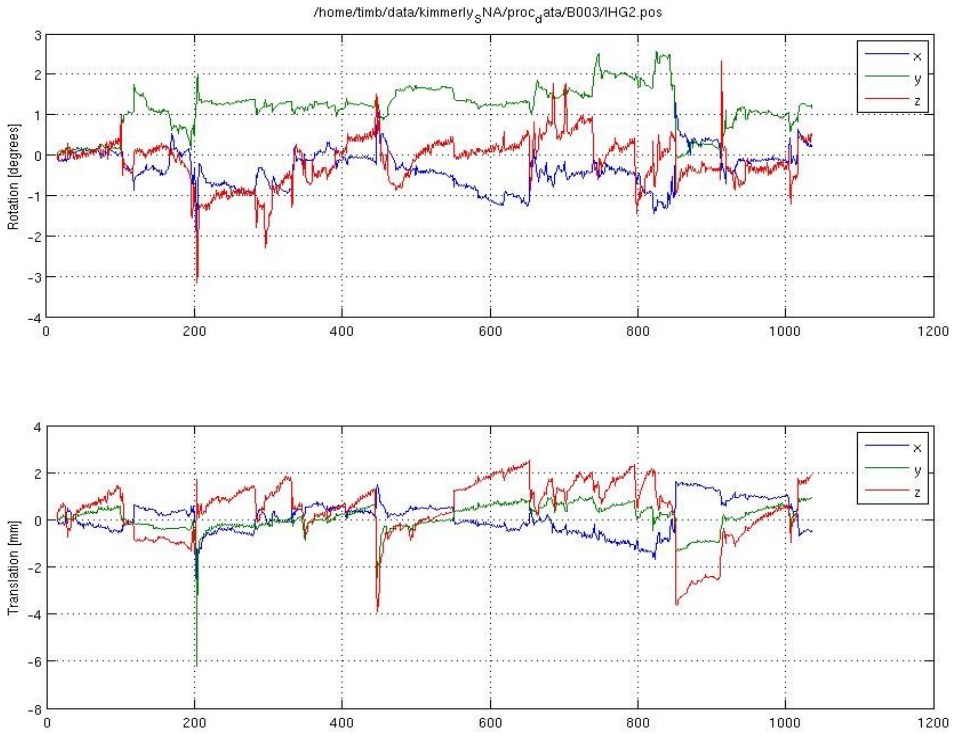
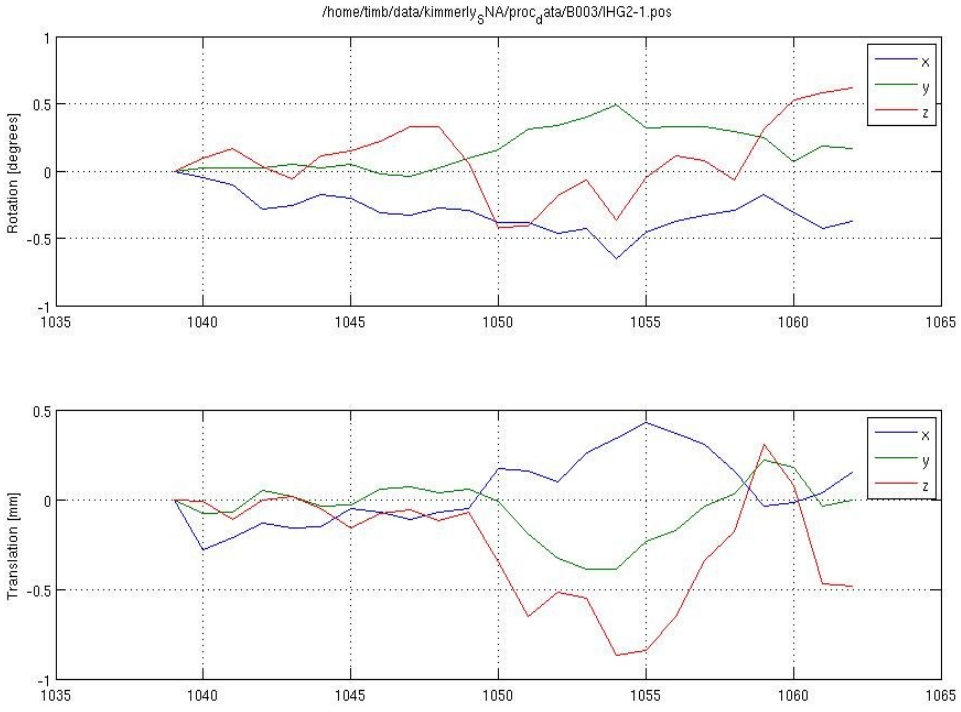
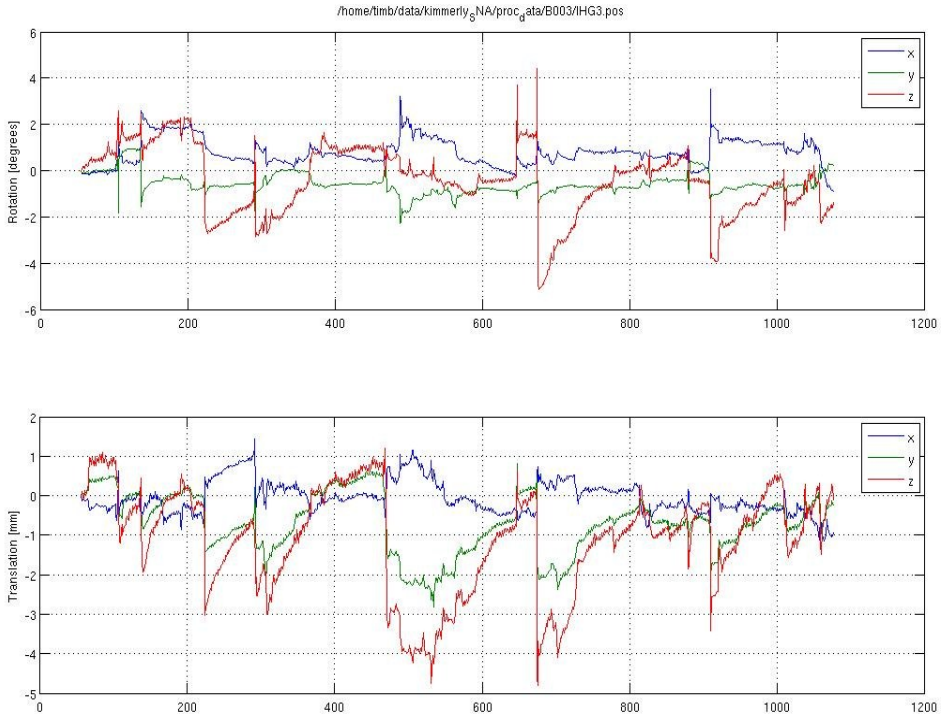


IHG2-1



IHG3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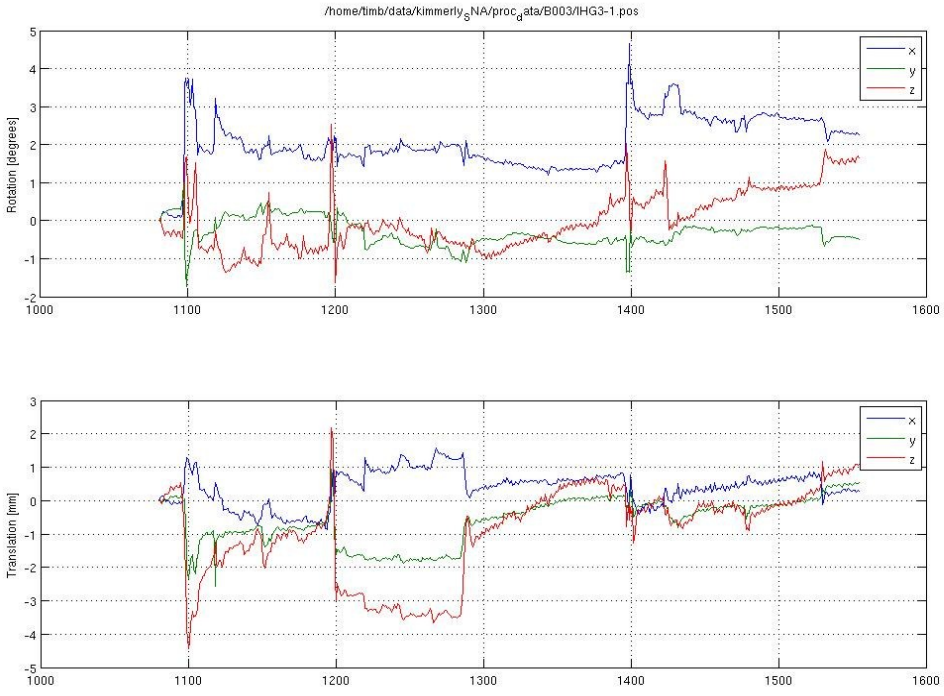
Translation [mm]
x
y
z

I

HG3-1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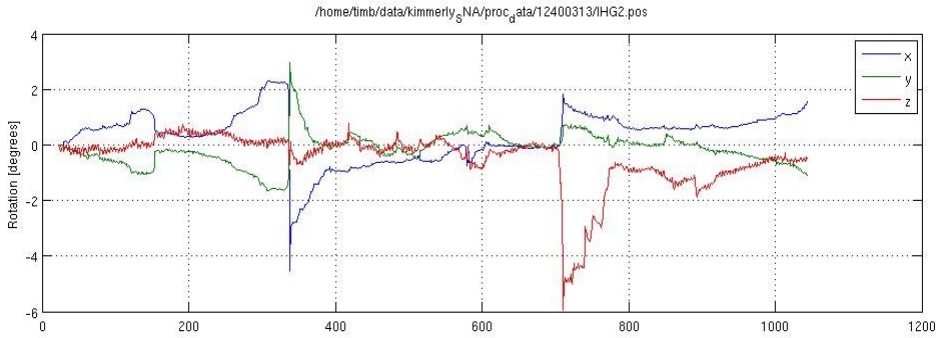
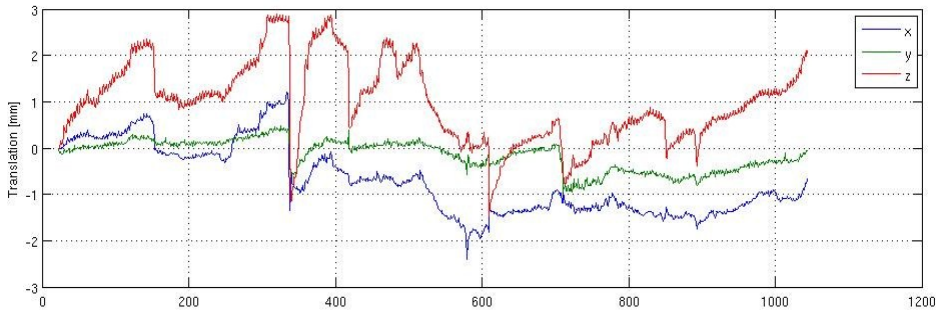
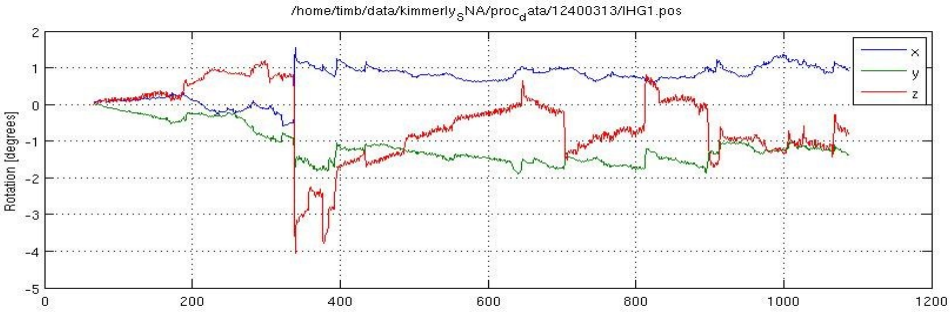
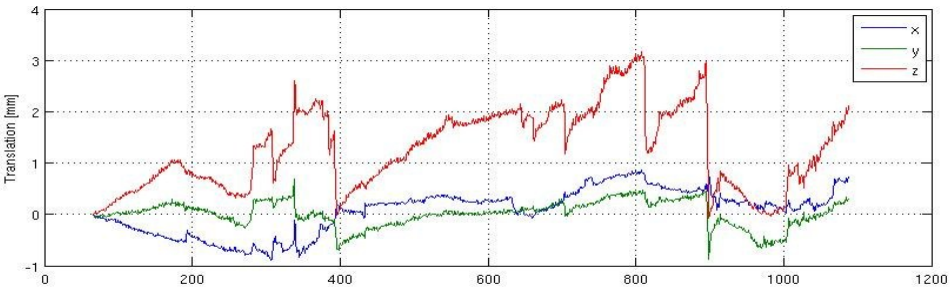
Participant B006:
IHG2





IHG3





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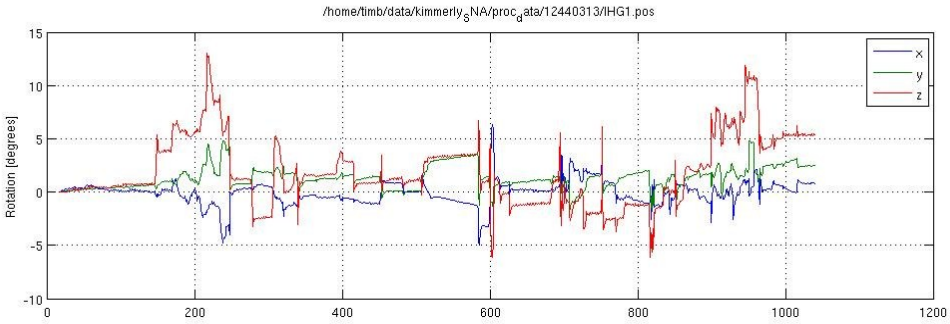
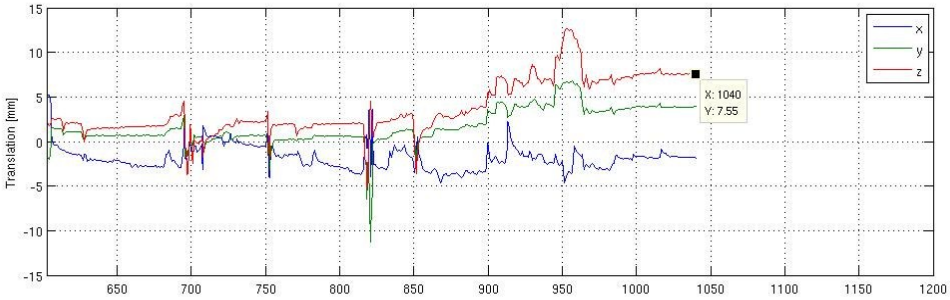
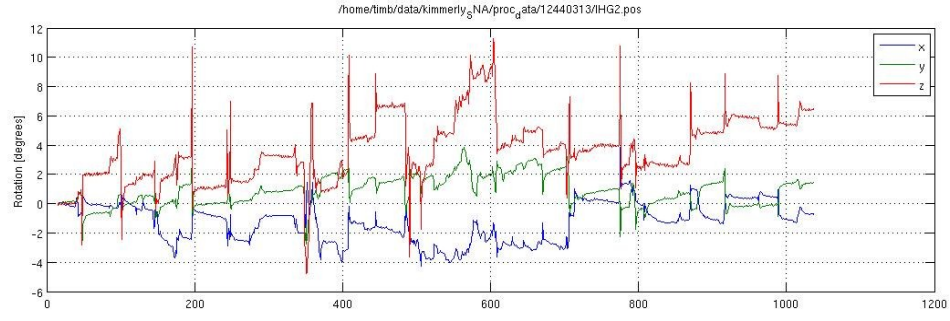
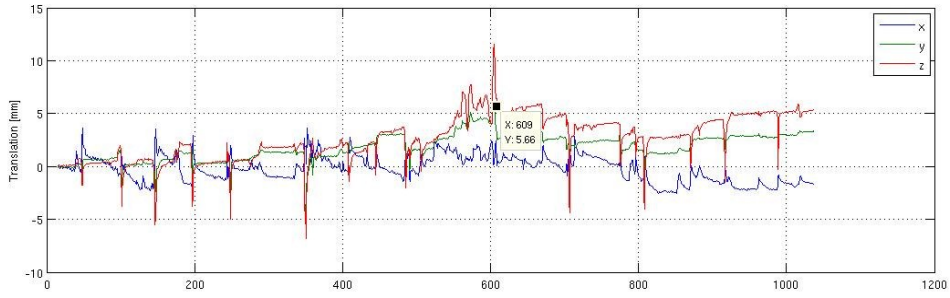
IHG1





IHG2





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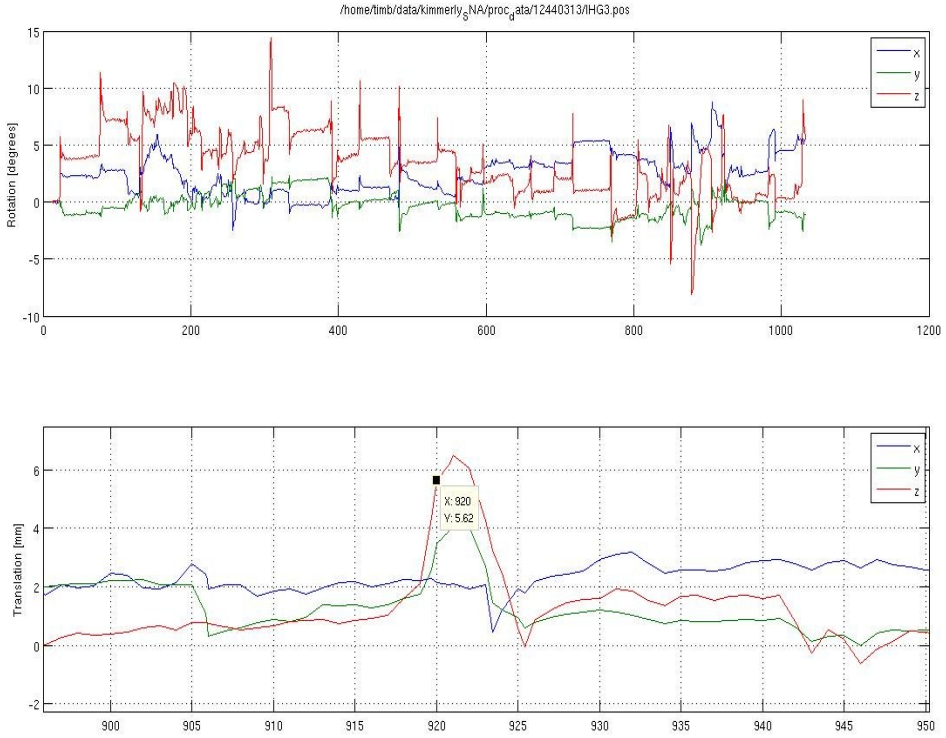
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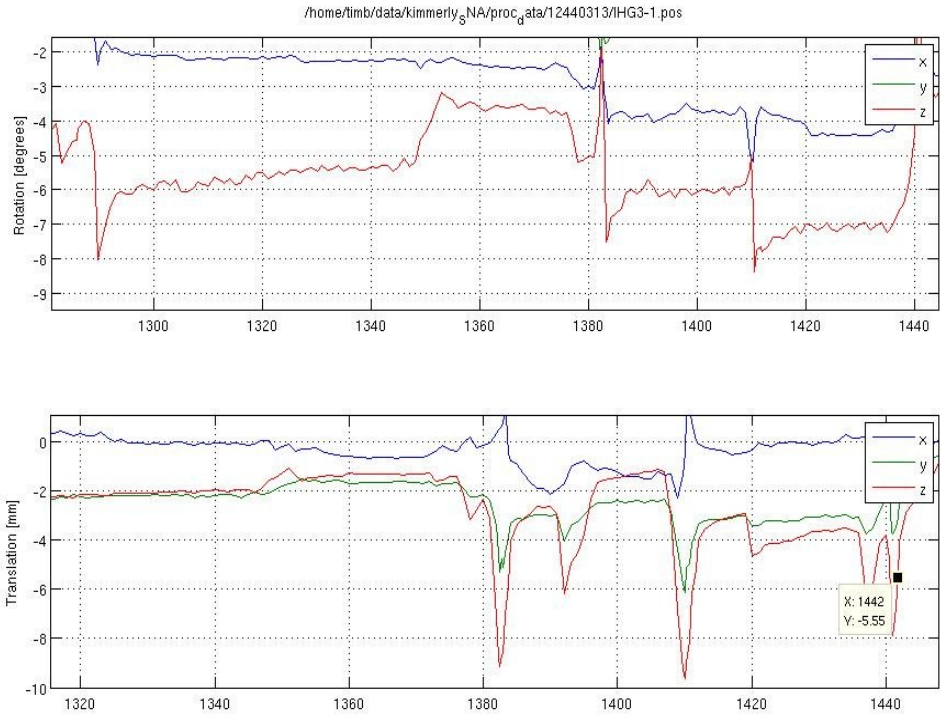
IHG3-1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Participant B009:
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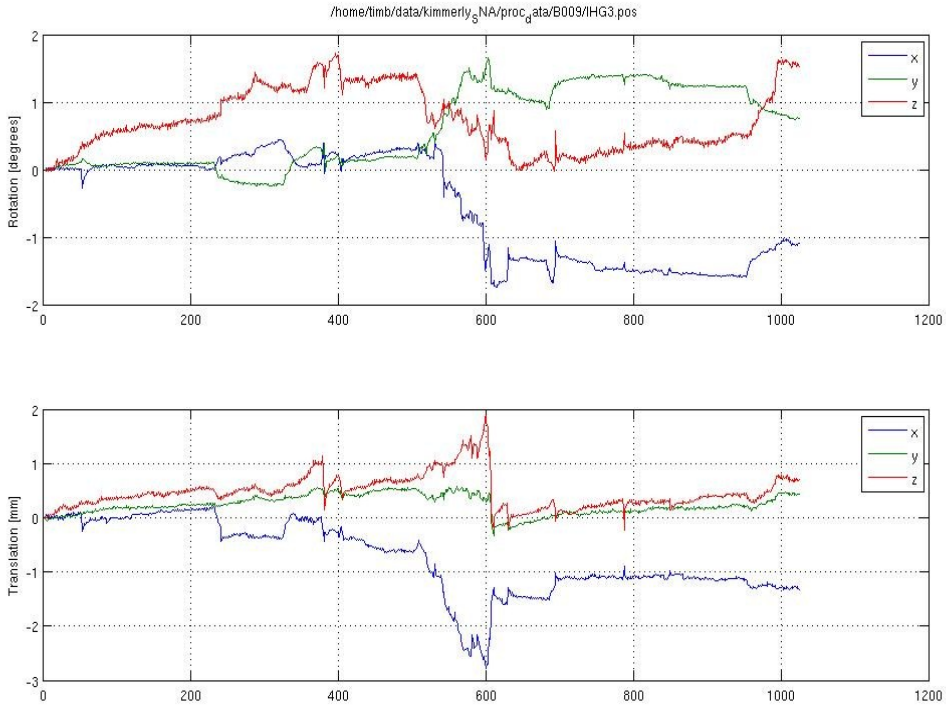
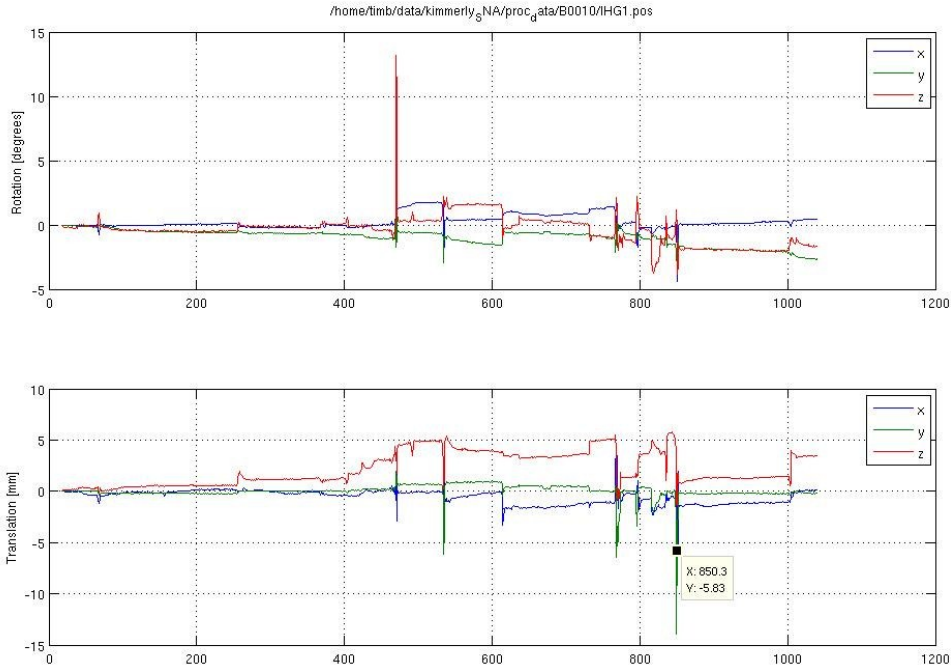
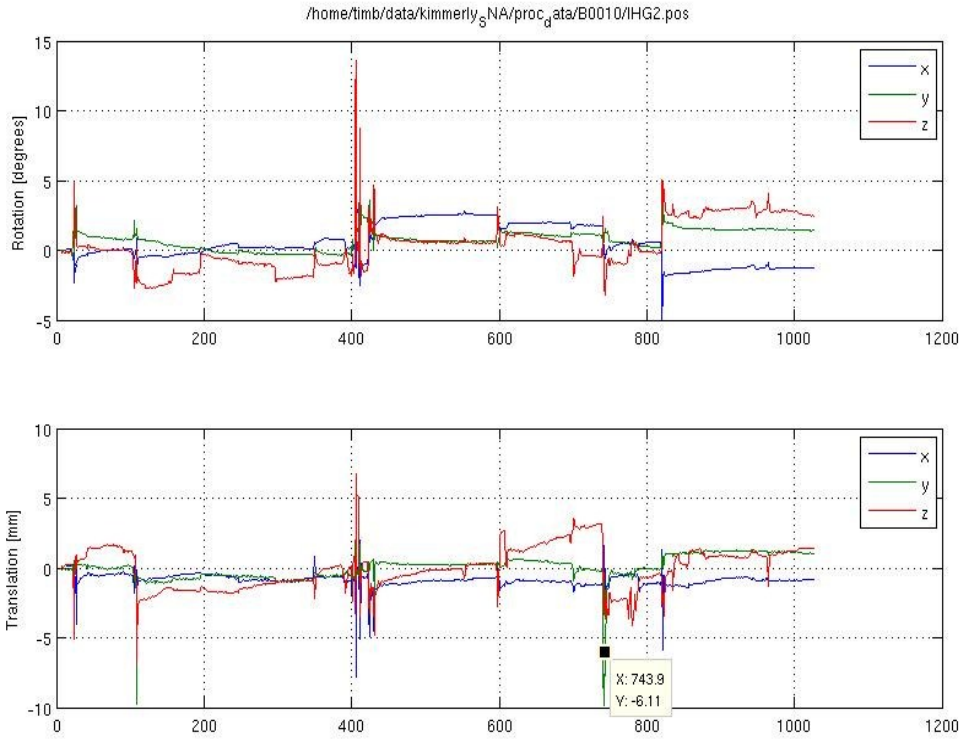


Participant B0010:
IHG1



IHG2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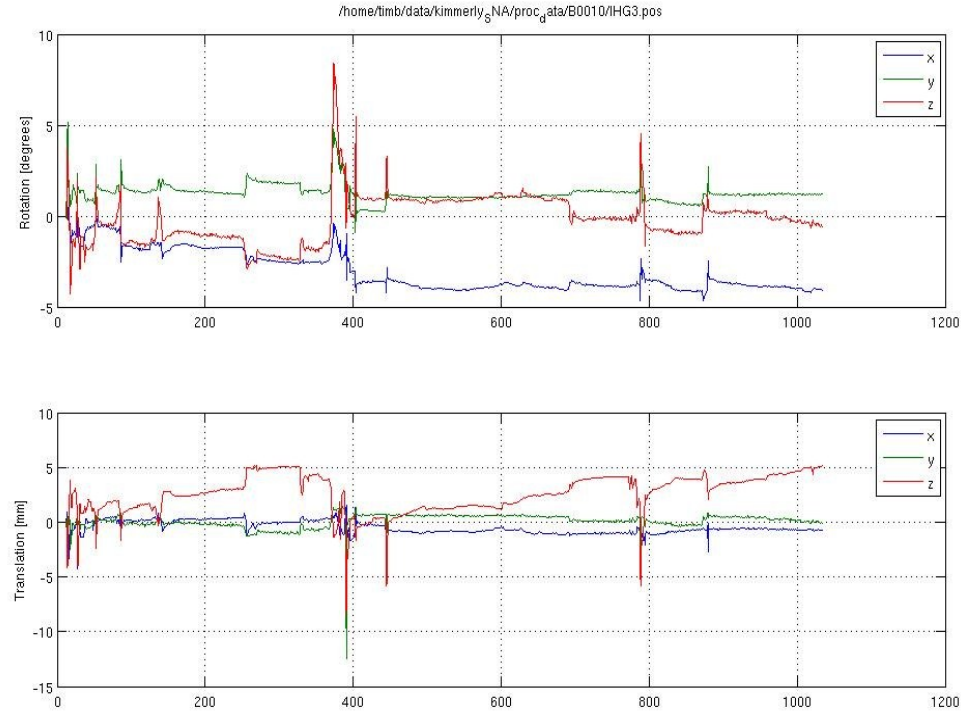
IHG3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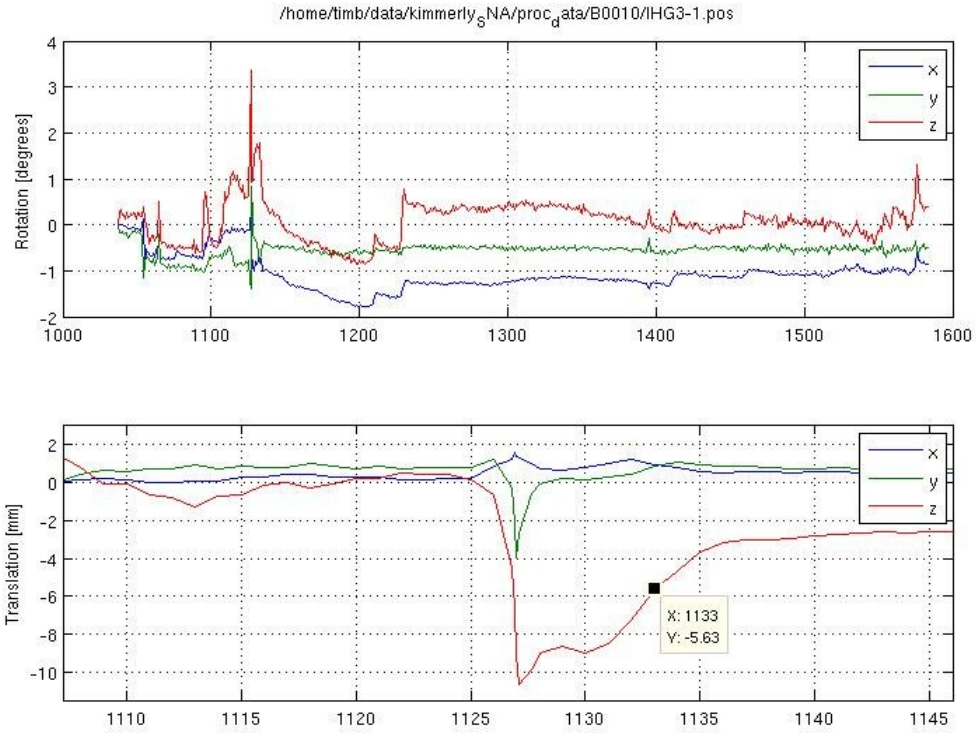
z

IHG3-1



Participant B0011:
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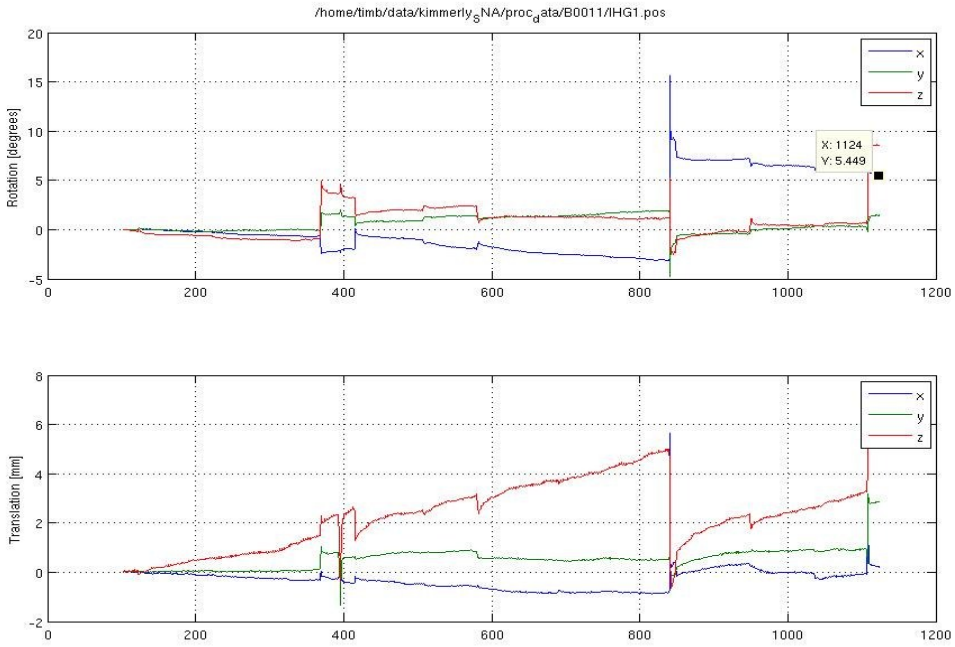

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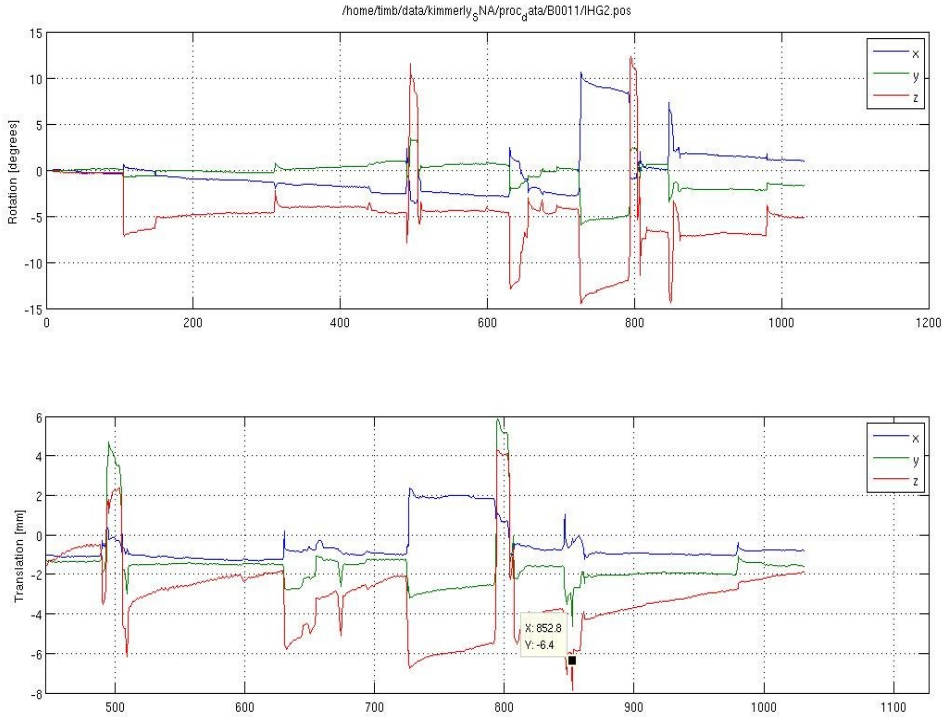

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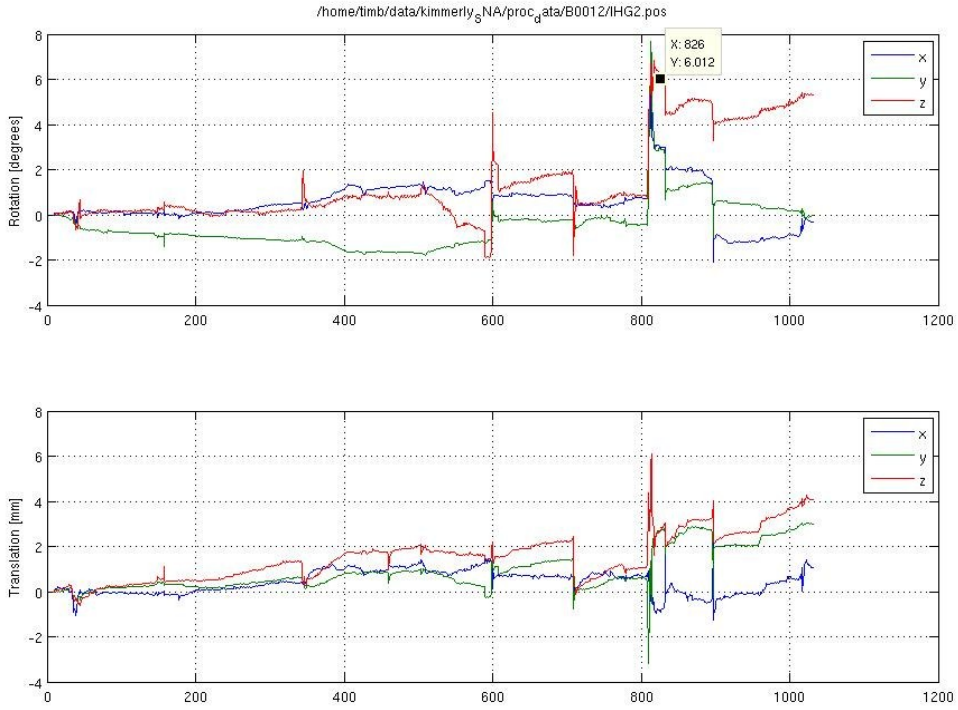

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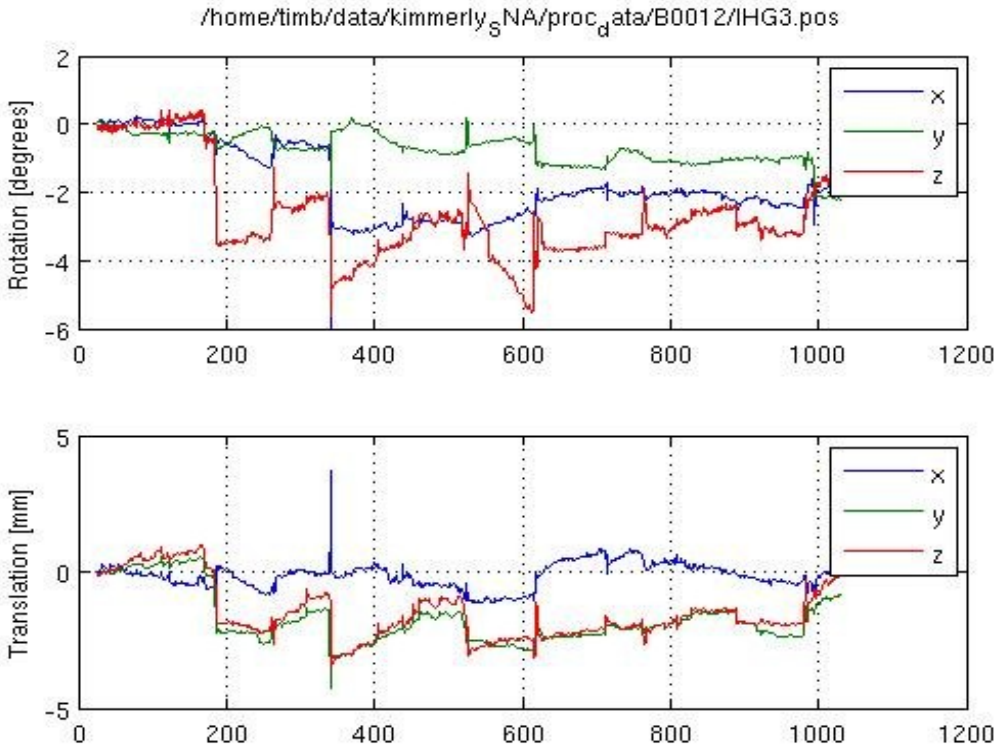

Participant B0013

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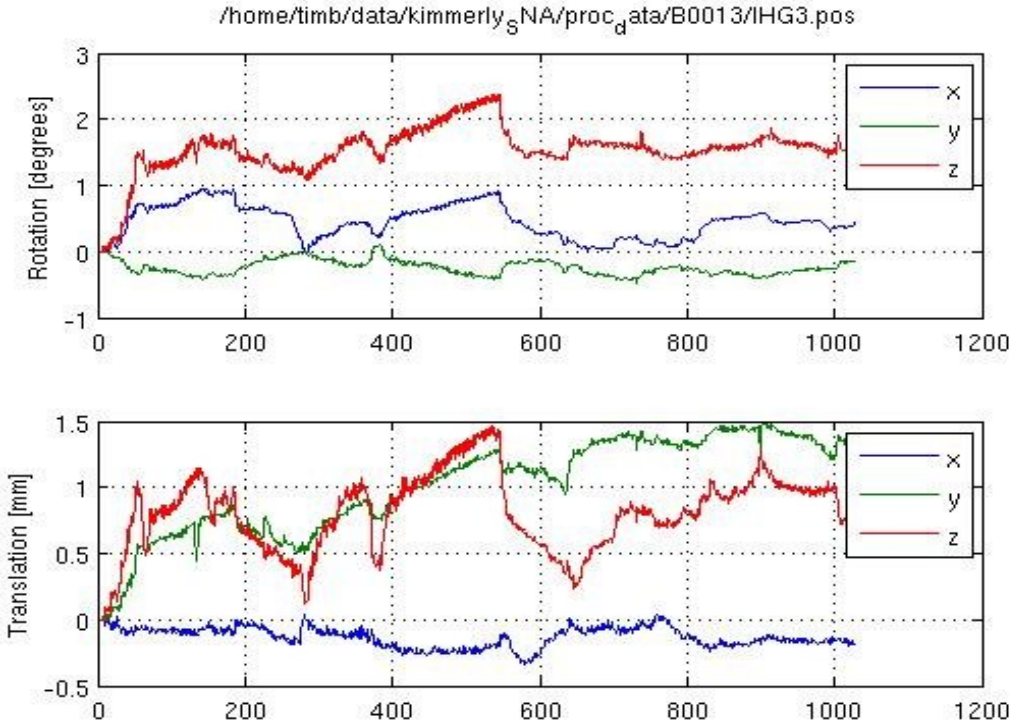

Participant B0015:
IHG1-1