

Electronic Thesis and Dissertation Repository

11-1-2021 9:30 AM

Direct electrical stimulation of prefrontal cortex modulates the transient heart rate response to exercise in conscious humans

Bartek Kulas, *The University of Western Ontario*

Supervisor: Shoemaker, Kevin, *The University of Western Ontario*

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

© Bartek Kulas 2021

Follow this and additional works at: <https://ir.lib.uwo.ca/etd>



Part of the [Integrative Biology Commons](#), [Kinesiology Commons](#), [Systems and Integrative Physiology Commons](#), and the [Systems Neuroscience Commons](#)

Recommended Citation

Kulas, Bartek, "Direct electrical stimulation of prefrontal cortex modulates the transient heart rate response to exercise in conscious humans" (2021). *Electronic Thesis and Dissertation Repository*. 8253. <https://ir.lib.uwo.ca/etd/8253>

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlsadmin@uwo.ca.

Abstract

Prefrontal cortical regions play an essential role in generating appropriate cardiovascular adjustments, particularly in cardio-vagally mediated heart rate (HR) responses to active tasks. Functional imaging studies provide correlational evidence that this region coordinates HR responses to exercise, however, direct experimental evidence of prefrontal cortical HR regulation in humans is not available. Seven persons with epilepsy implanted with intracranial electroencephalography (iEEG) completed 2-second isometric handgrip (IHG) contractions at no-stimulation (NO-STIM) or sham-stimulation (SHAM) conditions, and during direct electrical stimulation (STIM) of the orbitofrontal and medial prefrontal cortex. HR responses to IHG during NO-STIM and SHAM increased HR by $\Delta 4.9 \pm 2.7$ bpm, compared to an attenuated HR response of $\Delta 1.7 \pm 3.1$ bpm during STIM ($P = 0.024$). Using a novel electrical stimulation model, these preliminary data support the role of prefrontal cortices as a key node in the transient cardio-vagal response to IHG task, and are in agreement with previous neuroimaging and animal literature.

Keywords

heart rate; parasympathetic nervous system; medial prefrontal cortex; orbitofrontal cortex; cortical autonomic network; isometric handgrip, deep brain stimulation

Lay Summary

Changes to our hearts rate are modified on a beat to beat basis, by a branch of the nervous system that operates involuntarily – called the autonomic nervous system. Through this system, signals arriving at the heart can increase or decrease heart rate when necessary. For example, your heart rate might increase if you encounter a grizzly bear in the wild or if you have to deliver a presentation in front of a large crowd. On the contrary, laying down on the couch after a big meal may decrease your heart rate. Higher regions of your brain have been shown to influence changes in heart rate, but this “brain-heart” relationship is poorly understood in the context of health and disease. This dissertation provides new information on the ability of higher brain regions to modify heart rate. We showed that modifying activity in this region of the brain via direct electrical stimulation reduced the ability of heart rate to rapidly increase during exercise. This mode of study was possible thanks to volunteering persons with epilepsy with clinically indicated brain electrode implants.

Acknowledgements

I am grateful and fortunate for your mentorship these past two years, Dr. Kevin Shoemaker. Your passion and leadership style have inspired me to take on new challenges, think creatively, and pursue gratifying projects. Thank you for a priceless experience that has allowed me to grow as a researcher and as a person.

To all my lab mates, thank you for inspiring critical thinking, all the thought-provoking conversations, and always making our lab feel like a family and a home. Arlene, thank you for your technical support, kindness, and helping me stay organized. Steve, thank you for your mentorship and valuable insight on projects.

The opportunity to carry out research at the Epileptic Monitoring Unit was truly humbling, and I'm grateful for all the enthusiasm and support I received from colleagues. Thank you to Miguel Astrada, Seyed Mirsattari, Greydon Gilmore, Jenn De-Jonge, Rich Laing, nurses, surgeons, and all the clinicians involved, for the amazing work you do, and your collaboration that made this project possible.

Table of Contents

Abstract.....	ii
Lay Summary.....	iii
Acknowledgements.....	iv
Table of Contents.....	v
List of Tables.....	vii
List of Figures.....	viii
List of Appendices.....	ix
List of Abbreviations.....	x
Chapter 1.....	1
1 Introduction.....	1
Chapter 2.....	5
2 Literature Review.....	5
2.1 Heart Rate Response to Exercise.....	5
2.1.1 Central Command.....	6
2.1.2 Cardio-vagal control.....	7
2.2 Cortical Autonomic Network.....	7
2.2.1 Associated Neural Pathways.....	10
2.3 The Prefrontal Cortex.....	13
2.3.1 Electrical Stimulation of the Cortical Autonomic Network.....	15
2.3.2 Induced Lesions.....	16
2.3.3 Neuroimaging.....	16
2.4 Clinical Correlations.....	20
2.5 Summary & Purpose.....	21

Chapter 3.....	23
3 Methods.....	23
3.1 Participants.....	23
3.2 Experimental protocol.....	24
3.3 Data analysis.....	28
3.3 Statistical analysis.....	29
Chapter 4.....	31
4 Results.....	31
4.1 Participant Characteristics and Heart Rate Measurements.....	31
4.2 Excluded Subjects.....	36
4.2.1 Arrhythmia Patient.....	36
4.2.2 Prefrontal Seizure Patient.....	37
Chapter 5.....	42
5 Discussion.....	42
5.1 Limitations.....	47
5.2 Future Directions.....	49
5.3 Conclusion and Impact.....	50
References.....	51
Appendices.....	69
Appendix A: Ethics Approval.....	69
Appendix B: Letter of Information and Consent Forms.....	70
Curriculum Vitae.....	76

List of Tables

Table 4.1: Participant characteristics and medical history	31
Table 4.2: Mean and standard deviations of heart rate responses to isometric handgrip task ...	33

List of Figures

Figure 2.1: Schematic of the cortical autonomic network	9
Figure 2.2: The brain stem constituents of the central autonomic network	10
Figure 2.3: The medial prefrontal cortex and orbitofrontal cortex	14
Figure 3.1: Overview of the experimental design	26
Figure 3.2: Measurement of heart rate during brief isometric handgrip	29
Figure 4.1: Montreal Neurological Institute template brain of stimulated electrodes	32
Figure 4.2: Delta heart rate responses during isometric handgrip	34
Figure 4.3: Heart rate time series of averaged isometric handgrip trials	35
Figure 4.4: The arrhythmia of excluded patient	37
Figure 4.5: Hypothesized epileptogenic network of excluded patient	39
Figure 4.6: Hypotension response to electrical stimulation	40
Figure 4.7: Intracranial electroencephalogram of orbitofrontal electrical stimulation	41
Figure 5.1: Schematic of descending prefrontal neural pathways	45

List of Appendices

Appendix A: Ethics Approval	69
Appendix B: Letter of Information and Consent Forms	70

List of Abbreviations

HR	Heart Rate
BP	Blood Pressure
CAN	Cortical Autonomic Network
NTS	Nucleus Tractus Solitarii
PAG	Periaqueductal Grey Matter
NA	Nucleus Ambiguus
RVLM	Rostral Ventrolateral Medulla
SUDEP	Sudden Unexpected Death in Persons with Epilepsy
IC	Insular Cortex
VNS	Vagus Nerve Stimulation
MPFC	Medial Prefrontal Cortex
OFC	Orbitofrontal Cortex
IHG	Isometric Handgrip
iEEG	Intracranial Electroencephalography
ESM	Electrical Stimulation Mapping
PB	Parabrachial Nucleus
NO	Nitric Oxide
L-NAME	N ^G -nitro-L-arginine methyl ester
fMRI	Functional Magnetic Resonance Imaging
BOLD	Blood Oxygen Level Dependent
PET	Positron Emission Tomography
PECO	Post-Exercise Cuff Occlusion
MVC	Maximal Voluntary Contraction
EMU	Epileptic Monitoring Unit
ECG	Electrocardiogram

ASDs	Anti-seizure Drugs
MRI	Magnetic Resonance Imaging
CT	Computed Tomography
NO-STIM	No Stimulation Handgrip Condition
STIM	Stimulation Handgrip Condition
SHAM	Sham Handgrip Condition
ANOVA	Analysis of Variance
SD	Standard Deviation
MNI	Montreal Neurological Institute
DVN	Dorsal Vagal Motor Nucleus

Chapter 1

1 Introduction

Beat by beat cardiovascular adjustments are dependent on a healthy homeostatic balance between tonically-active parasympathetic and sympathetic outflow to the sinoatrial node of the heart (Saul, 1990; Shaffer et al., 2014), via the vagus nerve and the stellate ganglia respectively (Guo et al., 2002; J. H. Mitchell et al., 1989). These regulations in heart rate (HR) are influenced by well-defined reflex circuits in vasomotor sites of the brain stem, such as the continuous afferent feedback of baroreceptors from the carotid sinus and aortic arch involved in regulating blood pressure (BP) and HR baroreflex (Ciriello, 1983; Schreihofner & Guyenet, 2002; Spyer, 1981). Conversely, beat by beat modulations of HR also receive inputs from regions of the cortical autonomic network (CAN), which are believed to have descending influences on central sites of cardiac regulation, namely the nucleus tractus solitarius (NTS), periaqueductal grey matter (PAG), nucleus ambiguus (NA), and rostral ventrolateral medulla (RVLM) (Cechetto & Shoemaker, 2009; Critchley, Corfield, et al., 2000; Thayer et al., 2012). This widespread network is undoubtedly complex, and the role of CAN regions in the coupling of cardiovascular responses with high-order neural systems that integrate activity in perceptual, viscerosensory, emotional, and memory systems remains unknown.

From a clinical perspective, neurological disturbances and diseases of CAN regions may have detrimental consequences on autonomic function. In epilepsy, seizures arising from the prefrontal, hippocampal, and amygdalar regions may produce autonomic manifestations such as cardiac arrhythmias, asystole, syncope, viscerosensory phenomena, brief apnea, sweating, and piloerection (Devinsky, 2004; Devinsky et al., 1986; Oppenheimer et al., 1990; Tinuper et al., 2001). The rate of sudden unexpected death in epilepsy (SUDEP) is >20-fold greater than in the general population (Ficker, 2000; Shorvon & Tomson, 2011), and may be related to cardiac arrhythmias and eventual asystole in patients who have no previous history of cardiac disease (Devinsky, 2004; Oppenheimer et al., 1990; Verrier et al., 2020). Also, ischemic strokes involving the prefrontal and insular cortex (IC) regions can result in fatal cardiac arrhythmias that may explain sudden death early after stroke (Cheung & Hachinski, 2000; Colivicchi et al.,

2004; Oppenheimer et al., 1990). Some studies suggest that seizures or stroke may result in asymmetric activation of cardio-vagal and sympathetic outflow to the heart, causing an imbalance in the intrinsic electrophysiological properties of myocardium (Colivicchi et al., 2004; Hachinski et al., 1992; Oppenheimer et al., 1990, 1992; Talman, 1985). Interestingly, vagal nerve stimulation (VNS) is an effective therapy for reducing seizure frequency in drug-resistant epilepsy (DRE) (DeGiorgio et al., 2000; Handforth et al., 1998; Ronkainen et al., 2006), and in the treatment of drug-resistant depression (Rush et al., 2000, 2005; Sackeim et al., 2001), although the mechanisms of these VNS therapies on cortical neurocircuitry remain to be determined. Increasing our knowledge of the “brain-heart” relationship is a fundamental step towards understanding the CAN in health, and in advancing therapies and diagnostic tools.

Converging evidence from animal and human studies suggest the prefrontal cortical region may act as a key node of the CAN implicated in the modification of cardiovascular responses. Neuroanatomically, regions of the medial prefrontal (MPFC) and orbitofrontal cortex (OFC) project onto cingulate cortices, hippocampus, and the IC (Hurley et al., 1991; Neafsey et al., 1986; Thayer et al., 2009), forming a large interconnected network between the thalamus, PAG, NTS, and other brain stem sites with important roles in cardiovascular control (Benarroch, 1993; Öngür & Price, 2000; Verberne et al., 1997). Importantly, these prefrontal cortices also receive multimodal sensory information and are involved in high-order processing (Francis et al., 1999; King et al., 1999; Rolls et al., 2003; Rolls & Grabenhorst, 2008). Direct prefrontal cortex stimulation studies in both awake and anesthetized rats and rabbits report effects of bradycardia, hypotension, and gastro-intestinal motility (Buchanan et al., 1985; Buchanan & Powell, 1984; Burns & Wyss, 1985; Hurley-Gius & Neafsey, 1986; Owens et al., 1999). In rodents, the bradycardia and depressor responses observed during ventral hippocampal stimulation were abolished following bilateral lesions to the MPFC (K. G. Ruit & Neafsey, 1988). Furthermore, awake rats with lesioned MPFC had diminished tachycardia and behavioral (‘freeze’) responses to conditioned fear (Fryszak & Neafsey, 1991, 1994). In humans, functional neuroimaging data of the brain during performance of the isometric handgrip (IHG) task suggests a correlation between deactivation in the MPFC and the acute increase in HR associated with the withdrawal of cardio-vagal activity (Wong, Massé, et al., 2007). Other neuroimaging studies report ventral MPFC activity elevated in vegetative states in monkeys (Rolls et al., 2003), and during resting state in humans (Raichle et al., 2001), supporting the influence of this region on parasympathetic

efferent activity. Functionally, this region has been associated in the “default mode network”, in which a number of prefrontal regions have elevated activity at a passive resting state; this highly active neuronal state then becomes less active (deactivates) during a wide range of active tasks (Nagai et al., 2004; Raichle et al., 2001; Shulman et al., 1997). Therefore, these prefrontal cortices have an essential role in generating appropriate cardiovascular responses, which appear to be a mechanism of cardio-vagal outflow mediated by the parasympathetic branch of the nervous system.

During the onset of volitional exercise, the rapid cardiovascular response is appropriately matched to the intensity and effort of the exercise performed (Mitchell, 2012). These autonomic changes were first described by Krogh & Lindhard in 1913, and the contributing central control mechanisms have interested scientists for well over 100 years (Krogh & Lindhard, 1913; Leonard et al., 1985; Mitchell, 2012; Victor, Secher, Lyson, & Mitchell, 1995). Central command is classically defined as the feed-forward mechanism involving parallel activation of motor and cardiovascular centres (Williamson et al., 2006). These rapid HR increases at exercise onset appear to represent a feed-forward component of autonomic control, due to the fact it is associated with the perceptual effort of exercise (Krogh & Lindhard, 1913; Williamson et al., 2001, 2006). Furthermore, this early HR response is achieved primarily through the withdrawal of vagal activity to the heart. Studies of volitional IHG have found that the rapid increase in HR precedes the peripheral sympathetic responses (Mark et al., 1985), is not affected by beta-receptor blockade (Mitchell et al., 1989), and can be attenuated or entirely eliminated through atropine (vagal) blockade (Hollander & Bouman, 1975; Leonard et al., 1985; Victor et al., 1995).

Taken together, the available data suggest that high neuronal activity at baseline state of the prefrontal cortex leads to high cardio-vagal outflow, and that removal of this neural influence leads to vagal withdrawal at the heart. If the rise in HR during handgrip exercise is due to vagal withdrawal as well as reduced activity in the prefrontal cortex compared to baseline, then it follows that retention of neuronal activation in the prefrontal cortex during handgrip exercise should prevent the handgrip-induced tachycardia.

The primary **purpose** of this investigation was to examine the effect of direct electrical stimulation of the prefrontal cortical region on the HR response during a brief IHG task. The use of brief IHG task allows us to evaluate transient HR changes mediated by parasympathetic

withdrawal. We recruited patients undergoing intracranial electroencephalography (iEEG) monitoring, in whom electrical stimulation mapping (ESM) was indicated for clinical seizure localization. Our electrical stimulation experimental model is the first to assess whether small electrical currents conducted through the prefrontal cortex can disrupt the regular neural transmission believed to be associated with transient HR changes in the human brain. This research tested the **hypothesis** that direct electrical stimulation of the MPFC and OFC during IHG attenuates the HR response associated with the task.

Chapter 2

2 Literature Review

2.1 Heart Rate Response to Exercise

The rapid shortening of the R-R interval of the cardiac cycle characterizes the prominent autonomic response at the onset of exercise (Secher, 1985) to meet the increased metabolic demands of the exercise (Mitchell, Kaufman, & Iwamoto, 1983; Mitchell, 2013). Cardiovascular adjustments are coordinated through a series of reflexes at central brain sites associated with sensory afferents (Mitchell et al., 1983) from the working muscle as well as the volitional effort associated with the exercise (Williamson et al., 2006). However, differentiating the parasympathetic and sympathetic contributions presents a challenge because these two branches of the nervous system are tonically active and often respond simultaneously. Generally, if parasympathetic activity decreases, then sympathetic activity increases. For example, when high pressure sensitive arterial baroreceptors are disinhibited by moving from the supine to upright postures, the baroreflex centres in the brainstem increase sympathetic activity inducing arterial vasoconstriction while parasympathetic activity on the heart is reduced (Spyer, 1981).

However, certain manoeuvres can help dissociate the typical inverse relationships between vagal and sympathetic directional changes during stress. Carefully designed studies utilizing voluntary and static handgrip contractions have offered the opportunity to differentiate some of the parasympathetic and sympathetic contributions to the exercise response. Tachycardia is often observed in concert with sympathetic vasomotor components; however, these peripheral vasoconstrictor components of the response have a delayed course of onset that is associated with the gradual build-up of fatiguing muscle metabolites (Victor, Seals, & Mark, 1987). Sympathetic neural recordings have shown that moderate intensity handgrip produces a rapid tachycardia that precedes vasoconstrictive sympathetic nerve activity by ~ 30 seconds (Mark et al., 1985). Further, this immediate tachycardia response is found to be largely attenuated or eliminated by administration of vagal blockade (Hollander & Bouman, 1975; J. H. Mitchell et al., 1989; Vianna, Fadel, Secher, & Fisher, 2015; Victor et al., 1987). Mitchell et al. (1985) demonstrated that atropine (a muscarinic antagonist) substantially attenuated the tachycardia response to static quadriceps femoris exercise, whereas beta adrenoreceptor blockade did not

affect the response. Together, these observations demonstrate that the transient tachycardia response to exercise is mediated predominantly by the withdrawal of parasympathetic activity to the heart (Hollander & Bouman, 1975; Victor et al., 1987).

2.1.1 Central Command

Central command is classically defined as the descending neural signals from higher brain regions of the central nervous system, which are capable of coordinating muscular contractions, respiratory function and the cardiovascular response during exercise in parallel (Krogh & Lindhard, 1913; Williamson et al., 2002, 2006). Thus, the autonomic response to exercise is believed to be comprised of both ascending sensory signals from the working muscle, and descending signals from cortical regions associated with the perceptual effort of exercise (Macefield & Henderson, 2019a; Secher, 1985; Williamson et al., 2002, 2006). Investigations of the concept of central command have primarily revolved around associating an individual's perception of effort with their cardiovascular response, independent of the actual workload or force produced. For example, the use of partial neuromuscular blockade enables disassociation between an increase in central command from the feedback of metabolic (type III/VI) and muscle tension receptors (type I/II) during voluntary exercise (Leonard et al., 1985; Mitchell et al., 1989). This model was used to demonstrate that lowering work performed with partial-curarization (neuromuscular blockade) during static handgrip exercise did not eliminate the HR and BP responses (Mitchell et al., 1989). Furthermore, administration of atropine was found to attenuate this HR response that was preserved during neuromuscular blockade, suggesting that withdrawal of parasympathetic activity on the heart is an important mechanism mediated by central command during static exercise (Mitchell et al., 1989). This concept is also supported by observations that an imagined handgrip exercise can elicit the same cardiovascular response as actual exercise (Williamson et al., 2001, 2002).

While it remains generally accepted that an individual's perception of volitional effort during exercise is closely coupled with elevated cardiovascular responses, the direct mechanisms and higher region(s) of the central nervous system involved in generating these autonomic adjustments remain in question. A growing body of evidence has suggested this autonomic function may be represented in the prefrontal cortex, more specifically the MPFC, OFC, and IC. Contributions of these regions are discussed in detail later in the review (sections 2.2 and 2.3).

2.1.2 Cardio-vagal control

In 1921, Loewi first discovered the release of a neurotransmitter, later named acetylcholine, in regions of the heart during stimulation of the vagal nerve in frogs and toads. In the following years, acetylcholine was observed to have an inhibitory effect on heart rate (Loewi and Navratil, 1926). The cardio-inhibitory effects via cholinergic transmission are the result of: (i) a decreased rhythm of the sinus node; and (ii) decreased excitability of the atrial ventricular junctional fibers, and thus, slowing the of excitatory cardiac impulses through the ventricles (Blumenthal et al., 1968; Saul, 1990). In humans, when both cardiac vagal and sympathetic inputs are blocked pharmacologically, with atropine and propranolol respectively, the sinus node fires action potentials spontaneously at a frequency of about ~100 beats per minute in the absence of external influences (Saul, 1990). Intrinsic resting HR is significantly lower (~70 bpm), supporting the idea that influence of parasympathetic activity to the sinus node must have a net suppressive effect over sympathetic influence at rest. As a consequence, the withdrawal of tonically active vagal tone on the sinus node results in a prominent increase of HR (Fontollet et al., 2018; Leonard et al., 1985). Functionally, the ability to decrease vagal tone with low latency appears to be important in the onset of effortful tasks (Freyschuss, 1970; Krogh & Lindhard, 1913; Leonard et al., 1985; Wong, Massé, et al., 2007), and in variability of HR associated with attentional regulation and executive function (Thayer et al., 2012). The loss of normal autonomic nervous system regulation of HR and cardiac rhythm may result in diminished beat-to-beat HR variability (Van Ravenswaaij-Arts et al., 1993; Shaffer et al., 2014), a strong predictor of sudden cardiac death and fatal arrhythmias (Bigger et al., 1993; Dreifus et al., 1993).

2.2 Cortical Autonomic Network

The CAN includes the following components: the prefrontal cortices, IC, amygdala, hippocampus, stria terminalis, hypothalamus, midbrain PAG matter, NTS, and other functionally important medullary nuclei such as the NA and RVLM (Figure 2.1) (Cechetto, 2014; Öngür & Price, 2000; Thayer et al., 2012). MPFC, OFC, and IC regions of the neocortex are believed to be involved in the highest order of processing viscerosensory information and initiating integrated autonomic functions (Benarroch, 1993; Neafsey, 1991). The prefrontal cortex and neighbouring limbic regions (IC, amygdala) are intimately interconnected both with each other,

and with the hypothalamus, PAG, NTS, and ventrolateral medulla (Cechetto, 2014; Öngür et al., 1998; Öngür & Price, 2000). The prefrontal cortex and the hippocampus are ascribed similar functional roles in facilitating predictions about upcoming outcomes (G. Ruit & Neafsey, 1990; Wikenheiser & Schoenbaum, 2016), and may be involved in cortically-mediated HR responses to exercise (Norton et al., 2013). In the dorsolateral medulla, the NTS is supplied by baroreceptor afferents, and forms a critical connection with the ventrolateral medulla for baroreflex induced sympathoinhibition and cardio-vagal stimulation (Beckstead & Norgren, 1979; Guyenet et al., 1989; Torvik, 1956). Within the midbrain, the PAG is subdivided into four longitudinal tracts that coordinate autonomic, nociceptive, and motor mechanisms for psychological stressors, defensive, and reproductive behaviours (Bandler et al., 1991; Dampney, 2018). More specifically, the dorsolateral PAG region evokes changes in sympathetic drive and baroreflex sensitivity (Inui et al., 1994; Keay & Bandler, 2001; Sverrisdóttir et al., 2014). Discrete central brain sites such as the NTS and PAG participate in highly coordinated reflex arcs involved in responding to stimuli (i.e. somatosensory/ visual inputs) with the appropriate cardiovascular and respiratory effect: These regions appear to integrate some degree of descending cortical input when coding an autonomic response (Benarroch, 1993; Dampney, 2018).

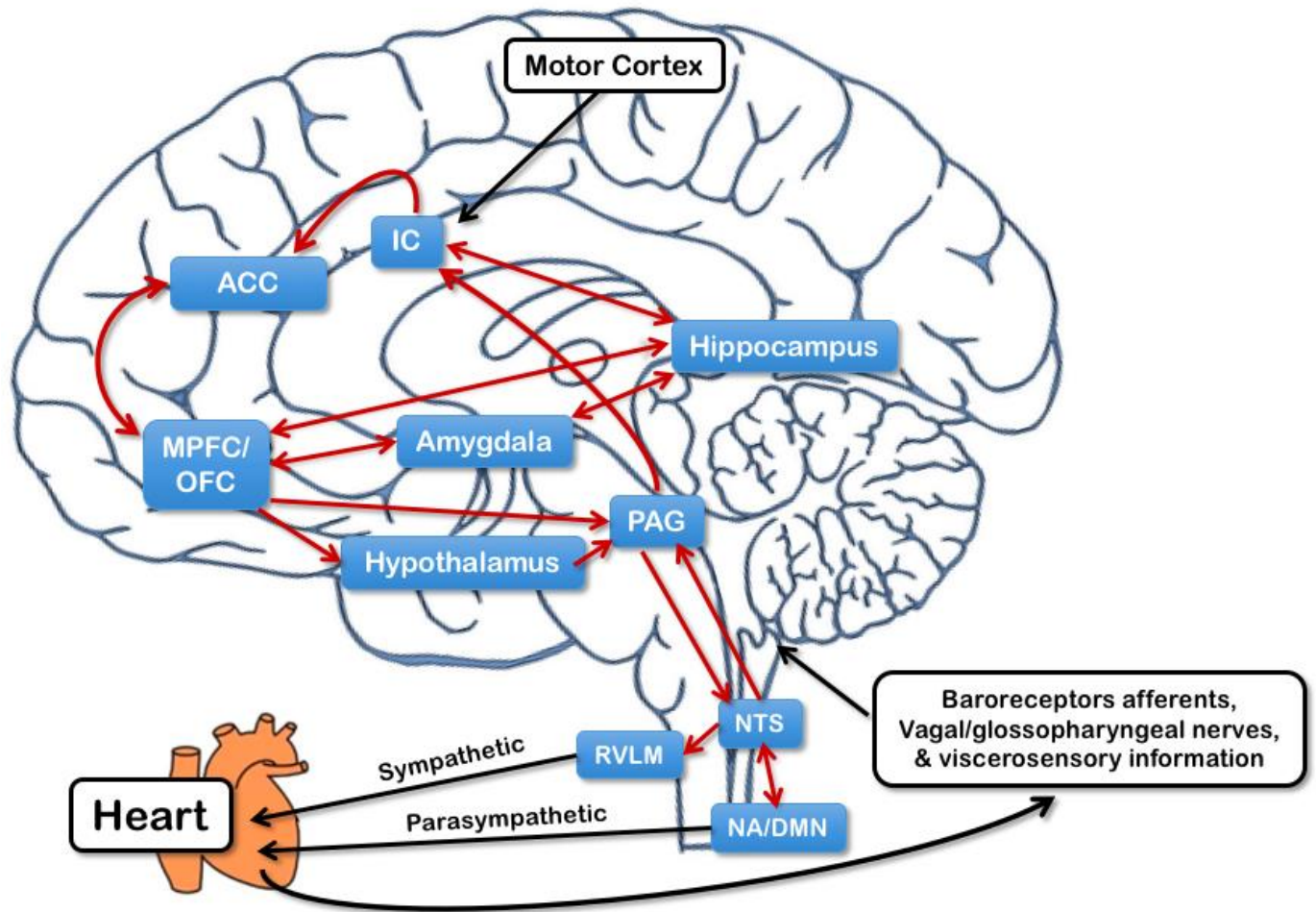


Figure 2.1: The major components of the cortical autonomic network (CAN). MPFC = medial prefrontal cortex; OFC = orbitofrontal cortex; ACC = anterior cingulate cortex; IC = insular cortex; PAG = periaqueductal grey; NTS = nucleus tractus solitarius; RVLM = rostral ventrolateral medulla; NA = nucleus ambiguus; DMN = dorsal motor nucleus. The CAN receives ascending inputs from brain stem sites, which reach the limbic system, and eventually the MPFC and OFC which are believed to be responsible for the highest order of processing and integration of multimodal sensory information. Descending CAN inputs from the prefrontal cortices project onto medullary sites via the PAG, where eventually the sympathetic and parasympathetic branches of the nervous system influence the heart rate and contractibility.

2.2.1 Associated Neural Pathways

The midbrain contains several key loci involved in autonomic function including the PAG, NTS, RVLM, NA, and parabrachial (PB) nuclei (Figure 2.2) (Benarroch, 1993; Öngür & Price, 2000; Owens & Verberne, 2001; Shoemaker & Goswami, 2015; Verberne, 1996). These lower regions of the brain form well-defined reflex arcs (i.e, baroreflex, chemoreflex), that produce highly coordinated responses to afferent viscerosensory inputs (Francis et al., 1999; Schreihöfer & Guyenet, 2002; Spyer, 1981). These neural circuits are located primarily at the medullary level which suggests they may not be entirely dependent on cortical inputs, and thus, are thought to operate subconsciously. This is an oversimplification of course, and the following section aims to cover the important relationships shared between the brainstem and higher cortical sites.

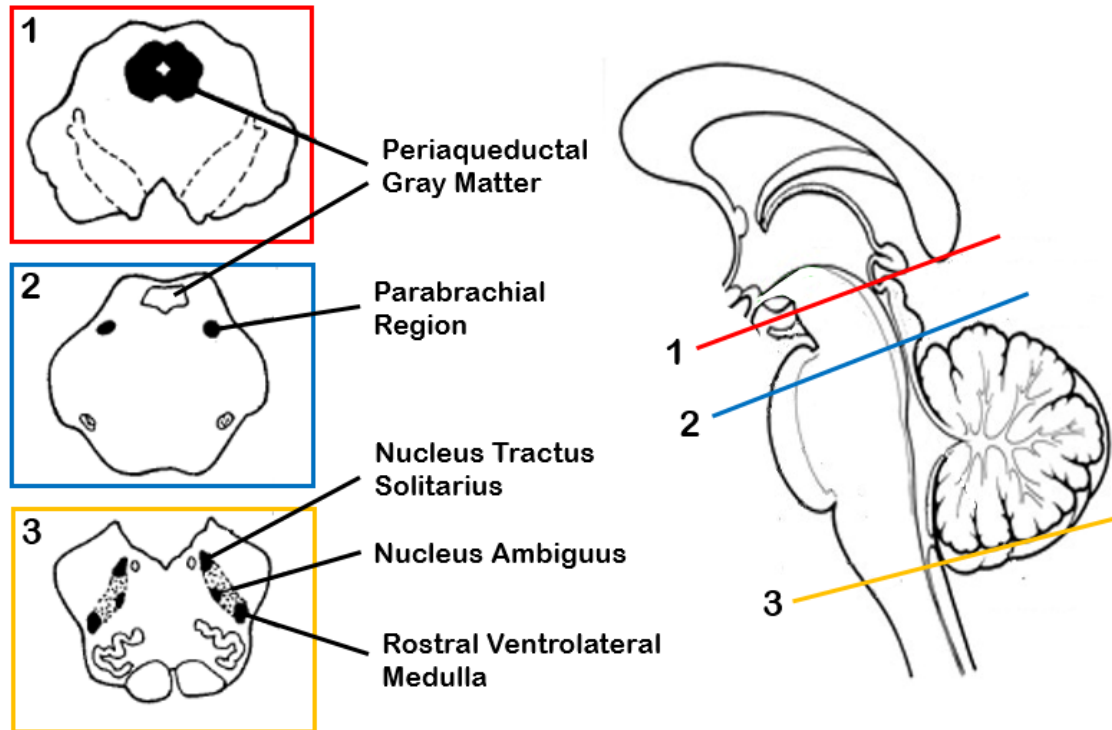


Figure 2.2. Simplified schematic of the major brain stem nuclei involved in the central autonomic network. Note, the labeled nuclei columns are highly interconnected with one another, and form neural circuits integrating afferents from mechanoreceptor, chemoreceptor, and respiratory control. For example, afferents from vagal (X) and glossopharyngeal (IX) cranial nerves terminate at the nucleus tractus solitarius, and form circuits with the nucleus ambiguus which is highly involved in cardiac control. This figure was recreated with permission, from a

figure originally published in *Mayo Clinic Proceedings*, 68(10), E. Benarroach, The Central Autonomic Network: Functional Organization, Dysfunction, and Perspective, 988-1001, Copyright Elsevier (1993).

In the midbrain, the PAG nuclei plays a critical role in autonomic function and behavioural responses to threatening stimuli as well as in pain inhibition (Behbehani, 1995; Benarroch, 1993; Dampney, 2018). In particular, the PAG is essential in cardiorespiratory responses in the context of fear responses, psychological stressors, and other defensive and emotional behaviors (Carrive & Bandler, 1991a, 1991b; Dampney, 2015, 2018; Inui et al., 1994; Keay & Bandler, 2001; Lovick, 1985). Recent anatomical studies have identified that midbrain PAG columns receive distinct sets of ascending (spinal and medullary) and descending (cortical and hypothalamic) afferents (Keay & Bandler, 2001). For example, stimulation of the cardiac sympathetic afferents in cats (Guo et al., 2002) and noxious visceral stimuli inducing a depressor response in rats (Clement *et al.*, 1996) increases *c-fos* expression in the PAG. While in humans, neuroimaging data reveals increased activity in the PAG during maximal inspiration and Valsalva manoeuvre (Topolovec et al., 2004). Interestingly, deep brain stimulation targeting the PAG reduced BP in hypertensive individuals (Green et al., 2005, 2006; Pereira et al., 2010), and modified muscle sympathetic nerve activity when targeting the dorsal lateral PAG (Sverrisdóttir et al., 2014). The prefrontal cortex also projects to the PAG, primarily the dorsal lateral PAG (Hurley et al., 1991; Neafsey et al., 1986). In rats, PAG projections originate from large regions of the prefrontal cortex and overlap with superior colliculus and NTS projections, suggesting the PAG is involved in a wide range of brain visceral and somatic functions (Neafsey et al., 1986; Terreberry & Neafsey, 1987; Van Der Kooy et al., 1982). These projections onto autonomic centers of the brainstem support the concept of prefrontal cortices functioning as a “visceral motor cortex” (Terreberry & Neafsey, 1987), in which cortical inputs are believed to be involved in coupling emotional stimuli to physiological responses such as bradycardia and tachycardia responses (Bandler et al., 2000; Benarroch, 1993; Keay & Bandler, 2001). However, few studies have investigated the function of direct projections from the prefrontal region to the PAG. One recent optogenetics study in mice found that selective inhibition of prefrontal projections to the dorsal PAG produced a behavioural response mimicking “social defeat” (Franklin et al., 2017).

Anatomically, animal models have indicated extensive connections of the prefrontal cortex to important brain stem autonomic regions. Tract tracing studies demonstrate that neurons in the medial and lateral prefrontal cortex terminate throughout the rostrocaudal NTS of the dorsal brainstem (Terreberry & Neafsey, 1983; Van Der Kooy et al., 1982, 1984) with high antidromic conduction velocities (0.7 m/s) (G. Ruit & Neafsey, 1990). Importantly, the NTS is the first relay site of the central nervous system for visceral afferents carried by the vagal (X) and glossopharyngeal (IX) cranial nerves (Beckstead & Norgren, 1979; Torvik, 1956), serving two key functions: i) initiating medullary autonomic reflexes, including cardiovascular and respiratory, and ii) supplying viscerosensory inputs to the CAN (Benarroch, 1993; Guyenet et al., 1989). The NTS has been shown to send output to the PB nucleus in the pons, and the PB nucleus projects to several cortical regions associated with autonomic control such as the IC and amygdala (Cechetto & Saper, 1987; Cechetto & Shoemaker, 2009). These findings have helped localize the representation of viscerosensory input in the prefrontal cortices (Neafsey, 1991; Terreberry & Neafsey, 1987; Van Der Kooy et al., 1982), establishing an important link and earning this region the nickname, “visceral motor cortex”. Importantly, CAN contributions to the sinoatrial node of the heart are under the tonic inhibitory control via the GABAergic neurons of the NTS (Owens et al., 1999; Potts, 2006; Thayer et al., 2012).

Within the medulla oblongata, the NTS activates efferent cardio-vagal neurons of the NA and the DVM nucleus (Benarroch, 1993; Van Der Kooy et al., 1982). Thus, inhibition of the NTS can result in a deactivation of the cardio-inhibitory effect at these primary preganglionic parasympathetic centers (Van Der Kooy et al., 1982). Another important relay of the NTS includes the inhibition of RVLM neurons in the brainstem, which constitute the primary circuitry of the vasomotor baroreflex (Guyenet, 1990; Verberne, 1996). The sympathoexcitatory neurons located in the RVLM are mediated in part by neighboring regions in the brain stem, such as the NTS (Thayer et al., 2012; Van Der Kooy et al., 1982). In addition to indirect mediation, light-microscopic anterograde tracing (Hurley et al., 1991) and retrograde tracing (Van Bockstaele et al., 1989) experiments in rats suggest direct projections exist from the MPFC to the RVLM (Verberne, 1996), which may also contribute to depressor and sympathoinhibitory responses.

These findings highlight the imperative role of midbrain neural circuitry in cardiac and vascular control. It is conceivable that a combination of these pathways is likely to participate in

the vasodepressor responses elicited from the prefrontal cortex. However, some evidence reveals that subdivisions of the prefrontal region do not share identical efferent projections (Öngür & Price, 2000), and this is further complicated when comparing across species (Bandler et al., 2000; Dampney, 2018; Seamans et al., 2008). The mechanisms and specific cortical inputs involved in these pathways remain poorly understood, especially in humans, and merit further investigation.

2.3 The Prefrontal Cortex

The prefrontal cortices most relevant in the CAN include the MPFC, OFC, and the IC which is also categorized as a limbic structure. Anatomically, MPFC is a region largely synonymous with the OFC, with the orbitofrontal cortices extending further ventro-laterally (Figure 2.3). The OFC and MPFC are linked to executive functions such as response planning, learning, predicting and evaluating outcomes, by supporting response inhibition, attention, and emotion behaviours (Franklin et al., 2017; Wallis, 2007). It has been proposed that these regions evaluate and interpret situational and environmental information while integrating past experiences, via working memory in response planning (Franklin et al., 2017; Wallis, 2007). The OFC receives processed information from all sensory modalities (Cavada et al., 2000; Öngür et al., 1998; Romanski et al., 1999; Wallis, 2007), whereas only weak motor connections exist in the area (Carmichael & Price, 1995). Using functional imaging, the MPFC has also been associated with an elevated “default” mode of brain activity during rest, and its level of neuronal activity decreases relative to baseline at the onset of volitional exercise or a non-specific task requiring attention (Raichle et al., 2001; Shulman et al., 1997; Wong, Massé, et al., 2007). Another prominent feature of the OFC and MPFC is the relative location, and the presence of a multitude of inputs from the limbic system, including the hippocampus, amygdala and cingulate cortex (Carmichael & Price, 1995; Cechetto, 2014). The limbic system is thought to have a role in coupling emotional stimuli to autonomic adjustments (Bandler et al., 2000; Dampney, 2016; Keay & Bandler, 2001). Furthermore, it shares a number of direct connections with autonomic sites, such as the hypothalamus and PAG (Dampney, 2018; Franklin et al., 2017; Öngür et al., 1998).

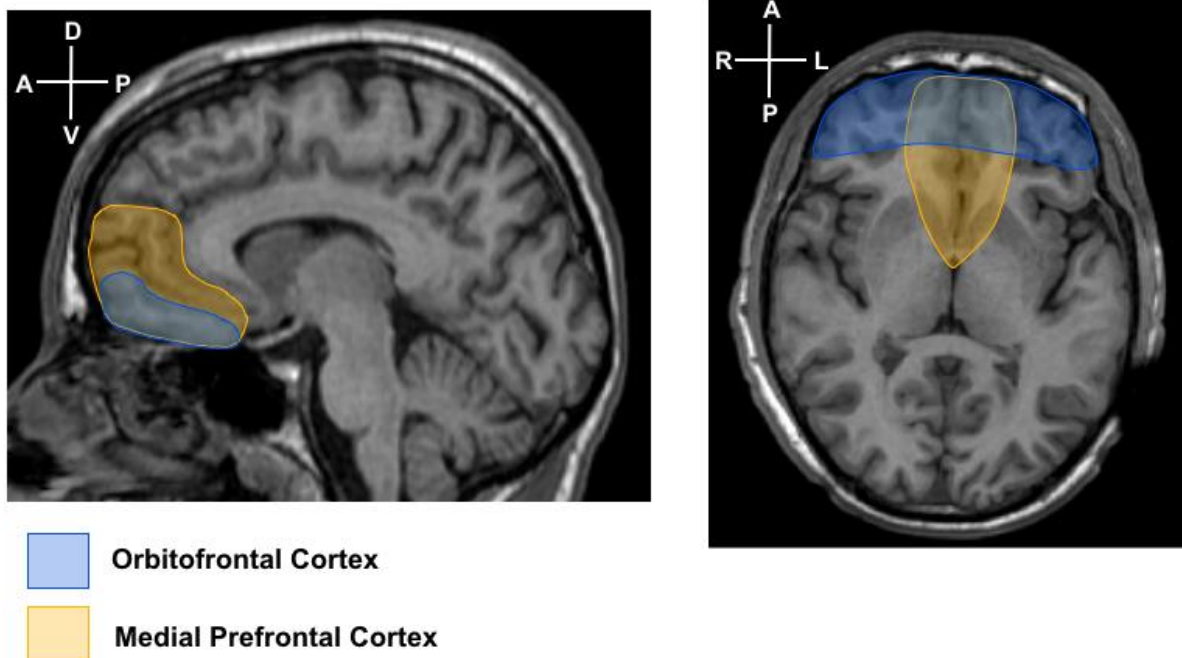


Figure 2.3. Approximated areas of regions recognized as the orbitofrontal cortex (OFC) and medial prefrontal cortex (MPFC) on a T1-weighted anatomical magnetic resonance imaging scan of a representative participant. The left image shows a centered sagittal slice, where much of the medial wall of the prefrontal cortex is regarded as the MPFC, whereas the OFC overlaps with the ventral MPFC. The right image shows an axial slice near the ventral surface of the prefrontal cortex, where the OFC overlaps with MPFC but also extends ventro-laterally.

Our understanding of this complex neocortical region in the functional role of autonomic control has relied primarily on animal models utilizing electrical stimulation, chemical stimulation, ablation studies, and tracing of neuronal tracts, while human studies have focused on functional neuroimaging techniques, as well as limited evidence from studying prefrontal brain injury patients. This section will cover the growing body of animal and human research supporting the representation of the prefrontal cortex in the autonomic nervous system and will attempt to parse out the role of the region in the CAN, specifically in cardiovascular control.

2.3.1 Electrical Stimulation of the Cortical Autonomic Network

A variety of autonomic responses can be elicited by electrical stimulation of the prefrontal cortex. The earliest electrical stimulation studies included a series of classical experiments in primates which reported that stimulation of the anterior cingulate, orbital, and insular regions of the prefrontal cortex could cause both pressor and depressor responses, large inhibitions in respiration, complete cessation of heart rate, and pupillary dilation and constriction (Kaada et al., 1949; Smith, 1945; Wall & Davis, 1951; Ward, 1948). These earlier investigations used large currents which may have co-activated neighboring regions or other neural tracts. More recently, studies using relatively small currents in the prefrontal cortex have reported effects of bradycardia, hypotension, and gastro-motor (gastro-intestinal motility) in both awake and anaesthetized rats and rabbits (Buchanan et al., 1985; Buchanan & Powell, 1984; Burns & Wyss, 1985; Hurley-Gius & Neafsey, 1986; Owens et al., 1999). In lower species, these effects were primarily localized to the MPFC. Direct cortical stimulation studies have also attempted to address hypotheses regarding which medullary neuronal groups mediate the observed MPFC parasympathetic responses. One such report, found that the depressor and sympathoinhibitory responses attained from low intensity stimulations in the rat MPFC were attenuated following the inhibition of NTS neurons using microinjections of the GABA_A receptor agonist muscimol (Owens et al., 1999). The vasomotor baroreflex was also inhibited during NTS neuron muscimol injection, although authors were unable to directly link this effect to the MPFC pathway (Owens et al., 1999). Furthermore, Verberne reported about half of the RVLM sympathoexcitatory neurons tested were inhibited by MPFC stimulation in rat, while no RVLM neurons were ever excited by MPFC stimulation, suggesting that a subpopulation of RVLM neurons may be under inhibitory control of cortical inputs (Verberne, 1996). To this date, there is little evidence from stimulation studies that has contributed to identifying local physiological mechanisms explaining the effects of MPFC stimulation. Owens & Verberne found that the administration of a nitric oxide (NO) synthesis inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), significantly reduced the MPFC stimulation depressor response, with large reductions in flow seen in the mesenteric and iliac vascular beds (Owens & Verberne, 2001). This suggests that an NO-mediated vasodilation mechanism may be associated with the depressor response seen from MPFC stimulation (Owens & Verberne, 2001), however, further investigations studying local mechanisms are merited.

2.3.2 Induced Lesions

Animal models involving ablations have conclusively demonstrated the involvement of the prefrontal cortex in cardiovascular control. Rodents with bilateral excitotoxin MPFC lesions did not show altered resting MAP and HR, however, the baroreceptor HR reflex responses to pressor and depressor agents (phenylephrine and sodium nitroprusside) were diminished (Verberne et al., 1987). Cardiovascular responses to conditioned emotional stimuli in rats also appear reduced following MPFC lesions (Fryszak & Neafsey, 1994). Fryszak & Neafsey reported that awake rats with lesioned MPFC had diminished tachycardia and behavioral (“freeze”) responses to conditioned fear (Fryszak & Neafsey, 1991, 1994), highlighting the functional relationship of the prefrontal and limbic regions. Connections between the MPFC and hippocampus appear to be imperative in eliciting cardiovascular responses. Bilateral lesions of the MPFC in rat were found to abolish the bradycardia and depressor response observed during ventral hippocampus stimulation (K. G. Ruit & Neafsey, 1988). Taken together, animal models with removal of the MPFC suggest the idea that the MPFC is not responsible for exerting tonic influence on vasomotor control, but instead, acts as a critical relay center for the autonomic nervous system during responsive cardiovascular adjustments.

Invasive animal models have broadened our understanding of the representation of the autonomic nervous system in the prefrontal cortex. However, as with studies of other cortical areas, the responses obtained have been variable in different species and varying conditions (i.e. anesthesia) (Kaada et al., 1949; Smith, 1945; Wall & Davis, 1951; Ward, 1948). The complexity of the human and primate prefrontal cortex must be taken into consideration (Öngür & Price, 2000). In the prefrontal cortices of lower species such as rodent, information processing and combining of elements likely occurs at a rudimentary level (Bandler et al., 2000; Seamans et al., 2008; Uylings et al., 1990). The following section continues into the review of the prefrontal cortex in the CAN, but in humans, which poses other unique challenges.

2.3.3 Neuroimaging

Functional magnetic resonance imaging (fMRI), particularly in humans, has offered new perspectives into understanding the functional anatomy of the CAN. The most common fMRI

uses blood-oxygen-level dependent (BOLD) contrast, discovered by Seiji Ogawa in 1990 (Ogawa et al., 1976). This technique enables mapping of neural activity in the brain by imaging changes in blood flow related to metabolic expenditure in brain cells (Ogawa et al., 1976). In the context of autonomic neuroanatomy, this approach provides strictly correlational data and does not offer the same degree of temporal resolution of electrophysiology or spatial resolution of tract tracing, but instead, is able to provide a complete view of the brain enabling the study of whether regions become more or less metabolically active compared to resting levels (Cechetto & Shoemaker, 2009; Raichle et al., 2001).

In 1999, King et al. were the first to utilize this imaging approach to examine regions of the forebrain involved in visceral function (King et al., 1999). They identified increased activation of the MPFC, IC, and thalamus during tasks that altered HR and BP such as maximal inspiration, Valsalva manoeuvre and isometric handgrip tasks (King et al., 1999). Similarly, Harper et al. used the Valsalva manoeuvre and cold compression to induce HR and BP changes, and observed widespread activation in the MPFC, anterior cingulate and insular cortices (Harper et al., 2000). However, they noted that these brain activation patterns were composed of both autonomic and noxious inputs (Harper et al., 2000). Another investigation using positron emission tomography (PET) imaging had participants perform short-duration handgrip and mental mathematics to induce acute and transient cardiovascular changes, and found that sympathetic nervous system activation was lateralized to the right anterior cingulate, and right insula, whereas parasympathetic nervous system activation was represented in the amygdala, orbitofrontal and ventromedial cortices, left insula, and cingulate (Critchley, Corfield, et al., 2000; Critchley, Elliott, et al., 2000). Furthermore, measures of spontaneous fluctuations in skin conductance (electrodermal activity), an index reflecting sympathetic tone, were correlated with activation in anterior cingulate, orbitofrontal, insular, and medial prefrontal cortices, whereas only the orbitofrontal activation appeared altered during arousal and relaxation tasks involving biofeedback (Critchley, Corfield, et al., 2000; Critchley, Elliott, et al., 2000; Nagai et al., 2004). These early fMRI experiments were essential in supporting the notion that autonomic responses enroll a complex network of cortical sites.

In volitional exercise, fMRI has successfully illustrated the network of forebrain regions with important influence over autonomic outflow and cardiovascular control, although it must be

considered that most volitional motor tasks involve a degree of motor, cognitive and/or sensory components (Cechetto & Shoemaker, 2009; Shoemaker & Goswami, 2015). Thus, functional imaging presents the issue of discerning regions responding to afferent signals from regions that are more so involved in the generation of autonomic responses. To address this complication of dual modality activation, investigators have used selective models with passive or active stimuli. An early investigation by Williamson et al. found significant changes in the left insula with dynamic exercise (active cycling), but not during passively induced exercise, and suggested that the left insula is responsible for reductions in cardio-vagal activity only during exercise involving a volitional component (Williamson et al., 1997). In another dynamic cycling study, they demonstrated that increasing the physical exertion in volitional exercise resulted in a greater magnitude of insular activation (Williamson et al., 1999). Further, with the use of a more controlled static handgrip exercise model, Williamson et al. attempted to assess the role of central command in exercise induced BP changes (Williamson et al., 2003). The initial BP increase associated with handgrip was compared to the sustained BP increase in the post-exercise cuff occlusion (PECO) protocol (Williamson et al., 2003). They postulated that the higher activation observed in insular and anterior cingulate cortices with the handgrip, compared to PECO, may be representative of regions with important influence over central command (Williamson et al., 2003). A hypnosis study by this group also reported activation in the insular and anterior cingulate cortices that occurred only when the imagined handgrip protocol elicited cardiovascular responses (Williamson et al., 2002).

Despite the clear role of the prefrontal cortex in modulating cardiovascular responses, there are a limited number of studies defining the specific role of the region in volitional exercise. In an fMRI investigation, Wong et al. looked at cortical correlates of the cardio-vagal withdrawal response during short handgrip exercise (Wong, Massé, et al., 2007). By correlating the acute HR and BP response to handgrip with the imaging data, they found that the increased HR response was associated with the deactivation of the ventral MPFC and elevated activity in the insular and motor cortices (Wong, Massé, et al., 2007). During a parallel microneurography experiment, they reported no peripheral sympathetic (MSNA) response in the handgrip protocol (Wong, Massé, et al., 2007). These findings suggest the deactivation of the ventral MPFC is involved in the transient cardio-vagal withdrawal response evoked by this effortful task, which likely precedes the sympathetic response to strenuous exercise (Wong, Massé, et al., 2007).

Interestingly, the ventral MPFC was still deactivated during handgrip trials at 5% maximum voluntary contraction (MVC) where no tachycardia response was seen, however, the deactivation was significantly higher when HR increased throughout 35% MVC trials (Wong, Massé, et al., 2007). In the context of health, Wood et al. showed that individuals with higher cardiorespiratory fitness had greater deactivation in the MPFC and hippocampus during handgrip task, even though cardiorespiratory fitness was not a predictor of HR responses (Wood et al., 2017). Conversely, during periods of sub-motor electrical stimulation of wrist flexors (targeting fast conducting type I and/or type II afferents), the somatosensory inputs appeared to be represented by increased activity in the ventral MPFC and insular cortex (Goswami et al., 2011). Moreover, the somatosensory stimulation represented in this region appears to have a neuromodulatory effect of sympathoinhibition on muscle sympathetic outflow during baroreflex unloading (Goswami et al., 2012). Thus, these prefrontal regions appear involved in the parasympathetic nervous system, and may also play a role in modulation of sympathetic regions associated with baroreceptor afferents, such as the NTS, RVLM, and NA (Benarroch, 1993; Macefield & Henderson, 2019b; Öngür & Price, 2000; Thayer et al., 2012).

Notably, the ventral MPFC has previously been implicated in the “default mode network”, in which a number of prefrontal cortical regions that have elevated activity at a passive resting state, appear to decrease in neural activity during active tasks, regardless of the task (Gusnard et al., 2001; Gusnard & Raichle, 2001; Nagai et al., 2004; Raichle et al., 2001; Shulman et al., 1997). A physiological account of this state was reported by Nagai et al., where a significant negative correlation was seen between skin conductance and activation in ventral MPFC and OFC during both relaxation and arousal tasks (Nagai et al., 2004). Other studies report ventral MPFC activity elevated in vegetative states in monkeys (Rolls et al., 2003), and during resting state in humans (Raichle et al., 2001), supporting the influence of parasympathetic activity in this region. Gusnard et al. theorize that the network is associated with continuous processing during the waking state, and the introduction of a goal-oriented task suspends the integration of emotional and cognitive processes (Gusnard et al., 2001; Gusnard & Raichle, 2001).

2.4 Clinical Correlations

The importance of the prefrontal cortex in autonomic control and health in humans is also reflected in clinical evidence. Seizures arising from the prefrontal, hippocampal, and amygdalar regions may produce autonomic manifestations such as cardiac arrhythmias, viscerosensory phenomena, brief apnea, sweating, and piloerection (Cheung & Hachinski, 2000; Devinsky, 2004; Devinsky et al., 1986; Terrence et al., 1981). Notably, cardiac arrhythmias may be responsible for SUDEP in patients who have no previous history of cardiac disease (Cheung & Hachinski, 2000; Devinsky, 2004; Oppenheimer et al., 1990; Terrence et al., 1981). In focal seizures, autonomic changes are commonly observed, including reports of asystole and syncope due to altered cardio-vagal activity (Oppenheimer et al., 1990; Tinuper et al., 2001). Also, ischemic strokes involving the IC and neighboring regions of the prefrontal cortex can lead to fatal cardiac arrhythmias that may explain sudden death early after stroke (Cheung & Hachinski, 2000; Colivicchi et al., 2004; Oppenheimer et al., 1990). These clinical investigations also reveal that seizures or stroke in the prefrontal cortex can result in asymmetric activation of cardio-vagal and sympathetic outflow on the heart, causing an imbalance in the intrinsic electrophysiological properties of the heart (Benarroch, 1993; Colivicchi et al., 2004; Hachinski et al., 1992; Oppenheimer et al., 1990, 1992; Talman, 1985).

In humans, damage to prefrontal regions may lead to a range of deficits in personality, social decision making, goal directed behavior, and impulse control, in spite of otherwise normal cognition and intellectual performance (Barrash et al., 2000; Damasio et al., 2013; Hilz et al., 2006; Wallis, 2007). These deficits may be related to autonomic dysfunction. A study of patients with prefrontal damage found that they were not able to generate anticipatory skin conductance responses to a risk evaluation task, supporting the idea that these patients suffer deficits in evaluating future outcomes and decision making (Bechara et al., 1996; Rolls & Grabenhorst, 2008). Clinical data also link the prefrontal cortex with mood, depression/anxiety, and stress disorders (W. C. Drevets et al., 1998; Wayne C. Drevets et al., 1997; Pezawas et al., 2005). Since visceral function and emotion are closely associated in the prefrontal cortex (LeDoux et al., 1983), it is conceivable that this region may in part be responsible for the link between emotional disorders and cardiovascular complications (Dampney, 2015; Hänsel & von Känel, 2008; Hilz et al., 2006; Jiang et al., 2005; Joynt et al., 2003; Liu & Ziegelstein, 2010). For example, Hilz et al.

found that compared to healthy controls, patients with right ventral MPFC lesions exhibited paradoxical cardiovascular responses to pleasant and unpleasant emotional stimuli (Hilz et al., 2006). A growing body of evidence has also proposed depression disorder as an independent risk factor for ischemic heart disease (Jiang et al., 2005; Joynt et al., 2003; Liu & Ziegelstein, 2010). Recently, an investigation in persons with epilepsy and depression, in whom iEEG implants were indicated for seizure monitoring, found that direct prefrontal cortex (specifically, lateral orbitofrontal) electrical stimulations caused acute, dose-dependent improvements in mood-state and depression symptoms (Rao et al., 2018). While the exact mechanisms still remain largely unclear, the cardiovascular complications observed in these patient populations may be related to prefrontal cortical damage causing altered generation of vagal outflow on the heart. Interestingly, VNS provides an effective therapy for patients with DRE (DeGiorgio et al., 2000; Handforth et al., 1998; Ronkainen et al., 2006), and clinical depression (Rush et al., 2000, 2005; Sackeim et al., 2001), although the exact effects of this approach on mid brain and CAN circuitry remain to be determined. Increasing our knowledge on the relationship between autonomic and emotional aspects of the prefrontal cortex will likely yield important diagnostic and therapeutic implications.

2.5 Summary & Purpose

The prefrontal cortical region plays an important role in mediating appropriate cardiovascular responses. Specifically, a reduction in MPFC neural activity is associated with rapid changes in HR at the onset of a volitional task, which is believed to reflect changes in cardio-vagal influence (Mitchell et al., 1983; Wong, Massé, et al., 2007). However, a specific role of this region in cardio-vagal activity remains difficult to validate in humans, and with that, the cardiac complications associated with impaired prefrontal cortex function remain poorly understood (Cheung & Hachinski, 2000; Oppenheimer et al., 1990; Terrence et al., 1981). Persons with epilepsy admitted for depth electrode monitoring, whom are indicated for cortical stimulation mapping for seizure localization, offer a unique opportunity to study the feed-forward cardiovascular contributions of the prefrontal cortex (Al-Otaibi et al., 2010; George et al., 2020). Therefore, the **purpose** of the present study was to investigate the effect of small electrical currents conducted through the MPFC and OFC on the transient HR response to brief

2-second bouts of isometric handgrip exercise. This investigation tested the **hypothesis** that stimulation of the MPFC and OFC will diminish the tachycardia response to voluntary handgrip exercise by modulating the normal reduction in neural transmission from these regions.

Chapter 3

3 Methods

3.1 Participants

All participants provided written informed consent and received detailed explanations of the experimental protocol prior to participation. The study was approved by the Health Sciences Research Ethics Board at Western University. The research procedures were performed in accordance with the Declaration of Helsinki.

Participants were included if they had electrodes implanted in brain regions of interest and were willing to cooperate with the study protocol. Patients were excluded if they declined to provide informed consent, or had significant cardiac arrhythmia or severe cardiovascular complications. A total of nine participants consented to the study. The nature of the exclusion criteria required that some data be collected to determine cardiac variability and epileptogenic sites of stimulation. More information on excluded patients is provided in the results.

Overall, the aggregate data reflect seven patients (38 ± 12 years old; 3 females, 176 ± 7 cm, 82 ± 13 kg, and a body mass index of 26 ± 3 kg/m²), who had been diagnosed with DRE and were undergoing iEEG surveillance for seizure onset localization at the *Epileptic Monitoring Unit* (EMU), at the University Hospital in London Ontario. Patients were between the ages of 24 and 57 years. Two patients who signed consent were studied but their data were not included in the aggregate report: one patient had a consistent arrhythmia, and the other patient had a seizure onset zone localized within the study region of interest, and thus would likely exhibit after discharges and seizure activity when stimulating the prefrontal cortical area. However, outcomes from each of these patients are described as case studies in this report. Patients were on various standard anti-seizure drugs (ASDs) prescriptions used to control seizures in DRE including lacosamide (n=5), lamotrigine (n=2), clobazam (n=1), levetiracetam (n=3), phenytoin (n=1), valporic acid (n=1), and carbamazepine (n=1).

Intracranial depth EEG electrodes (iEEG) were implanted on the day of admission to the EMU, about ~1-2 weeks before testing. All patients underwent comprehensive brain magnetic resonance imaging (MRI) prior to craniotomy operation, which included high resolution T1

weighted images (slice thickness = 1-3 mm, no interslice gap). MRI scans were co-registered with the post-operative volumetric computed tomography (CT) scan for localization and labeling of iEEG electrode coverage. Surgery was performed using a Leksell frame-based stereotactic approach with CT registration (Leksell Coordinate Frame G, Elekta AB, Stockholm, Sweden). Electrode (Ad-Tech Medical Instrument Corporation, Racine, WI) placement is planned using the pre-operative MRI with standard planning software (Neuroinspire™, Renishaw Inc, Gloucestershire, UK). Electrodes are implanted with the aid of the neuromate® stereotactic robot (Renishaw plc, Gloucestershire, UK), and placement is guided by clinical indications and hypotheses for seizure localization and monitoring, and varied somewhat across participants. Each electrode implanted consists of ten evenly spaced contacts, and typically included coverage across sites in the prefrontal cortical area implicated in controlling cardiovascular responses. As a consequence of the time windows for ESM sessions, patient tolerance, number of electrodes identified with prefrontal grey matter coverage, missing signals from electrode contacts, and other constraints related to the clinical environment, it was not possible to stimulate all the desired electrodes of interest in all participants.

Participant recruitment was limited to the flow of iEEG monitoring cases at the Epileptic Monitoring Unit. As a consequence, patient admission was largely disrupted through the course of the COVID-19 global pandemic (Cucinotta & Vanelli, 2020) when nearly all elective surgeries were either halted or postponed.

3.2 Experimental protocol

Following iEEG implantation, participants are monitored at the EMU for ~1-2 weeks for clinical seizures. Then, prior to removal of iEEG electrodes and discharge from the hospital, the clinical team will routinely study each patient using ESM to map eloquent cortical regions (George et al., 2020), and the relationship of the epileptogenic network.

The participants were brought to the testing room ahead of ESM for instrumentation, including live iEEG monitoring on Natus NeuroWorks. They were seated on a lazyboy recliner for the study. Heart rate was quantified using lead II from a standard three-lead electrocardiogram (ECG). Resting HR measurements were averaged from a 60-second recording in the same seated position as the experimental study, prior to testing. Three resting seated BP measurements (BPTRU™ sphygmomanometer cuff) were recorded and averaged prior to ESM.

A strain gauge (model 1132 Pneumotrace II; UFI, Morro Bay, CA) around the chest enabled for monitoring of spontaneous breathing. Participants were instructed to first complete two MVCs with their dominant hand, and the peak voltage output was used to calibrate handgrip intensity for the ensuing protocol. The participants were initially naïve to the protocol, and were then familiarized with the 2 second 50% MVC isometric handgrip (IHG) task. The IHG task involved a screen displaying real-time visual feedback representing the contraction strength, and practice trials were performed until participants were able to consistently hold 2 seconds within the target range. Respiration was monitored to ensure regular spontaneous breathing throughout the handgrip. The 2-second IHG duration was cued by a metronome sound following the investigator's instruction to squeeze. This approach allowed IHG contractions to be initiated during the low lung volume phase of the respiratory cycle (i.e., preceding inspiration). Any trials with irregular breathing were discarded to avoid respiratory contributions to heart rate variability.

The study includes data from the pilot trials (*experiment 1*) and the more rigorous randomized experimental trials (*experiment 2*) because the results were not affected. In *experiment 1* (n =4), participants completed a set of handgrip trials with no stimulation (NO-STIM) before and after the ESM session. During ESM, handgrip trials were performed during simultaneous stimulation (STIM) of prefrontal cortices of interest. The clinician delivering the electrical stimulation was guided by the same metronome queuing the participant to initiate the IHG task. In *experiment 2* (n = 3), participants were blinded by randomizing the order of each STIM handgrip trial with a sham-stim handgrip trial (SHAM), and thus completed sets of two trials. During SHAM trials, patients were not made aware whether the clinician conducted a stimulation during the trial. In both NO-STIM, SHAM and STIM trials, the queuing and visual feedback during the IHG task was identical. In both experiments, all IHG trials were separated by at least a 15 second period of rest. A detailed overview of the experimental design is shown in Figure 3.1.

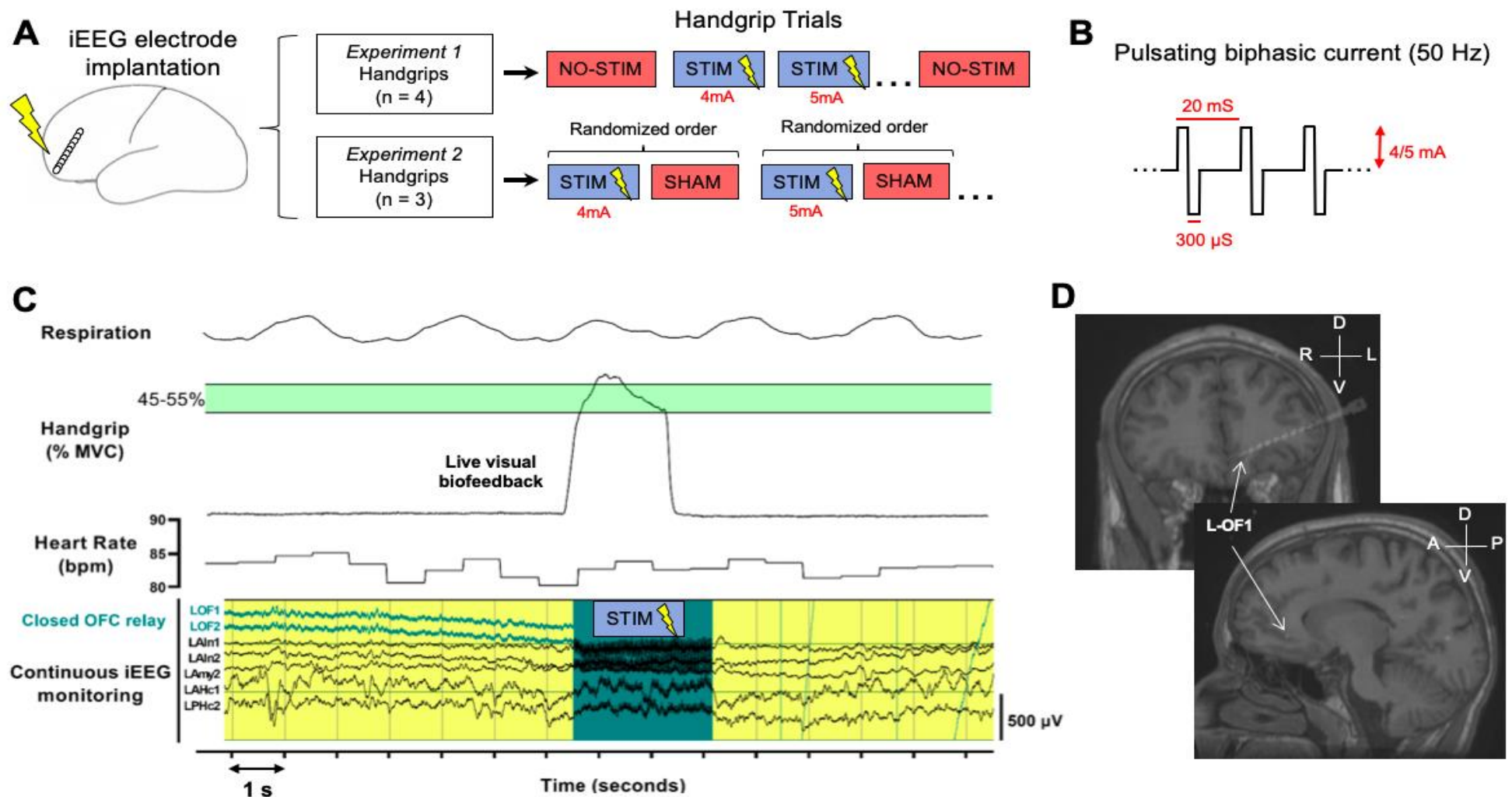


Figure 3.1: Overview of Experiment Design. (A) Experimental timeline: subjects received surgery for electrode implantation for seizure monitoring, and completed either *experiment 1* (n = 4) or *experiment 2* (n = 3). Handgrip interventions were performed during routine electrical stimulation mapping studies. (B) Details of STIM parameters: stimulations delivered during handgrip trials used a biphasic pulse current of 300 μ S pulse width, 50 Hz frequency, and amplitudes set to 4 and 5 mA. (C) Representative data illustrating

a participant performing a handgrip trial during STIM. Participants received a live visual display providing biofeedback of volitional contraction strength, and were instructed to maintain the contraction within the green shaded range indicator (45-55%). Heart rate was recorded, and continuous intracranial electroencephalography (iEEG) was monitored. The closed relay loop allowed for STIM between orbitofrontal cortex (OFC) electrodes. (D) Representative participant's co-registered CT and MRI scan illustrating the prefrontal cortex coverage of electrode points. The most proximal left-orbitofrontal (L-OF) electrode is labeled, each line contains 10 evenly spaced electrodes.

Brain stimulations were applied using the manually-operated Nicolet™ Cortical Stimulator (Natus Medical, Inc., Pleasanton, CA) and were delivered during the same time window as handgrip contractions. Stimulations deliver a biphasic, pulsating constant-current train with the following parameters: 4 and 5mA, 50 Hz, and 300 μ s pulse width. The average duration of biphasic currents delivered during data collection was 2.4 ± 0.6 s. These are standardized parameters for clinical study, where only milliamps are adjusted. Prior to the collection of STIM trials at 4 and 5mA, the region was typically stimulated at lower amplitudes (typically 1-3mA) to threshold the site, where we expect the effect of stimulation to be not as robust (George et al., 2020; Rao et al., 2018). We collected trials at both 4 and 5 mA to maximize the number of trials collected without extending the duration of the ESM study. Additionally, at these low amplitudes (4-5mA), none of the participants reported any sensation or feeling during stimulation periods. A clinical team of neurologists and stimulation technician monitored live iEEG recordings through the ESM session for stimulation-induced after discharges or onset of epileptogenic seizures which did not occur during prefrontal cortex stimulation handgrip trials.

3.3 Data analysis

The HR responses to IHG were measured by converting intervals between R-wave from the ECG tracing into HR (beats per minute). The peak of the transient HR response to IHG was consistently identified in the immediate 2 seconds following contraction. Thus, the delta values for the HR responses were derived using a 2 second onset window (prior to IHG contractions) and the 2 second response window following the contraction (Figure 3.2). Measures during these two HR windows, and the delta values collected during each IHG trial were then reported as an averaged value for each individual. During MVC calibration, patients generated a mean of 0.073 ± 0.022 V. The raw voltage generated during 50% IHG trials did not differ between NO-STIM and STIM trials (0.031 ± 0.013 V vs. 0.033 ± 0.012 V), or between SHAM and STIM trials (0.037 ± 0.012 V vs. 0.036 ± 0.011 V). The aggregate data displayed in results and used in analysis were the result of merging NO-STIM and SHAM conditions from *experiment 1* and *experiment 2* respectively, for comparison with the STIM condition. This was the result of a pilot protocol (*experiment 1*) which evolved into more rigorous study design (*experiment 2*).

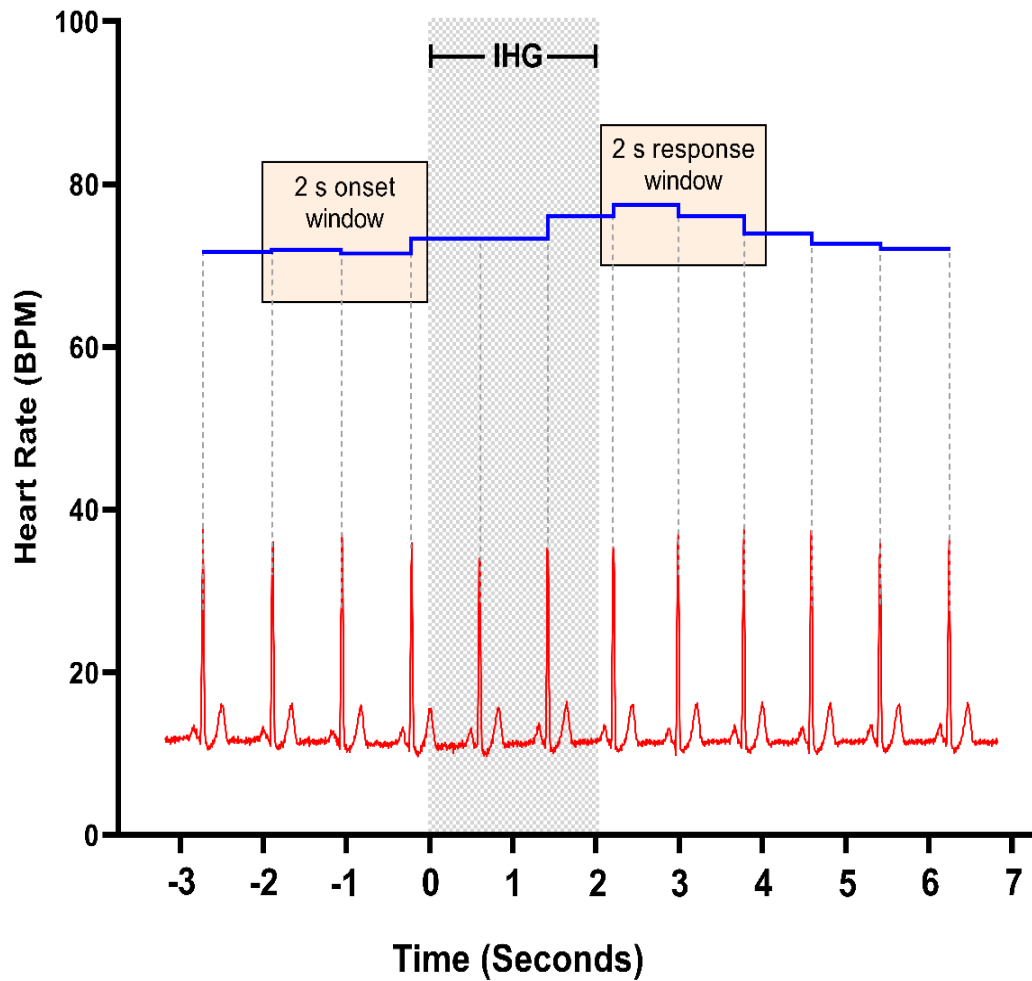


Figure 3.2: Measurement of instantaneous beat-to-beat changes in heart rate during isometric handgrip (IHG) exercise. The electrocardiogram (ECG) illustrates the progressive shortening of time in between beats with IHG, also represented by the increase of instantaneous heart rate. The sampling windows used to calculate delta heart rate are shown, two seconds prior to IHG (-2 to 0), and two seconds following IHG (0 to 2).

3.3 Statistical analysis

The data from *experiment 1* (NO-STIM vs STIM) and *experiment 2* (SHAM vs STIM) were combined for statistical analysis. The results of NO-STIM and SHAM trials were pooled together, and compared against the pooled STIM trials. The aggregate effect of IHG and STIM

on HR were assessed using a two-way repeated measure analysis of variance (ANOVA) test. Significant interactions were further assessed using paired t-tests with Bonferroni's correction. The effect of condition on the change in Δ HR was assessed with a t-test. Significance was set as $P \leq 0.05$. Effect size (Cohen's d) was calculated using HR response delta values and the pooled standard deviations (SD), which was then used to determine the post hoc statistical power. Statistical analysis was performed using Prism (version 8, GraphPad software, LLC, San Diego, CA) and GPower software (version 3.1).

Chapter 4

4 Results

4.1 Participant Characteristics and Heart Rate Measurements

Details and medical history descriptions of the seven participants who completed the protocol are presented in Table 4.1. Epilepsy duration and seizure frequency were approximated based on medical records, and the seizure onset zones were hypothesized by the clinical care team. Participants had an average seated BP of 123 / 81 mmHg (systolic / diastolic).

Table 4.1: Participant characteristics and medical information.

Age / Sex	Hemisphere Stimulated	Handedness	Seated BP (mmHg)	Epilepsy duration / seizure frequency	Seizure onset zone	Comorbidities
36 M	R	R	131 / 86	32 yrs / 1-3 per mo	Right frontal cortical tuber, right hippocampus and amygdala	Mosaic TBS
26 M	L	L	146 / 97	22 yrs / 1 per mo	Right hippocampus and amygdala	Perinatal stroke Hypertension
32 F	R	R	115 / 78	30 yrs / 3 per wk (clusters)	Unknown	Anxiety Right frontal lesionectomy
45 F	R	L	115 / 75	Since childhood / 15 per mo	Left hippocampus and amygdala	Depression Anxiety Right temporal lobectomy
24 M	L	R	113 / 82	20 yrs / 1 per mo	Left hippocampus and amygdala and temporal neocortex	TBI with subdural hematoma and SAH
45 F	L	R	114 / 67	14 yrs / 1-2 per wk	Left temporo-occipito-parietal	None
57 M	L	R	125 / 81	56 yrs / 2-3 per mo	Left hippocampus and bilateral temporal neocortex	Depression Hyponatremia Gout

Details of participants completing our protocol (n=7). BP, blood pressure; yrs, years; wk, week; mo, month; TSC, tuberous sclerosis complex; TBI, traumatic brain injury; SAH, subarachnoid hemorrhage. Participants were diagnosed with drug resistant epilepsy (DRE) and admitted to the Epileptic Monitoring Unit (EMU) at University Hospital for intracranial electroencephalography (iEEG) seizure monitoring. The information presented is based on the clinical study and expansive medical history available for each patient.

A total of 43 stimulations (STIM) were conducted during the IHG task, 18 in the right, and 25 in the left hemisphere of the prefrontal cortex. The stimulated iEEG electrode lines included 4 in the left and 3 in the right hemisphere, and covered regions of the prefrontal cortex including the MPFC, medial and lateral OFC, and pars orbitalis. Figure 4.1 illustrates electrode coordinates of the STIM condition in all 7 patients, projected onto the Montreal Neurological Institute (MNI) template brain space.

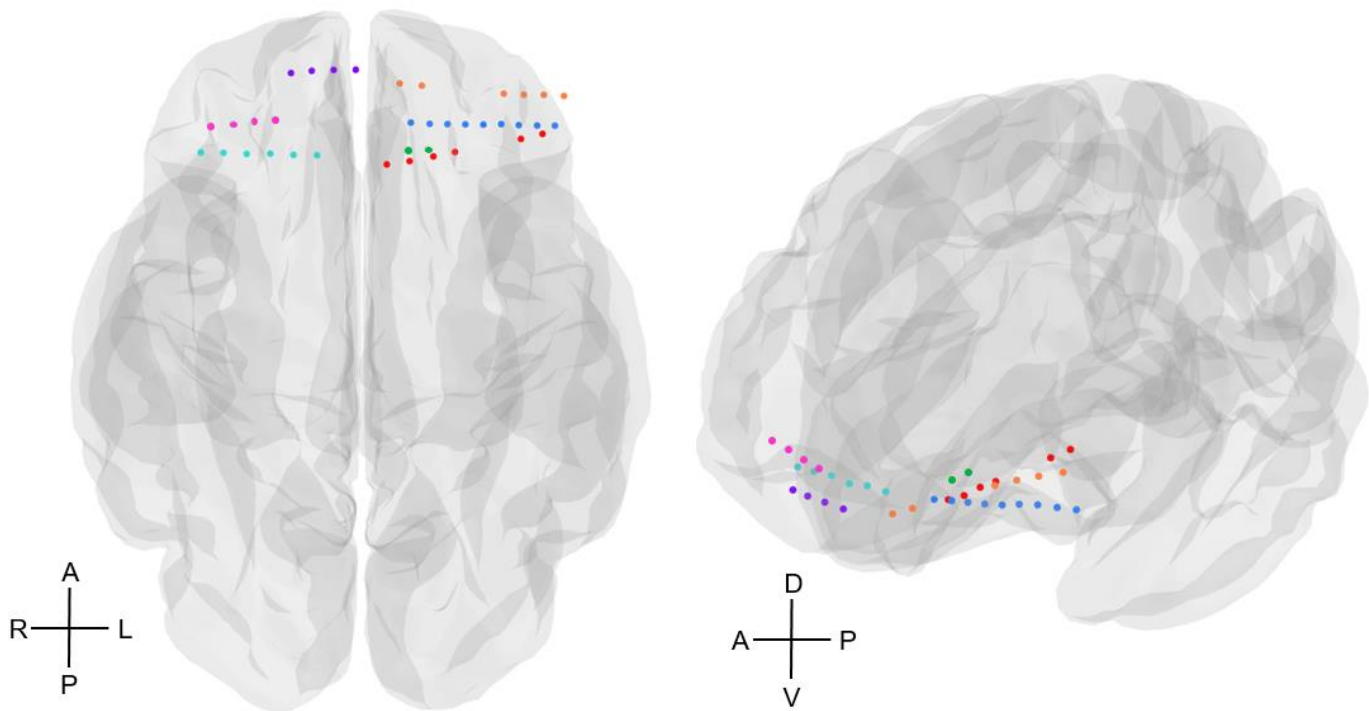


Figure 4.1: Montreal Neurological Institute (MNI) template brain with all electrodes used for stimulation IHG trials. The coordinate locations of electrodes were identified using co-registered CT and MRI scans, before being transformed to the MNI template brain. Each colour is representative of a different individual ($n = 7$).

Table 4.2 presents each participants averaged HR and standard deviation at the onset and during the transient response window, for all IHG trials performed. Notably, we report a large range of HR variability and resting HR within our sample of patients. This was anticipated with a

highly variable parameter like HR, but in our case, it may have been exaggerated in select individuals because of the nature of the patient population and the range of age differences.

Table 4.2: Age/Sex, resting heart rate (HR), and mean \pm standard deviation of HR responses to IHG (isometric hand grip) task trials of each participant. Resting HR obtained from 60-second recordings prior to testing in the study room. The two second interval windows used for HR at onset, and HR post IHG are described in the methods section.

Age/Sex	Resting HR	IHG			IHG + STIM		
		HR at onset	HR post IHG	Δ HR	HR at onset	HR post IHG	Δ HR
36 M	69	67 \pm 3	72 \pm 2	5 \pm 2	67 \pm 2	69 \pm 2	2 \pm 1
26 M	100	98 \pm 4	104 \pm 3	6 \pm 3	98 \pm 3	98 \pm 2	0 \pm 4
32 F	78	77 \pm 2	79 \pm 3	3 \pm 2	74 \pm 5	75 \pm 2	1 \pm 3
45 F	82	81 \pm 5	83 \pm 1	2 \pm 6	83 \pm 4	80 \pm 2	-3 \pm 4
24 M	88	84 \pm 13	93 \pm 7	10 \pm 8	90 \pm 4	97 \pm 4	7 \pm 1
45 F	84	84 \pm 3	90 \pm 4	5 \pm 1	83 \pm 1	86 \pm 1	3 \pm 0
57 M	77	76 \pm 2	79 \pm 1	3 \pm 3	78 \pm 1	79 \pm 2	1 \pm 3

There was a main effect of IHG on HR ($P = 0.021$), and an interaction was detected for the effect of IHG x STIM on HR ($P = 0.026$). Post-hoc t-tests with Bonferroni's correction determined that IHG increased HR during NO-STIM and SHAM conditions ($P = 0.006$), but IHG did not change HR during STIM conditions ($P = 0.421$). When comparing delta HR values shown in Figure 4.2, we found that IHG contractions at NO-STIM and SHAM increased HR by $\Delta 4.9 \pm 2.7$ bpm (80.9 \pm 8 to 85.8 \pm 11), compared to an attenuated HR response of $\Delta 1.7 \pm 3.1$ bpm (81.8 \pm 10 to 83.43 \pm 11) during STIM ($P = 0.024$). The calculated effect size (Cohen's d) for the sample was considered large ($d = 1.1$), and was used to compute a post hoc statistical power of 0.68 (1- β error probability). Importantly, these statistical tests were performed on aggregate data pooled from *experiment 1* (NO-STIM vs STIM) and *experiment 2* (SHAM vs STIM) protocols because the experiments were largely synonymous and the results were similar. Following testing, patients scored the 50% MVC IHG task on the BORG rating of perceived exertion scale at very light to fairly light, an average of 10 points (out of 20 total points).

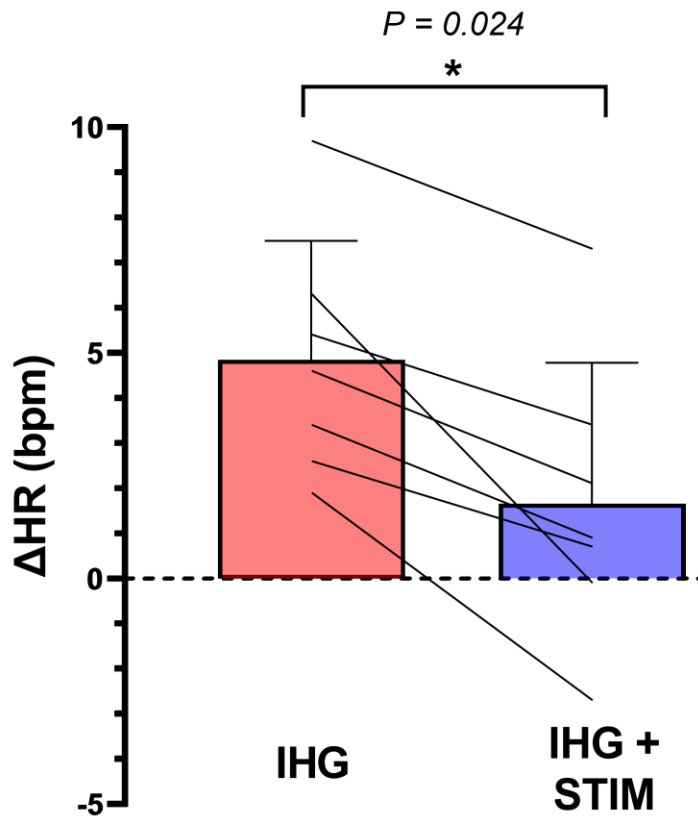


Figure 4.2: Delta heart rate (HR) responses during isometric handgrip (IHG) at SHAM and NO-STIM, versus IHG + STIM trials. Each participants ($n=7$) mean delta HR responses are shown. *Significantly different from IHG + STIM, $P = 0.024$.

Figure 4.3 presents the averaged transient HR responses to IHG trials of each participant plotted over a time series. Note, inter-individual variability was marked both in terms of the initial HR preceding IHG, and the timing of HR response peaks, which was most often observed to occur immediately following the two second IHG window.

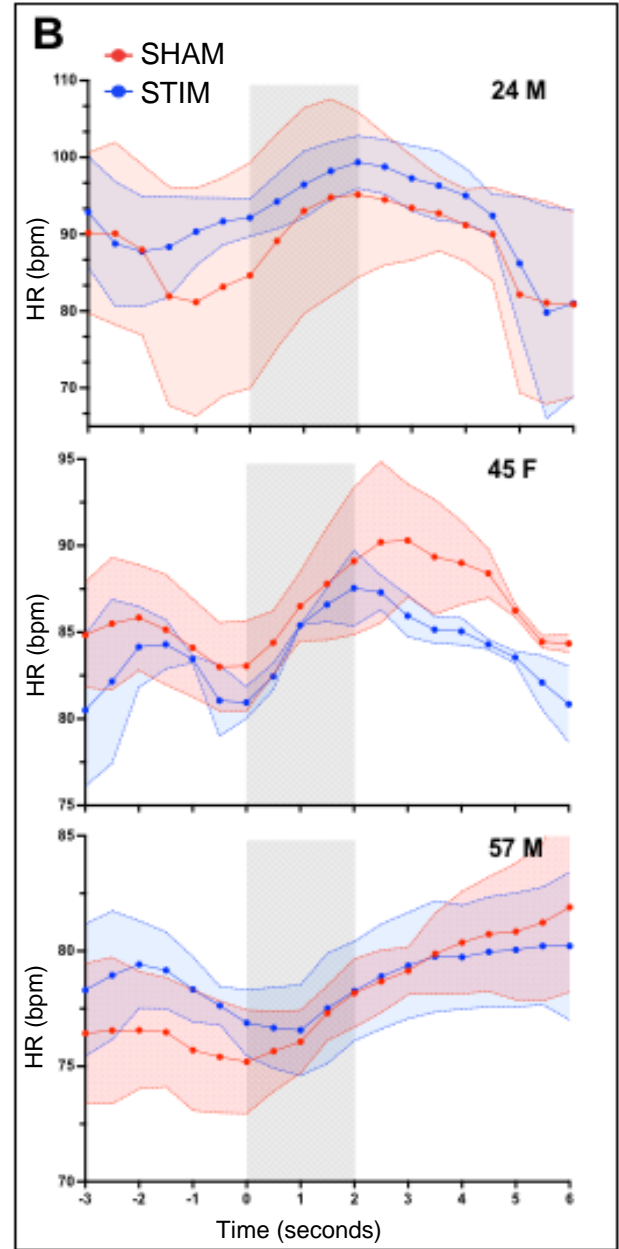
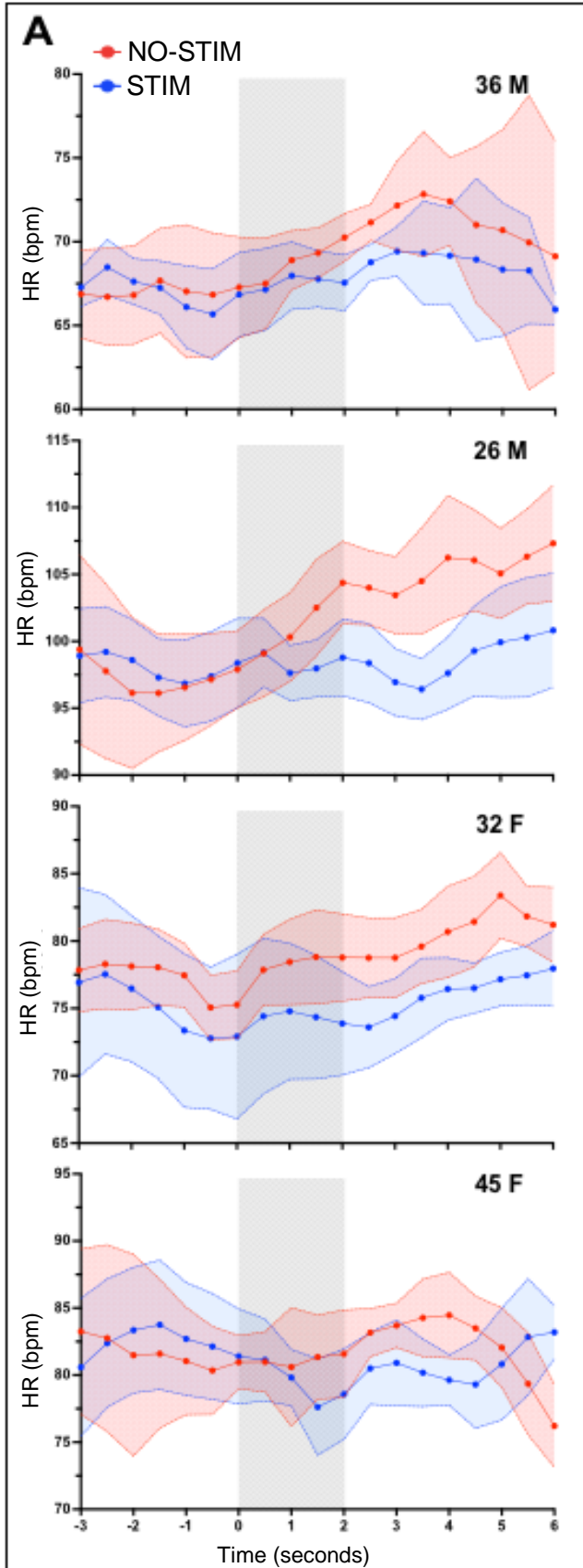


Figure 4.3: Heart rate (HR) time series of averaged isometric handgrip (IHG) trials for each participant, measurements of beats per minute (bpm) occur in 0.5 second intervals. Panel (A) includes participants (n = 4) completing no stimulation (NO-STIM) and STIM trials, and panel (B) includes participants (n = 3) completing sham-stimulation (SHAM) and STIM trials with randomized order. The IHG trials are represented the grey blocks from 0 to 2 seconds. STIM trials appeared to exhibit less HR variability during IHG, and tended to lack a defined peak HR response.

4.2 Excluded Subjects

4.2.1 Arrhythmia Patient

The first excluded subject was a 63 year-old female with DRE (2 seizures / month), presenting early as febrile convulsions at 9 months of age which progressed to tonic-clonic seizures. The patient underwent left anterior lobectomy when she was 38 but continued to have seizures originating bi-temporally. The patient was normotensive (125/74 mmHg), with an arrhythmia that was consistent during most of the ECG recording. Shown in Figure 4.4, her HR followed a repetitive irregular rhythm of two to three beats at a regular rate (~60-80 bpm), followed by a dramatically shortened R-R interval for one beat. This pattern persisted throughout most of the hour and a half recording during ESM, and no transient HR responses were observed during both MVC and 50% IHG trials. The arrhythmia appears to be involved in the patient's inability to generate hemodynamic response to exercise, and perhaps this irregularity of sino-atrial rhythm has further implications on her response to autonomic stress. Thus, we were unable to explain the cause of the heart arrhythmia and excluded the patient from study on this basis.

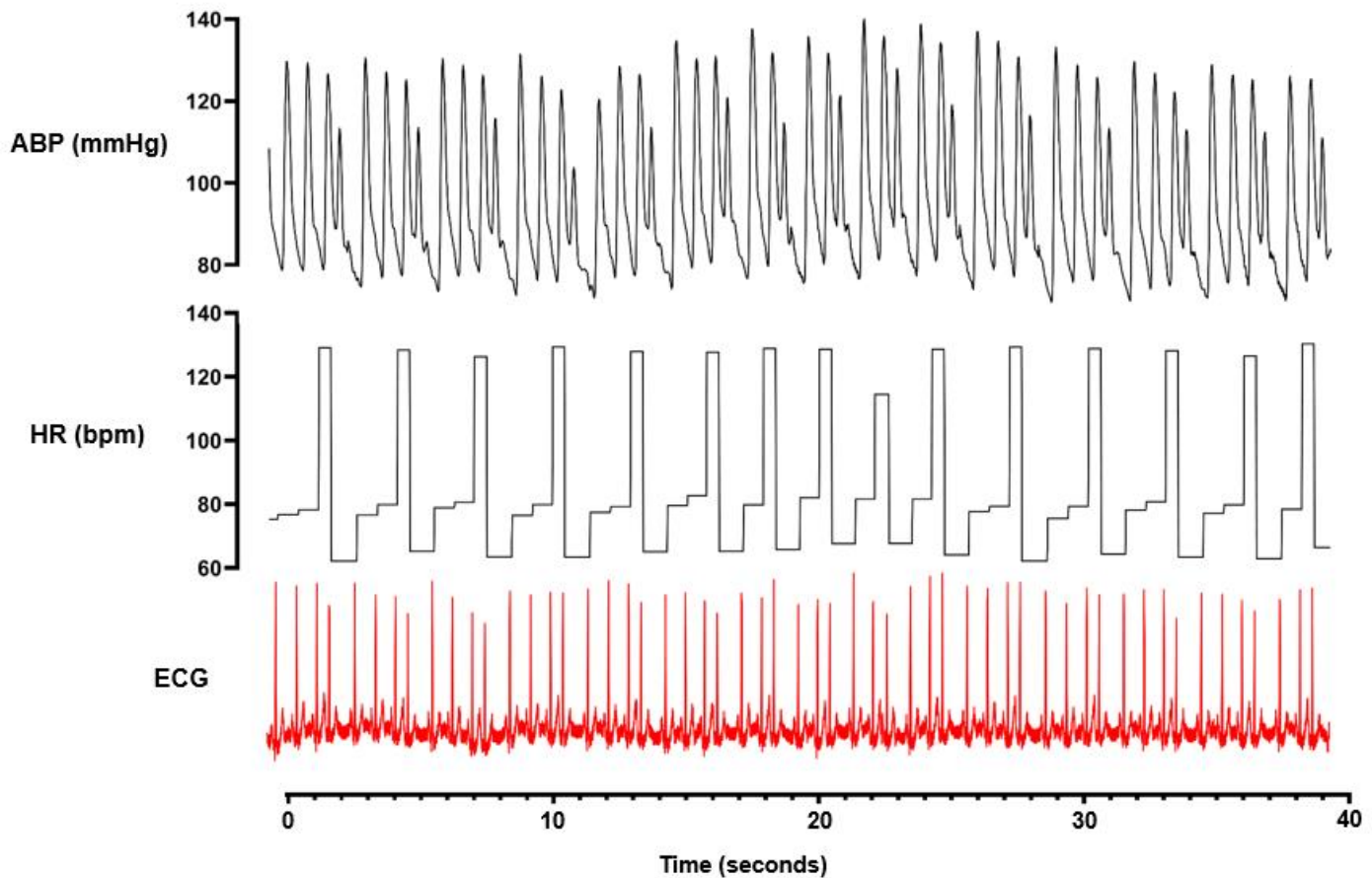


Figure 4.4: Arterial blood pressure (ABP), heart rate (HR), and electrocardiogram (ECG) recording of an excluded patient with a consistent arrhythmia. The patient's HR consistently exhibited the pattern illustrated, throughout the two-hour recording session. Note, the ECG signal was likely contaminated with noise from 60 Hz power line interference.

4.2.2 Prefrontal Seizure Patient

The second excluded subject was a 58-year-old female. The patient was normotensive (106/67 mmHg) with stable HR, and underwent callosotomy surgery when she was 37. Her seizures began at age 12, which progressed to bilateral tonic-clonic at age 36 when she was off all her medications. Notably, even when calibrating the maximal volitional effort IHG, the patient did not exhibit the expected transient HR response seen in older healthy individuals (Lalande et al., 2014). Following study of her clinical seizures in the EMU, the seizures were reported to onset from the orbitofrontal region (predominantly left) and immediately propagate to

the anterior insular region, followed by early progression to anterior cingulate, supplementary motor areas and spread to further recording contacts in both cerebral hemispheres. This progression was captured during clinical seizures recorded by iEEG lines at the EMU, and is illustrated in Figure 4.6. Importantly, her seizures appeared to onset independently and predominantly from the left, but also the right orbitofrontal regions, and she was referred for VNS therapy. In this case, the epileptogenic network was within our region of interest and was implicated in a circuit of neighboring regions also implicated in autonomic control. During orbitofrontal stimulation, no clinical seizures were elicited, but clinically silent seizures (i.e. no visible symptoms) and after discharges coincided with a marked hypotensive response. A hypotensive effect coinciding with a drop in HR occurred during multiple stimulations conducted in the left OFC, suggesting a sudden disruption in baroreflex regulation which may be associated with spreading electrical discharge (Figure 4.7). Another recent investigation in persons with epilepsy undergoing iEEG monitoring revealed that electrical stimulation of the MPFC (Brodmann 25) led to a near immediate drop in systolic blood pressure, without affecting diastolic or HR (Lacuey et al., 2018). After discharges resulting from left OFC stimulation during ESM are pictured in Figure 4.8.

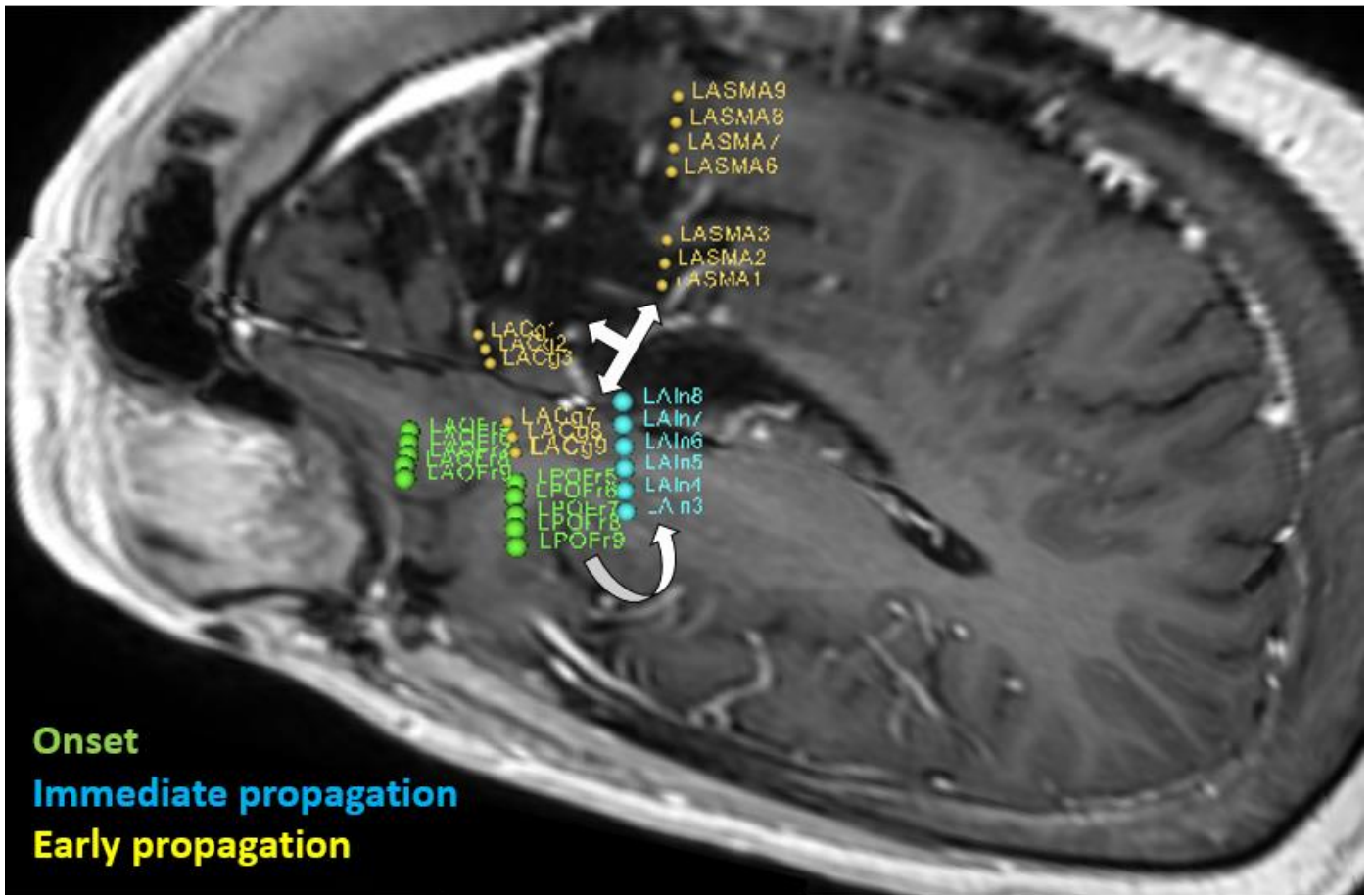


Figure 4.5: The proposed epileptogenic network in the brain of the excluded patient, based on clinical seizures observed during intracranial electroencephalography (iEKG) monitoring of clinical seizures. The seizures onset in the left anterior (LAOFr) and posterior orbitofrontal (LPOFr), with activity spreading to the left anterior insula (LAIn), left anterior cingulate (LACg), left anterior supplementary motor areas (LASMA), and eventually bilaterally. Note, while her seizures onset predominantly from the left hemisphere pictured here, the right orbitofrontal region was also responsible for independently producing seizures. Figure used with the permission of the Department of Neurological Sciences, University of Western Ontario, originally presented at epilepsy rounds (April 2021).

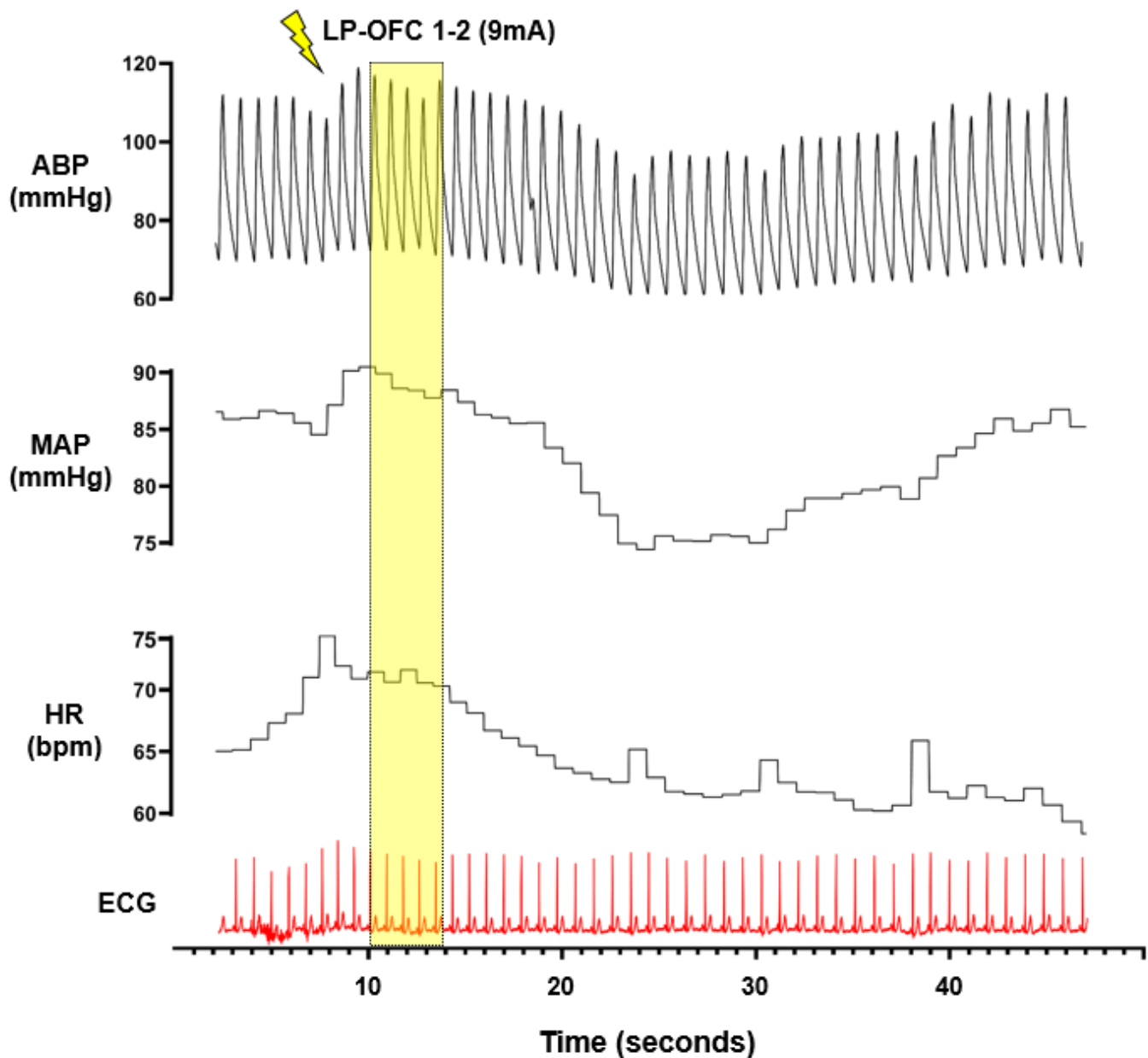


Figure 4.6: Stimulation (yellow shading) conducted in the left posterior orbitofrontal cortex line (LP-OFC), electrodes 1-2, at 9 mA. After-discharges following the stimulation coincided with a marked drop in arterial blood pressure (ABP) / mean arterial pressure (MAP). Interestingly, the baroreflex appears to be dysregulated during this period of spreading discharge, suggested by the concomitant drop in heart rate (HR).

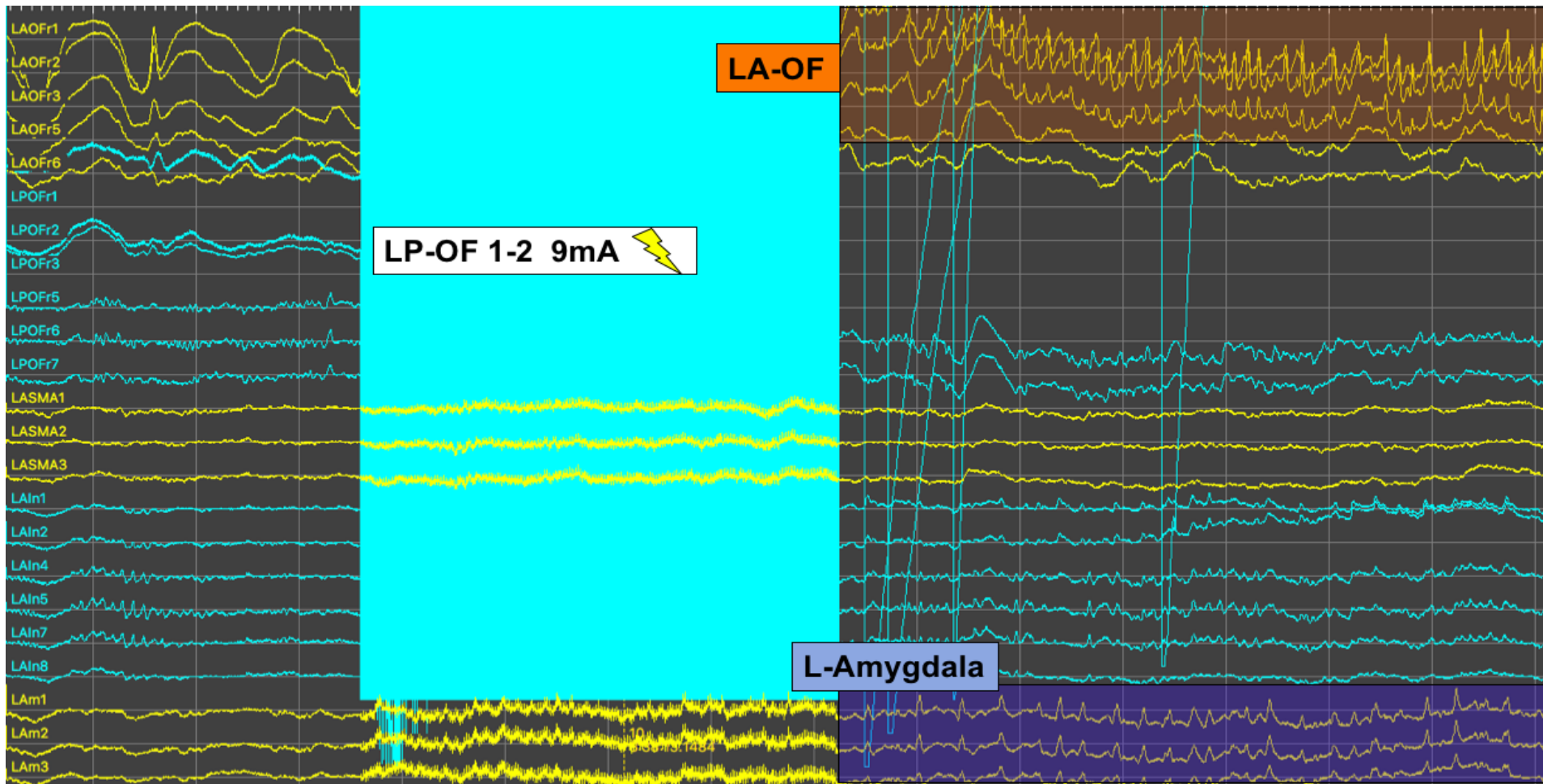


Figure 4.7: Intracranial EEG recording of stimulation conducted in the left posterior orbitofrontal cortex line (LP-OFC), electrodes 1-2, at an intensity of 9 mA. An immediate hypotension, and decrease in heart rate was simultaneously observed following stimulation, as well as synchronized spiking that appears to spread from left orbitofrontal to the left amygdala (L-Am) in this case. LA-OFR, left anterior orbitofrontal; LA-SMA, left anterior supplementary motor area; LA-In, left anterior insula.

Chapter 5

5 Discussion

The findings of the current investigation support the role of the prefrontal cortex in the transient cardiac response to IHG task, indicated by the diminished HR response to brief IHG during direct electrical stimulation of the OFC and MPFC regions in all subjects. Therefore, the present study is the first to describe that electrical stimulation of prefrontal cortical regions may modulate cardiac vagal outflow.

Past studies have identified prefrontal cortices as a key node involved in modulating cardiovascular responses within the widespread CAN (Al-Khazraji & Shoemaker, 2018; Cechetto & Shoemaker, 2009; Öngür & Price, 2000). Investigations of direct IC stimulation in persons with epilepsy with iEEG have reported cardiovascular responses of tachycardia and bradycardia (Al-Otaibi et al., 2010; Chouchou et al., 2019; Oppenheimer et al., 1992), but to our knowledge, no study has investigated HR responses of OFC/MPFC stimulation in humans. Neuroanatomically, the OFC and MPFC project onto the cingulate cortices, hippocampus, and the IC (Hurley et al., 1991; Neafsey et al., 1986; Thayer et al., 2009), forming a large interconnected network between the thalamus, PAG, and brain stem sites (i.e. NTS) with important roles in cardiovascular control (Benarroch, 1993; Öngür & Price, 2000; Verberne et al., 1997). One of the common problems with studying this network is identifying the specific function of discrete sites, which we addressed by conducting direct stimulations in the prefrontal cortical relay, albeit a widespread prefrontal region.

A significant role of prefrontal cortical regions in cardiac control has been demonstrated in functional neuroimaging studies (King et al., 1999; Norton et al., 2013; Ruiz Vargas et al., 2016; Thayer et al., 2012; Wong, Massé, et al., 2007), and experimental animal models using stimulation (Buchanan et al., 1985; Buchanan & Powell, 1984; Kaada et al., 1949; Owens et al., 1999; Smith, 1945), induced lesions (Fryszak & Neafsey, 1991, 1994; G. Ruit & Neafsey, 1990), and tracer studies (Hurley et al., 1991; Neafsey et al., 1986; Terreberry & Neafsey, 1983; Van Bockstaele et al., 1989; Van Der Kooy et al., 1982, 1984). However, this is the first study in humans to use direct electrical stimulation of the prefrontal region to modulate HR response to brief IHG exercise. Anatomically, invasive animal models have demonstrated the essential role

of connections between prefrontal cortical and subcortical autonomic structures (Öngür & Price, 2000), but it must be considered that these findings are limited to species with significantly underdeveloped prefrontal cortices (Floyd et al., 2000; Seamans et al., 2008). In humans, previous findings from functional neuroimaging investigations have reported that HR responses to handgrip exercise were correlated with deactivation of the MPFC from baseline levels (Wong, Massé, et al., 2007). The imaging studies suggest a highly reproducible relationship between a rapid deactivation of the MPFC region as HR increases in response to IHG. However, the mechanisms and contributions of cortical pathways mediating these changes in HR remain poorly understood. The rapid changes in HR associated with the onset of exercise are interpreted as a withdrawal of parasympathetic activity on the heart (Wong, Massé, et al., 2007). The immediate increase in HR observed at the onset of IHG exercise: i) can be largely diminished by vagal blockade (Hollander & Bouman, 1975; Mitchell et al., 1989; Victor et al., 1987), ii) is not altered by sympathetic (beta adrenoceptor) blockade (Mitchell et al., 1989), and iii) occurs before peripheral sympathetic activation (Mark et al., 1985; Wong, Massé, et al., 2007). Thus, we hypothesized that preventing the relative deactivation of this region by electrical stimulation to sustain focal neural activity should modulate the HR response. To emphasize the cardio-vagal mechanism, we used the brief two second static contraction task, allowing us to assess the rapid parasympathetic mediations associated with the HR response. Indeed, the novel direct stimulation approach prevented the HR response in the current participants. Taken together, we speculate that the attenuated HR responses observed during STIM were the result of a retention in vagal withdrawal associated with the IHG task.

Another factor related to the attenuation of HR during STIM could include the central command components associated with the perceptual aspect of the volitional IHG exercise (Williamson, 2010; Williamson et al., 2002). The concept of central command is described as the feed-forward mechanism involving concomitant activation of motor and cardiovascular centers (Williamson, 2010; Williamson et al., 2002), which appears to originate from supramedullary regions (Dampney, 2016; Goodwin et al., 1972). In the current investigation, the IHG exercise task did not produce a notable hyperemic response because of the relatively dismissible metabolic demands of the exercising muscle. For this reason, the tachycardia response might be interpreted as the anticipation or preparation for exercise (Krogh & Lindhard, 1913; Williamson et al., 2006). Although the IHG was an active task, the brief contraction used does not cause

increased arterial blood pressure responsible for activating visceral afferents observed in longer duration (~20 to 30 s) or fatiguing handgrip exercise (Al-Khazraji & Shoemaker, 2018; Macefield & Henderson, 2019b; Shoemaker, 2017; Topolovec et al., 2004). Thus, we may also discount contributions from the exercise pressor response (Mitchell et al., 1983). Similarly, the IHG task is likely too short for the accumulation of any significant chemical end-products of muscle contraction that are suggested to contribute to sympathetic activity (Batman et al., 1994; Victor & Seals, 1989; Williamson et al., 2003).

The isometric muscle contraction task used to elicit the cardiac response involved several constituents. While the IHG task neither is a strenuous or dynamic exercise, it has been suggested that cardiovascular responses to static exercises are dependent on the activity of fast twitch muscle fibres (Fallentin et al., 1985; Juhlin-Dannfelt et al., 1979), as well as slow twitch muscle fibres (Iwamoto & Botterman, 1985). In addition to the static muscle contraction component, the task also involved a degree of cognition and interpretation of real-time visual biofeedback used to gauge volitional effort with the required level of handgrip force. Interestingly, the OFC receives highly processed multimodal sensory information, such as visual inputs from inferior temporal visual regions (Thorpe et al., 1983), and somatosensory inputs from somatosensory, parietal, and insular cortices (Barbas, 1988; Carmichael & Price, 1995; Wallis, 2007). The OFC and MPFC regions have been labeled as sites of sensory integration linked to autonomic response planning (Bechara et al., 1996; Thayer et al., 2009; Wallis, 2007), and thus, it may be conceivable that STIM disrupted high-order processing responsible for initiating the cardiac autonomic response. Ongur and Price (2000) proposed that within the OFC and MPFC network, the OFC regions receive viscerosensory afferents, while the more medial wall of the prefrontal region sends visceral motor efferents to lower autonomic regions like the hypothalamus and brainstem (Öngür & Price, 2000). While we cannot draw any conclusions from the current data, the region covered by STIM include highly connected relays, and therefore, it is important to consider the numerous functions likely involved in the IHG task.

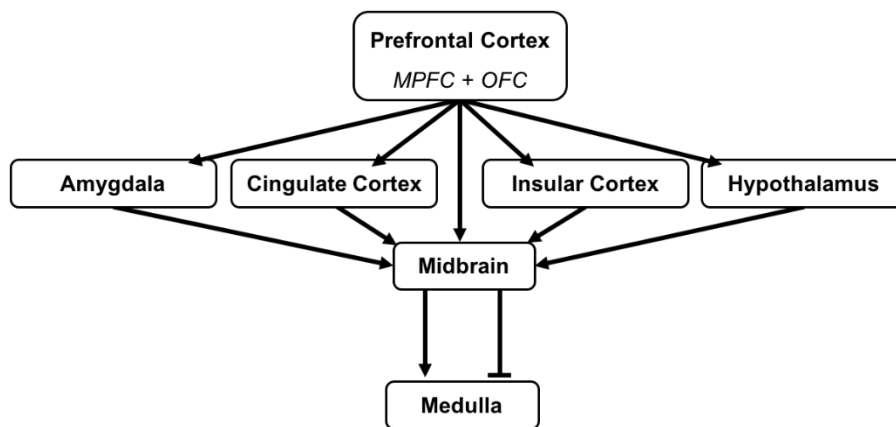


Figure 5.1: Simple schematic diagram showing the pathways by which the prefrontal cortices, including the medial prefrontal cortex (MPFC) and orbitofrontal cortex (OFC), may influence medullar regions controlling heart rate. It has been proposed that the prefrontal cortex is responsible for the highest order of processing, and is interconnected to the network through bidirectional pathways.

The ventro-medial region of the prefrontal cortex has been associated with high baseline metabolic activity at rest (Shulman et al., 1997), which exhibits decrease from this baseline during a wide range of nonspecific tasks in functional imaging studies (Gusnard et al., 2001; Gusnard & Raichle, 2001; Raichle et al., 2001; Wong, Massé, et al., 2007). This ‘default’ mode of brain function was hypothesized as the continuous processing of monitoring and integrating emotional and cognitive processes, which are suspended during goal-oriented behavior (Gusnard et al., 2001; Gusnard & Raichle, 2001; Raichle et al., 2001). From an autonomic perspective, reduced activity in the MPFC has a strong inverse correlation with a rise in HR (Wong, Massé, et al., 2007), and directly correlates with heart rate variability (a predictor of PNS influence on the heart) (Ruiz Vargas et al., 2016; Thayer et al., 2012). Furthermore, Nagai et al. demonstrated that sympathetic skin conductance negatively correlates with ventral-MPFC and OFC activity during both arousal and relaxation tasks (Nagai et al., 2004). Together, these findings indicate that reduced activity in the prefrontal region may have a direct influence in peripheral physiological adjustments during active tasks.

In the current study, the descending neural mechanisms of STIM are unclear, but perhaps may involve the modulation of direct projections from the OFC and MPFC on central autonomic sites such as the NTS (Terreberry & Neafsey, 1983; Van Der Kooy et al., 1982), PAG (Hurley et al., 1991; Neafsey et al., 1986), and hypothalamus (Öngür & Price, 2000; Van Der Kooy et al., 1984; Verberne et al., 1997). The NTS appears to be important in healthy cardiac control, serving as the initial site of termination for vagal and glossopharyngeal afferents (Beckstead & Norgren, 1979; Torvik, 1956), as well as other subnuclei which project to higher centers responsible for autonomic, endocrinic, and behavioural responses (Owens et al., 1999). Interestingly, a neuroimaging study of coronary artery disease patients reported that MPFC activity did not decrease from baseline during the IHG task (Norton et al., 2015), suggesting the function of this network may be altered in disease states.

The neuromodulation of cardio-vagal activity reported in the current and future studies may provide insight into clinical implications of the “brain-heart” connection. We provide evidence of contributions from prefrontal areas of the CAN involved in cardio-vagal mediated HR responses during IHG task. Importantly, CAN contributions to the sinoatrial node of the heart are under the tonic inhibitory control via the GABAergic neurons of the NTS (Owens et al., 1999; Potts, 2006; Thayer et al., 2012). The NTS shares direct interneuron connections with the NA and dorsal vagal motor nucleus (DVN) (Van Der Kooy et al., 1982), which provide input to cardio-vagal neurons (Benarroch, 1993; Thayer et al., 2012; Zubcevic & Potts, 2010). Vagal nerve activity flows in both directions, where efferents are important in parasympathetic innervation of the heart, lungs, kidneys, and liver (Levy, 1990), and afferents are involved in inflammatory response, visceral information, and blood pressure control via the baroreflex (Thayer & Sternberg, 2006). This bi-directional connection means efferent outflow from the brain affects the heart, and afferent outflow from the heart affect the brain. Unraveling these “brain-heart” pathways is essential in advancing the use of therapies including VNS used to reduce seizure frequency (DeGiorgio et al., 2000; Handforth et al., 1998; Ronkainen et al., 2006), and VNS therapy for treatment-resistant depression (Rush et al., 2000, 2005; Sackeim et al., 2001), or even assess the effectiveness of top-down electrical stimulation therapies such as the recently discovered benefits in mood-state and depression symptoms following stimulation of lateral OFC (Rao et al., 2018).

Therefore, the present investigation is the first to describe evidence on the neuromodulation of cardio-vagal HR response to IHG via direct electrical stimulation of the prefrontal cortices including the MPFC and OFC, which is in agreement with the previously proposed role of this cortical region. We suggest that these prefrontal cortices operate in the CAN as a key node in modulating transient cardio-vagally mediated HR adjustments, specifically in response to an IHG task involving viscerosensory arousal. However, care must be taken when interpreting these preliminary results. As discussed earlier, the MPFC and OFC are undoubtedly complex regions that integrate multimodal sensory information associated with the IHG task, and thus, our conclusions merit further investigation.

5.1 Limitations

The novelty and uniqueness of the current investigation presented methodological challenges and limitations that must be acknowledged. Although reductions of the HR response to IHG during STIM were consistent across all subjects, there were noticeable differences with respect to resting HR at the onset of IHG, the time course of the response, and most importantly, the magnitude of HR response. Patients were in the seated position for the duration of the clinical stimulation study, which in turn involves a degree of baroreflex unloading and reduces the magnitude of hemodynamic responses compared to the supine position (Lalande et al., 2014), likely due to a consistent reduction in overall cardio-vagal influence. Whilst the HR parameter is influenced by the interplay of various inputs to the sino-atrial node resulting in the complex variability characterized in HR time series data (Saul, 1990; Van Ravenswaaij-Arts *et al.*, 1993; Thayer *et al.*, 2012; Shaffer *et al.*, 2014), we expect these contributing factors to be similar across NO-STIM/SHAM and STIM trials which were all performed under the same environmental conditions. Also, our subject recruitment was dependent on the flow of patients admitted for iEEG monitoring at University Hospital, and as a consequence, we were unable to be selective of phenotypes in our recruited sample. Previous studies have reported both age and sex differences in the magnitude of HR responses to static handgrip exercise (Lalande et al., 2014; Wong, Kimmerly, et al., 2007). Thus, inter-subject variability was to be expected and unavoidable in our investigation.

We must also take into consideration and acknowledge the constraints imposed by the clinical nature of the experiment. The electrodes selected for STIM trials were limited by clinical procedure. Every patient recruited had iEEG monitoring coverage in the OFC. However, the laterality and exact location of electrodes within the prefrontal region was related to the epilepsy case being investigated, as well as surgical planning (i.e. avoiding blood vessels). Additionally, not all contacts on the prefrontal electrode lines were stimulated. This is because the co-registration of CT and MRI scans allows for careful planning to selectively target contacts placed in grey matter, which are more functionally relevant for mapping of eloquent cortex and localizing the epileptogenic zone during ESM (George et al., 2020). For other reasons such as limited time windows for ESM sessions, missing signals at electrode contacts, patient tolerance, and other constraints related to the clinical environment, it was not possible to stimulate all the electrodes of interest in subjects.

Lastly, the current investigation included a patient population diagnosed with DRE, and for this reason, we must mention the necessary considerations to keep in mind when discussing our findings and conclusions in the context of healthy individuals. In DRE patients, partial and generalized seizures may cause temporary instability in cardiac autonomic function, typically manifested by an over activation of sympathetic nervous system activity (Devinsky, 2004; Devinsky et al., 1986; Nass et al., 2019; Poh et al., 2010). However, during our data collection, the clinical team and trained EEG technicians continuously monitored iEEG recordings for any seizures or after discharges resulting from electrical stimulations, ensuring that no activity spread through the epileptogenic network during our data sampling periods. Also of note, these patients are prescribed therapeutic ASDs to suppress seizures, which may alter autonomic function (Devinsky, 2004; Lotufo et al., 2012). For example, one patient included in our study sample was prescribed carbamazepine (57 M), which has anticholinergic properties (Alrashood, 2016; Hennessy et al., 2001). Abrupt withdrawal of carbamazepine may cause increased sympathetic activity during sleep (Hennessy et al., 2001), and in some other ASDs, over dosage may cause fatal cardiac arrhythmias (Tomson & Kennebäck, 1997). In the weeks preceding our study, patients were under close care of nurses and practitioners, and are maintained on regular ASD dosage during testing. In the context of cardiovascular response to IHG exercise, we suggest that the reported hemodynamic responses to the IHG task should closely resemble those of healthy individuals.

It is also worthwhile mentioning that data collection was done at a hospital during the COVID-19 pandemic (Cucinotta & Vanelli, 2020), with periods of disrupted patient access or laboratory access. Because iEEG monitoring of patients is considered an elective surgery, patient flow was intermittently halted which had a large impact in limiting our sample size.

5.2 Future Directions

It is our hope that our preliminary work promotes future studies utilizing direct electrical stimulation as a model of investigating cardiovascular reflexes and relays to higher brain sites of visceral sensory information, to further aid our understanding of autonomic regulation within the central nervous system. This work has been limited in humans due to the invasive nature and the need for clinical models (Al-Otaibi et al., 2010; Oppenheimer et al., 1992). Future experiments may be a fundamental step in understanding the functional brain pathways related to cardiac control within the human CAN in health and disease.

The stimulations in the current investigation used biphasic current parameters preset for clinical neuroscientific study, which are optimized for defining eloquent brain areas and delimiting seizure foci for the purpose of epilepsy neurosurgery planning. For example, during clinical stimulation mapping of eloquent hippocampal cortex, patients performing a simple neurocognitive naming task may experience an inability to name an object during stimulation of hippocampal electrodes (Aron et al., 2021). The disruption of neurocognitive tasks may vary on the amplitude of current used, which usually ranges from 1 to 10 mA, and depends largely on the region's after-discharge threshold (George et al., 2020; Rao et al., 2018). It remains unknown whether a dose response profile exists between electric stimulation current amplitude and cardiovascular outcomes. Also, we used a standard pulse frequency of 50 Hz, and thus, the impact of lower frequency stimulations remains to be explored (Valentín et al., 2002). Similarly, the duration of the biphasic stimulation can be extended beyond the ~2-3 seconds used in the present study, or alternatively, a single-pulse stimulation can be conducted (Rao et al., 2018; Valentín et al., 2002), enabling approaches that study potential neural adaption phenomenon, or if prolonged stimulation exerts delayed cardiovascular outcomes.

The statistical power of the current study was 68% indicating that a larger sample size is needed to verify the current results. Further, sex-based differences analysis should be explored in the future to follow up on previous work on cardio-vagal contributions to hemodynamic

responses varying with sex (Wong, Kimmerly, et al., 2007). Similar questions should be addressed regarding age, and comorbidities with high prevalence in persons with epilepsy such as depression/anxiety (Kwon & Park, 2014; Rao et al., 2018). Furthermore, our work stimulated range of sites within the prefrontal cortex. Although the prefrontal region exhibits high intrinsic interconnectivity (Kahnt et al., 2012; Öngür & Price, 2000; Verberne & Owens, 1998), follow-up studies should aim to determine more specific roles related to cardio-vagal control in sub regions such as the MPFC which receives extensive inputs from limbic structures (Verberne & Owens, 1998), and OFC regions known for viscerosensory processing and a major role in ‘goal’ selection behaviour (Rolls & Grabenhorst, 2008).

While there are many future studies needed to take advantage of the iEEG model to explain cortical networks and cardiovascular control, it must be understood that the interpretability of the iEEG model is limited because electrical stimulations could affect cell body activation, synaptic junctions and/or axonal tracts and fibers of additional neurons passing through the stimulated region. Also, conducting direct electrical stimulations is not a “naturally” occurring stimulus and should be regarded as an external disruption to neurocircuitry in the brain.

5.3 Conclusion and Impact

Our study was the first to demonstrate attenuated HR response to IHG task during direct electrical stimulation of the prefrontal cortex in humans. This finding has significant impact on the understanding of central autonomic pathways regulating cardio-vagal activity. We provide knowledge useful in understanding conditions in which cardiac autonomic regulation is modulated, including fatal cardiac outcomes following stroke, and cardiac complications associated with epilepsy (i.e. SUDEP). It is our hope that our findings serve to underpin the “brain-heart” relationship and help guide future studies in this unique field such as VNS therapy in DRE and depression patients. Given the vast range of functions of the prefrontal region, STIM may have modified the relay involved in the coupling of cardio-vagal mediated HR responses with complex high-order neural systems that integrate activity in perceptual, viscerosensory, emotional, and memory systems. We have proposed that prefrontal cortical stimulations prevent normal reduction in cardio-vagal activity during IHG.

References

- Al-Khazraji, B. K., & Shoemaker, J. K. (2018). The human cortical autonomic network and volitional exercise in health and disease. *Applied Physiology, Nutrition and Metabolism*, *43*(11), 1122–1130. <https://doi.org/10.1139/apnm-2018-0305>
- Al-Otaibi, F., Wong, S. W. H., Shoemaker, J. K., Parrent, A. G., & Mirsattari, S. M. (2010). The cardioinhibitory responses of the right posterior insular cortex in an epileptic patient. *Stereotactic and Functional Neurosurgery*, *88*(6), 390–397. <https://doi.org/10.1159/000321182>
- Alrashood, S. T. (2016). Carbamazepine. *Profiles of Drug Substances, Excipients and Related Methodology*, *41*, 133–321. <https://doi.org/10.1016/bs.podrm.2015.11.001>
- Aron, O., Jonas, J., Colnat-Coulbois, S., & Maillard, L. (2021). Language Mapping Using Stereo Electroencephalography: A Review and Expert Opinion. *Frontiers in Human Neuroscience*, *15*(March), 1–12. <https://doi.org/10.3389/fnhum.2021.619521>
- Bandler, R., Carrive, P., & Zhang, S. P. (1991). Chapter 13 Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: Viscerotopic, somatotopic and functional organization. *Progress in Brain Research*, *87*(C), 269–305. [https://doi.org/10.1016/S0079-6123\(08\)63056-3](https://doi.org/10.1016/S0079-6123(08)63056-3)
- Bandler, R., Keay, K. A., Floyd, N., & Price, J. (2000). Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. *Brain Research*, *53*(1), 95–104.
- Barbas, H. (1988). Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. *Journal of Comparative Neurology*, *276*(3), 313–342. <https://doi.org/10.1002/cne.902760302>
- Barrash, J., Tranel, D., & Anderson, S. W. (2000). Acquired personality disturbances associated with bilateral damage to the ventromedial prefrontal region. *Developmental Neuropsychology*, *18*(3), 355–381. <https://doi.org/10.1207/S1532694205Barrash>
- Batman, B. A., Hardy, J. C., Leuenberger, U. A., Smith, M. B., Yang, Q. X., & Sinoway, L. I. (1994). Sympathetic nerve activity during prolonged rhythmic forearm exercise. *Journal of Applied Physiology*, *76*(3), 1077–1081. <https://doi.org/10.1152/jappl.1994.76.3.1077>
- Bechara, A., Tranel, D., Damasio, H., & Damasio, A. R. (1996). Failure to respond autonomically to anticipated future outcomes following damage to prefrontal cortex. *Cerebral Cortex*, *6*(2), 215–225.

<https://doi.org/10.1093/cercor/6.2.215>

- Beckstead, R. M., & Norgren, R. (1979). An autoradiographic examination of the central distribution of the trigeminal, facial, glossopharyngeal, and vagal nerves in the monkey. *Journal of Comparative Neurology*, *184*(3), 455–472. <https://doi.org/10.1002/cne.901840303>
- Behbehani, M. M. (1995). Functional characteristics of the midbrain periaqueductal gray. In *Progress in Neurobiology* (Vol. 46, Issue 6, pp. 575–605). Elsevier Science Ltd. [https://doi.org/10.1016/0301-0082\(95\)00009-K](https://doi.org/10.1016/0301-0082(95)00009-K)
- Benarroch, E. E. (1993). The Central Autonomic Network: Functional Organization, Dysfunction, and Perspective. *Mayo Clinic Proceedings*, *68*(10), 988–1001. [https://doi.org/10.1016/S0025-6196\(12\)62272-1](https://doi.org/10.1016/S0025-6196(12)62272-1)
- Bigger, J. T., Fleiss, J. L., Rolnitzky, L. M., & Steinman, R. C. (1993). Frequency domain measures of heart period variability to assess risk late after myocardial infarction. *Journal of the American College of Cardiology*, *21*(3), 729–736. [https://doi.org/10.1016/0735-1097\(93\)90106-B](https://doi.org/10.1016/0735-1097(93)90106-B)
- Blumenthal, M. R., Wang, I., Markee, S., & Wang, S. C. (1968). Effects of acetylcholine on the heart. *American Journal of Physical Medicine*, *214*(6), 1280–1287.
- Buchanan, S. L., & Powell, D. A. (1984). 3H-2-Deoxyglucose uptake after electrical stimulation of cardioactive sites in insular cortex and mediodorsal nucleus of the thalamus in rabbits. *Brain Research Bulletin*, *19*(4), 439–452. [https://doi.org/10.1016/0361-9230\(87\)90148-1](https://doi.org/10.1016/0361-9230(87)90148-1)
- Buchanan, S. L., Valentine, J., & Powell, D. A. (1985). Autonomic responses are elicited by electrical stimulation of the medial but not lateral frontal cortex in rabbits. *Behavioural Brain Research*, *18*, 51–62.
- Burns, S. M., & Wyss, M. J. (1985). The involvement of the anterior cingulate cortex in blood pressure control. *Brain Research*, *340*(1), 71–77. [https://doi.org/10.1016/0006-8993\(85\)90774-7](https://doi.org/10.1016/0006-8993(85)90774-7)
- Carmichael, S. T., & Price, J. L. (1995). Sensory and premotor connections of the orbital and medial prefrontal cortex of macaque monkeys. *Journal of Comparative Neurology*, *363*(4), 642–664. <https://doi.org/10.1002/cne.903630409>
- Carrive, P., & Bandler, R. (1991a). Control of extracranial and hindlimb blood flow by the midbrain periaqueductal grey of the cat. *Experimental Brain Research*, *84*(3), 599–606. <https://doi.org/10.1007/BF00230972>

- Carrive, P., & Bandler, R. (1991b). Viscerotopic organization of neurons subserving hypotensive reactions within the midbrain periaqueductal grey: a correlative functional and anatomical study. *Brain Research*, *541*(2), 206–215. [https://doi.org/10.1016/0006-8993\(91\)91020-2](https://doi.org/10.1016/0006-8993(91)91020-2)
- Cavada, C., Compañy, T., Tejedor, J., Cruz-Rizzolo, R. J., & Reinoso-Suárez, F. (2000). The anatomical connections of the macaque monkey orbitofrontal cortex. A review. *Cerebral Cortex*, *10*(3), 220–242. <https://doi.org/10.1093/cercor/10.3.220>
- Cechetto, D. F. (2014). Cortical control of the autonomic nervous system. *Experimental Physiology*, *99*(2), 326–331. <https://doi.org/10.1113/expphysiol.2013.075192>
- Cechetto, D. F., & Saper, C. B. (1987). Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. *Journal of Comparative Neurology*, *262*(1), 27–45. <https://doi.org/10.1002/cne.902620104>
- Cechetto, D. F., & Shoemaker, J. K. (2009). Functional neuroanatomy of autonomic regulation. *NeuroImage*, *47*(3), 795–803. <https://doi.org/10.1016/j.neuroimage.2009.05.024>
- Cheung, R. T. F., & Hachinski, V. (2000). The insula and cerebrogenic sudden death. *Archives of Neurology*, *57*(12), 1685–1688. <https://doi.org/10.1001/archneur.57.12.1685>
- Chouchou, F., Mauguière, F., Vallayer, O., Catenoix, H., Isnard, J., Montavont, A., Jung, J., Pichot, V., Rheims, S., & Mazzola, L. (2019). How the insula speaks to the heart: Cardiac responses to insular stimulation in humans. *Human Brain Mapping*, *40*(9), 2611–2622. <https://doi.org/10.1002/hbm.24548>
- Ciriello, J. (1983). Brainstem projections of aortic baroreceptor afferent fibers in the rat. *Neuroscience Letters*, *36*, 37–42.
- Colivicchi, F., Bassi, A., Santini, M., & Caltagirone, C. (2004). Cardiac autonomic derangement and arrhythmias in right-sided stroke with insular involvement. *Stroke*, *35*(9), 2094–2098. <https://doi.org/10.1161/01.STR.0000138452.81003.4c>
- Conny M. A. Van Ravenswaaij-Arts, Louis A. A. Kolleé, Hopman, J. C. W., Stoeltinga, G. B. A., & Geijn, H. P. van. (1993). Heart Rate Variability. In *Annals of Internal Medicine* (Vol. 17, Issue 6, pp. 436–447). <https://doi.org/10.1111/j.1540-8167.2006.00501.x>
- Critchley, H. D., Corfield, D. R., Chandler, M. P., Mathias, C. J., & Dolan, R. J. (2000). Cerebral correlates of autonomic cardiovascular arousal: a functional neuroimaging investigation in humans. *Journal of Physiology*, *523*(1), 259–270.

- Critchley, H. D., Elliott, R., Mathias, C. J., & Dolan, R. J. (2000). Neural activity relating to generation and representation of galvanic skin conductance responses: A functional magnetic resonance imaging study. *Journal of Neuroscience*, *20*(8), 3033–3040. <https://doi.org/10.1523/jneurosci.20-08-03033.2000>
- Cucinotta, D., & Vanelli, M. (2020). WHO declares COVID-19 a pandemic. *Acta Biomedica*, *91*(1), 157–160. <https://doi.org/10.23750/abm.v91i1.9397>
- Damasio, A. R., Tranel, D., & Damasio, H. (2013). Individuals with sociopathic behavior caused by frontal damage fail to respond autonomically to social stimuli. *The Science of Mental Health: Volume 7: Personality and Personality Disorder*, *41*, 297–310.
- Dampney, R. A. L. (2015). Central mechanisms regulating coordinated cardiovascular and respiratory function during stress and arousal. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *309*(5), R429–R443. <https://doi.org/10.1152/ajpregu.00051.2015>
- Dampney, R. A. L. (2016). Central neural control of the cardiovascular system: Current perspectives. *Advances in Physiology Education*, *40*(3), 283–296. <https://doi.org/10.1152/advan.00027.2016>
- Dampney, R. A. L. (2018). Emotion and the cardiovascular system: Postulated role of inputs from the medial prefrontal cortex to the dorsolateral periaqueductal gray. *Frontiers in Neuroscience*, *12*(MAY), 1–8. <https://doi.org/10.3389/fnins.2018.00343>
- DeGiorgio, C. M., Schachter, S. C., Handforth, A., Salinsky, M., Thompson, J., Uthman, B., Reed, R., Collin, S., Tecoma, E., Morris, G. L., Vaughn, B., Naritoku, D. K., Henry, T., Labar, D., Gilmartin, R., Labiner, D., Osorio, I., Ristanovic, R., Jones, J., ... Heck, C. (2000). Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures. *Epilepsia*, *41*(9), 1195–1200. <https://doi.org/10.1111/j.1528-1157.2000.tb00325.x>
- Devinsky, O. (2004). Effects of Seizures on Autonomic and Cardiovascular Function. *Epilepsy Currents*, *4*(2), 43–46. <https://doi.org/10.1111/j.1535-7597.2004.42001.x>
- Devinsky, O., Price, B. H., & Cohen, S. I. (1986). Cardiac manifestations of complex partial seizures. *The American Journal of Medicine*, *80*(2), 195–202. [https://doi.org/10.1016/0002-9343\(86\)90009-4](https://doi.org/10.1016/0002-9343(86)90009-4)
- Dreifus, L. S., Agarwal, J. B., Botvinick, E. H., Ferdinand, K. C., Fisch, C., Fisher, J. D., Kennedy, J. W., Kerber, R. E., Lambert, C. R., Okike, O. N., Prystowsky, E. N., Saksena, S. V., Schroeder, J. S., & Williams, D. O. (1993). Heart rate variability for risk stratification of life-threatening arrhythmias. *Journal of the American College of Cardiology*, *22*(3), 948–950. <https://doi.org/10.1016/0735->

1097(93)90217-O

- Drevets, W. C., Öngür, D., & Price, J. L. (1998). Neuroimaging abnormalities in the subgenual prefrontal cortex: Implications for the pathophysiology of familial mood disorders. *Molecular Psychiatry*, 3(3), 220–226. <https://doi.org/10.1038/sj.mp.4000370>
- Drevets, Wayne C., Price, J. L., Simpson, J. R., Todd, R. D., Reich, T., Vannier, M., & Raichle, M. E. (1997). Subgenual prefrontal cortex abnormalities in mood disorders. In *Nature* (Vol. 386, Issue 6627, pp. 824–827). <https://doi.org/10.1038/386824a0>
- Fallentin, N., Sidenius, B., & Jorgensen, K. (1985). Blood pressure, heart rate and EMG in low level static contractions. *Acta Physiologica Scandinavica*, 125(2), 265–275. <https://doi.org/10.1111/j.1748-1716.1985.tb07715.x>
- Ficker, D. M. (2000). Sudden unexplained death and injury in epilepsy. *Epilepsia*, 41(6 SUPPL. 2), 0–5. <https://doi.org/10.1111/j.1528-1157.2000.tb01519.x>
- Floyd, N. S., Price, J. L., Ferry, A. T., Keay, K. A., & Bandler, R. (2000). Orbitomedial prefrontal cortical projections to distinct longitudinal columns of the periaqueductal gray in the rat. *Journal of Comparative Neurology*, 422(4), 556–578. [https://doi.org/10.1002/1096-9861\(20000710\)422:4<556::AID-CNE6>3.0.CO;2-U](https://doi.org/10.1002/1096-9861(20000710)422:4<556::AID-CNE6>3.0.CO;2-U)
- Fontollet, T., Pichot, V., Bringard, A., Fagoni, N., Adami, A., Tam, E., Furlan, R., Barthélémy, J. C., & Ferretti, G. (2018). Testing the vagal withdrawal hypothesis during light exercise under autonomic blockade: A heart rate variability study. *Journal of Applied Physiology*, 125(6), 1804–1811. <https://doi.org/10.1152/jappphysiol.00619.2018>
- Francis, S., Rolls, E. T., Bowtell, R., McGlone, F., O’Doherty, J., Browning, A., Clare, S., & Smith, E. (1999). The representation of pleasant touch in the brain and its relationship with taste and olfactory areas. *NeuroReport*, 10(3), 453–459. <https://doi.org/10.1097/00001756-199902250-00003>
- Franklin, T. B., Silva, B. A., Perova, Z., Marrone, L., Masferrer, M. E., Zhan, Y., Kaplan, A., Greetham, L., Verrechia, V., Halman, A., Pagella, S., Vyssotski, A. L., Illarionova, A., Grinevich, V., Branco, T., & Gross, C. T. (2017). Prefrontal cortical control of a brainstem social behavior circuit. *Nature Neuroscience*, 20(2), 260–270. <https://doi.org/10.1038/nn.4470>
- Freyschuss, U. (1970). Elicitation of heart rate and blood pressure increase on muscle contraction. *Journal of Applied Physiology*, 28(6), 758–761. <https://doi.org/10.1152/jappl.1970.28.6.758>
- Fryszak, R. J., & Neafsey, E. J. (1991). The effect of medial frontal cortex lesions on Respiration,

- “Freezing,” and Ultrasonic Vocalizations during Conditioned Emotional Responses in Rats. *Brain Research*, 643(1–2), 181–193. [https://doi.org/10.1016/0006-8993\(94\)90024-8](https://doi.org/10.1016/0006-8993(94)90024-8)
- Fryszak, R. J., & Neafsey, E. J. (1994). The effect of medial frontal cortex lesions on cardiovascular conditioned emotional responses in the rat. *Brain Research*, 643(1–2), 181–193. [https://doi.org/10.1016/0006-8993\(94\)90024-8](https://doi.org/10.1016/0006-8993(94)90024-8)
- George, D. D., Ojemann, S. G., Drees, C., & Thompson, J. A. (2020). Stimulation Mapping Using Stereoelectroencephalography: Current and Future Directions. *Frontiers in Neurology*, 11(May), 1–7. <https://doi.org/10.3389/fneur.2020.00320>
- Goodwin, G. M., McCloskey, D. I., & Mitchell, J. H. (1972). Cardiovascular and Respiratory Responses to Changes in Central Command During Isometric Exercise at Constant Muscle Tension. *Journal of Physiology*, 226, 173–190.
- Goswami, R., Frances, M. F., & Shoemaker, J. K. (2011). Representation of somatosensory inputs within the cortical autonomic network. *NeuroImage*, 54(2), 1211–1220. <https://doi.org/10.1016/j.neuroimage.2010.09.050>
- Goswami, R., Frances, M. F., Steinback, C. D., & Kevin Shoemaker, J. (2012). Forebrain organization representing baroreceptor gating of somatosensory afferents within the cortical autonomic network. *Journal of Neurophysiology*, 108(2), 453–466. <https://doi.org/10.1152/jn.00764.2011>
- Green, A. L., Wang, S., Owen, S. L. F., Xie, K., Bittar, R. G., Stein, J. F., Paterson, D. J., & Aziz, T. Z. (2006). Stimulating the human midbrain to reveal the link between pain and blood pressure. *Pain*, 124(3), 349–359. <https://doi.org/10.1016/j.pain.2006.05.005>
- Green, A. L., Wang, S., Owen, S. L. F., Xie, K., Liu, X., Paterson, D. J., Stein, J. F., Bain, P. G., & Aziz, T. Z. (2005). Deep brain stimulation can regulate arterial blood pressure in awake humans. *NeuroReport*, 16(16), 1741–1745. <https://doi.org/10.1097/01.wnr.0000183904.15773.47>
- Guo, Z. L., Li, P., & Longhurst, J. C. (2002). Central pathways in the pons and midbrain involved in cardiac sympathoexcitatory reflexes in cats. *Neuroscience*, 113(2), 435–447. [https://doi.org/10.1016/S0306-4522\(02\)00173-2](https://doi.org/10.1016/S0306-4522(02)00173-2)
- Gusnard, D. A., Akbudak, E., Shulman, G. L., & Raichle, M. E. (2001). Medial prefrontal cortex and self-referential mental activity: Relation to a default mode of brain function. *Proc. Natl. Acad. Sci.*, 98(7), 4259–4264.
- Gusnard, D. A., & Raichle, M. E. (2001). Searching for a baseline: Functional imaging and the resting

- human brain. *Nature Reviews Neuroscience*, 2(10), 685–694. <https://doi.org/10.1038/35094500>
- Guyenet, P. G., Haselton, J. R., & Sun, M. K. (1989). Sympathoexcitatory neurons of the rostroventrolateral medulla and the origin of the sympathetic vasomotor tone. *Progress in Brain Research*, 81(C), 105–116. [https://doi.org/10.1016/S0079-6123\(08\)62002-6](https://doi.org/10.1016/S0079-6123(08)62002-6)
- Hachinski, V. C., Oppenheimer, S. M., Wilson, J. X., Guiraudon, C., & Cechetto, D. F. (1992). Asymmetry of Sympathetic Consequences of Experimental Stroke. *Archives of Neurology*, 49(7), 697–702. <https://doi.org/10.1001/archneur.1992.00530310039010>
- Handforth, A., DeGiorgio, C. M., Schachter, S. C., Uthman, B. M., & Naritoku, D. K. (1998). Vagus nerve stimulation therapy for partial-onset seizures. *Neurology*, 51(1), 48–55. <https://doi.org/10.1097/ANA.0b013e31815b7df1>
- Hänsel, A., & Von Känel, R. (2008). The ventro-medial prefrontal cortex: A major link between the autonomic nervous system, regulation of emotion, and stress reactivity? *BioPsychoSocial Medicine*, 2, 1–5. <https://doi.org/10.1186/1751-0759-2-21>
- Harper, R. M., Bandler, R., Spriggs, D., & Alger, J. R. (2000). Lateralized and widespread brain activation during transient blood pressure elevation revealed by magnetic resonance imaging. *Journal of Comparative Neurology*, 417(2), 195–204. [https://doi.org/10.1002/\(SICI\)1096-9861\(20000207\)417:2<195::AID-CNE5>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1096-9861(20000207)417:2<195::AID-CNE5>3.0.CO;2-V)
- Hennessy, M. J., Tighe, M. G., Binnie, C. D., & Nashef, L. (2001). Sudden withdrawal of carbamazepine increases cardiac sympathetic activity in sleep. *Neurology*, 57(9), 1650–1654. <https://doi.org/10.1212/WNL.57.9.1650>
- Hilz, M. J., Devinsky, O., Szczepanska, H., Borod, J. C., Marthol, H., & Tutaj, M. (2006). Right ventromedial prefrontal lesions result in paradoxical cardiovascular activation with emotional stimuli. *Brain*, 129(12), 3343–3355. <https://doi.org/10.1093/brain/awl299>
- Hollander, A. P., & Bouman, L. N. (1975). Cardiac acceleration in man elicited by a muscle-heart reflex. *Journal of Applied Physiology*, 38(2), 395–403.
- Hurley-Gius, K. M., & Neafsey, E. J. (1986). The medial frontal cortex and gastric motility: Microstimulation results and their possible significance for the overall pattern of organization of rat frontal and parietal cortex. *Brain Research*, 365(2), 241–248. [https://doi.org/10.1016/0006-8993\(86\)91635-5](https://doi.org/10.1016/0006-8993(86)91635-5)
- Hurley, K. M., Herbert, H., Moga, M. M., & Saper, C. B. (1991). Efferent projections of the infralimbic

- cortex of the rat. *Journal of Comparative Neurology*, 308(2), 249–276.
<https://doi.org/10.1002/cne.903080210>
- Inui, K., Murase, S., & Nosaka, S. (1994). Facilitation of the arterial baroreflex by the ventrolateral part of the midbrain periaqueductal grey matter in rats. *Journal of Physiology*, 477(1), 89–101.
- Iwamoto, G. A., & Botterman, B. R. (1985). Peripheral factors influencing expression of pressor reflex evoked by muscular contraction. *Journal of Applied Physiology*, 58(5), 1676–1682.
<https://doi.org/10.1152/jappl.1985.58.5.1676>
- Jiang, W., Glassman, A., Krishnan, R., O'Connor, C. M., & Califf, R. M. (2005). Depression and ischemic heart disease: What have we learned so far and what must we do in the future? *American Heart Journal*, 150(1), 54–78. <https://doi.org/10.1016/j.ahj.2005.04.012>
- Joynt, K. E., Whellan, D. J., & O'Connor, C. M. (2003). Depression and cardiovascular disease: Mechanisms of interaction. *Biological Psychiatry*, 54(3), 248–261. [https://doi.org/10.1016/S0006-3223\(03\)00568-7](https://doi.org/10.1016/S0006-3223(03)00568-7)
- Juhlin-Dannfelt, A., Frisk-Holmberg, M., Karlsson, J., & Tesch, P. (1979). Central and peripheral circulation in relation to muscle-fibre composition in normo- and hyper-tensive man. *Clinical Science*, 56(4), 335–340. <https://doi.org/10.1042/cs0560335>
- Kaada, B. R., Primbram, K. H., & Epstein, J. (1949). Respiratory and vascular responses in monkeys from temporal pole, insula, orbital surface and cingulate gyrus. *Journal of Neurophysiology*, 347–356.
- Kahnt, T., Chang, L. J., Park, S. Q., Heinzle, J., & Haynes, J. D. (2012). Connectivity-based parcellation of the human orbitofrontal cortex. *Journal of Neuroscience*, 32(18), 6240–6250.
<https://doi.org/10.1523/JNEUROSCI.0257-12.2012>
- Keay, K. A., & Bandler, R. (2001). Parallel circuits mediating distinct emotional coping reactions to different types of stress. *Neuroscience and Biobehavioral Reviews*, 25(7–8), 669–678.
[https://doi.org/10.1016/S0149-7634\(01\)00049-5](https://doi.org/10.1016/S0149-7634(01)00049-5)
- King, A. B., Menon, R. S., Hachinski, V., & Cechetto, D. F. (1999). Human forebrain activation by visceral stimuli. *Journal of Comparative Neurology*, 413(4), 572–582.
[https://doi.org/10.1002/\(SICI\)1096-9861\(19991101\)413:4<572::AID-CNE6>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1096-9861(19991101)413:4<572::AID-CNE6>3.0.CO;2-S)
- Krogh, A., & Lindhard, J. (1913). The regulation of respiration and circulation during the initial stages of muscular work. *The Journal of Physiology*, 47(1–2), 112–136.

- Kwon, O. Y., & Park, S. P. (2014). Depression and anxiety in people with epilepsy. *Journal of Clinical Neurology (Korea)*, *10*(3), 175–188. <https://doi.org/10.3988/jcn.2014.10.3.175>
- Lacuey, N., Hampson, J. P., Theeranaew, W., Zonjy, B., Vithala, A., Hupp, N. J., Loparo, K. A., Miller, J. P., & Lhatoo, S. D. (2018). Cortical structures associated with human blood pressure control. *JAMA Neurology*, *75*(2), 194–202. <https://doi.org/10.1001/jamaneurol.2017.3344>
- Lalande, S., Sawicki, C. P., Baker, J. R., & Shoemaker, J. K. (2014). Effect of age on the hemodynamic and sympathetic responses at the onset of isometric handgrip exercise. *Journal of Applied Physiology*, *116*(2), 222–227. <https://doi.org/10.1152/jappphysiol.01022.2013>
- LeDoux, J. E., Sakaguchi, A., & Reis, D. J. (1983). Subcortical Conditioned Projections of the Medial Nucleus Mediate Emotional Responses. *Journal of Neuroscience*, *4*(3), 683–698.
- Leonard, B., Mitchell, J. H., Mizuno, M., Rube, N., Saltin, B., & Secher, N. H. (1985). Partial neuromuscular blockade and cardiovascular responses to static exercise in man. *The Journal of Physiology*, *359*(1), 365–379. <https://doi.org/10.1113/jphysiol.1985.sp015590>
- Levy, M. N. (1990). Autonomic Interactions in Cardiac Control. *Annals of the New York Academy of Sciences*, *601*(1), 209–221. <https://doi.org/10.1111/j.1749-6632.1990.tb37302.x>
- Liu, S. S., & Ziegelstein, R. C. (2010). Depression in patients with heart disease: the case for more trials. *Future Cardiology*, *6*(4), 547–556. <https://doi.org/10.2217/fca.10.18.Depression>
- Lotufo, P. A., Valiengo, L., Benseñor, I. M., & Brunoni, A. R. (2012). A systematic review and meta-analysis of heart rate variability in epilepsy and antiepileptic drugs. *Epilepsia*, *53*(2), 272–282. <https://doi.org/10.1111/j.1528-1167.2011.03361.x>
- Lovick, T. A. (1985). Ventrolateral medullary lesions block the antinociceptive and cardiovascular responses elicited by stimulating the dorsal periaqueductal grey matter in rats. *Pain*, *21*(3), 241–252. [https://doi.org/10.1016/0304-3959\(85\)90088-0](https://doi.org/10.1016/0304-3959(85)90088-0)
- Macefield, V. G., & Henderson, L. A. (2019a). Identification of the human sympathetic connectome involved in blood pressure regulation. *NeuroImage*, *202*(August). <https://doi.org/10.1016/j.neuroimage.2019.116119>
- Macefield, V. G., & Henderson, L. A. (2019b). Identification of the human sympathetic connectome involved in blood pressure regulation. *NeuroImage*, *202*, 116119. <https://doi.org/10.1016/j.neuroimage.2019.116119>

- Mark, A. L., Victor, R. G., Nerhed, C., & Wallin, B. G. (1985). Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circulation Research*, 57(3), 461–469. <https://doi.org/10.1161/01.RES.57.3.461>
- Mitchell, J. H., Kaufman, M. P., & Iwamoto, G. A. (1983). The exercise pressor reflex: its cardiovascular effects, afferent mechanisms, and central pathways. *Annual Review of Physiology*, 45(75), 229–242. <https://doi.org/10.1146/annurev.ph.45.030183.001305>
- Mitchell, J. H., Reeves, D. R., Rogers, H. B., Secher, N. H., & Victor, R. G. (1989). Autonomic blockade and cardiovascular responses to static exercise in partially curarized man. *The Journal of Physiology*, 413(1), 433–445. <https://doi.org/10.1113/jphysiol.1989.sp017662>
- Mitchell, J. H. (2012). Neural control of the circulation during exercise: Insights from the 1970-1971 Oxford studies. *Experimental Physiology*, 97(1), 14–19. <https://doi.org/10.1113/expphysiol.2011.058156>
- Mitchell, J. H. (2013). Neural circulatory control during exercise: Early insights. *Experimental Physiology*, 98(4), 867–878. <https://doi.org/10.1113/expphysiol.2012.071001>
- Nagai, Y., Critchley, H. D., Featherstone, E., Trimble, M. R., & Dolan, R. J. (2004). Activity in ventromedial prefrontal cortex covaries with sympathetic skin conductance level: A physiological account of a “default mode” of brain function. *NeuroImage*, 22(1), 243–251. <https://doi.org/10.1016/j.neuroimage.2004.01.019>
- Nass, R. D., Hampel, K. G., Elger, C. E., & Surges, R. (2019). Blood pressure in seizures and epilepsy. *Frontiers in Neurology*, 10(MAY), 1–10. <https://doi.org/10.3389/fneur.2019.00501>
- Neafsey, E. J. (1991). Chapter 7 Prefrontal cortical control of the autonomic nervous system: Anatomical and physiological observations. *Progress in Brain Research*, 85(C), 147–166. [https://doi.org/10.1016/S0079-6123\(08\)62679-5](https://doi.org/10.1016/S0079-6123(08)62679-5)
- Neafsey, E. J., Hurley-Gius, K. M., & Arvanitis, D. (1986). The topographical organization of neurons in the rat medial frontal, insular and olfactory cortex projecting to the solitary nucleus, olfactory bulb, periaqueductal gray and superior colliculus. *Brain Research*, 377(2), 261–270. [https://doi.org/10.1016/0006-8993\(86\)90867-X](https://doi.org/10.1016/0006-8993(86)90867-X)
- Norton, K. N., Badrov, M. B., Barron, C. C., Suskin, N., Heinecke, A., & Shoemaker, J. K. (2015). Coronary artery disease affects cortical circuitry associated with brain-heart integration during volitional exercise. *Journal of Neurophysiology*, 114(2), 835–845.

<https://doi.org/10.1152/jn.00008.2015>

- Norton, K. N., Luchyshyn, T. A., & Shoemaker, J. K. (2013). Evidence for a medial prefrontal cortex-hippocampal axis associated with heart rate control in conscious humans. *Brain Research, 1538*, 104–115. <https://doi.org/10.1016/j.brainres.2013.09.032>
- Ogawa, S., Lee, T. M., Kay, A. R., & Tank, D. W. (1976). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Biophysics, 419*(2), 223–228. [https://doi.org/10.1016/0005-2736\(76\)90348-5](https://doi.org/10.1016/0005-2736(76)90348-5)
- Öngür, D., AN, X., & Price, J. L. (1998). Prefrontal cortical projections to the hypothalamus in Macaque monkeys. *The Journal of Comparative Neurology, 401*(4), 480–505. [https://doi.org/10.1002/\(sici\)1096-9861\(19981130\)401:4<480::aid-cne4>3.0.co;2-f](https://doi.org/10.1002/(sici)1096-9861(19981130)401:4<480::aid-cne4>3.0.co;2-f)
- Öngür, D., & Price, J. L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral Cortex, 10*(3), 206–219. <https://doi.org/10.1093/cercor/10.3.206>
- Oppenheimer, S. M., Cechetto, D. F., & Hachinski, V. C. (1990). Cerebrogenic Cardiac Arrhythmias: Cerebral Electrocardiographic Influences and Their Role in Sudden Death. *Archives of Neurology, 47*(5), 513–519. <https://doi.org/10.1001/archneur.1990.00530050029008>
- Oppenheimer, S. M., Gelb, A., Girvin, J. P., & Hachinski, V. C. (1992). Cardiovascular effects of human insular cortex stimulation. *Neurology, 42*(9), 1727–1727. <https://doi.org/10.1212/WNL.42.9.1727>
- Owens, N. C., Sartor, D. M., & Verberne, A. J. M. (1999). Medial prefrontal cortex depressor response: Role of the solitary tract nucleus in the rat. *Neuroscience, 89*(4), 1331–1346. [https://doi.org/10.1016/S0306-4522\(98\)00389-3](https://doi.org/10.1016/S0306-4522(98)00389-3)
- Owens, N. C., & Verberne, A. J. M. (2001). Regional haemodynamic responses to activation of the medial prefrontal cortex depressor region. *Brain Research, 919*(2), 221–231. [https://doi.org/10.1016/S0006-8993\(01\)03017-7](https://doi.org/10.1016/S0006-8993(01)03017-7)
- Pereira, E. A. C., Wang, S., Paterson, D. J., Stein, J. F., Aziz, T. Z., & Green, A. L. (2010). Sustained reduction of hypertension by deep brain stimulation. *Journal of Clinical Neuroscience, 17*(1), 124–127. <https://doi.org/10.1016/j.jocn.2009.02.041>
- Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., Egan, M. F., Mattay, V. S., Hariri, A. R., & Weinberger, D. R. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: A genetic susceptibility mechanism for depression.

Nature Neuroscience, 8(6), 828–834. <https://doi.org/10.1038/nn1463>

- Poh, M. Z., Loddenkemper, T., Swenson, N. C., Goyal, S., Madsen, J. R., & Picard, R. W. (2010). Continuous monitoring of electrodermal activity during epileptic seizures using a wearable sensor. *2010 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBC'10*, 4415–4418. <https://doi.org/10.1109/IEMBS.2010.5625988>
- Potts, J. T. (2006). Inhibitory neurotransmission in the nucleus tractus solitarii: Implications for baroreflex resetting during exercise. *Experimental Physiology*, 91(1), 59–72. <https://doi.org/10.1113/expphysiol.2005.032227>
- Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proc. Natl. Acad. Sci.*, 98(2), 676–682. <https://doi.org/10.1016/j.msea.2006.02.358>
- Rao, V. R., Sellers, K. K., Wallace, D. L., Lee, M. B., Bijanzadeh, M., Sani, O. G., Yang, Y., Shanechi, M. M., Dawes, H. E., & Chang, E. F. (2018). Direct Electrical Stimulation of Lateral Orbitofrontal Cortex Acutely Improves Mood in Individuals with Symptoms of Depression. *Current Biology*, 28(24), 3893–3902.e4. <https://doi.org/10.1016/j.cub.2018.10.026>
- Rolls, E. T., & Grabenhorst, F. (2008). The orbitofrontal cortex and beyond: From affect to decision-making. *Progress in Neurobiology*, 86(3), 216–244. <https://doi.org/10.1016/j.pneurobio.2008.09.001>
- Rolls, E. T., Inoue, K., & Browning, A. (2003). Activity of primate subgenual cingulate cortex neurons is related to sleep. *Journal of Neurophysiology*, 90(1), 134–142. <https://doi.org/10.1152/jn.00770.2002>
- Romanski, L. M., Bates, J. F., & Goldman-Rakic, P. S. (1999). Auditory belt and parabelt projections to the prefrontal cortex in the rhesus monkey. *Journal of Comparative Neurology*, 403(2), 141–157. [https://doi.org/10.1002/\(SICI\)1096-9861\(19990111\)403:2<141::AID-CNE1>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1096-9861(19990111)403:2<141::AID-CNE1>3.0.CO;2-V)
- Ronkainen, E., Korpelainen, J. T., Heikkinen, E., Myllylä, V. V., Huikuri, H. V., & Isojärvi, J. I. T. (2006). Cardiac autonomic control in patients with refractory epilepsy before and during vagus nerve stimulation treatment: A one-year follow-up study. *Epilepsia*, 47(3), 556–562. <https://doi.org/10.1111/j.1528-1167.2006.00467.x>
- Ruit, G., & Neafsey, E. J. (1990). Hippocampal input to a “visceral motor” corticobulbar pathway: an anatomical and electrophysiological study in the rat. *Experimental Brain Research*, 82(3), 606–616. <https://doi.org/10.1007/BF00228802>
- Ruit, G., & Neafsey, E. J. (1988). Cardiovascular and respiratory responses to electrical and chemical

- stimulation of the hippocampus in anesthetized and awake rats. *Brain Research*, 457(2), 310–321.
[https://doi.org/10.1016/0006-8993\(88\)90701-9](https://doi.org/10.1016/0006-8993(88)90701-9)
- Ruiz Vargas, E., Sörös, P., Shoemaker, J. K., & Hachinski, V. (2016). Human cerebral circuitry related to cardiac control: A neuroimaging meta-analysis. *Annals of Neurology*, 79(5), 709–716.
<https://doi.org/10.1002/ana.24642>
- Rush, A. J., George, M. S., Sackeim, H. A., Marangell, L. B., Husain, M. M., Giller, C., Nahas, Z., Haines, S., Simpson, R. K., & Goodman, R. (2000). Vagus nerve stimulation (VNS) for treatment-resistant depressions: A multicenter study. *Biological Psychiatry*, 47(4), 276–286.
[https://doi.org/10.1016/S0006-3223\(99\)00304-2](https://doi.org/10.1016/S0006-3223(99)00304-2)
- Rush, A. J., Marangell, L. B., Sackeim, H. A., George, M. S., Brannan, S. K., Davis, S. M., Howland, R., Kling, M. A., Rittberg, B. R., Burke, W. J., Rapaport, M. H., Zajecka, J., Nierenberg, A. A., Husain, M. M., Ginsberg, D., & Cooke, R. G. (2005). Vagus nerve stimulation for treatment-resistant depression: A randomized, controlled acute phase trial. *Biological Psychiatry*, 58(5), 347–354.
<https://doi.org/10.1016/j.biopsych.2005.05.025>
- Sackeim, H. A., Rush, A. J., George, M. S., Marangell, L. B., Husain, M. M., Nahas, Z., Johnson, C. R., Seidman, S., Giller, C., Haines, S., Simpson, R. K., & Goodman, R. R. (2001). Vagus nerve stimulation (VNS™) for treatment-resistant depression: Efficacy, side effects, and predictors of outcome. *Neuropsychopharmacology*, 25(5), 713–728. [https://doi.org/10.1016/S0893-133X\(01\)00271-8](https://doi.org/10.1016/S0893-133X(01)00271-8)
- Saul, J. (1990). Beat-To-Beat Variations of Heart Rate Reflect Modulation of Cardiac Autonomic Outflow. *Physiology*, 5(1), 32–37. <https://doi.org/10.1152/physiologyonline.1990.5.1.32>
- Schreihofer, A. M., & Guyenet, P. G. (2002). The baroreflex and beyond: Control of sympathetic vasomotor tone by gabaergic neurons in the ventrolateral medulla. *Clinical and Experimental Pharmacology and Physiology*, 29(5–6), 514–521. <https://doi.org/10.1046/j.1440-1681.2002.03665.x>
- Seamans, J. K., Lapish, C. C., & Durstewitz, D. (2008). Comparing the Prefrontal Cortex of Rats and Primates: Insights from Electrophysiology. *Neurotoxicity Research*, 14(604), 249–262.
- Secher, N. H. (1985). Heart rate at the onset of static exercise in man with partial neuromuscular blockade. *The Journal of Physiology*, 368(1), 481–490.
<https://doi.org/10.1113/jphysiol.1985.sp015870>

- Shaffer, F., McCraty, R., & Zerr, C. L. (2014). *A healthy heart is not a metronome : an integrative review of the heart ' s anatomy and heart rate variability*. 5(September), 1–19.
<https://doi.org/10.3389/fpsyg.2014.01040>
- Shoemaker, J. K. (2017). Recruitment strategies in efferent sympathetic nerve activity. *Clinical Autonomic Research*, 27(6), 369–378. <https://doi.org/10.1007/s10286-017-0459-x>
- Shoemaker, J. K., & Goswami, R. (2015). Forebrain neurocircuitry associated with human reflex cardiovascular control. *Frontiers in Physiology*, 6(SEP), 1–14.
<https://doi.org/10.3389/fphys.2015.00240>
- Shorvon, S., & Tomson, T. (2011). Sudden unexpected death in epilepsy. *The Lancet*, 378(9808), 2028–2038. [https://doi.org/10.1016/S0140-6736\(11\)60176-1](https://doi.org/10.1016/S0140-6736(11)60176-1)
- Shulman, G. L., Corbetta, M., Buckner, R. L., Fiez, J. A., Miezin, F. M., Raichle, M. E., & Petersen, S. E. (1997). Common blood flow changes across visual tasks: I. Increases in subcortical structures and cerebellum but not in nonvisual cortex. *Journal of Cognitive Neuroscience*, 9(5), 624–647.
<https://doi.org/10.1162/jocn.1997.9.5.624>
- Smith, W. K. (1945). The functional significance of the rostral cingular cortex as revealed by its responses to electrical excitation. *Journal of Neurophysiology*, 8(4), 241–255.
<https://doi.org/10.1152/jn.1945.8.4.241>
- Spyer, K. M. (1981). Neural organisation and control of the baroreceptor reflex. *Reviews of Physiology, Biochemistry and Pharmacology*, 88, 24–124. <https://doi.org/10.1007/bfb0034536>
- Sverrisdóttir, Y. B., Green, A. L., Aziz, T. Z., Bahuri, N. F. A., Hyam, J., Basnayake, S. D., & Paterson, D. J. (2014). Differentiated baroreflex modulation of sympathetic nerve activity during deep brain stimulation in humans. *Hypertension*, 63(5), 1000–1010.
<https://doi.org/10.1161/HYPERTENSIONAHA.113.02970>
- Talman, W. T. (1985). Cardiovascular regulation and lesions of the central nervous system. *Annals of Neurology*, 18(1), 1–12. <https://doi.org/10.1002/ana.410180102>
- Terreberry, R. R., & Neafsey, E. J. (1983). Rat medial frontal cortex: a visceral motor region with a direct projection to the solitary nucleus. *Brain Research*, 278(1–2), 245–249. [https://doi.org/10.1016/0006-8993\(83\)90246-9](https://doi.org/10.1016/0006-8993(83)90246-9)
- Terreberry, R. R., & Neafsey, E. J. (1987). The rat medial frontal cortex projects directly to autonomic regions of the brainstem. *Brain Research Bulletin*, 19(6), 639–649. <https://doi.org/10.1016/0361->

- Terrence, C. F., Rao, G. R., & Perper, J. A. (1981). Neurogenic pulmonary edema in unexpected, unexplained death of epileptic patients. *Annals of Neurology*, *9*(5), 458–464.
<https://doi.org/10.1002/ana.410090508>
- Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience and Biobehavioral Reviews*, *36*(2), 747–756.
<https://doi.org/10.1016/j.neubiorev.2011.11.009>
- Thayer, J. F., Hansen, A. L., Saus-Rose, E., & Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: The neurovisceral integration perspective on self-regulation, adaptation, and health. *Annals of Behavioral Medicine*, *37*(2), 141–153.
<https://doi.org/10.1007/s12160-009-9101-z>
- Thayer, J. F., & Sternberg, E. (2006). Beyond heart rate variability: Vagal regulation of allostatic systems. *Annals of the New York Academy of Sciences*, *1088*, 361–372.
<https://doi.org/10.1196/annals.1366.014>
- Thorpe, S. J., Rolls, E. T., & Maddison, S. (1983). The Orbitofrontal Cortex: Neuronal Activity in the Behaving Monkey. *Experimental Brain Research*, *49*, 93–115.
- Tinuper, P., Bisulli, F., Cerullo, A., Carcangiu, R., Marini, C., Pierangeli, G., & Cortelli, P. (2001). Ictal bradycardia in partial epileptic seizures: Autonomic investigation in three cases and literature review. *Brain*, *124*(12), 2361–2371. <https://doi.org/10.1093/brain/124.12.2361>
- Tomson, T., & Kennebäck, G. (1997). Arrhythmia, heart rate variability, and antiepileptic drugs. *Epilepsia*, *38*(SUPPL.11), 48–51. <https://doi.org/10.1111/j.1528-1157.1997.tb06128.x>
- Topolovec, J. C., Gati, J. S., Menon, R. S., Shoemaker, J. K., & Cechetto, D. F. (2004). *Human Cardiovascular and Gustatory Brainstem Sites Observed by Functional Magnetic Resonance Imaging*. *461*(December 2003), 446–461. <https://doi.org/10.1002/cne.20033>
- Torvik, A. (1956). Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures. An experimental study in the rat. *Journal of Comparative Neurology*, *106*(1), 51–141. <https://doi.org/10.1002/cne.901060104>
- Uylings, H. B., van Eden, C. G., de Bruin, J. P., Corner, M. A., & Feenstra, M. G. (1990). Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. *The*

Prefrontal Cortex: Its Structure, Function and Pathology, 85(1940), 31–62.

- Valentín, A., Anderson, M., Alarcón, G., García Seoane, J. J., Selway, R., Binnie, C. D., & Polkey, C. E. (2002). Responses to single pulse electrical stimulation identify epileptogenesis in the human brain in vivo. *Brain*, 125(8), 1709–1718. <https://doi.org/10.1093/brain/awf187>
- Van Bockstaele, E. J., Pieribone, V. A., & Aston-Jones, G. (1989). Diverse afferents converge on the nucleus paragigantocellularis in the rat ventrolateral medulla: Retrograde and anterograde tracing studies. *Journal of Comparative Neurology*, 290(4), 561–584. <https://doi.org/10.1002/cne.902900410>
- Van Der Kooy, D., Koda, L. Y., McGinty, J. F., Gerfen, C. R., & Bloom, F. E. (1984). The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat. *Journal of Comparative Neurology*, 224(1), 1–24. <https://doi.org/10.1002/cne.902240102>
- Van Der Kooy, D., McGinty, J. F., Koda, L. Y., Gerfen, C. R., & Bloom, F. E. (1982). Visceral cortex: A direct connection from prefrontal cortex to the solitary nucleus in rat. *Neuroscience Letters*, 33(2), 123–127. [https://doi.org/10.1016/0304-3940\(82\)90238-5](https://doi.org/10.1016/0304-3940(82)90238-5)
- Verberne, A. J. M. (1996). Medullary sympathoexcitatory neurons are inhibited by activation of the medial prefrontal cortex in the rat. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 270(4 39-4). <https://doi.org/10.1152/ajpregu.1996.270.4.r713>
- Verberne, A. J. M., Lam, W., Owens, N. C., & Sartor, D. (1997). Supramedullary modulation of sympathetic vasomotor function. *Clinical and Experimental Pharmacology and Physiology*, 24, 748–754.
- Verberne, A. J. M., Lewis, S. J., Worland, P. J., Beart, P. M., Jarrott, B., Christie, M. J., & Louis, W. J. (1987). Medial prefrontal cortical lesions modulate baroreflex sensitivity in the rat. *Brain Research*, 426(2), 243–249. [https://doi.org/10.1016/0006-8993\(87\)90878-X](https://doi.org/10.1016/0006-8993(87)90878-X)
- Verberne, A. J. M., & Owens, N. C. (1998). Cortical modulation of the cardiovascular system. *Progress in Neurobiology*, 54(2), 149–168. [https://doi.org/10.1016/S0301-0082\(97\)00056-7](https://doi.org/10.1016/S0301-0082(97)00056-7)
- Verrier, R. L., Pang, T. D., Nearing, B. D., & Schachter, S. C. (2020). The Epileptic Heart: Concept and clinical evidence. *Epilepsy and Behavior*, 105, 106946. <https://doi.org/10.1016/j.yebeh.2020.106946>
- Vianna, L. C., Fadel, P. J., Secher, N. H., & Fisher, J. P. (2015). A cholinergic contribution to the circulatory responses evoked at the onset of handgrip exercise in humans. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 308(7), R597–R604.

<https://doi.org/10.1152/ajpregu.00236.2014>

- Victor, R. G., & Seals, D. R. (1989). Reflex stimulation of sympathetic outflow during rhythmic exercise in humans. *American Journal of Physiology - Heart and Circulatory Physiology*, 257(6).
<https://doi.org/10.1152/ajpheart.1989.257.6.h2017>
- Victor, R. G., Seals, D. R., & Mark, A. L. (1987). Differential control of heart rate and sympathetic nerve activity during dynamic exercise. Insight from intraneural recordings in humans. *Journal of Clinical Investigation*, 79(2), 508–516. <https://doi.org/10.1172/JCI112841>
- Victor, Ronald G., Secher, N. H., Lyson, T., & Mitchell, J. H. (1995). Central command increases muscle sympathetic nerve activity during intense intermittent isometric exercise in humans. *Circulation Research*, 76(1), 127–131. <https://doi.org/10.1161/01.res.76.1.127>
- Wall, P. D., & Davis, G. D. (1951). Three Cerebral Cortical Systems Affecting Autonomic Function. *Journal of Neurophysiology*, 14(6), 507–517.
- Wallis, J. D. (2007). Orbitofrontal cortex and its contribution to decision-making. *Annual Review of Neuroscience*, 30, 31–56. <https://doi.org/10.1146/annurev.neuro.30.051606.094334>
- Ward, A. A. (1948). The Cingular Gyrus: Area 24. *Journal of Neurophysiology*, 11(1), 13–23.
- Wikenheiser, A. M., & Schoenbaum, G. (2016). Over the river, through the woods: Cognitive maps in the hippocampus and orbitofrontal cortex. *Nature Reviews Neuroscience*, 17(8), 513–523.
<https://doi.org/10.1038/nrn.2016.56>
- Williamson, J. W. (2010). The relevance of central command for the neural cardiovascular control of exercise. *Experimental Physiology*, 95(11), 1043–1048.
<https://doi.org/10.1113/expphysiol.2009.051870>
- Williamson, J. W., Fadel, P. J., & Mitchell, J. H. (2006). New insights into central cardiovascular control during exercise in humans: A central command update. *Experimental Physiology*, 91(1), 51–58.
<https://doi.org/10.1113/expphysiol.2005.032037>
- Williamson, J. W., McColl, R., & Mathews, D. (2003). Evidence for central command activation of the human insular cortex during exercise. *Journal of Applied Physiology*, 94(5), 1726–1734.
<https://doi.org/10.1152/jappphysiol.01152.2002>
- Williamson, J. W., McColl, R., Mathews, D., Ginsburg, M., & Mitchell, J. H. (1999). Activation of the insular cortex is affected by the intensity of exercise. *Journal of Applied Physiology*, 87(3), 1213–

1219. <https://doi.org/10.1152/jappl.1999.87.3.1213>

- Williamson, J. W., Mccoll, R., Mathews, D., Mitchell, J. H., Raven, P. B., & Morgan, W. P. (2001). Hypnotic manipulation of effort sense during dynamic exercise: Cardiovascular responses and brain activation. *Journal of Applied Physiology*, *90*(4), 1392–1399. <https://doi.org/10.1152/jappl.2001.90.4.1392>
- Williamson, J. W., McColl, R., Mathews, D., Mitchell, J. H., Raven, P. B., & Morgan, W. P. (2002). Brain activation by central command during actual and imagined handgrip under hypnosis. *Journal of Applied Physiology*, *92*(3), 1317–1324. <https://doi.org/10.1152/jappphysiol.00939.2001>
- Williamson, J. W., Nobrega, A. C. L., McColl, R., Mathews, D., Winchester, P., Friberg, L., & Mitchell, J. H. (1997). Activation of the insular cortex during dynamic exercise in humans. *Journal of Physiology*, *503*(2), 277–283. <https://doi.org/10.1111/j.1469-7793.1997.277bh.x>
- Wong, S. W., Kimmerly, D. S., Massé, N., Menon, R. S., Cechetto, D. F., & Shoemaker, J. K. (2007). Sex differences in forebrain and cardiovagal responses at the onset of isometric handgrip exercise: A retrospective fMRI study. *Journal of Applied Physiology*, *103*(4), 1402–1411. <https://doi.org/10.1152/jappphysiol.00171.2007>
- Wong, S. W., Massé, N., Kimmerly, D. S., Menon, R. S., & Shoemaker, J. K. (2007). Ventral medial prefrontal cortex and cardiovagal control in conscious humans. *NeuroImage*, *35*(2), 698–708. <https://doi.org/10.1016/j.neuroimage.2006.12.027>
- Wood, K. N., Luchyshyn, T. A., & Shoemaker, J. K. (2017). High cardiorespiratory fitness in early to late middle age preserves the cortical circuitry associated with brain-heart integration during volitional exercise. *Journal of Neurophysiology*, *117*(4), 1831–1840. <https://doi.org/10.1152/jn.00592.2016>
- Zubcevic, J., & Potts, J. T. (2010). Role of GABAergic neurones in the nucleus tractus solitarii in modulation of cardiovascular activity. *Experimental Physiology*, *95*(9), 909–918. <https://doi.org/10.1113/expphysiol.2010.054007>

Appendices

Appendix A: Ethics Approval



Date: 13 April 2020
To: Seyed Mirsattari
Project ID: 106711
Study Title: Susceptibility to Sudden Death in Epilepsy and Stroke
Reference Number/ID: N/A
Application Type: HSREB Amendment Form
Review Type: Delegated
Full Board Reporting Date: 05May2020
Date Approval Issued: 13/Apr/2020 09:56
REB Approval Expiry Date: 28/Mar/2021

Dear Seyed Mirsattari,

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
Letter of Information Healthy Volunteers, amendment Apr 11 2020	Consent Form	11/Apr/2020	6
Letter of Information Patients, amendment Mar 5 2020	Consent Form	05/Mar/2020	5
Susceptibility to sudden death in epilepsy and stroke, amendment Mar 5 2020	Protocol	05/Mar/2020	6

Documents Acknowledged:

Document Name	Document Type	Document Date	Document Version
Susceptibility to sudden death in epilepsy and stroke, amendment Mar 5 2020	Summary of Changes	05/Mar/2020	6

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Nicola Geoghegan-Morphet, Ethics Officer on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix B: Letter of Information and Consent Forms

Version 5 – March 5, 2020

SEYED M. MIRSATTARI, M.D., Ph.D., F.R.C.P.(C)
Epilepsy, General Neurology
Dept. of Clinical Neurological Sciences



339 Windermere Rd
London, Ontario, N6A 5A5

LETTER OF INFORMATION

Susceptibility to Sudden Death in Epilepsy and Stroke

Principal Investigator: Dr. Seyed M. Mirsattari

PURPOSE

The purpose of this letter is to provide you with the information that you require in order to make an informed decision for your participation in this research project. The pronouns 'you' and 'your' should be read as referring to the participant. The purpose of this study is to investigate the patterns of the brain and heart functioning in normal control population and patients with stroke or epilepsy to discover sudden death prone patterns in susceptible individuals with stroke or epilepsy. Sudden death in epilepsy and stroke has become a focus of attention as it is one of the most disturbing aspects of patient care. The data collected as your routine clinical procedure in epilepsy or stroke units of the University Hospital will form a database to develop a sudden death risk index and guidelines for preventative action for patients with epilepsy or stroke. Some of the data is part of your routine clinical practice and some will be collected only for research purposes of this investigation. You are being invited to participate to obtain data that will be used to provide such risk index and guidelines. This project requires 180 patients.

Standard of Care

- Clinical visits to neurologists, neurosurgeons, psychologists, and clinical neuropsychologists for diagnosis and treatment.
- Outpatient EEGs, MRI.
- Admission to the Epilepsy Monitoring Unit (EMU) for Video-EEG telemetry.
- Patients may undergo functional MRI (fMRI) as part of routine clinical practice.

STUDY PROCEDURES

During your admission in the EMU you will perform simple physiological maneuvers (breath-holds and hand grip exercise) and will undergo Microneurography and Transcutaneous Electrical Nerve Stimulation (TENS) tests to measure your autonomic balance as part of the interest for this research study. These measures are not part of the routine clinical practice.

The following data collected will be used in this study under your consent:

- Your vital signs (blood pressure, heart rate, oxygen saturation and respiration)

1 of 6

Participant's Initials _____

- Your imaging data including MRI and fMRI
- Your Microneurography, TENS and autonomic maneuvers tests results.

These data will be used to:

- Detect the seizure onset zone
- Detect brain functional connectivity alterations (FCA)
- Determine the extent of the involvement of specific brain regions in seizure onset zone and FCA maps
- Extract the brain's patterns of cardiovascular control
- Determine a risk factor by conducting statistical analysis to the results of the previous parts.

The study procedures consist in:

- **Microneurography:** The nervous system that regulates your blood pressure will be examined with a technique called "microneurography". This procedure has two phases. First, the position of a nerve that runs very close to your skin just on the outside of the knee will be located using a small electrical pulse that will cause your foot to twitch. This will be a strange sensation but will not be uncomfortable and carries no risk at the levels of electrical current that are being used. Second, a thin needle-like electrode (made out of tungsten and about the size of a large human hair, 200 microns) will be inserted through the intact skin and positioned just under the skin about 2-3 cm from the nerve site. You may feel a pin-prick sensation when the electrode is passed through the skin, much like the feeling you get when your blood is being taken. This will be followed by the placement of a second electrode through the intact skin into this nerve (called the peroneal nerve). This second electrode will be manipulated by the researcher until the appropriate recording site is found; this search will not last longer than 60-min. The microelectrodes are sterilized before use and the area of skin around the knee is cleaned with alcohol before and after the procedure. To confirm an acceptable recording of nervous system activity we will perform a series of breath holds for about 30 sec.
- **TENS:** An electrical stimulator will be placed on your skin. The amount of stimulation will be set just below that needed to cause discomfort or a very small muscle contraction. Therefore, this type of muscle stimulation is painless and you may not feel anything. Before placement of the stimulation pads, the skin is numbed using a lidocaine cream. This TENS will be applied intervals up to 5 min at a time.
- **Breath Holds (also called Apnea).** You will be asked to hold your breath for as long you can or up to a maximal duration of 30 seconds. You will be asked to perform this test up to three times with 1-2 minutes of rest between each breath hold.
- **Hand grip exercise:** This is another physiological test which is done to increase the arterial pressure so we can measure this variation. First, you will squeeze a device as hard as you can so we can record your maximum handgrip strength. You will then perform a series of lighter squeezes that will last from 30-sec to 2

minutes in length. Following the 2 min test, a cuff will be placed around your upper arm and inflated to levels that will feel like your blood pressure is being measured. This level of cuff pressure stops blood from going into or out of your arm and will be maintained for up to 2 minutes. This maneuver allows us to study the role of nerves in your arm that elevate blood pressure when muscles get tired during exercise. Some of these handgrip tasks will be performed together with TENS.

DURATION OF THE STUDY

No session is required outside of your routine clinical sessions. All required data will be collected during your routine clinical sessions.

REASONS THAT WILL EXCLUDE YOU FROM THIS STUDY

If you are less than 18 years old or more than 85 years old. For the autonomic maneuvers, Microneurography and TENS the maximum age is 65 years old. Also, if you have: coronary heart disease, cardiac arrhythmias, cerebrovascular disease, hypertension, cerebrovascular disease, kidney or liver disease, Raynaud's Disease, movement disorders such as Parkinson's Disease, severe mental health impairments or metabolic disorders such as diabetes, any history of head or eye injury involving metal fragments, have ever worked in a metal shop, been a soldier, have some type of implanted electrical device (such as a cardiac pacemaker), have severe heart disease (including susceptibility to arrhythmias), or are wearing metal braces on your teeth, you should not have an MRI scan. Additionally, if you have any of the following, you should not have an MRI scan:

- (1) claustrophobia,
- (2) any metallic implants, such as a pacemaker or cerebral aneurysm clips,
- (3) have been injured anywhere in your body by a metallic object that was not removed,
- (4) If you are pregnant or trying to conceive.

RISKS AND DISCOMFORTS

Finometer

There are no risks of using the finger cuff method (Finometer) of examining arterial blood pressure. With the finger cuff the finger tip may turn a little blue and feel numb during the prolonged test sessions but this resolves immediately when the cuff is removed.

Portable ECG

This device called "Firstbeat Bodyguard" has an adhesive gel on the patch sensors that rarely causes skin irritation.

MRI

The Food & Drug Administration (USA) has indicated that for clinical diagnosis an 'insignificant' risk is associated with human MRI exposure at the intensities used in this project. Current Canadian guidelines follow the USA guidelines. Although very rare, injury

and deaths have occurred in MRI units from unsecured metal objects being drawn at high speeds into the magnet or from internal body metal fragments of which the subject was unaware or not had not informed MRI staff. To minimize this latter possibility it is essential that you complete a screening questionnaire. Other remote but potential risks involve tissue burns and temporary hearing loss from the loud noise inside the magnet. The latter can be avoided with ear plugs protection that also allows continuous communication between the subject and staff during the study.

Risks to participants in this study are minimal. Magnetic resonance imaging at 1.5 Tesla is a routine clinical tool available in most hospitals, and is performed on thousands every year. So far, no adverse side effects or complications have occurred during or following magnetic resonance examinations involving patients who are not claustrophobic and do not have any cranial clips, pins, cardiac pacemaker, or other metallic implants. At the present time there have been no reliable research reports that have found any immediate or long-term effects to human health from being in MRI scanners from 1.5 up to 8 Teslas. However, there is a small chance that an as yet unknown problem may occur due to a relatively limited experience with this imaging modality.

Isometric hand grip

The longer periods of handgrip exercise will produce forearm fatigue. Also, the post-exercise period of forearm occlusion may be uncomfortable. This fatigue and discomfort vanish quickly following the protocol. No major risks of this task or continuous cuff inflation have been reported.

Breath Hold

You will feel breathless at the end of this test but there are no known risks to this task.

Microneurography and Transcutaneous Electrical Nerve Stimulation

During these procedures you may experience a small pinch similar to that of a small needle when the electrode crosses the skin. You may feel transient paresthesias (pins and needles) or involuntary muscle twitches during the process of locating an adequate recording site. There are no residual consequences. Transient tenderness over the microelectrode insertion site has been reported for ~7 days following the procedure in 0.5% of the more than 1200 cases studied by the Neurovascular Research Laboratory under Prof. Shoemaker (who will do the procedures). This risk will be minimized by limiting the search for an adequate signal to 60 minutes. Patients will be advised to restrain from heavy physical exercise with the involved limb and that no pressure is placed over it for 24-hours after the test.

BENEFITS

The information obtained may increase the understanding of sudden death in epilepsy and stroke and which may be beneficial for the participant and may benefit future patients with these conditions.

CONFIDENTIALTY

The study doctor will keep any personal health information about you in a secure and

confidential location for a minimum of 25 years. A list linking your study number with your name will be kept by the study doctor in a secure place, separate from your study file. Representatives of the University of Western Ontario Health Sciences Research Ethics Board and representatives of Lawson Quality Assurance Education Program may look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines. Incidental findings are highly unlikely to exist as all will undergo routine clinical evaluation before hand, however, if they do occur the principal investigator will approach them and provide them with the appropriate information/care.

WITHDRAWING FROM THIS STUDY

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw your data from this study at anytime with no effect on your future care.

COMPENSATION

You will not be paid to take part in the study.

CONTACT INFORMATION

If you have any questions about this study, please feel free to contact Dr. Seyed M. Mirsattari. If you have any questions about your rights as a research participant or the conduct of this study, you may contact the Patient Relations Office at LHSC at (519) 685-8500 ext. 52036 or access the online form at: <https://apps.lhsc.on.ca/?q=forms/patient-relations-contact-form>.

Please Note: You do not waive any legal rights by signing the consent form. You will be given a copy of this letter of information and consent form once it has been signed.

Consent Form for Participation

Title of Study: Susceptibility to Sudden Death in Epilepsy and Stroke

Principal Investigator: Seyed M. Mirsattari, MD, PhD, FRCP(C)

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

Dated in London, this _____ day of _____, 20____

(Signature of Participant)

(Print name of Participant)

(Signature of Researcher)

(Signature of Person Obtaining the Consent of the Participant)

(Print name of Person Obtaining the Consent of the Participant)

Date Obtaining the Consent form

Curriculum Vitae

Bartek Kulas

EDUCATION

The University of Western Ontario

MSc Kinesiology, Integrative Biosciences, 2021 (expected)

Thesis: Neuromodulation of the tachycardia response to exercise: Direct electrical stimulation of prefrontal cortex in conscious humans.

Advisor: Dr. J. Kevin Shoemaker, PhD

The University of Western Ontario

BSc Honors Specialization in Kinesiology, 2019

HONOURS & AWARDS

- 2020-2021 Natural Sciences and Engineering Research Council (NSERC) Canada Graduate Scholarships - Masters. (\$17,500)
- 2020-2021 Ontario Graduate Scholarship (OGS) Masters Award. (\$15,000)
- 2019-2021 Western Graduate Research Scholarship (WGRS) Masters Award. (\$5,368)

RESEARCH CONTRIBUTIONS

Kulas. B., et al. (2021). Neuromodulation of the tachycardia response to exercise: Direct electrical stimulation of prefrontal cortex in conscious humans. *American Autonomic Society International Symposium*. November 3-6, 2021.

Kulas. B., et al. (2020). Exploring the interactive effects of apneic and baroreflex stress on neural coding strategies in human muscle sympathetic nerve activity. *American Autonomic Society International Symposium*. November 6-7, 2020. Poster.

PRESENTATIONS & GUEST LECTURES

“Neuromodulation of the transient tachycardia response to exercise via cortical stimulation of the prefrontal cortex”, Kinesiology Graduate Student Research Conference. The University of Western Ontario, April 12, 2021. Conference oral talk.

“Effect of prefrontal cortical brain stimulation on transient heart rate responses”, Epilepsy Rounds, Department of Clinical Neurological Sciences. University Hospital, The University of Western Ontario, January 8, 2021.

“A direct electrical stimulation model: the neuromodulation of cardiovascular responses”, Epilepsy Mini-Series, Department of Clinical Neurological Sciences. London Health Sciences Centre / BrainsCAN, January 29, 2021.

Kulas. B., et al. (2020). Exploring the interactive effects of apneic and baroreflex stress on neural coding strategies in human muscle sympathetic nerve activity. *American Autonomic Society International Symposium*. November 6-7, 2020. Poster Presentation.

“Nervous System Development in Infancy”, Human Growth & Development (KIN 3347A), School of Kinesiology. The University of Western Ontario, October 7, 2020. Guest Lecture.

TEACHING ASSISTANTSHIPS

Medical Issues in Sport (KIN 4437B), School of Kinesiology. The University of Western Ontario, January-April 2021.

Human Growth & Development (KIN 3347A), School of Kinesiology. The University of Western Ontario, September-December 2020.

Human Cadaver Anatomy Lab (ANATCELL 2221B), Department of Cell Anatomy. The University of Western Ontario, January-April 2020.

Biomechanics of Human Locomotion (KIN 3353B), School of Kinesiology. The University of Western Ontario, January-April 2021.

Human Cadaver Anatomy Lab (ANATCELL 2221A), Department of Cell Anatomy. The University of Western Ontario, September-December 2021.