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Improving the Assessment and Understanding of Neurogenic Orthostatic Hypotension

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ABSTRACT

Neurogenic Orthostatic Hypotension (NOH) is a cardinal feature of autonomic failure. Patients with NOH experience a persistent and consistent drop in blood pressure when standing due to failure of the autonomic nervous system to reflexively increase sympathetic outflow. NOH affects individuals worldwide, presenting as both a primary feature (i.e. Multiple Systems Atrophy, Pure Autonomic Failure) and secondary to several common disorders including diabetes and Parkinson's Disease. However, there are still several gaps in our overall understanding and assessment of patients with NOH. Therefore, the six studies presented in this thesis aimed to address some of these gaps in our current knowledge.

Study 1 and 2 aimed to investigate activity within the central autonomic network (CAN) both at rest and during standardized autonomic challenges to determine whether patients have reduced activity relative to healthy controls. In this study we found patients had reduced activation in several CAN structures including the cingulate cortices, thalamus, hippocampus and cerebellum.

Based on study 1 and 2 results, study 3 and 4 aimed to determine whether patients also had reduced functional connectivity in two structures involved in postural blood pressure regulation: the brainstem and cerebellum. We found patients had significantly less connectivity between the brainstem and several CAN structures including the cerebellum, insula and cingulate cortices. Additionally, patients had significantly less intracerebellar connectivity, less cerebellar-brainstem connectivity and reduced connectivity to CAN structures including the insula, anterior cingulate, hippocampus, thalamus and putamen.

Finally, symptoms associated with NOH include postural light-headedness, dizziness and syncope. Proper diagnosis rests in the ability to accurately distinguish these non-specific symptoms as either orthostatic (postural) or non-orthostatic (non-postural). The purposes of studies 5 and 6 were to create a simple instrument capable of making this distinction, demonstrate its validity and reliability, sensitivity and specificity, and to test its ability to assess individuals based on symptomatology. In these studies, I found our questionnaire was valid, reliable and capable of positively predicting individuals with orthostatic intolerance related to autonomic dysfunction.

Overall, this thesis greatly expands our understanding of NOH pathophysiology and provides a new tool for assessing orthostatic symptomatology related to autonomic dysfunction.

SUMMARY FOR LAY AUDIENCE

The autonomic nervous system (ANS) is a branch of the nervous system that regulates processes that occur without conscious effort, including: heart rate, breathing, blood pressure, sweating, digestion, sexual function, respiration, urination and defecation. The ANS has two major divisions: the sympathetic nervous system, primarily active during the "fight-or-flight" response, and the parasympathetic nervous system, primarily active during "rest-and-digest".

When we stand up, the ANS ensures gravity does not pull our blood into our legs. However, when the ANS fails, this function is lost and as a result, individuals experience a significant drop in blood pressure when standing. ANS failure affects individuals worldwide, presenting as both a primary disorder (i.e. Pure Autonomic Failure) and secondary to several common disorders including diabetes and Parkinson's Disease. However, there are still several gaps in our overall understanding and assessment of patients with autonomic failure. This thesis aimed to address these gaps.

The first half of the thesis focused to improve our understanding of autonomic failure by investigating brain activity in structures known to contribute to proper autonomic control. In addition, I investigated how these brain regions may be functionally connected to help regulate heart rate and blood pressure. I found patients with autonomic failure showed reduced activity in several brain structures involved in blood pressure regulation and these structures showed reduced functional connectivity among each other.

The second half of the thesis aimed to improve the assessment of patients with ANS failure. When patients experience a significant blood pressure drop while standing, common symptoms include light-headedness and dizziness. Proper diagnosis rests in the ability to accurately distinguish these non-specific symptoms as either postural or non-postural. To identify and assess patients, we created a questionnaire capable of making this distinction. I evaluated several important parameters and found the questionnaire to be valid, reliable and capable of positively predicting individuals with autonomic dysfunction based on a symptom assessment.

This thesis greatly expands our understanding of autonomic failure and provides a new tool for assessing symptomatology related to autonomic dysfunction.

KEYWORDS

Autonomic dysfunction, Neurogenic orthostatic hypotension, orthostatic intolerance, functional magnetic resonance imaging, central autonomic network, functional connectivity, cerebellum, brainstem connectivity, symptom assessment

CO-AUTHORSHIP STATEMENT

This thesis contains material from published manuscripts (Chapters 2-7). On all manuscripts, Jacquie Baker was the first author and Dr. Kurt Kimpinski was a co-author. Justin Paturel was co-author on Chapters 2, 3, 6 and 7. David Sletten and Phillip Low were co-authors on Chapter 6 and 7. All experimental data presented in this thesis were collected, analyzed and interpreted by Jacquie Baker.

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List of Abbreviations

ACC – Anterior Cingulate Cortex
ACh - Acetylcholine
ANS – Autonomic Nervous System
ARS – Autonomic Reflex Screen
ASP – Autonomic Symptom Profile
AUC – Area Under the Curve
AV – Anterior Vermis
BMI – Body Mass Index
BOLD – Blood-Oxygen Level Dependent
CAN – Central Autonomic Network
CASS – Composite Autonomic Scoring Scale
COMPASS – Composite Autonomic Symptom Scale
dHb – Deoxygenated Hemoglobin
CVLM – Caudal Ventrolateral Medulla
ECG – Electrocardiography
FDR – False Discovery Rate
fMRI – Function Magnetic Resonance Imaging
FN – Fastigial Nucleus
FOV – Field of View
FEW – Family-wise Error
FWHM – Full-Width Half-Maximum
Hb – Hemoglobin
HR – Heart Rate
HRDB – Heart Rate to Deep Breathing
HUT – Head-up Tilt
IC – Insular Cortex
IML – Intermediolateral Cell Column
LBD – Lewy Body Dementia
LBNP – Lower-body Negative Pressure
MNI – Montreal Neurological Institute
MRI – Magnetic Resonance Imaging
MSA – Multiple System Atrophy
NA – Nucleus Ambiguus
NE – Norepinephrine
NOH – Neurogenic Orthostatic Hypotension
NS – Non-Orthostatic Symptom Score
NTS – Nucleus Tractus Solitarius
ODSS – Orthostatic Discriminant and Severity Scale
OHDAS – Orthostatic Hypotension Daily Activity Score

OHQ – Orthostatic Hypotension Questionnaire
OHSA – Orthostatic Hypotension Symptom Assessment
OI – Orthostatic Intolerance
OS – Orthostatic Symptom Score
PAF – Pure Autonomic Failure
PBN – Parabrachial Nucleus
POTS – Postural Orthostatic Tachycardia Syndrome
PCC – Posterior Cingulate Cortex
PD – Parkinson's Disease
Q – Cardiac Output
QSART – Quantitative Sudomotor Axon Reflex Test
rCBF – Regional Cerebral Blood Flow
rCBV – Regional Cerebral Blood Volume
ROC – Receiver Operator Characteristic Curve
ROI – Regions of Interest
RVLM – Rostral Ventrolateral Medulla
SBP – Systolic Blood Pressure
TE – Echo Time
TI – Inversion Time
TR – Repetition Time
VM – Valsalva Maneuver
VNC – Vestibular Nucleus Complex
VSR – Vestibulo-sympathetic Reflex

CHAPTER 1

1 General Introduction

The idea that the body exists as two distinct systems, an “animal” (somatic) and an “organic” (autonomic) originated with the ancient Greeks[1]. However, it would be Galen and Vesalius who would be among the first to probe into the true nature of this division. In an attempt to understand and characterize the complex neural network that is the Autonomic Nervous System (ANS), the next several hundred years would see the rise and fall of theories and descriptions put forth by Willis, Whytt, Bichat and others throughout the 17th, 18th, and 19th centuries[1]. Finally, in the 19th C Gaskell would develop the term “involuntary nervous system” and, along with J.N. Langley, discern that two sets of fibers, with opposite effects, supply each tissue. Eventually, Langley coined the term "autonomic" nervous system and classified this system into “sympathetic” and “parasympathetic” divisions[2].

Despite the enormous contributions that have brought us to our current understanding of autonomic anatomy and physiology, many aspects of this complex system remain to be fully elucidated. Among the current gaps in our knowledge is that regarding the causes and consequences of autonomic failure. It is without question that a deeper understanding of diseases of the ANS could lead to improved diagnostic evaluations, treatments and management and improved understanding of basic pathophysiology contributing to ANS impairments.

1.1 The Autonomic Nervous System

The Autonomic Nervous System (ANS) is highly integrated with virtually all organs and systems of the human body. The ANS functions autonomously i.e. without conscious effort, to regulate many internal processes including, but not limited to, heart rate, breathing, blood pressure, sweating, digestion, sexual function, respiration, urination and defecation. The ANS has two major complementary subdivisions; the sympathetic nervous system is primarily active during the “fight-or-flight” response, while the parasympathetic (vagal) nervous system is primarily active during periods of “rest-and-digest”.

1.2 The Brainstem

The arterial baroreflex provides beat-to-beat control of arterial blood pressure at rest and especially in response to postural changes. The brainstem contains clusters of nuclei that maintain reflex control of blood pressure, total peripheral resistance and cardiac output. These regions include the periaqueductal gray (PAG) in the midbrain, the parabrachial nucleus (PBN) in the pons and several medullary sites such as the nucleus tractus solitarius (NTS) and caudal/rostral portions of the ventrolateral medulla (CVLM and RVLM, respectively). A considerable amount of work has established these brainstem regions and their associated circuitry in cardiovascular autonomic control of the arterial baroreflex. The PBN serves as a major relay center for afferent information to subcortical sites, including the hypothalamus, thalamus and amygdala. The NTS is the primary site to receive afferent information, which is then relayed, either directly or indirectly, through the PBN to rostral brainstem sites or forebrain regions. Additionally, the NTS is the primary relay for medullary reflexes such as the arterial baroreflex, chemoreflex and mechanoreflexes. The RVLM is integral for blood pressure regulation. The RVLM provides tonic sympatho-excitation to sympathetic preganglionic neurons in the intermediolateral cell column, which synapse with post-ganglionic axons to provide peripheral sympathetic innervation to the heart and blood vessels to increase cardiac output and total peripheral resistance[3,4]. The RVLM also receives modulatory inputs from brainstem nuclei including inhibitory baroreceptor signals from the NTS and CVLM, as well as forebrain regions[5], such as the hypothalamus. The CVLM maintains tonic gamma aminobutyric acid (GABA)-ergic inhibition of the RVLM and relays the arterial baroreflex sympatho-inhibitory inputs from the NTS.

1.3 Peripheral Network

Peripheral control of autonomic responses is primarily regulated by the sympathetic and parasympathetic systems. Both branches consist of preganglionic neurons located in the brainstem or spinal cord that synapse with autonomic ganglia prior to innervating target organs.

1.3.1 Sympathetic Nervous System

Most pre-ganglionic neurons of the sympathetic nervous system occupy the intermediolateral cell (IML) column expanding the thoracolumbar spine from T1-L3. Short preganglionic sympathetic axons enter the paravertebral ganglia (sympathetic chain ganglia) found on either side of the spinal cord. Pre-ganglionic fibers can either stay at the same level or travel up or down the chain. They synapse with post-ganglionic cell bodies and leave as long post-ganglionic sympathetic fibers to their target tissue. Sympathetic chain ganglia innervate all organs and tissues, except those of the abdomen, pelvis and perineum. Alternatively, some pre-ganglionic fibers synapse in the prevertebral ganglia, which provides innervation to all viscera and blood vessels of the abdomen and pelvis. The celiac and superior mesenteric ganglia receive inputs from T5-T11, which provides innervation to abdominal viscera along with mesenteric and renal vessels. Sympathetic innervation to pelvic organs originates from T11-L3. Pre-ganglionic axons synapse with inferior mesenteric ganglia, which give rise to post-ganglionic neurons[6].

1.3.2 Parasympathetic Nervous System

The most important cranial preganglionic axon of the parasympathetic nervous system is carried by the vagus nerve. The vagus nerve innervates the heart, respiratory tract, gastrointestinal tract, liver, pancreas and gallbladder[6]. Parasympathetic pre-ganglionic axons are long, release acetylcholine (ACh) and synapse with ganglia close to the target tissue. Most vagal pre-ganglionic axons originate from neurons within the dorsal motor nucleus of the vagus within the medulla. These axons innervate the respiratory tract, esophagus, stomach and intestines. Pre-ganglionic innervation to the heart primarily originates from the nucleus ambiguus in the medulla[6]. Alternatively, sacral parasympathetic outflow originates from the sacral parasympathetic nucleus of the spinal cord (S2-S4). Sacral parasympathetic outflow controls defecation, micturition and sexual organ function[6].

Together, the parasympathetic and sympathetic systems, along with arterial baroreceptor nuclei within the brainstem facilitate a coordinated response to ensure autonomic homeostasis. Even a task as simple as standing results in remarkably coordinated and intricate responses to maintain postural normotension.

1.4 Maintenance of Postural Normotension

When we stand up the pull of gravity causes an estimated 500-1000 mL redistribution of blood to the lower extremities[7]. In healthy adults, ~50% of this shift occurs within the first 10 seconds and is almost complete within 3-5 minutes of an orthostatic stress. Most of the pooled blood is contained within the deep veins of the legs and splanchnic capacitance beds. Blood pooling plus reduced plasma volume results in decreased venous return, reduced stroke volume and a subsequent fall in cardiac output (~20%). Despite a reduction in cardiac output, a drop in arterial blood pressure is prevented by compensatory vasoconstriction of the resistance and capacitance vessels in the splanchnic, musculo-cutaneous and renal vascular beds. Mechanistically, when there is a transient reduction in arterial blood pressure, reduced afferent inputs to cardiovascular segments within the caudal NTS results in disinhibition of the RVLM, which in turn, results in a reflexive increase in sympathetic efferent activity to the heart and vasculature to increase cardiac output and total peripheral resistance, respectively. In contrast, during hypertension, increased baroreceptor firing sends afferent information to the NTS. This information is relayed to the nucleus ambiguus and/or the dorsal motor nucleus of the vagus to elicit direct parasympathetic outflow. Additionally, afferent baroreceptor information is relayed to the CVLM, to facilitate inhibitory modulation of the RVLM.

1.5 The Central Autonomic Network

In addition to afferent inputs, baroreceptor brainstem nuclei receive efferent signals from cortical and subcortical structures. Benarroch (1993) was the first to propose an integrated model of cortical, subcortical and brainstem structures involved in regulating autonomic function, known as the central autonomic network (CAN)[8]. The concept of a central autonomic network has been further corroborated through recent advances in neuroimaging, specifically, functional magnetic resonance imaging (fMRI). As a result, several cortical and subcortical regions such as the cingulate cortex, insula, hypothalamus, thalamus and cerebellum, have been highly implicated in autonomic regulation. Although there are several important cortical and subcortical structures that make up the CAN, here I will further discuss the insula (Figure 1.1), cingulate (Figure 1.2) and cerebellum (Figure 1.3) as they are highly prevalent in autonomic literature.

1.5.1 Insular Cortex

Anatomically, the insula has reciprocal projections to brainstem autonomic nuclei[3,9], which provides an anatomical basis for autonomic regulation. Functionally, the insula is extremely complex. The insula can be structurally and functionally partitioned into anterior and posterior portions as well as lateralized into left and right. For example, stimulation of the rostral posterior insula in rats induced tachycardia while bradycardia was elicited via caudal stimulation[10]. Furthermore, Zhang et al., (1998) demonstrated that damage to the left insula increased cardiac baroreceptor gain with no effect on heart rate or blood pressure, while right insular lesions caused increased baseline heart rate and blood pressure with no effect on gain[11]. Finally, neuroimaging studies involving humans support insular activation in response to sympatho-excitatory tasks such as Valsalva, handgrip, maximal inspirations and lower-body negative pressure[12–14] (Figure 1.1). fMRI with simultaneous peripheral sympathetic recordings also showed increased anterior insular activity corresponded with increase muscle sympathetic nerve activity[14,15].

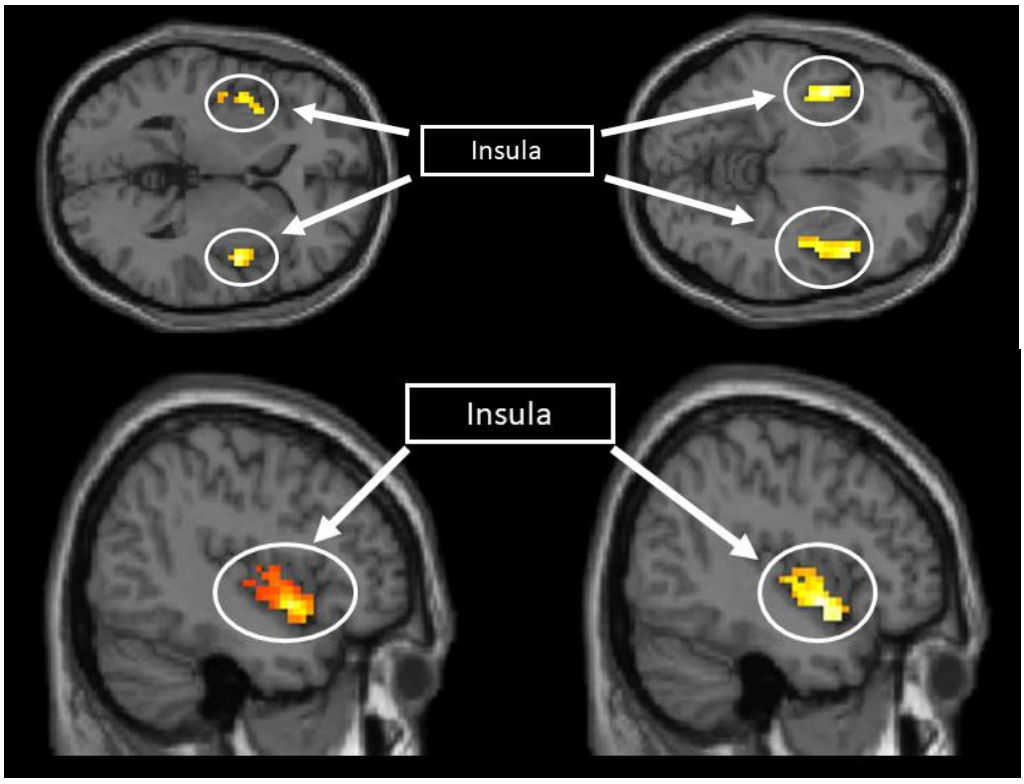

Figure 1.1. Insula Cortex

1.5.2 Cingulate Cortex

Similar to the insula cortex, the cingulate cortex is often parcellated into posterior and anterior segments (Figure 1.2) with both contributing to autonomic regulation, specifically modulations of heart rate and blood pressure[12,16,17]. The anterior cingulate cortex (ACC) is interconnected with the anterior insula[18], and therefore it is not surprising that the ACC is also commonly activated during maneuvers that elicit an increase in sympathetic activity such as the Valsalva maneuver, maximal inspiratory apneas and lower-body negative pressure[19]. Furthermore, like the anterior insular cortex, increased ACC activation has been coupled with direct recordings of sympathetic nerve activity[14,15]. In contrast, sub-motor somatosensory stimulation of small Type III and IV muscle afferents, along with corresponding changes in parasympathetic indicators (i.e. high frequency heart rate variability) have produced posterior cingulate activation[20].

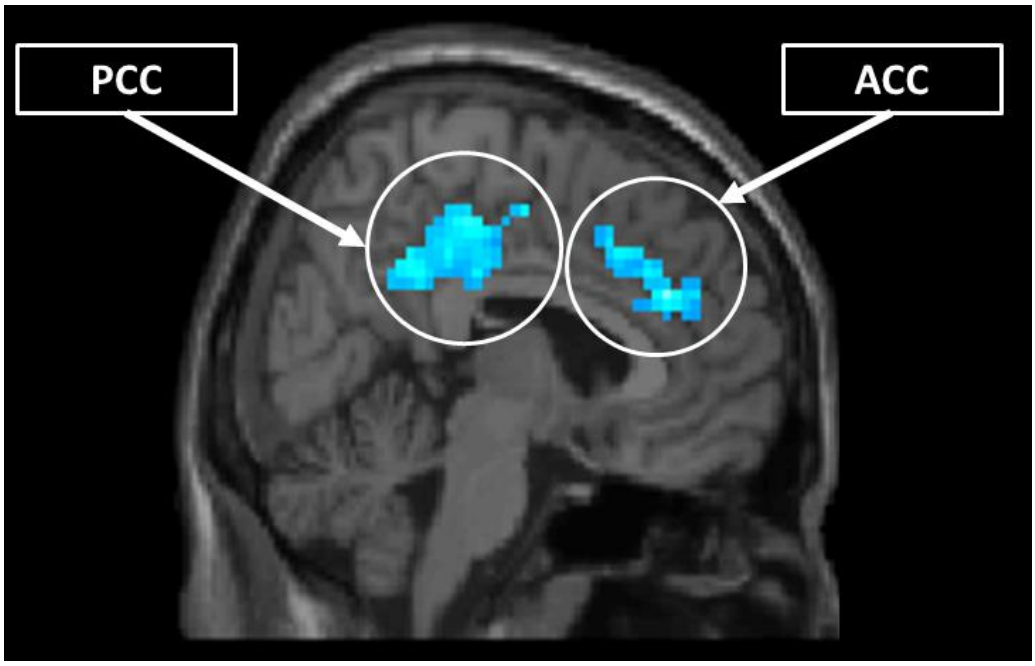

Figure 1.2. Anterior (ACC) and Posterior (PCC) Cingulate Cortices

1.5.3 Cerebellum

Dating back to the 17th C, in an attempt to understand the ANS, Thomas Willis found that some neural pathways appeared to take place without cerebral involvement. He postulated that the cerebrum controlled voluntary movement, whereas the cerebellum (Figure 1.3) was the originating point for involuntary movement. This theory regarding sympathetic control stemming from the brainstem and cerebellum was later altered by Robert Whytt[1]. This three-century-old idea would only be superficially discussed until nearly 80 years ago when Giuseppe Moruzzi showed that electrical stimulation of the cerebellum could affect various autonomic reflexes, including vasomotor, respiratory and carotid sinus reflexes[21]. Since then, research regarding cerebellar influences on autonomic processes has grown considerably. Yet, in 2016 the recommendations of a consensus panel still identified the contributions of the human cerebellum to autonomic control as an area deserving more attention and further investigation[22].

Anatomically, the cerebellum is connected to the brainstem via three cerebellar (inferior, middle and superior) peduncles. Furthermore, tracing studies have revealed the structural network of neurons projecting from the cerebellum to the NTS, RVLM, PBN and NA[23–25]. These anatomical networks support a functional role for the cerebellum in cardiovascular autonomic regulation. Functionally, the cerebellum integrates vestibular and somatosensory information regarding movement and postural adjustment. More specifically, the cerebellum integrates positional changes related to head-up tilt and upright posture. In response to postural changes to an upright position, the cerebellum integrates vestibular information and facilitates an early increase in efferent sympathetic outflow[26]. The integration of both vestibular and autonomic signals, together, establishes the vestibulo-sympathetic reflex (VSR). Cerebellar involvement in the VSR has been established in both animal and human models. For example, cerebellar stimulation in animal models produces significant cardiovascular responses including increased cerebral blood flow, tachycardia and arterial pressor responses with measurable increases in muscle, splanchnic and renal sympathetic nerve activity[26–31]. In healthy individuals, neuroimaging studies have demonstrated cerebellum and deep cerebellar nuclei activation during challenges involving increased sympathetic activity such as the Valsalva maneuver, inspiratory capacity apnea and lower-body negative pressure[14,15,32]. Furthermore, increase activity in the cerebellum/deep cerebellar nuclei was demonstrated to occur with concomitant increases in muscle sympathetic

nerve activity[14]. Finally, damage to the cerebellum has been shown to result in long-term impairment of cardiovascular responses including orthostatic hypotension and autonomic dysregulation[33–35].

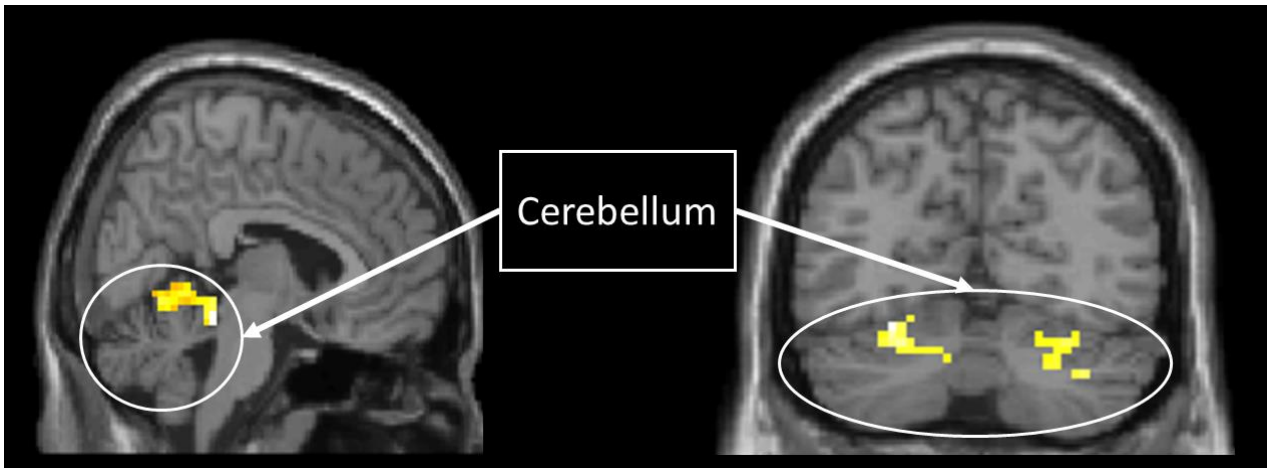

Figure 1.3. Cerebellum

1.6 Evaluation of autonomic function: clinically validated tests

Due to the widespread nature of the autonomic network and its complex integration within a

number of organs and systems, the impact of autonomic dysfunction or failure can be extremely disabling. Therefore, reliable and validated methods of evaluation are of utmost importance. The Autonomic Reflex Screen (ARS) is a battery of non-invasive, standardized autonomic tests to evaluate the presence, severity and distribution of autonomic dysfunction. The ARS is comprised of four evaluations including: 1) Quantitative sudomotor axon reflex test (QSART), 2) heart rate responses to deep breathing (HR_{DB}), 3) heart rate and blood pressure responses to Valsalva maneuver (VM) and 4) heart rate and blood pressure responses to head-up tilt (HUT)[36,37].

1.6.1 Quantitative Sudomotor Axon Reflex Test (QSART)

Quantitative Sudomotor Axon Reflex Test (QSART) evaluates the integrity of postganglionic sympathetic axons by means of transdermal iontophoresis of acetylcholine (ACh) for 5 minutes with a constant current generator set to 2 mA. The neural pathway consists of an axon reflex mediated by the postganglionic sympathetic sudomotor axon. The axon terminal is activated by ACh, creating an antidromic impulse that travels to a branch point. Subsequently, an orthodromic impulse travels to a secondary nerve terminal whereby ACh is released. ACh released from the secondary axon terminal binds to muscarinic receptors on eccrine sweat glands to evoke a reflexive sweat response. Following stimulation, an additional 5-minutes of recording provides a measurable residual sweat response. QSART is performed at four standard sites, the forearm, proximal leg, distal leg and foot, to provide a measure of axon integrity of the ulnar, peroneal, saphenous and sural, respectively. Total sweat volumes are measured by a sudorometer and calculated based on an integrated area under the curve of the entire 10-minutes. Reduced or absent sweat responses can be indicative of impaired postganglionic sympathetic axon integrity (Figure 1.4)[36,37].

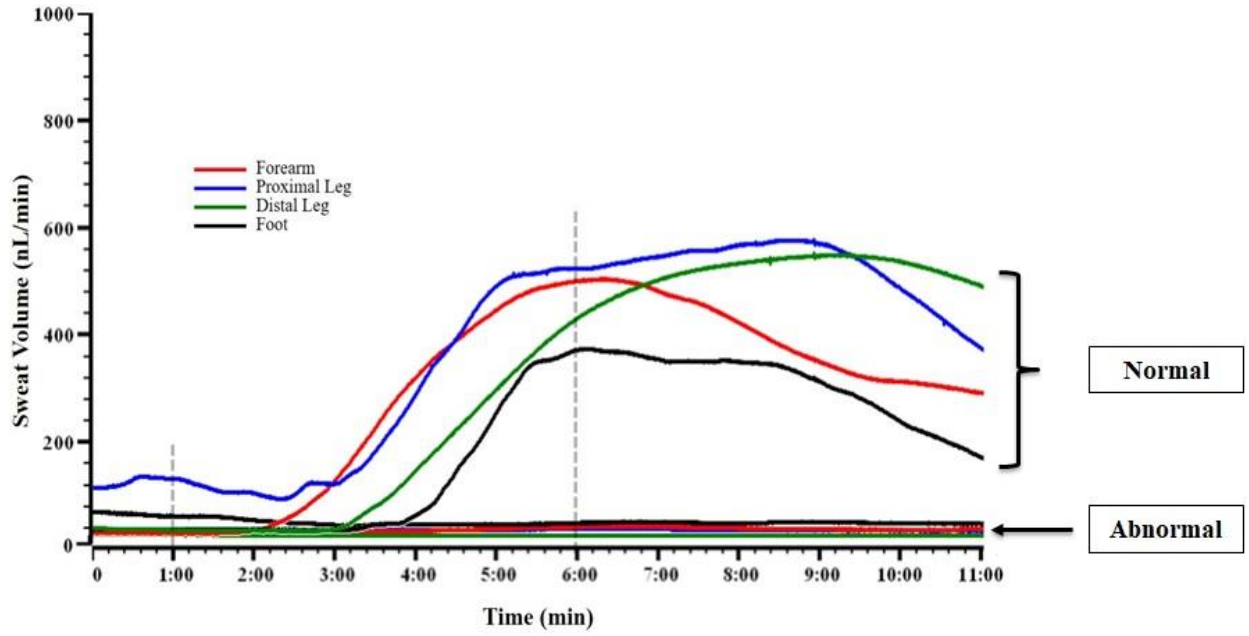

Figure 1.4 Quantitative Sudomotor Axon Reflex Test (QSART).

1.6.2 Heart Rate Response to Deep Breathing (HRDB)

Breathing naturally causes a pattern of discharge from the vagus nerve, and the rate of discharge can be influenced by the rate and depth of respiration. The vagal innervation to the heart contains both the afferent and efferent pathways for this reflex arc, and therefore the maneuver is regarded as a measure of cardiovagal function. To produce the maximal variation between breaths, individuals are asked to complete eight breathing cycles at a rate of 6 breaths/minute[38]. During analysis, the five highest consecutive peak-to-trough heart rate differences are calculated and averaged to provide an average heart rate response to deep breathing. A healthy cardiovagal response will show HR fluctuations in response to the maneuver (Figure 1.5A), whereas reduced heart rate responses are indicative of cardiovagal impairment (Figure 1.5B).

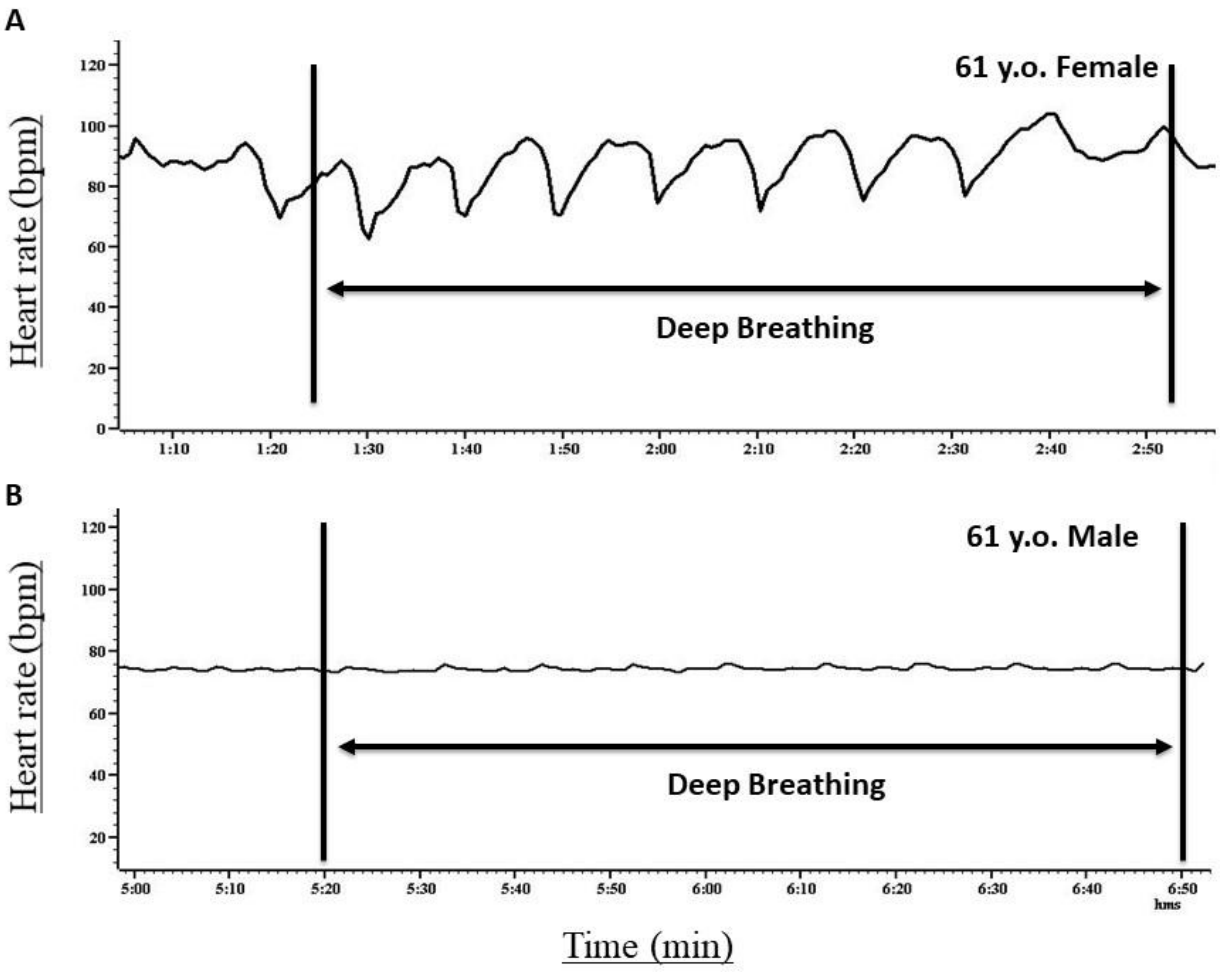

Figure 1.5 Heart Rate Responses to Deep Breathing (HRDB).

Normal (A) and severely reduced (B) heart rate responses to deep breathing. (B) represents cardiovagal impairment

1.6.3 Valsalva Maneuver (VM)

Valsalva maneuver (VM) is a simple and non-invasive clinical test ideal for providing important information regarding both sympathetic and cardiovagal functioning. The maneuver reflex is mediated by the baroreflex and changes in intra-thoracic pressures. In practice, participants are asked to exhale at an expiratory pressure of 40 mmHg held for 15 seconds. In doing so, a classic quadri-phasic change in blood pressure is produced in healthy individuals to provide insight into adrenergic function (Figure 1.6A).

Phase I: Phase I is mechanical in nature, upon inhalation there is a large increase in intra-thoracic pressure that causes compression of the aorta and a subsequent small increase in systolic blood pressure (SBP).
Phase II_Early: In early phase II there is a transient decline in SBP due to a reduced stroke volume and consequently a reduction in cardiac output (Q).
Phase II_Late: Within approximately 4 seconds, the drop in SBP is arrested, and starts to increase again. This occurs as a result of increased plasma concentration of norepinephrine (NE) and increased sympathetic discharge, which together results in an increase in total peripheral resistance (TPR).
Phase III: Phase III, similar to Phase I, is mechanically mediated. Upon release of the maneuver there is a drop in intra-thoracic pressure that results in a rapid drop in SBP for about 1-2 seconds.
Phase IV: As a result of the sudden drop in SBP in Phase III, there is a burst in sympathetic activity that increases Q. Increased Q in conjunction with the increased TPR from Phase II_Late, results in a large SBP overshoot above baseline levels. In contrast, reduced or absent adrenergic phases (late phase II and phase IV) provide evidence of adrenergic failure (Figure 1.6B).

An additional measure of cardiovagal functioning can be derived from the heart rate response to the VM. The Valsalva ratio (VR) is calculated from the highest heart rate generated from the maneuver divided by the lowest heart rate achieved following maneuver release. Physiologically, this occurs as there is a progressive compensatory tachycardia due to the decrease in SBP starting from Phase II_Early. Subsequently, the Phase IV SBP overshoot is accompanied by a transient bradycardia following release of the maneuver.

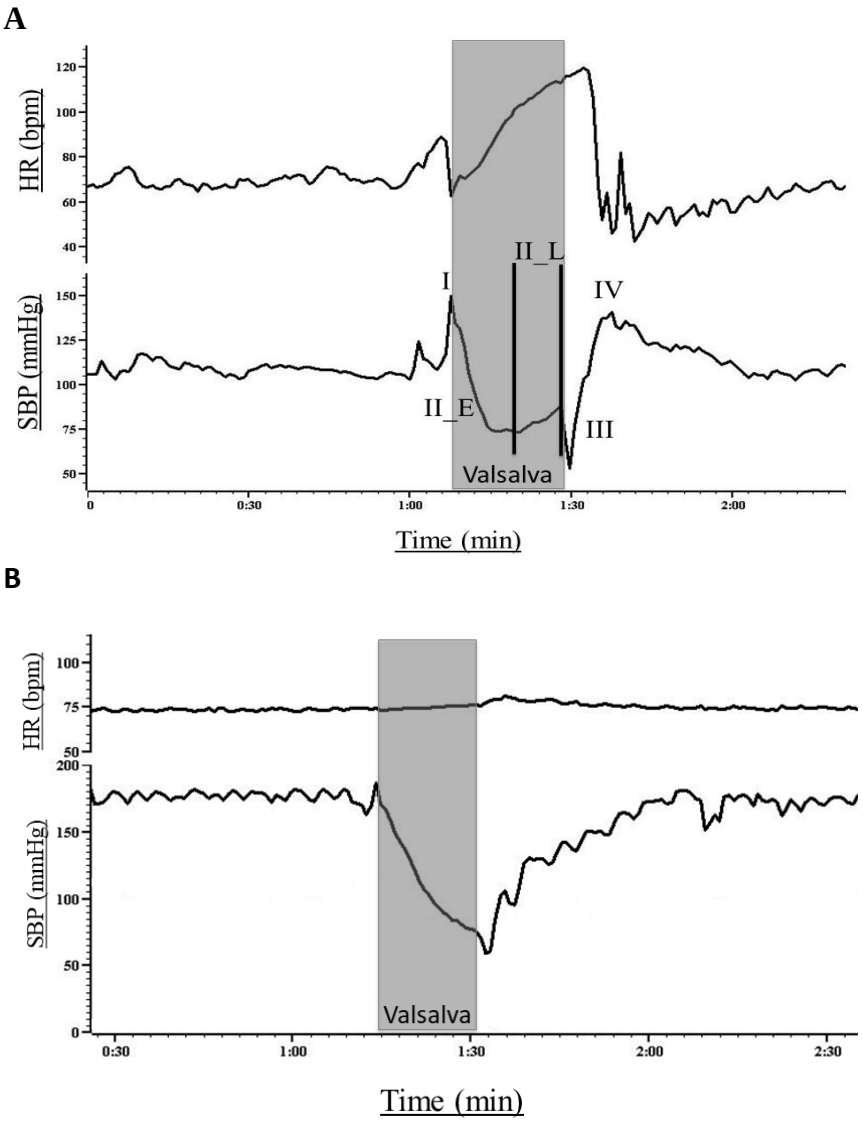

Figure 1.6. Heart rate and blood pressure responses to Valsalva maneuver

(A) Healthy individuals show reproducible quadri-phasic blood pressure responses to Valsalva and large tachycardic response. (B) Patients with autonomic failure will have absent late phase II and phase IV blood pressure responses, along with impaired heart rate responses.
Abbreviations: HR, Heart rate; bpm, beats per minute; SBP, systolic blood pressure; mmHg, millimeters of mercury; II_E, Phase II_Early; II_L, Phase II_Late

1.6.4 Head-up Tilt (HUT):

In response to an orthostatic challenge such as a passive head-up tilt (HUT), there are natural mechanisms to counteract the effects of gravity to maintain adequate blood pressure at the head and heart level. HUT is commonly the last test of the ARS as individuals should remain supine for a minimum of 20 minutes prior to tilt to allow for equal redistribution of intravascular volume, stable basal sympathetic nerve output and to maximize the degree of orthostatic stress. Following baseline, individuals are slowly (~10 seconds) and passively tilted to 70° from the horizontal. (Note: The 70° angle was found to produce a maximal orthostatic stress while minimizing the effects of muscular contraction.) Within the first 30 seconds of HUT it is common for healthy individuals to experience a transient and modest (<10 mmHg, mean BP) decline in systolic, diastolic and mean blood pressures followed by recovery within the first minute (Figure 1.7A). This recovery is mediated by sympathetic activation resulting in reflexive tachycardia, increased release of norepinephrine and increased TPR via vasoconstriction. Therefore, measuring the changes in HR and SBP in response to HUT provides a measure of sympathetic function. The duration of HUT is variable depending on the clinical investigation. For example, the clinical criterion for diagnosing postural orthostatic tachycardia syndrome is minimum tilt duration of 10 minutes. In contrast, individuals with evidence of neurogenic orthostatic hypotension (Figure 1.7B) may only last 1-2 minutes before tilt needs to be aborted due rapid and severe orthostatic hypotension.

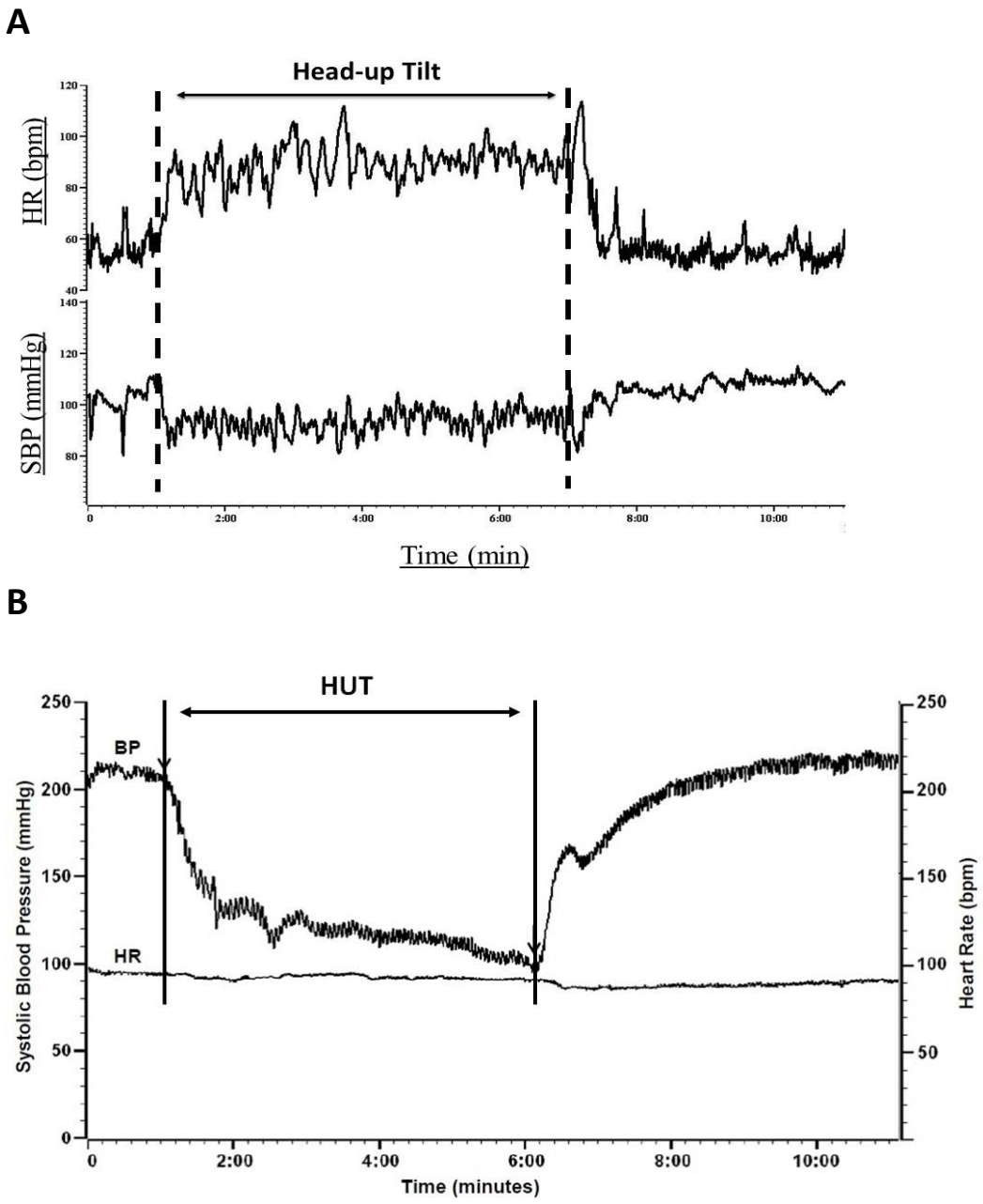

Figure 1.7 Hemodynamic response to Head-up Tilt (HUT).

(A) Healthy individuals show transient decline in systolic blood pressure at onset of head-up tilt (HUT). This is accompanied by an appropriate compensatory postural tachycardia (black line) and subsequent BP recovery. (B) Clear evidence of Neurogenic Orthostatic Hypotension with no compensatory postural tachycardia.

1.6.5 Composite Autonomic Severity Score

The composite autonomic severity score (CASS) is derived from the autonomic reflex screen and is normalized for age and sex[39]. The CASS is a validated score used to quantify autonomic dysfunction and provide a measure of the severity and distribution of autonomic failure[40]. The 11-point CASS (0-10) is divided into three indices: Sudomotor Index (0–3), Cardiovagal Index (0–3), and Adrenergic Index (0–4). Patients with a composite score ranging from 1-3 are considered to have mild autonomic dysfunction, 4-6 are considered moderate, and 7-10 have severe autonomic failure. A score of 0 would indicate no autonomic dysfunction.

1.7 Patient Self-report Instruments

In addition, there are a number of self-report questionnaires that have been validated for clinical autonomic populations. Two common questionnaires include the Autonomic Symptom Profile and the Orthostatic Hypotension Questionnaire.

1.7.1 Autonomic Symptom Profile

The autonomic symptom profile (ASP) is a validated self-report instrument designed to provide an index of autonomic symptom severity[41]. The ASP is comprised of 169 questions to yield a Composite Autonomic Symptom Scale (COMPASS) reflecting overall severity of autonomic symptoms. There are 10 subscale scores (11 for men) that assess severity of symptoms within the following domains: orthostatic intolerance, bladder dysfunction, diarrhea, gastroparesis, secretomotor dysfunction, syncope, sleep disorder, constipation, vasomotor symptoms, and pupillomotor symptoms and sexual dysfunction for men. The highest possible overall score for men is 200 and 170 for women, with higher scores indicating more autonomic symptomatology. Newer and briefer versions of the COMPASS (COMPASS 31) are currently available[42].

1.7.2 Orthostatic Hypotension Questionnaire

The Orthostatic Hypotension Questionnaires (OHQ) is a 10 question self-report questionnaire to assess symptoms related to a low blood pressure problem. The OHQ yields the following two sub-scores: Part I: the orthostatic hypotension symptoms assessment (OHSA), and Part II: the orthostatic hypotension daily activity scale (OHDAS). The OHSA consists of six questions to

measure the presence and severity of orthostatic symptoms, while the OHDAS consists of four questions to assess the impact of orthostatic symptoms on daily activities[43]. Each item is scored on an 11-point scale from 0–10, with 0 indicating no symptoms/no interference and 10 indicating the worst symptoms/complete interference. Included in the questionnaire is an additional option of "cannot do for other reasons".

1.8 Evaluation of autonomic function: additional tests

1.8.1 Lower Body Negative Pressure (LBNP):

Lower body negative pressure (LBNP) investigates the effects of blood volume displacement to reliably activate the baroreflex through baroreceptor unloading. In the supine position, individuals are sealed from the waist down in an airtight container connected to a vacuum. Negative pressure applied to the lower half of the body redistributes blood from the upper to the lower extremities resulting in relative central hypovolemia. The level of suction (i.e. negative pressure) can be precisely monitored using a manometer and rapidly controlled through valves that open to room pressure. When applied at lower levels (< 15 mmHg), LBNP reduces central blood volume and increases peripheral sympathetic activity primarily by unloading cardiopulmonary baroreceptors. At higher levels of suction (>30 mmHg), there is additional arterial baroreceptor unloading, to cause further reductions in CBV, accompanied by reduced cardiac filling and stroke volume. As a result, these changes produce reflex tachycardia and even greater levels of peripheral sympathetic activity[44–46]. LBNP provides a technique, especially for patients with limited mobility and functional imaging studies, as the testing can be executed entirely in the supine position. Clinically, LBNP provides an orthostatic stress equivalent to that of head-up tilt (Figure 1.8).

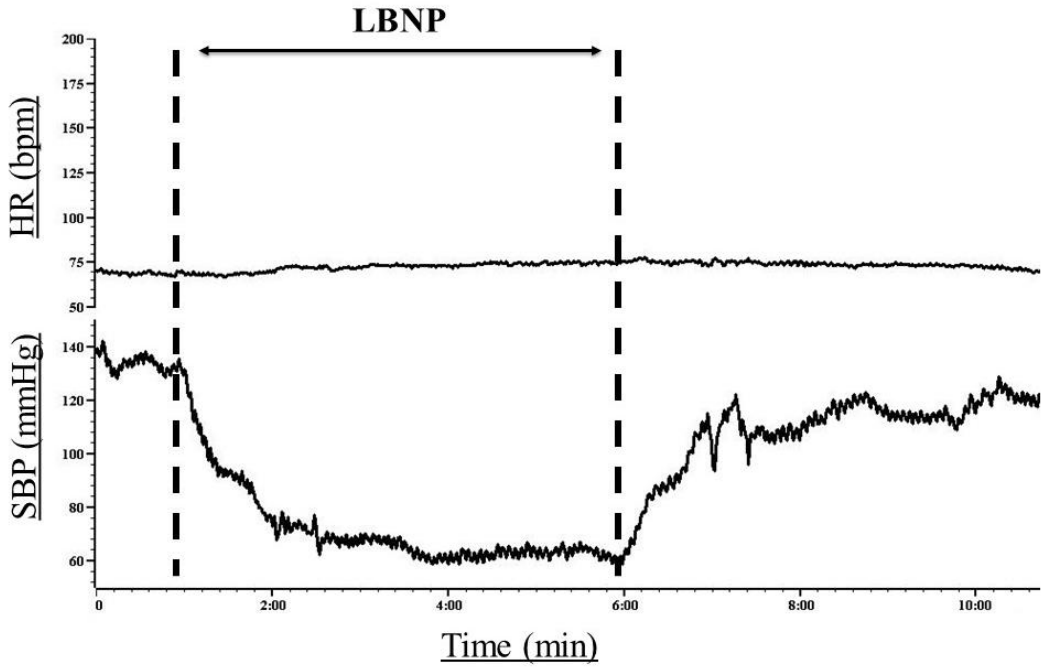

Figure 1.8 Lower Body Negative Pressure (LBNP) in patient with autonomic failure.

Abbreviations: HR, Heart rate; bpm, beats per minute; SBP, systolic blood pressure; mmHg, millimeters of mercury; LBNP, lower body negative pressure.

1.8.2 Functional Magnetic Resonance Imaging (fMRI)

Functional magnetic resonance imaging (fMRI) provides a non-invasive modality for mapping patterns of brain activation and therefore has emerged as a leading tool in both clinical and research settings. In brief, the blood-oxygen-level-dependent (BOLD) response, generally measured by means of a T2*-weighted sequence, takes advantage of the tight coupling between neural activity, cerebral blood flow and the change in magnetic properties as hemoglobin shifts from oxygenated (Hb) to deoxygenated (dHb). The MR signal intensity is distorted by the magnetic properties of dHb. Therefore, a relative change in dHb produces a change in MR signal. The BOLD response to a short stimulus shows the following three phases: 1) Fast response/early dip, 2) Main BOLD response and, 3) Post-stimulus undershoot (Figure 1.9).

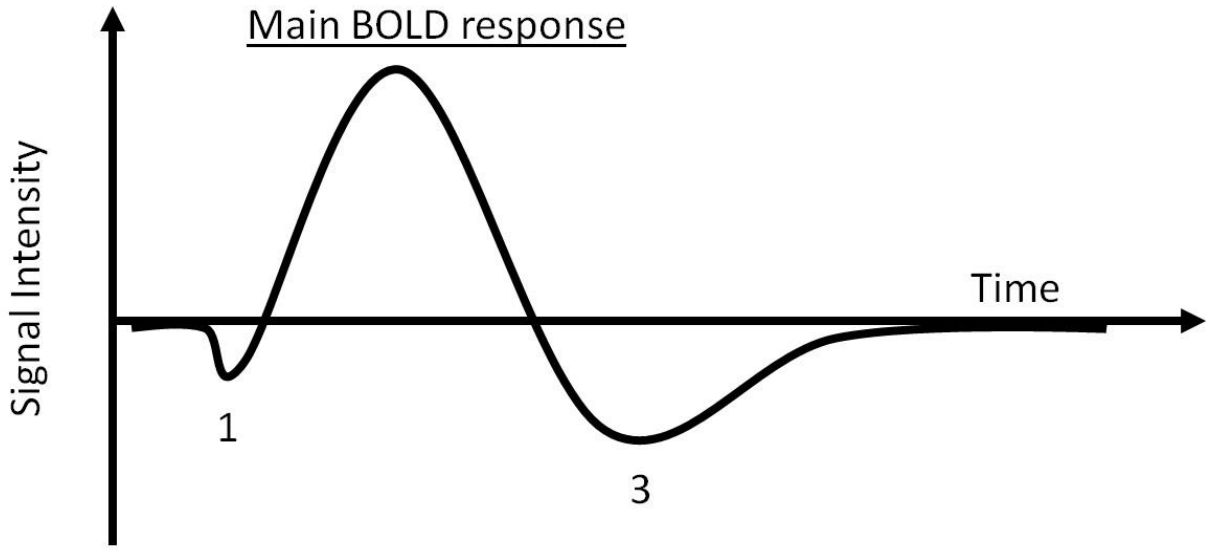

Figure 1.9 Blood Oxygen Level Dependent (BOLD) response to a stimulus

Adapted from Norris D. (2006) Principles of magnetic resonance assessment of brain function. *Journal of Magnetic Resonance Imaging.* 23(6): 794-807.

The BOLD contrast is influenced by three physiological parameters: the rate of oxygen consumption, regional cerebral blood flow (rCBF) and regional cerebral blood volume (rCBV).

The initial negative dip in signal intensity following a stimulus is due to increased oxygen consumption (i.e. increase in dHb) without any appreciable changes to rCBF and rCBV. The main BOLD response reflects an increase in rCBF and rCBV causing a reduction in the amount of dHb, and subsequent large MR signal. Finally, post-stimulus undershoot is thought to be due to a maintained rate of oxygen consumption once blood flow has returned to baseline. Overall, the BOLD response provides an indirect measure of brain activity, as an increase in neural activity will increase all three parameters facilitating a measurable BOLD response[47–49].
Since the emergence of functional imaging, brain mapping has focused largely on localizing brain functions. However, in the last decade the neurosciences has seen a shift from functional segregation to functional integration. fMRI data has been analyzed to greater depths to reveal how neural systems may be coupled to perform specific functions. The organization and integration of different brain regions is commonly referred to as "functional connectivity": an attempt to look at the functional architecture of the brain. Functional connectivity attempts to quantify the interactions of distinct brain regions that are simultaneously engaged during a specific task using correlations or covariances of activity derived from the BOLD data.

1.9 Autonomic Failure

Orthostatic hypotension (OH) is present in 5-30% of persons ≥65 years[50,51]. Neurogenic OH (NOH) can be differentiated from other causes of OH, such as hypotension due to endocrine issues, generalized low blood pressure, low blood volume, etc., in that NOH is associated with impairment of the sympathetic nervous system[52–54]. NOH is clinically defined as a sustained reduction in systolic blood pressure (SBP) ≥20 mmHg or diastolic blood pressure of ≥10 mmHg within 3 minutes of standing or head-up tilt without an appropriate compensatory tachycardia[55] (Figure 1.7B). To improve the false positive detection rate from 5% to 1%, and in patients with autonomic failure plus supine hypertension, a SBP reduction ≥30 mmHg or a diastolic drop ≥15 mmHg is recommended[54,56]. NOH is a cardinal feature of autonomic failure. The term "autonomic failure" represents a broad description of generalized pan-dysautonomia that can occur independently or accompany a number of disorders. Generally, clinical classifications of NOH secondary to autonomic failure can be made based on where failure of the sympathetic efferent signaling pathway occurs i.e. before or after the autonomic ganglia. For example, NOH occurs in disorders of the central nervous system /pre-ganglionic lesions including: Multiple

System Atrophy (MSA) and Lewy Body Dementia (LBD). In contrast, clinical populations such as Parkinson's Disease (PD) with autonomic failure, Pure Autonomic Failure (PAF), Diabetic Autonomic Neuropathies, etc., the lesion site is considered to be "post-ganglionic"/peripheral.

1.9.1 Pre-ganglionic Disorders

Multiple System Atrophy: Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder involving progressive deterioration of three specific regions of the brain and preganglionic nerves. Degeneration of the basal ganglia, cerebellum and pons give MSA a clinical triad of symptomatology resembling Parkinson's Disease, cerebellar ataxia and autonomic failure. MSA is considered pre-ganglionic and as such patients will typically have intact post-ganglionic sympathetic nerves. However, as the disease progresses some patients can acquire post-ganglionic denervation resulting in more widespread autonomic failure affecting blood pressure regulation, bowel and bladder dysfunction, respiration, sleep, etc. The prognosis of MSA is poor relative to other forms of autonomic dysfunction, with life expectancy ranging from 7-10 years following symptoms onset[57]. Particularly, following the development of severe autonomic failure, including NOH, survival is reduced by 2.3 years[58].

Lewy Body Dementia: Lewy Body Disorders (LBD) are identified by an accumulation of a protein called alpha-synuclein, also known as Lewy bodies, around the neuronal cell body and synaptic terminals[53]. Clinical features vary depending on the location of accumulation; however, autonomic failure and NOH are prominent in most Lewy body disorders. Unlike MSA, dementia, cognitive impairment and visual hallucinations are more prominent in LBD due to Lewy body accumulation in the basal forebrain and cerebral cortex.

1.9.2 Post-ganglionic Disorders

Pure Autonomic Failure: Pure Autonomic Failure (PAF) is a sporadic and chronic peripheral degenerative disorder characterized by autonomic failure without any other neurological deficits, including central degeneration, motor and sensory deficits. A clinical diagnosis of PAF is based on widespread and persistent sympathetic and parasympathetic dysfunction without any evidence of other pathology for an extended period of time. Despite significantly autonomic dysfunction,

PAF patients typically have normal life expectancies. Autonomic issues include: neurogenic orthostatic hypotension, erectile dysfunction, impaired sweating, bowel and bladder dysfunction and dry mouth.

Parkinson's Disease plus Autonomic Failure: NOH and autonomic dysfunction is present in approximately 30% of patients with Parkinson's disease (PD)[59]. Despite neuronal death within the substantia nigra, which accounts for many Parkinsonian symptoms, clinical literature provides robust evidence that supports PD plus Autonomic failure as a post-ganglionic disorder. Studies involving various testing of post-ganglionic sympathetic nerve integrity, including neuroimaging, pharmacological and neurochemical, have repeated revealed post-ganglionic sympathetic denervation in PD plus autonomic failure patients[52,60–62]. The most common autonomic problems include, neurogenic orthostatic hypotension, bowel and bladder dysfunction, gastrointestinal dysmotility and sexual dysfunction[63].

Idiopathic NOH: Approximately 1/3 of patients with NOH have no identifiable underlying cause for dysfunction[64] and as such NOH can occur as an independent entity, or the course of their disease has not become clear. Patients diagnosed with idiopathic NOH typically have considerable orthostatic hypotension, along with gastrointestinal issues or other questionable phenomenon such as olfactory impairment, but do not meeting criteria for other alpha-synucleinopathies. Patients with idiopathic NOH may develop a clearer diagnose over time.

1.10 Neurogenic Orthostatic Hypotension

Neurogenic Orthostatic Hypotension (NOH) is a disorder affecting individuals globally, but arguably has received considerably less attention than its counterpart, hypertension. Presently, there are several gaps in our overall understanding and assessment of patients with NOH related to autonomic failure.

First, regardless of the underlying etiology (i.e. pre- versus post-ganglionic), impairment of higher cortical and subcortical regions has not been investigated in the pathophysiology of NOH. Specific areas of the brain such as the cingulate cortex, insula, hippocampus and cerebellum, which have all been highlighted as key cortical structures in autonomic regulation. Importantly,

these structures have been shown to contribute to the proper regulation of basic autonomic functions such as heart rate and blood pressure; the same key autonomic functions that fail in patients with NOH[12,20,32,65,66].

Second, NOH is a debilitating condition associated with reduced quality of life, impaired function and is an independent predictor of mortality[67–69]. Common symptoms include: fatigue, weakness, head and neck pain, dizziness, lightheadedness, pre-syncope, and in some cases, full syncope[55]. Unfortunately, these symptoms are typically regarded as 'constitutional' or non-specific, that is, symptoms that can be related to many different systems and the results of a plethora of underlying causes. For this reason, a proper diagnosis of NOH can be challenging, especially for clinicians without significant experience in disorders of the ANS. Furthermore, roughly 50% of patients with NOH also have supine hypertension, which can distract practitioners who may then fail to obtain upright blood pressure measurements. Accurate identification of autonomic dysfunction lies in the ability to appropriately discern these symptoms on the basis of position. Autonomic symptomatology is posturally-related; patients can be extremely symptomatic in the upright position, but these can often be completely relieved by resuming a seated or lying position. Therefore, clinicians must identify these symptoms as being either orthostatic or non-orthostatic to help guide them towards a proper diagnosis of NOH. Unfortunately, clinicians do not have available to them a simple, non-invasive tool that they can administer to help make this distinction. Therefore, under these circumstances, more common syndromes and disorders associated with light-headedness, dizziness, etc., such as inner ear or vestibular issues, vertigo, migraines, hypoglycemia, anemia and even certain medication side effects, may be considered as differential diagnoses prior to orthostatic intolerance that accompanies NOH.

1.11 Purposes and Hypotheses

Therefore, the primary purpose of the studies described herein (Chapters 2-7) was to improve our assessment and understanding of Neurogenic Orthostatic Hypotension. More specific objectives and hypotheses are as follows:

Study 1 Objective: Evaluate brain activation patterns in NOH patients using functional MRI during standard tests of autonomic functioning.

Hypothesis: Relative to healthy controls, patients with NOH will demonstrate reduced activity in central autonomic structures during autonomic maneuvers.

Study 2 Objective: Evaluate functional connectivity between the brainstem and central autonomic structures at rest and during autonomic challenges in NOH patients.

Hypothesis: NOH patients will demonstrate reduced brainstem connectivity relative to their healthy counterparts

Study 3 Objective: Based on the results of study 1, we aimed to investigate cerebellar functional connectivity in NOH patients at rest and during autonomic maneuvers.

Hypothesis: Patients with NOH will demonstrate reduced cerebellar connectivity to key central autonomic structures.

Study 4 Objective: Create a self-report questionnaire to identify and assess patients with autonomic dysfunction based on symptomatology; evaluate validity and reliability.

Hypothesis: The questionnaire will demonstrate preliminary validity and reliability within a sample population of patients with orthostatic intolerance associated with autonomic dysfunction.

Study 5 Objective: Assess sensitivity and specificity of the questionnaire, inter-item reliability and the ability to positively predict patients with orthostatic dysfunction secondary to autonomic dysfunction.

Hypothesis: The questionnaire will produce symptom scores that are sensitive and specific and will be capable of positively identifying patients with orthostatic symptoms related to autonomic dysfunction.

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CHAPTER 2

2 Impaired cortical autonomic responses during sympathetic activation in Neurogenic Orthostatic Hypotension characterized by post-ganglionic autonomic dysfunction[1]

2.1 Introduction

Neurogenic orthostatic hypotension (NOH) is a debilitating condition associated with reduced quality of life, impaired function and is also an independent predictor of mortality[1–3]. NOH is clinically defined as a sustained reduction in systolic blood pressure (SBP) ≥20 mmHg or diastolic blood pressure of ≥10 mmHg within 3 minutes of standing or head-up tilt performed at 60° without an appropriate compensatory postural tachycardia[4]. However, to improve the false positive detection rate from 5% to 1%, in patients with autonomic failure, a SBP reduction ≥30 mmHg or a diastolic drop ≥15 mmHg is recommended[5,6]. Specifically, neurogenic OH can be differentiated from other causes of orthostatic hypotension, such as hypotension due to endocrine issues, generalized low blood pressure and low blood volume, in that a lesion is present in a specific region of the nervous system, known as the autonomic nervous system. Specifically, dysfunction of reflexive responses mediated by the sympathetic nervous system[7,8]. Autonomic dysfunction can be further defined as either central or peripheral. For example, in Multiple System Atrophy, atrophy of the pons results in significant autonomic failure[9]. In contrast, peripheral autonomic disorders such as, pure autonomic failure, Parkinson's Disease with autonomic failure and idiopathic NOH are characterized by lesions in the post-ganglionic sympathetic fibres[8]. Regardless of the underlying etiology, both central and peripheral autonomic lesions result in a significant drop in blood pressure as patients move from sitting to standing, which can lead to inadequate cerebral blood flow and reduced cerebral perfusion pressure in various cortical regions[10]. As cerebral blood flow and perfusion pressure are crucial factors in normal brain function, failure to adequately control these variables may significantly impact the

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Baker J, Paturel J and Kimpinski K. (2018). Impaired cortical autonomic responses during sympathetic activation in neurogenic orthostatic hypotension characterized by post-ganglionic autonomic dysfunction. *J. Appl. Physiol.* 125: 1210-1217

functionality of cortical networks. Recent advances in neuroimaging, specifically, functional magnetic resonance imaging (fMRI), has facilitated research mapping brain activation patterns by exploiting the relationship between neural activity, energy metabolism and cerebral blood flow. As a result, specific areas of the brain such as the cingulate cortex, insula, hippocampus and other cortical regions have been implicated in autonomic regulation. Furthermore, these structures have extensive cortical-cortical and cortical-subcortical projections to structures that regulate autonomic responses[11], and in animal and human studies alike, have been shown to influence autonomic parameters including heart rate and blood pressure[12–16]. Finally, in a clinical context, damage to these areas due to stroke or lesion has been shown to disrupt regular autonomic functioning[17–19]. Together these areas make up part of the central autonomic network[11,13,14].

Given the clinical context of autonomic failure, and the prominent feature of NOH leading to alterations in cerebral blood flow and perfusion pressure, the objective of this study was to evaluate brain activation patterns of NOH patients in response to standard tests of autonomic function. Patient blood oxygen-level dependent activation patterns were compared against healthy age-matched participants to investigate whether impairment of central autonomic structures is involved in the pathophysiology of NOH.

2.2 Methods

2.2.1 Study participants

The current study included 15 healthy, age-matched controls (63±13 years) and 15 patients diagnosed with Neurogenic Orthostatic Hypotension (NOH) (67±6 years) ($p=0.12$) related to a peripheral autonomic lesion. Patients were recruited from the Autonomic Disorders Laboratory in the Department of Clinical Neurological Sciences, London Health Sciences Centre, London, Ontario, Canada. Patients with evidence of central autonomic dysfunction were excluding to remove potentially confounding variables that would directly affect specific regions within the Central Autonomic Network (CAN). Prior to testing, all diagnoses were clinically confirmed by a Neurologist with specialty training in autonomic dysfunction (KK). Patients were clinically evaluated as having peripheral forms of autonomic failure based on neurological examination,

patient history (i.e. no evidence of REM sleep behaviour disorder, olfactory dysfunction, etc.) and diagnostic work-up (i.e. blood work, brain MRIs, and responsiveness to treatment). The NOH patient cohort was comprised of patients with evidence of peripheral autonomic denervation (pure autonomic failure, n=3; Parkinson's Disease + NOH, n=7; idiopathic NOH, n=5). Patients were excluded if there was evidence of any peripheral nerve injury unrelated to their diagnosis of autonomic dysfunction including diabetic neuropathies in any form. The patient cohort reported an average symptom duration of 7±5 years. Healthy participants were also examined to confirm the absence of any neurological conditions including autonomic dysfunction. Healthy participants were recruited from the general population, including recruitment from aging activity centers in London, Ontario. In addition, healthy participants were excluded if they fell under any one of the following categories: i) clinically significant coronary artery disease, ii) concomitant therapy with anticholinergic, alpha- and beta-adrenergic antagonists or other medications which could interfere with autonomic functioning, and iii) failure of other organ systems or systemic illness that could affect autonomic function or participants' ability to cooperate. All laboratory data were collected in the Autonomic Disorders Laboratory at University Hospital, London, Ontario. All functional imaging data were collected at Robart's Research Institute Centre for Functional and Metabolic Imaging at The University of Western Ontario. Ethical approval was obtained from the Health Science Research Ethics Board at Western University, and informed consent was obtained from all participants prior to any and all testing.

2.2.2 Autonomic testing

All participants underwent a battery of standardized and validated tests of autonomic function, namely the autonomic reflex screen (ARS)[20,21]. In brief, quantitative sudomotor axon reflex test (QSART) provided an assessment of post-ganglionic sympathetic function from four standard sites (forearm, proximal leg, distal leg and foot). Adrenergic function was assessed by the beat-to-beat blood pressure (BP) and heart rate (HR) responses to head-up tilt (HUT) performed at 70° from the horizontal for a maximum of 5 minutes and the Valsalva maneuver (VM) performed at an expiratory pressure of 40 mmHg for 15 seconds. Cardiovagal function was evaluated by the Valsalva ratio, and the HR responses to deep breathing. The composite

autonomic scoring scale (CASS) was derived as a quantitative measure of the ARS[22]. In the lab, beat-to-beat BP and HR responses were measured using a BMEYE Nexfin device (Amsterdam, The Netherlands) and an electrocardiography (ECG) device (Model 3000 Cardiac Trigger Monitor, IVY Biomedical Systems, Inc., Branford, CT) with ECG electrodes (Ambu® Blue Sensor SP, Glen Burnie, MD), respectively. All recordings were made using WR TestWorksTM software (WR Medical Electronics Co., Stillwater, MN). Participants repeated deep breathing and Valsalva during a functional MRI with the same aforementioned parameters. However, as opposed to only performing two trials of each task, participants were instructed to perform four deep breathing exercises and three VMs.

2.2.3 Neuroimaging data acquisition

All imaging data were collected using a whole body 3T imaging system (Magnetom Primsa, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil. A high-resolution T1-weighted structural volume was acquired with a 3D MPRAGE sequence at the beginning of the scanning session (sagittal, matrix 256x240 mm, voxel resolution 1.0x1.0x1.0 mm, 1 mm slice thickness, no gap, flip angle 9 degree, TE: 2.98 ms, TI: 900 ms, TR: 2300 ms). Blood oxygen level-dependent (BOLD) signals were acquired to provide an indirect measure of brain activation. Due to the paramagnetic properties of deoxygenated haemoglobin (dHb), there is distortion of the acquired magnetic signal that is related to changes in the amount of dHb present in the tissue. Therefore, changes in the magnetic signal as a result of changes in the amount of oxygenated Hb versus dHb provide an indirect measure of tissue oxygen extraction and thus activation[23]. BOLD signals were acquired using a T2- weighted gradient echo-echo planar imaging pulse sequence with the following parameters: TE: 3 0ms; FOV: 240x240 mm; flip angle: 40 degrees; multiband acceleration factor: 4. Forty-eight interleaved axial slices (3.0x3.0 mm in-plane voxel resolution, TR: 1000 ms) were acquired in each volume.

2.2.4 MRI experimental paradigm

Participants completed 4 deep breathing exercises (120 seconds each) with 60 seconds of rest in between each trial (620 volumes) and 3 VMs (15 seconds each) with a 60-second baseline and 120 seconds of rest in between each trial (465 volumes). The first 2 volumes of each test were

discarded from analysis to allow for an equilibrated MRI signal. To minimize head movement, each participant's head was placed in a cradle packed with foam padding. In addition, all participants practiced the protocol prior to scanning to help minimize head movements during each experimental protocol and were instructed to avoid head movements as much as possible. Beat-to-beat heart rate was recorded from the continuous signal derived from an MRI-compatible pulse oximeter (Nonin Medical, 8600FO MRI, Plymouth, MN) attached to the index finger of each participant's left hand when possible. In the presence of a significant tremor i.e. in PD+NOH patients, pulse oximetry was obtained from the hand with less potential for movement. All hemodynamic recordings were collected using WR TestWorks™ software (WR Medical Electronics Co., Stillwater, MN).

2.2.5 Neuroimaging data analysis

Raw fMRI data were analyzed using SPM12 (Wellcome Department of Imaging Neuroscience, London, UK). All functional images were realigned using a rigid body transformation to correct for head motion using the mean functional image. All images were co-registered with the T1-weighted scan, normalized to standard stereotaxic space (Montreal Neurological Institute; MNI) and smoothed with a 6mm FWHM Gaussian kernel. To reduce low frequency noise, a high pass filter with 128s cut-off was applied.

Two levels of analysis were performed. First, individual design matrices were constructed for each experimental protocol (HRDB and VM) modeled by a box-car and convolved with a canonical hemodynamic response function. The General Linear Model was used to create a statistical parametric map on a voxel-by-voxel basis[24]. Second, the average contrast image from each individual, for each experiment protocol [VM and HRDB] was entered into a 2-sample independent t-test. Significant changes in signal intensity from rest were determined for each paradigm (DB and VM). An additional contrast of phase IV of VM (10 seconds following release of the maneuver) was compared against rest. In contrast to the entire VM that incorporates both parasympathetic and sympathetic components of the ANS, 10-seconds following the immediate release of the maneuver was chosen to capture a phase that is primarily adrenergically-mediated[25]. Signal intensity differences between controls and patients were

compared using subtraction analysis. These contrasts included: Control VM>Patient VM [1, -1], Patient VM>Control VM [1, -1], Control VM Phase IV>Patient VM Phase IV [1, -1], Patient VM Phase IV> Control VM Phase IV [1, -1], Control HRDB>Patient HRDB [1, -1] and Patient HRDB>Control HRBD [1, -1]. Comparisons of the BOLD responses were corrected for multiple comparisons (family-wise error (FWE) <0.05) with a cluster threshold of 10 voxels. In some cases, a more lenient threshold of p<0.001, uncorrected was used.

2.2.6 Regions-of-Interest analysis

Regions of interest (ROI) were determined based on previous work highlighting regions of the central autonomic network. These a priori ROI included the bilateral insula, bilateral anterior and posterior cingulate, bilateral hippocampus and bilateral thalamus. All ROI masks were created using WFU_Pick Atlas toolbox version 1.2[26,27].

2.2.7 Statistical analysis

Physiological data are presented as mean ± standard deviation. Autonomic parameters between NOH patients and age-matched controls were compared using an independent t-test. All tests were 2-tailed with a p-value <0.05 to denote significance. All statistical analyses were performed using SPSS statistical software, Version 22.0. Manufactured by International Business Management (IBM) Corporation (SPSS Inc. Chicago, IL).

2.3 Results

2.3.1 Hemodynamic responses

Quantitative Sudomotor Axon Reflex Test: There was evidence of significantly smaller sweat volumes at the proximal and distal leg, and a trend toward smaller sweat volumes at the forearm (p=0.07) in patient with NOH. The foot showed no significant sweat volume differences between patients and controls. Even though the sweat response at the foot was not significantly different from the control group, these values were still considered reduced relative to normative data, suggesting impairment in the sweat response at this site[8] (Table 2.1). Overall, these data are still in keeping with autonomic dysfunction related to peripheral (post-ganglionic) autonomic impairment. Cardiovagal Index: Compared to controls, patients had significantly smaller heart

rate (HR) responses to deep breathing and Valsalva ratios ($p<0.001$). This was evident in both the lab and MRI protocols. Adrenergic Index: Patients had significantly larger SBP drops during HUT with significantly smaller compensatory tachycardias ($p<0.001$) (Table 2.1). Qualitative analysis of the VM demonstrated that patients with NOH had absent adrenergic phases in response to the maneuver, resulting in a significantly larger Adrenergic Index as evaluated by the CASS (Table 2.1). The Cardiovagal Index of the CASS showed a similar significant difference between controls and patients (Table 2.1). Hemodynamic changes to autonomic maneuvers showed no significant differences between the lab and MRI testing. The hemodynamic responses to standard autonomic testing clearly support pan-dysautonomia with NOH, related to a peripheral autonomic lesion.

2.3.2 Functional imaging

Cardiovagal Index: Healthy individuals demonstrated significant reductions in brain activity relative to rest in the bilateral thalamus, bilateral insula, left posterior cingulate cortex (PCC), and right parahippocampus (Table 2.2; Figure 2.1). Similarly, patients with NOH had significant reductions in cortical activity relative to rest in the (PCC), right anterior CC and bilateral insula (Table 2.2; Figure 2.1). Figure 5.2 provides a 3D render to better visualize insular deactivation in both controls and patients during the parasympathetically mediated maneuver, deep breathing (Figure 2.2). Furthermore, there was evidence of deactivation within the bilateral thalamus; however, these regions did not reach significance. A subtraction analysis [controls>patients; 1, -1] [patients>controls, 1, -1] revealed no significant differences between controls and patients in response to deep breathing.

Adrenergic Index: In contrast to cardiovagal function, the Valsalva maneuver revealed significant differences between healthy controls and patients. A subtraction analysis [controls>patients, 1, -1] revealed that controls had significantly greater activation during VM, relative to rest, in the bilateral thalamus, left PCC, right ACC and right hippocampus. Furthermore, during phase IV of the VM, the right hippocampus remained significantly more activated in healthy controls relative to rest compared to NOH patients (Table 2.3; Figure 2.3). Patients showed no significant activation during VM or Phase IV when the analysis was reversed [patients>controls, 1, -1].

Table 2.1. Anthropometric data and autonomic testing results

Anthropometric	**Control (n=15) Mean ± SD**	**Patient (n=15) Mean ± SD**	**p-value**
Age (years)	61±14	67±6	=0.2
Range (years)	48-79	59-77	
Sex M: F	7:8	9:6	
BMI	26.3±3.4	25.5±5.1	=0.6
Autonomic Testing:			
QSART			
Forearm	1.31±0.72	0.81±0.69	0.07
Proximal Leg	1.23±0.93	0.47±0.47	0.009
Distal Leg	1.23±0.86	0.44±0.40	0.004
Foot	0.91±0.68	0.65±0.50	0.24
Lab Deep Breathing (bpm)	15.2±8.3	3.7±2.0	<0.001
MRI Deep Breathing (bpm)	15.3±9.6	5.7±2.1	=0.002
Lab Valsalva Maneuver	1.9±0.4	1.21±0.17	<0.001
MRI Valsalva Maneuver	2.1±0.47	1.22±0.11	<0.001
Head-up Tilt			
Resting Heart Rate (bpm)	62±9.7	70.5±11.3	0.03
ΔHeart Rate	19.4±8.6	8.9±6.7	0.002
Resting SBP (mmHg)	117.1±14.9	146.3±25.2	0.001
ΔSBP (mmHg)	-18.1±5.9	-79.7±25	<0.001
ΔHR/ΔSBP ratio during HUT	1.1±0.5	0.15±0.2	<0.001
CASS			
Sudomotor Index	0.2±0.7	0.87±1.1	=0.06
Cardiovagal Index	0.1±0.4	1.7±1.0	<0.001
Adrenergic Index	0.0±0.0	4.0±0.0	<0.001
Total	0.3±1.0	6.5±1.8	<0.001

Abbreviations: BMI; body mass index; QSART, quantitative sudomotor axon reflex test; SBP, systolic blood pressure; Δ, change; MRI, magnetic resonance imaging; HR, heart rate; ΔHR, heart rate change; SD, standard deviation; CASS, Composite autonomic scoring scale

Table 2.2 Brain regions of deactivation in response to deep breathing in healthy controls and patients with NOH

Control Group	**Deep breathing Deactivation**						
Region	**Side**	**Coordinates**			**Voxel #**	**T-score**	**p-value**
		x	y	z			
Thalamus	L	-18	-22	8	165	8.34	p<0.05
Thalamus	R	15	-19	8	149	7.19	p<0.05
PCC	L	-3	-37	38	681	7.31	p<0.05
Parahippocampus	R	30	-25	-19	59	6.72	p<0.05
Insula	L	-30	23	-4	218	6.39	p<0.05
Insula	R	39	-19	2	195	6.29	p<0.05
Patient Group							
PCC		0	-37	35	365	8.5	p<0.05
ACC	R	6	35	26	173	6.89	p<0.05
Insula	L	-42	-16	2	50	6.91	p=0.007*
Insula	R	39	-19	5	55	6.81	p<0.05

*p-values uncorrected. Abbreviations: NOH, Neurogenic Orthostatic Hypotension; PCC, posterior cingulate cortex; ACC anterior cingulate cortex; L, left; R, right.

Table 2.3 Brain regions of activation in response to Valsalva maneuver [controls-patients].

Valsalva Activation Control - Patient							
Region	**Side**	**Coordinates**			**Voxel #**	**T-score**	**p-value**
		x	y	z			
ACC	R	15	50	14	57	4.29	p<0.009*
Hippocampus	R	42	-16	-16	11	8.03	p<0.05
PCC	L	-6	-46	26	19	7.6	p<0.05
Thalamus	L	-15	-10	11	46	8.45	p<0.05
Thalamus	R	15	-10	11	45	7.41	p<0.05
Valsalva Phase IV Activation Control - Patient							
Hippocampus	R	24	-19	-16	48	5.78	p<0.05

*p-values uncorrected. Abbreviations: ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; R, right; L, left

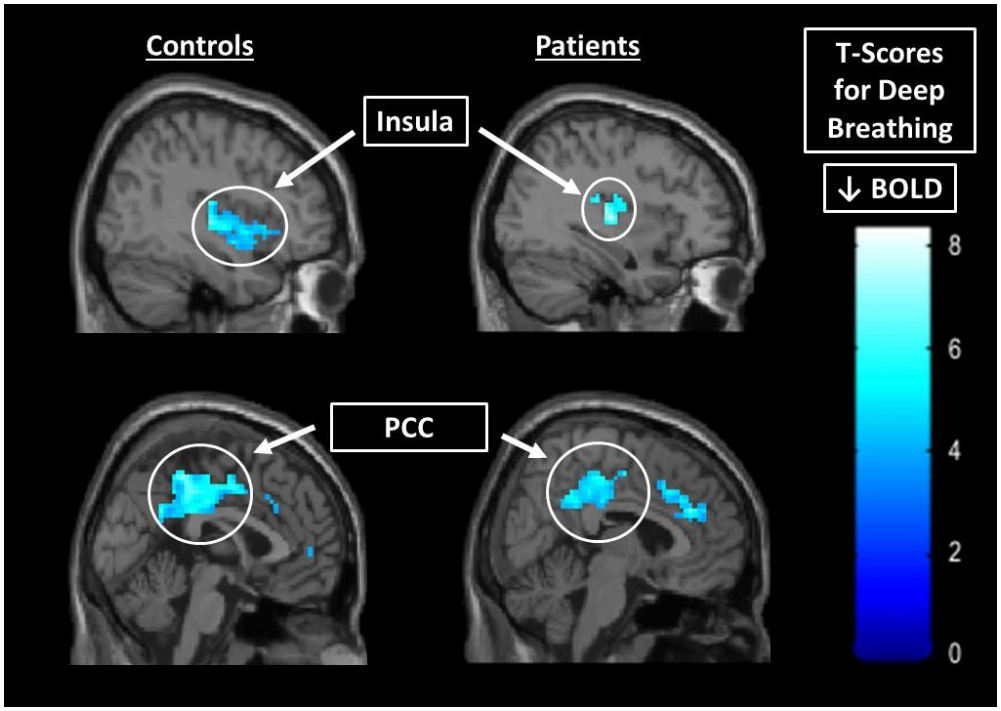

Figure 2.1 Cortical deactivation during deep breathing.

Cortical deactivation patterns relative to rest in healthy controls (n=15) and patients with NOH (n=15) during Deep breathing. Abbreviation: PCC, posterior cingulate cortex; BOLD, blood-oxygen-level-dependent.

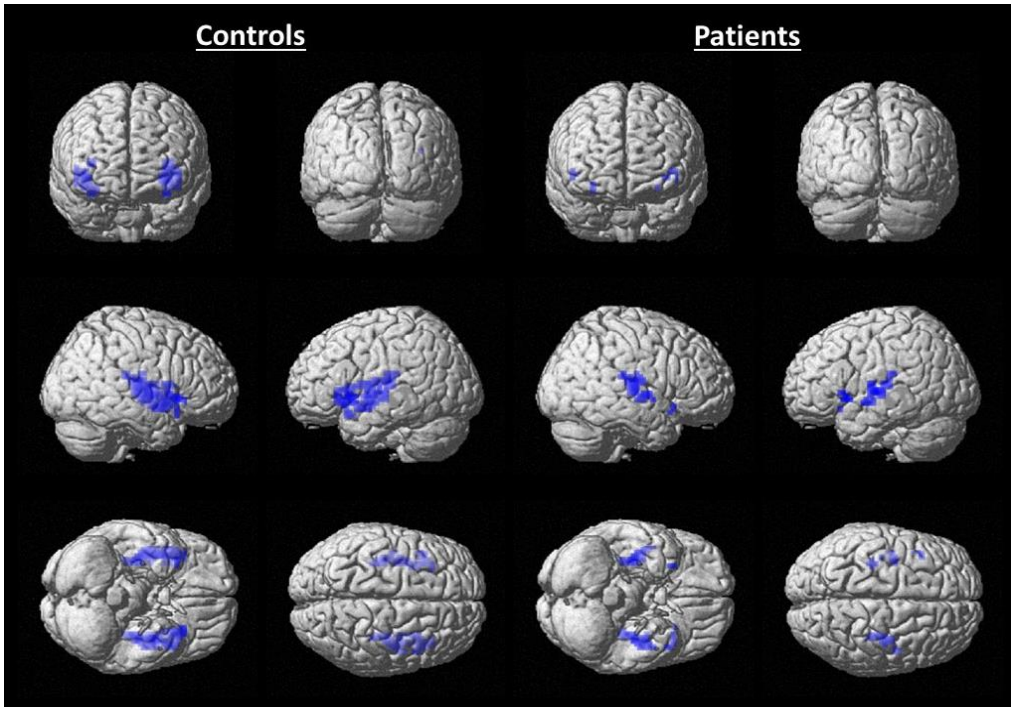

Figure 2.2 3D visualization of insular deactivation

Insular deactivation in healthy controls (n=15) and NOH patients (n=15) during deep breathing.

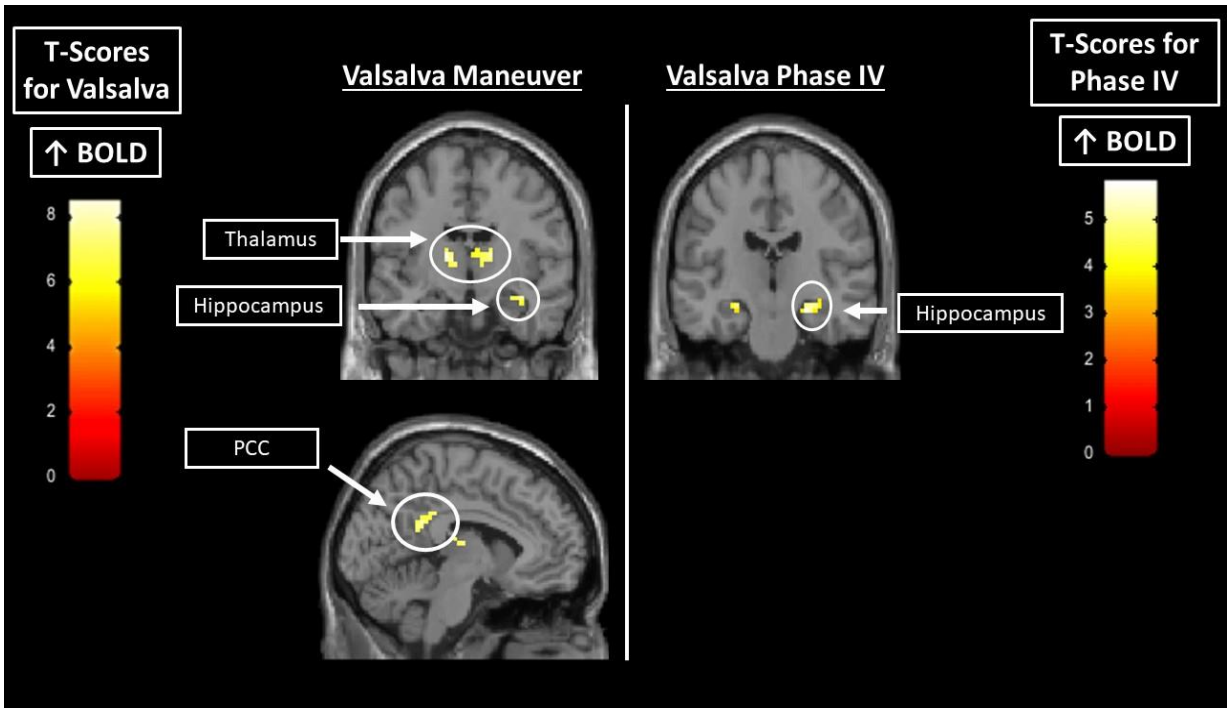

Figure 2.3 Cortical and subcortical activation during Valsalva maneuver

Controls had significantly more brain activation relative to rest during the Valsalva maneuver and during Phase IV following subtraction analysis [controls-patients]. Abbreviations: PCC, posterior cingulate cortex; BOLD, blood oxygen level dependent.

2.4 Discussion

Neurogenic orthostatic hypotension (NOH) is a cardinal feature of autonomic failure that results from dysfunction of the reflexive regulation mediated by the sympathetic nervous system. As a result, patients experience a considerable blood pressure reduction in the upright position. Furthermore, an abundance of literature has highlighted specific autonomic brain regions in what is commonly referred to as the central autonomic network (CAN). Our results reveal that patients with NOH demonstrate similar cortical activation patterns in response to deep breathing - a test of cardiovagal functioning. However, in response to Valsalva - an adrenergic indicator in its later phase – patients with NOH are more profoundly affected with considerably less cortical activation relative to healthy controls. These data further support dysfunction of the sympathetic nervous system in the pathophysiology of NOH. However, they also add to the current understanding by revealing additional impairment of the central autonomic network. The relationship between NOH and changes in cortical autonomic regions during an adrenergic maneuver such as the Valsalva may be due to impairment of cortical autonomic regions involved in sympathetic/ baroreflex mediated pathways and reduced autonomic afferent signaling.

2.4.1 Impaired sympathetic/baroreflex mediated pathways

In autonomic failure, regardless of central or peripheral autonomic lesions, both clinical populations demonstrate loss of baroreflex restraint/baroreflex buffering. In a study of primary autonomic dysfunction, patients demonstrated significant baroreflex-adrenergic dysfunction relative to healthy controls[28]. Our results go on to further suggest that cortical autonomic regions associated with sympathetic and baroreflex activation may also be significantly affected in NOH patients. Our results reveal that healthy controls have significantly more activation in the thalamus, cingulate (bilateral PCC and right ACC) and hippocampus in response to VM relative to patients. Functional imaging research has highlighted similar cortical and subcortical regions to be involved in sympathetic and baroreflex mediated responses. For example, increased activation of the hippocampus[29] and thalamus[16] have been demonstrated in response to Valsalva in healthy individuals. Furthermore, right anterior cingulate activation has been well established in the context of sympathetic activation, stress and tasks that facilitate a tachycardic response[12,30,31]. In the context of clinical populations, Critchley et al., tested patients with focal

damage involving the ACC and revealed that each patient had abnormal autonomic cardiovascular responses with blunted autonomic arousal[31]. In the study by Critchley et al., the level of cortical activity and morphology in the cingulate cortices also appeared to be affected in patients with pure autonomic failure[32]. Moreover, in the current study, during the blood pressure overshoot of phase IV of the Valsalva (an adrenergic phase of the maneuver), the hippocampus remained significantly more activated in healthy controls relative to NOH patients. Interestingly, in another study, regional cerebral blood flow as measured by MRI arterial spin labelling also highlighted a significant role of the hippocampus during phase IV. The results revealed that regional cerebral perfusion of the hippocampus was significantly correlated with baroreflex sensitivity in that impaired baroreflex sensitivity was related to brain hypoperfusion[33]. Overall, the data in the current study support current literature highlighting a role of the cingulate, thalamus and hippocampus in autonomic regulation, and go on to further suggest impairment of these regions in autonomic failure.

2.4.2 Altered autonomic afferent signals

Reduced functional cortical activation in these patients may be evidence of reduced autonomic afferent information to structures involved in autonomic control. Representation of afferent information within the central autonomic network has been established in both animal and human research. In animals, the use of axonal radiotracers such as horseradish peroxidase has revealed direct and extensive brainstem-cortical and cortical-cortical projections between structures intricately related to autonomic regulation[34–37]. Furthermore, in humans, somatosensory stimulation of forearm muscle afferents revealed afferent representation within the anterior and posterior cingulate cortices, and bilateral posterior insula[12]. In the current study, altered sympathetic afferent information in patients with peripheral autonomic lesions may result in reduced central representation of those signals. Though the suggestion of reduced afferent inputs within the central autonomic network is speculation, as this was not measured, the possibility is strengthened by a lack of significant thalamic activation during Valsalva and deep breathing. As a primary relay center in the brain, impaired activation of the thalamus may be evidence of reduced afferent input in autonomic dysfunction, which in turn could further disrupt thalamocortical projections to other cortical autonomic effector sites. The possibility of altered

autonomic afferent signaling has also been posited in other studies involving individuals with pure autonomic failure (PAF); a clinical population with selective peripheral denervation (i.e. post-ganglionic) of the autonomic nervous system. Analysis of voxel-based morphology in PAF patients identified regional reductions in grey matter volume in autonomic cortices relative to controls[32]. This potential mechanism may be supported because despite the considerable level of morbidity, these patients in general still have normal life expectancies. Therefore, as a direct result of long-term peripheral autonomic denervation, patients with peripheral autonomic denervation may experience cortical reorganization secondary to prolonged deafferentation, similar to that previously reported[38].

Overall, in the current study there is evidence to suggest that even in peripheral autonomic disorders, there is also impairment of higher central autonomic networks specifically related to sympathetic regulation. Conversely parasympathetic regulation did not produce any specific differences in the controls versus the NOH group with respect to the functioning of the CAN. In itself these data would argue that this is an important pathophysiological finding in patients with NOH. Issues such as compensation or de-compensation within the very complex CAN would be one important reason for our differential findings. For example, it may be that compensation from a presumed normal CAN with respect to parasympathetic innervation to cardiovascular structures is less profound than that of sympathetic function. In these patients the importance of adrenergic function to attempt to maintain postural normotension is an important factor especially from a clinical perspective as orthostatic blood pressure reduction has severe clinical consequences (i.e. syncope). Finally, these data are significant as these patients are an important clinical group with severe neurological impairment in the ANS. Such studies will be important to inform this clinical situation potentially from both a prognostic and diagnostic standpoint. Ultimately these data provide important insight into our understanding of the pathophysiology of NOH in peripheral autonomic disorders.

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CHAPTER 3

3 Cerebellar impairment during an orthostatic challenge in patients with neurogenic orthostatic hypotension[2]

3.1 Introduction

Neurogenic Orthostatic Hypotension (NOH) is a cardinal feature of autonomic dysfunction. NOH is clinically defined as a sustained reduction in systolic blood pressure (SBP) ≥30 mmHg or diastolic blood pressure of ≥15 mmHg within 3 minutes of standing or head-up tilt performed at 60° without an appropriate compensatory postural tachycardia[1]. NOH is unique in that the *orthostatic* component relates to an excessive BP drop associated with an upright or standing position and the *neurogenic* component highlights a failure of the autonomic nervous system to reflexively increase sympathetic outflow to counteract the BP drop. Regulation of arterial BP has been well established. In brief, blood pressure is mediated through an intricate arterial baroreflex-mediated circuit initiated through baroreceptor stretch receptors primarily located in the carotid sinus and aortic arch. During a state of hypotension, reduced afferent signaling to the nucleus tractus solitarius in the brainstem facilitates a cascade of inhibitory and excitatory signals, which ultimately increase sympathetic vasoconstrictor tone and tachycardia to help maintain blood pressure [2]. In addition to feedback mechanisms, feedforward or "central command" mechanisms also contribute to long- and short-term regulation of the cardiovascular system[3]. Specifically, the central autonomic network (CAN) includes a network of cortical, subcortical and brainstem regions that have been implicated in neurovascular control. Regions such as the cingulate cortices, insula, hippocampus, cerebellum and medial prefrontal have all demonstrated significant contributions to the cardiovascular changes that occur in response to various stressors[4,5].

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Additionally, in clinical models such as stroke and lesion studies, damage to these areas results in autonomic dysfunction[6–8]. Finally, a number of cortical and subcortical regions within the CAN have specifically been implicated in baroreflex functioning. For example, clusters of baroreceptor cells have been identified in the insula of rats[9] and monkeys[10], and posterior insular lesions result in altered baroreceptor gain[11]. In humans, CAN regions such as the insula, cingulate cortices, thalamus and cerebellum have also been evident during an orthostatic challenge elicited through lower-body negative pressure[12]. Therefore, the purpose of the current study was to compare activation patterns within the CAN in patients with NOH versus healthy age-matched controls during an orthostatic challenge.

3.2 Methods

3.2.1 Patient and control groups

Fifteen healthy, age-matched controls (61±14 years; females: 8) and 15 patients diagnosed with Neurogenic Orthostatic Hypotension (NOH) (67±6 years; females: 6) (p=0.12) completed the following study. NOH was defined as a reduction in SBP ≥30 mmHg within 3 minutes of head-up tilt (HUT) without an appropriate compensatory postural tachycardia as determined by the ΔHR/ΔSBP ratio[13]. As an additional assessment of autonomic dysfunction, all patients also had absent adrenergic phases (late phase II and phase IV) in response to the Valsalva maneuver. Prior to testing, all diagnoses were clinically confirmed by a Neurologist with specialty training in autonomic dysfunction (KK). Patients with central autonomic neurodegenerative disorders were not included in the present study to eliminate any potentially confounding variables associated with such central pathologies. Therefore, the NOH cohort was comprised of patients with evidence of peripheral autonomic denervation only (pure autonomic failure, n=3; Parkinson's Disease + NOH, n=7; idiopathic NOH, n=5). In the current study, patients were categorized as idiopathic NOH if there was considerable orthostatic hypotension, along with gastrointestinal issues or other questionable phenomenon such as olfactory impairment, while not meeting criteria for other alpha-synucleinopathies. As such, the latter diagnosis over time may be clearer as the patient can develop a more specific diagnosis. In contrast, those diagnosed with PAF have maintained a purely peripheral autonomic failure without any evidence of other pathology for an extended period of time. Quantitative sudomotor axon reflex testing was performed on all

patients to provide clinical evidence of peripheral denervation. Patients were excluded if there was evidence of any peripheral nerve injury unrelated to their diagnosis of autonomic dysfunction including diabetic neuropathies in any form. Healthy participants were examined to confirm the absence of any neurological conditions including any autonomic dysfunction. Healthy participants were also excluded if they fell under any one of the following categories: i) pregnant or lactating females, ii) clinically significant coronary artery disease, iii) concomitant therapy with anticholinergic, alpha- and beta-adrenergic antagonists or other medications which could interfere with autonomic functioning, and iv) failure of other organ systems or systemic illness that could affect autonomic function or participants' ability to cooperate. All laboratory data were collected in the Autonomic Disorders Laboratory at University Hospital, London, Ontario. All functional imaging data were collected at Robart's Research Institute Centre for Functional and Metabolic Imaging at The University of Western Ontario. Ethical approval was obtained from the Health Science Research Ethics Board at Western University, and informed consent was obtained from all participants prior to any and all testing.

3.2.2 Autonomic testing

All participants underwent a battery of standardized and validated tests of autonomic function, namely the autonomic reflex screen (ARS)[14,15]. Quantitative sudomotor axon reflex test (QSART): QSART provided an assessment of post-ganglionic sympathetic function from four standard sites (forearm, proximal leg, distal leg and foot). Beat-to-beat blood pressure and heart rate responses to Valsalva and head-up tilt provided an assessment of adrenergic function. In addition, all participants underwent Lower-body negative pressure (LBNP) as an additional orthostatic challenge. Following a minimum baseline period of 15 minutes in the supine position, LBNP was conducted at a pressure of -35 mmHg for 5 minutes, followed by a 5-minute recovery period. All healthy participants completed 5-minutes of LBNP at -35 mmHg. In contrast, due to the nature of the disease and the marked blood pressure drops in the patient group, in some cases the negative pressure needed to be reduced to ensure blood pressure did not drop below a certain threshold. On average, patients with NOH completed 5-minutes of LBNP at a negative pressure of 27 mmHg. All patient started at -35mmHg, however if SBP dropped <65 mmHg, negative pressure was reduced to ensure BP would plateau and not continue to drop. In the lab, beat-to-

beat blood pressure (BP) and heart rate (HR) responses during all tests were continuously measured and recorded using a BMEYE Nexfin device (Amsterdam, The Netherlands) and an electrocardiography (ECG) device (Model 3000 Cardiac Trigger Monitor, IVY Biomedical Systems, Inc., Branford, CT) with ECG electrodes (Ambu® Blue Sensor SP, Glen Burnie, MD), respectively. All recordings were made using WR TestWorksTM software (WR Medical Electronics Co., Stillwater, MN). Participants repeated the LBNP and VM protocol during a functional MRI.

3.2.3 Neuroimaging data acquisition

All imaging data were collected using a whole body 3T imaging system with a 32-channel head coil (Magnetom Primsa, Siemens Medical Solutions, Erlangen, Germany). A 3D MPRAGE sequence was used to acquire a high-resolution T1-weighted structural at the beginning of the scanning session (sagittal, matrix 256x240 mm, voxel resolution 1.0x1.0x1.0 mm, 1 mm slice thickness, no gap, flip angle 9 degree, TE: 2.98 ms, TI: 900 ms, TR: 2300 ms). Blood oxygen level-dependent (BOLD) signals were acquired using a T2- weighted gradient echo-echo planar imaging pulse sequence with the following parameters: TE: 30ms; FOV: 240x240 mm; flip angle: 40 degrees; multiband acceleration factor: 4. Forty-eight interleaved axial slices (3.0x3.0 mm in-plane voxel resolution, TR: 1000 ms) were acquired in each volume. Participant completed one round of LBNP and 3 Valsalva maneuvers (VM) during a functional scan of their brain. LBNP: Following a 60 second baseline, LBNP was initiated for 5-minutes following by a 5-minute period with LBNP off (660 volumes). Valsalva maneuver: Following a 60-second baseline, participants completed 3 VM's (15 seconds each), with 120 seconds of rest in between each trial (465 volumes). The first 2 volumes of each test were discarded from analysis to allow for an equilibrated MRI signal. To minimize head movement, each participant's head was placed in a cradle packed with foam padding. In addition, all participants practiced stabilizing themselves on the foot plates within the lower-body negative pressure box, to minimize movement when negative pressure was manipulated. Finally, all participants practiced performing the VM while being supervised to ensure minimal head movement during the maneuver. Beat-to-beat heart rate was recorded from a continuous signal derived from an MRI-compatible pulse oximeter (Nonin Medical, 8600FO MRI, Plymouth, MN) attached to the index

finger of each participant's left hand when possible. In the presence of a significant tremor (i.e. in PD+NOH patients), pulse oximetry was obtained from the hand with less potential for movement. All hemodynamic recordings were collected using WR TestWorks™ software (WR Medical Electronics Co., Stillwater, MN).

3.2.4 Neuroimaging data analysis

Raw fMRI data were analyzed using SPM12 (Wellcome Department of Imaging Neuroscience, London, UK). All functional images were realigned using a rigid body transformation to correct for head motion using the mean functional image. All images were co-registered with the T1-weighted scan, normalized to Montreal Neurological Institute (MNI) space and smoothed with a Gaussian kernel (FWHM=6 mm). A high pass filter with 128-second cut-off was applied to reduce low frequency noise.

Two levels of analysis were performed. In the first level of analysis, individual design matrices of each protocol (LBNP and VM) were constructed modelled by a box-car and combined with a canonical hemodynamic response function. A statistical parametric map was created on a voxel-by-voxel basis using the General Linear Model [16]. The LBNP protocol was broken down into periods of rest, LBNP and recovery. Rest periods included the first minute prior to LBNP and the last minute of the protocol. Brain activation patterns during LBNP were assessed during the final 60 seconds, when sympathetic activation should be the greatest. Finally, the first 30-seconds following LBNP were analyzed as a recovery phase. Similarly, the VM protocol was assessed as periods of rest and VM. All contrasts (VM, LBNP and LBNP-recovery) were compared against their respective rest periods. In a second-level analysis, each individual's contrast for each protocol was entered into a 2-sample independent t-test to compare differences between patients and controls. Comparisons of the BOLD responses were corrected for multiple comparisons (family-wise error (FWE) <0.05). In some cases, a more lenient threshold of $p<0.001$, uncorrected was used with a cluster threshold of 10 voxels.

3.2.5 Regions-of-Interest analysis

Regions of interest (ROI) were determined based on previous work highlighting regions of the central autonomic network. These a priori ROI included the bilateral insula, bilateral anterior and

posterior cingulate, bilateral hippocampus, bilateral thalamus and bilateral cerebellum. All ROI masks were created using WFU_Pick Atlas toolbox version 1.2[17,18].

3.2.6 Statistical analysis

Physiological data are presented as mean ± standard deviation. Autonomic parameters between patients and age-matched controls were compared using an independent t-test. All tests were 2-tailed with a p-value <0.05 to denote significance. All statistical analyses were performed using SPSS statistical software, Version 22.0. Manufactured by International Business Management (IBM) Corporation (SPSS Inc. Chicago, IL).

3.3 Results

3.3.1 QSART and hemodynamic findings

Compared to healthy controls, patients had significantly lower average sweat volumes at the proximal (1.23±0.93 µL vs. 0.47±0.47 µL, respectively) and distal leg (1.23±0.86 µL vs. 0.44±0.40 µL, respectively) ($p<0.01$), and a trend toward lower sweat volumes at the forearm (1.31±0.72 µL vs. 0.81±0.69 µL, respectively) ($p=0.07$). Sweat volumes at the foot were not significantly different (0.91±0.68 µL vs. 0.65±0.50 µL, respectively) ($p=0.24$). During both HUT and LBNP in the lab session, patients with NOH had significantly larger blood pressure drops with significantly smaller compensatory tachycardias versus healthy controls (Table 3.1) ($p<0.01$). LBNP during the functional imaging session revealed similar significantly different HR responses. Hemodynamic changes in response to the LBNP between LAB and MRI sessions revealed no significant differences (Table 3.1).

3.3.2 Functional BOLD responses

During LBNP, healthy controls showed significant activation relative to rest in the bilateral insula (Figure 3.1), bilateral thalamus, anterior cingulate cortex, and bilateral cerebellum (Table 3.2) ($p<0.05$). Similarly, patients with NOH had significant activation in the bilateral insula (Figure 3.1) and left thalamus (Table 3.2) ($p<0.05$). During LBNP, controls had significantly greater activation in the bilateral cerebellum compared to patients (Table 3.2; Figure 3.2) ($p<0.05$). To investigate the role of the cerebellum in a different test of baroreflex regulation,

both groups completed a series of VM. Similar to LBNP, controls also had significantly greater activation in the bilateral cerebellum in response to Valsalva maneuver (Figure 3.2) ($p<0.05$). Finally, during the recovery phase of LBNP, both controls and patients had significant activation in the bilateral insula and right cerebellum (Table 3.3; Figure 3.3) ($p<0.05$). Patients also had significant activation in the right anterior and midline posterior cingulate cortices (Table 3.3). No significant differences were found between controls and patients during the LBNP-recovery phase.

Table 3.1 Laboratory and MRI autonomic testing.

Orthostatic Testing:	**Control (n=15) Mean ± SD**	**Patient (n=15) Mean ± SD**	**p-value**
LAB Head-up Tilt			
Resting Heart Rate (bpm)	61.6±9.7	70.5±11.3	0.03
ΔHeart Rate	20.2±7.9	8.9±6.7	0.002
Resting SBP (mmHg)	117.1±14.9	146.3±25.2	0.001
ΔSBP (mmHg)	-21.0±8.2	-79.7±25	<0.001
ΔHR/ΔSBP ratio during HUT	1.1±0.5	0.14±0.2	<0.001
LAB LBNP			
Resting Heart Rate (bpm)	65.9±8.8	73.6±9.1	=0.1
ΔHeart Rate	19.3±8.5	7.0±4.3	<0.001
Resting SBP (mmHg)	105.3±11.7	148.4±27.8	=0.003
ΔSBP (mmHg)	-23±6	-57.7±22.6	<0.001
ΔHR/ΔSBP ratio during HUT	0.87±0.4	0.14±0.14	<0.001
MRI LBNP			
Resting Heart Rate (bpm)	69.9±11.6	74.8±8.4	=0.228
ΔHeart Rate	17.6±8.9	7.1±3.2	<0.001

Abbreviations: SBP, systolic blood pressure; LBNP, lower-body negative pressure; MRI, magnetic resonance imaging; Δ, change; QSART, quantitative sudomotor axon reflex test; SD, standard deviation

Table 3.2 Brain regions activated during LBNP in healthy controls and NOH patients. Controls had significantly greater activation in the cerebellum relative to patients.

Region	**Side**	**Voxel #**	**T-score**	**p-value**
Controls LBNP				
Thalamus	L	85	11.55	P<0.05
	R	126	8.74	P<0.05
Insula	L	163	9.36	P<0.05
	R	141	5.81	P<0.05
ACC	R	153	6.27	P<0.05
Cerebellum	Midline	235	7.33	P<0.05
	R		6.78	P<0.05
	L		6.67	P<0.05
Patient LBNP				
Insula	L	81	6.83	P<0.05
	R	73	5.51	P<0.05
Thalamus	L	64	7.20	P<0.05
Controls activation>Patients activation during LBNP				
Cerebellum	L	65	4.58	P<0.05
	R	65	4.53	P<0.05
Controls activation>Patients activation during VM				
Cerebellum	L	49	8.37	P<0.05
Cerebellum	R	186	8.28	P<0.05
Cerebellum	L	80	8.74	P<0.05

Abbreviations: LBNP, Lower body negative pressure; NOH, Neurogenic Orthostatic Hypotension; VM, Valsalva Maneuver; L, Left; R, Right.

Table 3.3 Brain regions of activation post-LBNP during recovery phase in healthy controls and NOH patients. No significant differences between controls and patients were found**.**

Region	**Side**	**Voxel #**	**T-score**	**p-value**
Controls – LBNP recovery				
Insula	R	82	6.48	P<0.05
	L	78	6.22	P<0.05
Cerebellum	R	131	6.66	P<0.05
Patients – LBNP recovery				
Insula	L	125	6.40	P<0.05
	R	101	5.81	P<0.05
ACC	R	228	6.73	P<0.05
PCC	Midline	63	5.40	P<0.05
Cerebellum	R	193	6.80	P<0.05

Abbreviations: LBNP, Lower body negative pressure; NOH, Neurogenic Orthostatic Hypotension; L, Left; R, Right.

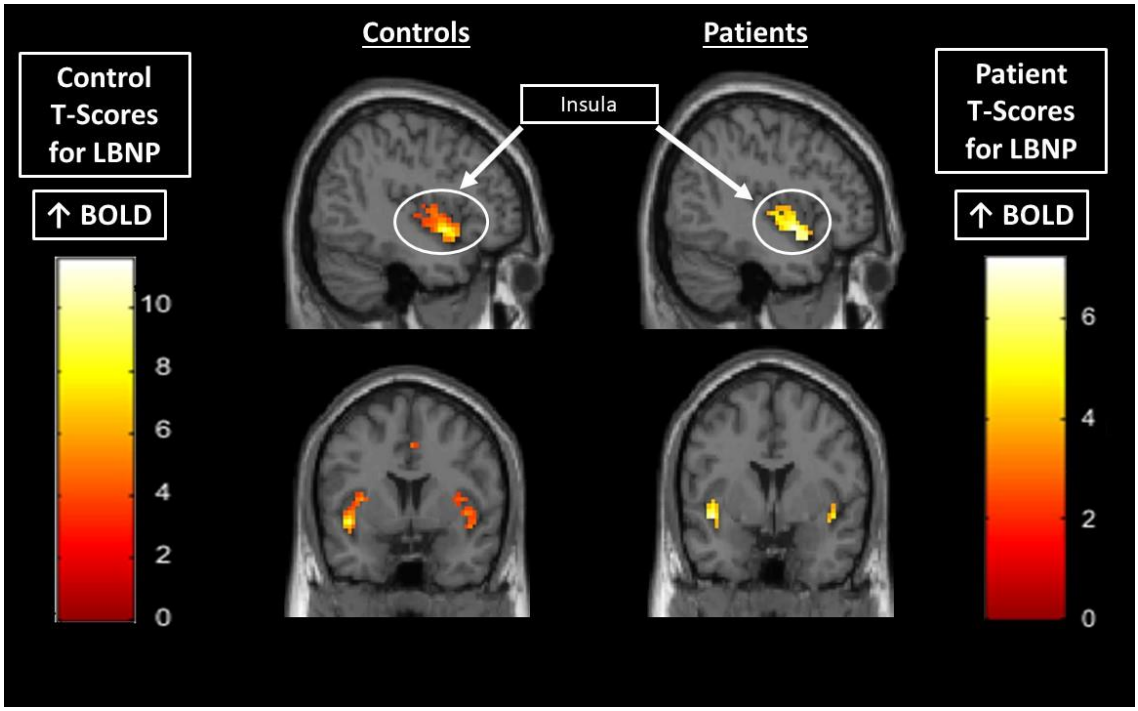

Figure 3.1 Cortical activation patterns during LBNP.

No significant differences between controls and patients during LBNP. Both groups had similar activation in the bilateral insula. Abbreviations: LBNP, Lower body negative pressure; BOLD, blood oxygen level dependent.

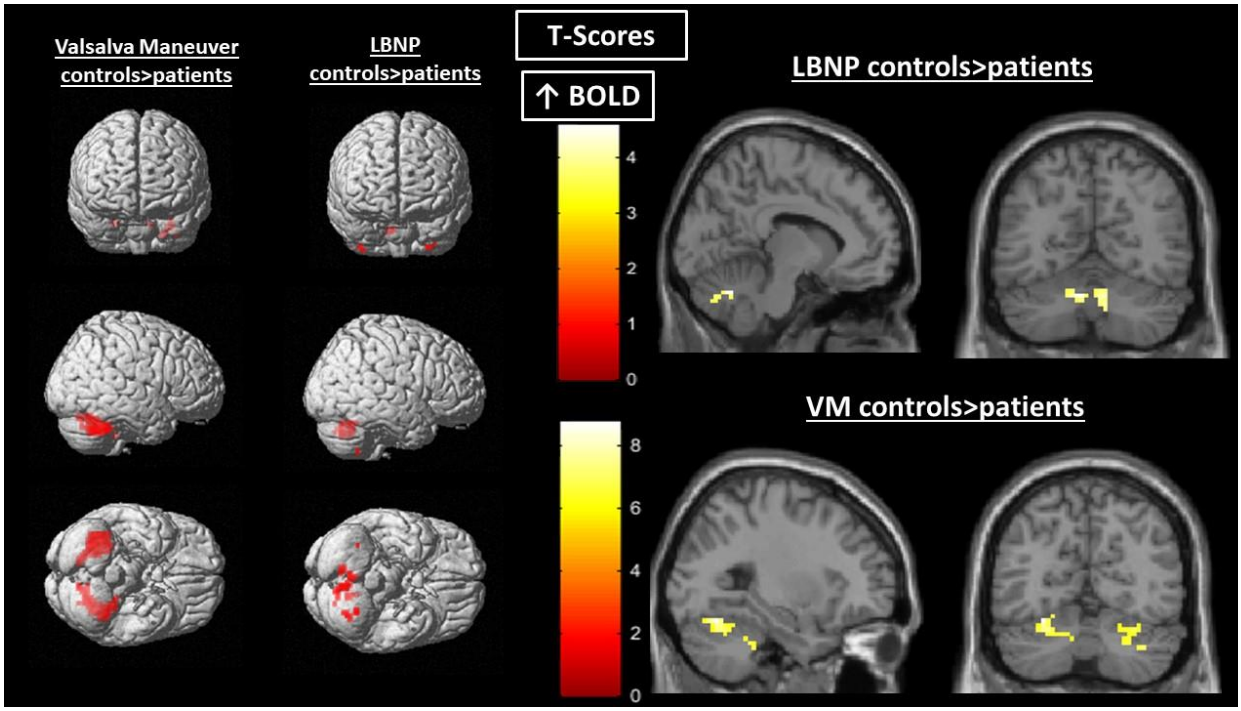

Figure 3.2 Comparison of cerebellar changes during VM and LBNP.

Controls had greater activation in the cerebellum during LBNP and VM relative to patients.

Abbreviations: LBNP, Lower body negative pressure; BOLD, blood oxygen level dependent; VM, Valsalva maneuver.

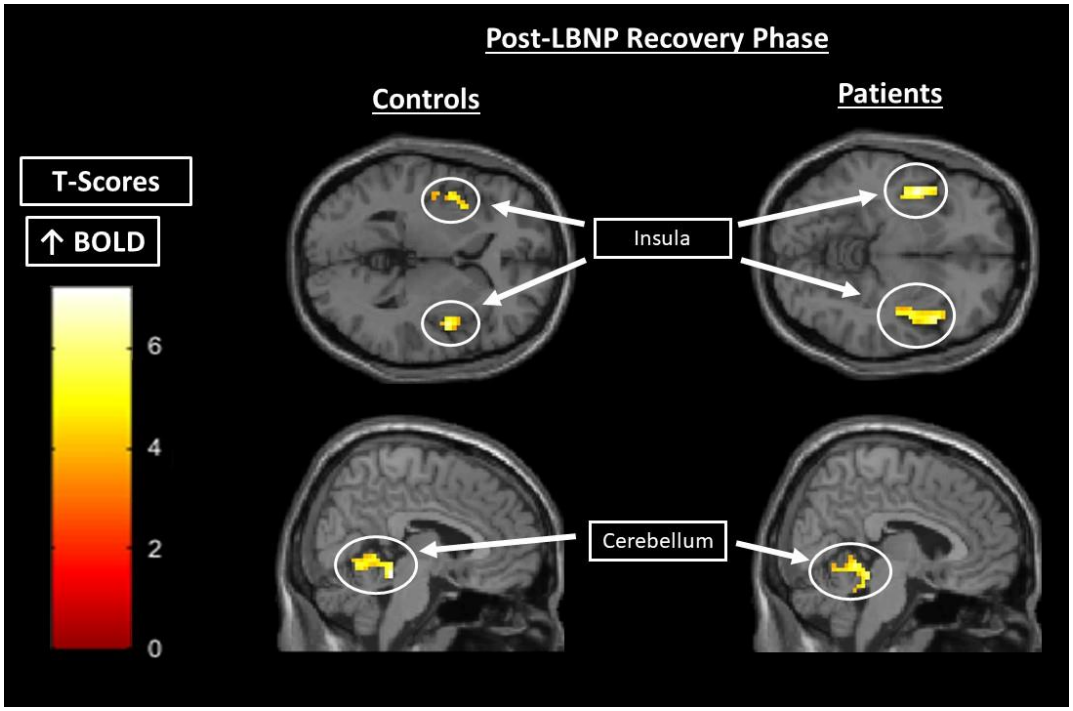

Figure 3.3 Brain activation during post-LBNP recovery phase.

During the post-LBNP recovery no significant differences were found between healthy controls and patients. Both controls and patients had activation in the bilateral insula and cerebellum. Abbreviations: LBNP, Lower body negative pressure; BOLD, blood oxygen level dependent.

3.4 Discussion

Neurogenic orthostatic hypotension (NOH) is a cardinal feature of autonomic failure. During an orthostatic challenge such as head-up tilt or lower-body negative pressure (LBNP), patients experience a significant and persistent blood pressure drop without evidence of an appropriate compensatory sympathetic response to counter the blood pressure fall. During an orthostatic stressor such as LBNP, regions within the central autonomic network (CAN) such as the insula and cerebellum have been largely implicated for their involvement in mediating cardiovascular responses. Our results reveal three important findings in the context of NOH and CAN activation: 1) Central activation patterns in healthy older controls are consistent with previous literature highlighting a role of the insula and cerebellum during an orthostatic challenge such as LBNP. 2) Interestingly, patients with autonomic dysfunction revealed similar insular activation patterns; however, there was significantly less cerebellar activation during LBNP. 3) To investigate the role of the cerebellum in a different test of baroreflex regulation, both groups completed a series of Valsalva maneuvers. Similar to LBNP, patients had significantly less cerebellar activation during VM compared to healthy controls.

The role of the cerebellum in movement coordination and balance/vestibular regulation has been well established. However, in the context of the central autonomic network, the cerebellum has arguably received less attention than cortical sites. In human and animal studies alike, a growing body of literature has emerged highlighting a number of autonomic functions that appear to involve pathways through the cerebellum[19], including postural control of blood pressure and heart rate[20]. In the present study, the cerebellum along with the insular cortex and thalamus were significantly activated in healthy individuals during a LBNP. These finding corroborate much of the previous work that has highlighted these same areas during both an orthostatic challenge[12,21] and during other mental and physical stressors that facilitate blood pressure changes. For example, in an exercise of mental and physical stress (hand-grip), increased regional cerebral blood flow (rCBF) in the cerebellum, right anterior cingulate and right insula covaried with mean arterial pressure. Similarly, rCBF in the pons, cerebellum and right insula covaried with heart rate[22]. Interestingly, cerebellar activation was not evident in patients with NOH during the same orthostatic challenge. The cerebellum and vestibular system are important to maintaining a stable

blood pressure and respiration during postural changes. The cerebellum projects to several brainstem structures, including the nucleus tractus solitarius (NTS), parabrachial nucleus [23] and rostral ventrolateral medulla (RVLM)[24], in addition to, the rostral portion of the inferior and medial vestibular nuclei[25,26]. Together, these brainstem sites integrate vestibular information and modulate sympathetic reflexes, which in turn regulate postural control of blood pressure.

The properties of the vestibulo-sympathetic reflex have been studied in several animal models. For example, electrical and chemical stimulation of cerebellar regions that process vestibular signals, including the fastigial nucleus[27,28] and posterior cerebellar cortex[29,30] elicit marked cardiovascular responses via direct or indirect connections with the aforementioned brainstem structures. Furthermore, during a postural change following removal of vestibular inputs, animals with cerebellar lesions experienced more severe orthostatic hypotension than cerebellum-intact animals[31]. Despite the severe blood pressure drops that patients with NOH experience, paradoxically, approximately 50% of patients also have supine hypertension[32]. The fastigial nuclei (FN) and cerebellar areas, play an important role in limiting blood pressure extremes such as that seen in hypo- and hypertension, and damage to these areas results in hypotension [33]. Furthermore, in evaluating the FN neural activity during blood pressure alterations via the modified oxford method, FN neural activity increases during hypotension[34]. These findings suggest that the FN may play an important compensatory role during large blood pressure changes by sympatho-excitatory and inhibitory processes. Given the propensity of the extreme blood pressures seen in NOH patients, the lack of significant cerebellar activation and the evidence supporting a role of cerebellar structures in attenuating such blood pressure extremes, it remains plausible that sympatho-excitatory reflexes facilitated by the cerebellum during a postural change may be absent or disrupted in NOH. Together, these data may provide some insight and evidence for this region and its involvement in the pathophysiology of NOH.

Finally, to investigate the role of the cerebellum in a different test of blood pressure regulation, both groups completed a series of Valsalva maneuvers. Similar to an orthostatic challenge, during the VM there is a precipitous drop in blood pressure that would normally be arrested via reflexive sympathetic vasoconstriction and tachycardia. In patients with NOH, the adrenergically-mediated phases of the maneuver are absent. As a result, patients demonstrate a

blood pressure profile that reveals a similar precipitous and persistent drop in blood pressure to that of Head-up Tilt, until the maneuver is completed. Similar to LBNP, patients had significantly less cerebellar activation during VM as compared to healthy controls. Overall, these data further support a role of the cerebellum in mediated important sympatho-excitatory processes during significant blood pressure perturbations.

3.4.1 Study limitations

Our results reveal important findings regarding the role of the cerebellum in the pathophysiology of NOH. Despite these unique findings, the current study contains the following limitations: 1) Due to the nature of blood pressure dysfunction seen in this clinical group, in some cases the negative pressure that was used during LBNP was less than that in healthy controls. Despite a reduced negative pressure, patients still showed activation within the insula and thalamus, similar to that of healthy controls. Furthermore, despite the lower negative pressure in some cases, all patients still demonstrated a significant blood pressure drop even at a reduced pressure. 2) Blood pressure was not measured during the MRI session. MRI compatible blood pressure monitoring typically involves invasive techniques such as insertion of an arterial line. Due to the invasive nature of this technique we opted to use heart rate changes as a surrogate indicator of the autonomic changes. Heart rate changes in response to LBNP and Valsalva were not significantly different between lab and MRI recording sessions. Furthermore, all study participants experienced the same negative pressure during the MRI as was performed during the lab. Therefore, despite the lack of a direct blood pressure measure in the MRI, we assume the cardiovascular and autonomic changes were similar. 3) In previous studies, LBNP is typically applied in several repeated bouts ranging from 30-45 seconds. In the current study, we applied a single-epoch design to replicate the head-up tilt protocol that has been validated and standardized in clinical autonomic disorders. Even though, multiple epochs are typically used, single-epoch studies have been previously used with certain protocols that cannot use repeated stimuli, such as pain studies [35]. Furthermore, single-epoch fMRI has been previously validated against multiple epochs of stimulation, with results that yield similar activation patterns[36]. 4) The sweat responses to QSART did not reveal a significant difference between patients and controls at the foot. However, these values are still considered reduced relative to normative data, and thus these data

support the presence of post-ganglionic sympathetic denervation in the pathophysiology of NOH. 5) Other more direct measures of cerebral circulation (i.e. transcranial doppler, regional cerebral blood flow, etc.) were not implemented in the current study. Certainly, these additional measurements would have been helpful to address questions pertaining to changes in brain blood flow and perfusion and could be considered in future studies.

3.5 Conclusion

The purpose of the current study was to compare activation patterns within the CAN in patients with Neurogenic Orthostatic Hypotension versus healthy age-matched controls during an orthostatic challenge. Our results reveal that patients with NOH have significantly less activation in the cerebellum during an orthostatic challenge compared to healthy controls. Furthermore, patients also had significantly less cerebellar activation during VM, which also involves baroreflex-mediated increases in sympathetic tone. Therefore, the results suggest that regions of the cerebellum that modulate vestibulo-sympathetic reflexes, which are important in blood pressure adjustments during postural alterations, as well as baroreflex mediated influences on sympathetic activation may be disrupted in patients with NOH.

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CHAPTER 4

4 Reduced brainstem functional connectivity in patients with peripheral autonomic failure[3]

4.1 Introduction

Neurogenic Orthostatic Hypotension (NOH) is a cardinal feature of autonomic failure. NOH is clinically defined as a sustained reduction in blood pressure ≥30 mmHg during an orthostatic challenge such as standing from a lying or seated position or during head-up tilt performed at a minimum 60° angle from the horizontal without an appropriate compensatory postural tachycardia[1]. NOH occurs due to a failure of the sympathetic reflexes that would normally counteract blood pressure perturbations through reflexive tachycardia and vasoconstriction. General classifications of NOH are made based on where failure of the sympathetic efferent signaling pathway occurs i.e. before or after the autonomic ganglia. For example, in Parkinson's Disease (PD) with autonomic failure, Pure Autonomic Failure (PAF) and diabetic autonomic neuropathies, the lesion site is considered to be post-ganglionic.

Autonomic homeostasis is dependent upon several brainstem nuclei, including the nucleus tractus solitarius (NTS) and rostral and caudal portions of the ventrolateral medulla. For example, the NTS receives afferent input and through a cascade of excitatory and inhibitory signaling makes beat-to-beat adjustments to efferent autonomic outflow. Moreover, various autonomic brainstem nuclei have been shown to project to both cortical and subcortical regions and have also been shown to receive input from higher cortical structures [2–4]. Through advancements in neuroimaging, several cortical and subcortical structures including the insula, hippocampus, cerebellum, thalamus and cingulate cortices, have been well established as components of the central autonomic network (CAN) [5–8].

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[3] A version of this chapter is currently under review for publication.
Baker J and Kimpinski K (2019). Reduced brainstem functional connectivity in patients with peripheral autonomic failure. NeuroImage: Clinical (under second review)

Importantly, studies have not only tested different modalities, but have also correlated brain activation patterns to hemodynamic responses and direct measures of sympathetic activity. Together, these studies have identified structures of the CAN that contribute specifically to sympathetic and parasympathetic regulation.

Based on the aforementioned pre- versus post-ganglionic classification of autonomic failure, it could be thought that brainstem nuclei and structures of the CAN would remain functionally intact. However, our laboratory recently demonstrated that despite post-ganglionic pathology, patients with NOH show evidence of reduced activation in regions of the CAN during autonomic maneuvers[9,10].

Despite the brainstem being the main region for central integration of baro- and chemoreceptor afferents, brainstem-to-brain connectomes have not been fully investigated. Specifically, the evaluation of functional connectivity between the brainstem and regions of the CAN in individuals with autonomic failure has yet to be studied. Therefore, the aim was to investigate whether functional connectivity from the brainstem to cortical and subcortical structures differs in patients with NOH secondary to autonomic failure as compared to their healthy counterparts.

4.2 Methods

4.2.1 Patient and control groups

The current study was comprised of fifteen healthy, age-matched controls (61±14 years; females: 8) and 15 patients diagnosed with NOH (67±6 years; females: 6; p=0.12). The NOH cohort consisted of patients with evidence of peripheral autonomic denervation only (PAF, n=4; PD with autonomic failure, n=6; idiopathic NOH, n=5). All patients underwent a standard head-up tilt (HUT) test and met the clinical criteria for NOH. On average patients had a resting heart rate (HR) and systolic blood pressure (SBP) of 71±11 bpm and 146±25 mmHg, respectively. On average, SBP dropped by 80±25 mmHg during HUT with an average HR change of 9±7 bpm. As an additional assessment of autonomic dysfunction, all patients demonstrated absent adrenergic phases (late phase II and phase IV) in response to the Valsalva maneuver and cardiovagal impairment evidenced by a reduced Valsalva ratio (1.2±0.1). Furthermore, to provide clinical evidence of post-ganglionic impairment, all patients underwent quantitative sudomotor axon

reflex testing (QSART) from four standard sites (forearm, proximal leg, distal leg and foot). Sudomotor dysfunction was scored as follows: 1= reduced sweat response at a single site, 2=absent response at a single site, 3=absent response at 2 or more sites. The composite autonomic scoring scale (CASS) was used to quantify the severity and distribution of autonomic failure across three domains: sudomotor (0-3), cardiovagal (0-3) and adrenergic (0-4)[11]. On average patients scored a 2/3, 2/3 and 4/4, respectively, resulting in a total CASS of 8/10 indicative of severe and widespread autonomic dysfunction.

Patients with neurodegenerative disorders related to a central autonomic pathology (i.e. Multiple System Atrophy) were excluded from the present study to eliminate any potentially confounding variables associated with such central pathologies (i.e. brain atrophy). Moreover, patients were excluded if there was evidence of any peripheral nerve injury unrelated to their diagnosis of autonomic dysfunction including diabetic neuropathies in any form. In the current study, Pure autonomic failure (PAF) was characterized by orthostatic hypotension along with more widespread autonomic failure, including sympathetic and parasympathetic dysfunction. In addition, PAF patients showed no clear identifiable underlying cause, no other neurological features present and no features to suggest central involvement. PAF patients had maintained a purely peripheral autonomic failure without any evidence of other pathology for an extended period of time. In contrast, a diagnosis of idiopathic NOH was given if, again, there was evidence of orthostatic hypotension, along with gastrointestinal issues or other questionable phenomenon such as olfactory impairment, but not meeting criteria for other alpha-synucleinopathies. A Neurologist (KK) with specialty training in autonomic dysfunction clinically confirmed all testing and made the final diagnoses.

All healthy participants were examined to confirm the absence of any neurological conditions including autonomic dysfunction. Additional exclusion criteria including the following categories: i) pregnant or lactating females, ii) clinically significant coronary artery disease, iii) concomitant therapy with anticholinergic, alpha- and beta-adrenergic antagonists or other medications which could interfere with autonomic functioning, and iv) failure of other organ systems or systemic illness that could affect autonomic function or participants' ability to cooperate.

All laboratory data were collected in the Autonomic Disorders Laboratory at University Hospital, London, Ontario. All functional imaging data were collected at Robart's Research Institute Centre for Functional and Metabolic Imaging at The University of Western Ontario. Ethical approval was obtained from the Health Science Research Ethics Board at Western University, and informed consent was obtained from all participants prior to testing.

4.2.2 Neuroimaging data acquisition

All imaging data were collected using a whole body 3T imaging system with a 32-channel head coil (Magnetom Primsa, Siemens Medical Solutions, Erlangen, Germany). At the beginning of the scanning session, a 3D MPRAGE sequence was used to acquire a high-resolution T1-weighted structural (sagittal, matrix 256x240 mm, voxel resolution 1.0x1.0x1.0 mm, 1 mm slice thickness, no gap, flip angle 9°, TE: 2.98 ms, TI: 900 ms, TR: 2300 ms). Blood oxygen level-dependent (BOLD) signals were acquired using a T2- weighted gradient echo-echo planar imaging pulse sequence with the following parameters: TE: 30 ms; FOV: 240x240 mm; flip angle: 40 degrees; multiband acceleration factor: 4. Forty-eight interleaved axial slices (3.0x3.0 mm in-plane voxel resolution, TR: 1000 ms) were acquired in each volume. To help minimize head movement each participant's head was placed in a cradle packed with foam padding.

4.2.3 Neuroimaging protocol

Participants completed 5 minutes of rest followed by three Valsalva maneuvers (VM) during a functional scan of their brain. Rest: For all participants, the resting period consisted of 5 minutes during which, all participants were instructed to remain still with their eyes closed, but not to fall asleep. Valsalva maneuver: The Valsalva session was modeled as a blocked design switching between periods of rest and the maneuver. Following a 1-min baseline, participants were instructed to take a deep breath in, followed immediately by an exhalation to be maintained at an expiratory pressure of 40 mmHg, held for 15-seconds. The maneuver was repeated three times separated by a 2-min (120 sec) rest in between trials. The first 10 seconds immediately following release of the maneuver was recorded as a recovery period. The remaining 110 seconds were recorded as a rest period to allow for hemodynamics to return to baseline prior to performing another Valsalva. Together, the Valsalva scanning duration was 465 seconds. All participants

were provided real-time visual feedback of their expiratory pressure to ensure the maneuver was performed correctly. To further minimize head movement during VM, all participants practiced the maneuver prior to scanning while being supervised. In addition, during the scanning session, an MRI technician provided feedback if there was excessive movement, in which case the maneuvers were repeated. Beat-to-beat heart rate was recorded from a continuous signal derived from an MRI-compatible pulse oximeter (Nonin Medical, 8600FO MRI, Plymouth, MN). All hemodynamic recordings were collected using WR TestWorks™ software (WR Medical Electronics Co., Stillwater, MN).

4.2.4 Neuroimaging analysis

All imaging data were analyzed using the Conn functional connectivity toolbox (v18a) available through SPM12 (Wellcome Department of Imaging Neuroscience, London, UK) using a MATLAB R2016b interface (Mathworks, Natick, MA). Preprocessing steps included realignment, unwarping and slice-time correction. All structural and functional images were segmented in grey matter, white matter and cerebral spinal fluid, normalized to Montreal Neurological Institute (MNI) space and smoothed with a Gaussian kernel (Full-Width Half-Max=6 mm). In addition to realignment, the ART-based scrubbing method was further used to detect outlier volumes with high motion (ART parameters: 2-mm subject motion threshold and a global signal threshold set at Z=9). Nuisance variables including: 6 realignment parameters, first 5 principle components from the white matter and CSF and the outlier volumes from the scrubbing procedure were then regressed out of the signal. The data were linearly detrended and a band-pass filter of 0.008 to 0.09Hz was applied. A brainstem mask was used as the brainstem seed source, and all areas for connectivity were defined on a regions-of-interest (ROI) basis using an ROI-to-ROI approach at rest and during Valsalva maneuver. Cortical (91 ROIs) and subcortical (15 ROIs) atlases from the Harvard-Oxford Atlas and cerebellum parcellation (26 ROIs) atlas from AAL atlas were used in the ROI analysis. In the first level analysis, ROI-to-ROI maps were generated for each individual during the predefined conditions. Individual connectivity maps were created using the General Linear Model convolved with a canonical hemodynamic response function. In the second-level analysis, a between-subjects contrast (controls>patients [1, -1]; patients>controls [-1, 1]) was performed on the basis of a random-

effects General Linear Model, with a seed-level correction for multiple comparisons (false-discovery rate: $p<0.05$).

4.3 Results

4.3.1 ROI-to-ROI functional connectivity

Rest: Compared to patients with NOH, at rest controls had significantly greater brainstem connectivity to the anterior cingulate cortex (ACC) (T-value: 4.29; p-FDR<0.001), left anterior insula (T-value: 3.31; p-FDR<0.001), left putamen (T-value: 3.31; p-FDR<0.005) and bilateral thalamus (T_R-value: 3.83; T_L-value: 4.25; p-FDR<0.001) (Figure 4.1). The effect sizes for the aforementioned brainstem-to-ROI connectivities ranged from small to moderate (Figure 4.1).

Valsalva (VM): During VM, controls also showed significantly more connectivity between the brainstem and both the left anterior (cerebellum 4/5) and bilateral posterior cerebellum (cerebellar 9 and left cerebellar 6). Other cerebellar regions included brainstem-to-vermis (Vermis 4/5, 6, 8, 9 and 10). Other brainstem-to-cortical and subcortical regions included: bilateral putamen, posterior cingulate cortex, amygdala and medial prefrontal cortex (Figure 4.2). The effect sizes were moderate-to-strong for each brainstem-to-ROI (Table 4.1). Moreover, there was a significant negative correlation between the brainstem-cerebellar connectivity and the total CASS (Table 4.2).

Valsalva recovery: During the recovery phase of the VM, controls had greater brainstem connectivity to the left thalamus (4.17; p-FDR=0.02); PCC (3.32; p-FDR<0.05); right putamen (3.28; p-FDR<0.05); right paracingulate gyrus (3.25; p-FDR<0.05) and left posterior cerebellum (C9: 3.21; p-FDR<0.05). Similar to VM, the effect sizes for each brainstem-to-ROI was moderate-strong (Figure 4.3).

Table 4.1 Targets with greater brainstem functional connectivity during VM in healthy controls

Brainstem Target	**Side**	**T-value**	**P-FDR corrected**	**Effect Size**
Cerebellum Lobule 9	R	5.29	<0.005	0.75
Cerebellum Lobule 6	L	4.82	<0.005	0.90
Cerebellum Lobule 9	L	4.53	<0.005	0.74
Vermis Lobule 4/5		4.31	<0.01	0.76
Cerebellum Lobule 4/5	L	3.97	<0.01	0.82
Vermis Lobule 6		3.88	<0.01	0.72
Vermis Lobule 8		3.86	<0.01	0.74
Putamen	L	3.78	<0.01	0.63
PCC		3.70	<0.01	0.68
Putamen	R	3.42	<0.01	0.59
Vermis Lobule 9		3.15	<0.05	0.64
Vermis Lobule 10		2.95	<0.05	0.47
Amygdala	L	2.91	<0.05	0.60
MPFC		2.82	<0.05	0.54

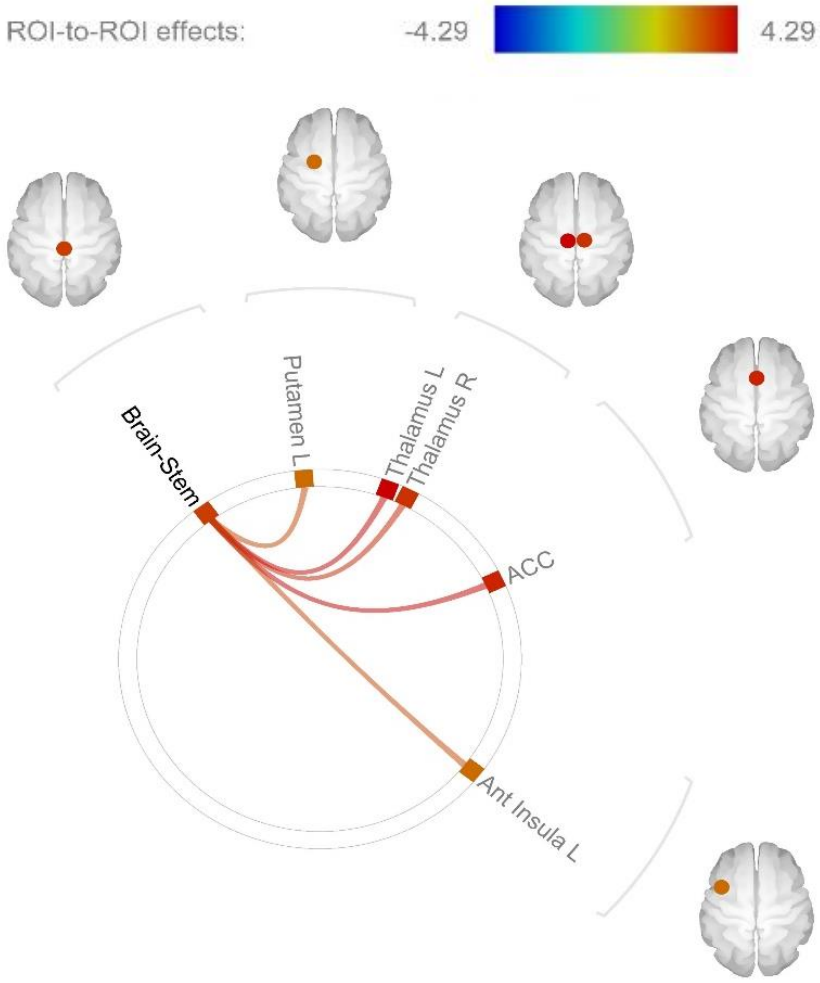
Abbrev. VM, Valsalva maneuver; L/R, left/right; PCC, posterior cingulate cortex

Table 4.2. Brainstem-cerebellar connectivity during VM correlates negatively with total CASS.

	C9-R	**C9-L**	**C6-L**	**C4/5-L**	**V4/5**	**V6**	**V8**	**V9**	**V10**
Total CASS*	-0.725	-0.674	-0.738	-0.592	-0.626	-0.559	-0.589	-0.529	-0.594
P-value	<0.001	=0.001	<0.001	=0.006	=0.003	=0.01	=0.006	=0.016	=0.006

* values represent r values

Abbrev: VM, Valsalva maneuver; CASS, composite autonomic scoring scale; C, Cerebellum; V, Vermis;



Brainstem Target	Effect Size
(L) Putamen	0.26
(L) Thalamus	0.41
(R) Thalamus	0.34
ACC	0.36
(L) Ant. Insula	0.26

Figure 4.1. Brainstem functional connectivity at rest [controls>patients].

At rest, controls had significantly greater brainstem connectivity compared to patients with NOH. Strength of connectivity is represented across a colour spectrum with red representing larger T-values and stronger connectivity. Abbrev. L/R, left/right; ACC, anterior cingulate cortex; Ant, anterior; Thal, Thalamus; ROI, region of interest

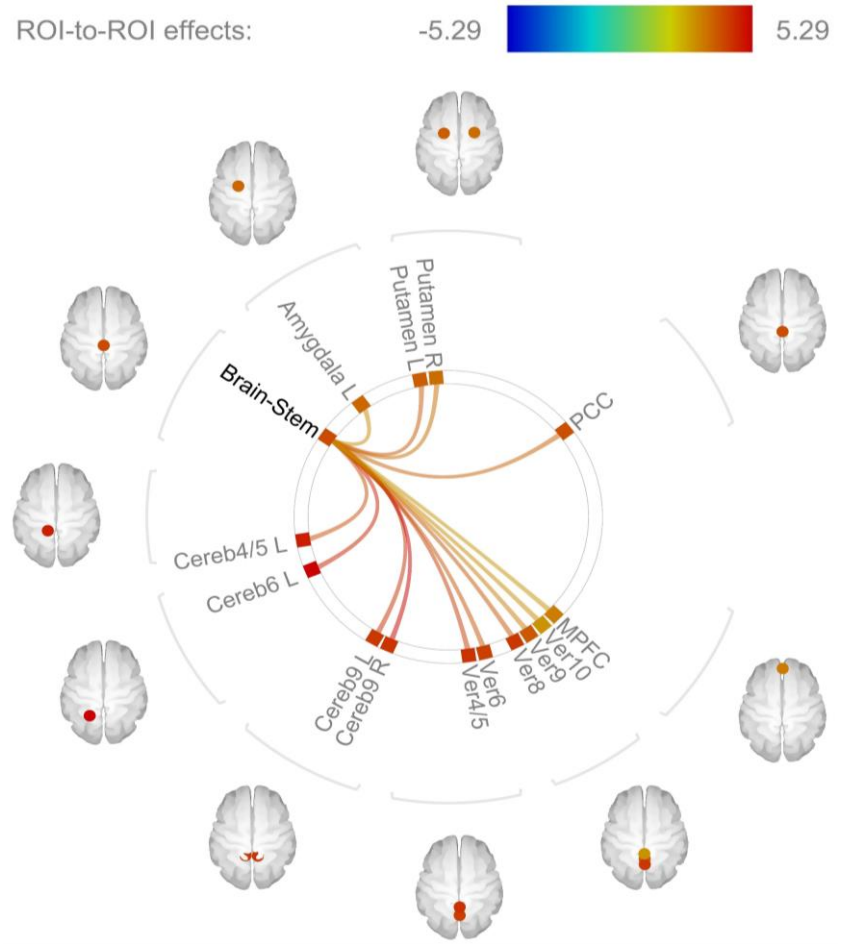
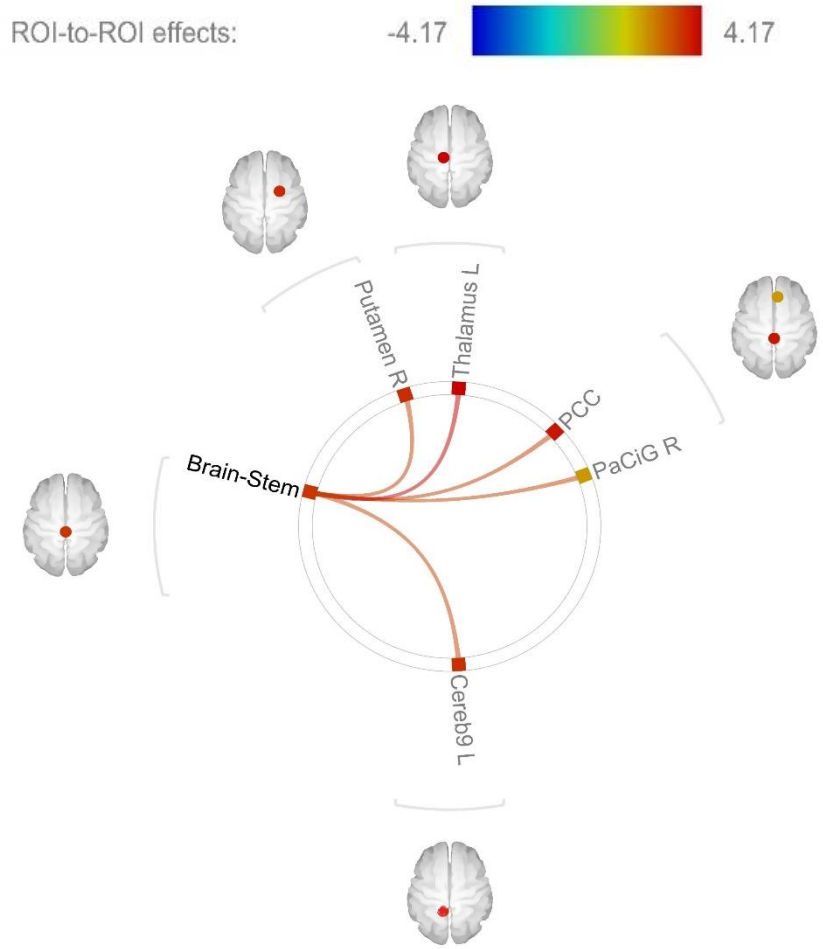


Figure 4.2. Brainstem functional connectivity during Valsalva maneuver [controls>patients].

During Valsalva maneuver, controls had significantly more brainstem connectivity to the amygdala, bilateral putamen, PCC, MPFC and cerebellum compared to patients. Strength of connectivity is represented across a colour spectrum with red representing larger T-values and stronger connectivity. Abbrev. L/R, left/right; PCC, posterior cingulate cortex; ROI, region of interest; MPFC, medial prefrontal cortex



Brainstem Target	Effect Size
(R) Putamen	0.58
(L) Thalamus	0.68
PCC	0.63
(R) PaCiG	0.34
(L) Cerebellum 9	0.57

Figure 4.3. Brainstem functional connectivity during recovery phase of Valsalva maneuver [controls>patients].

Controls had significantly more brainstem functional connectivity during the recovery phase of Valsalva. Strength of connectivity is represented across a colour spectrum with red representing larger T-values and stronger connectivity. Abbrev. L/R, left/right; PaCiG, paracingulate gyrus; PCC, posterior cingulate cortex; ROI, region of interest

4.4 Discussion

In the current study we compared functional connectivity measures between patients with autonomic failure and age-matched controls at rest and during an autonomic challenge. Using the brainstem as a seed source we found the following significant findings: 1) Patients with autonomic failure showed significantly less brainstem connectivity to structures of the central autonomic network throughout a state of rest and in response to a Valsalva maneuver. 2) Brainstem-to-cerebellum connectivity was negatively correlated with the total CASS suggesting individuals with more severe and widespread autonomic dysfunction have reduced brainstem-cerebellar connectivity.

The cortical and subcortical structures found functionally linked to the brainstem represent well recognized findings from neuroimaging and autonomic literature. In the current study, patients had significantly less brainstem connectivity to key autonomic structures including the cerebellum, thalamus, cingulate cortices, medial prefrontal and insula. These regions have all been highly implicated in autonomic regulation both in animals and humans, and in health and disease[5,12]. The thalamus plays a pivotal role as the primary relay site for information and has an abundance of anatomical projections to cortical, subcortical and brainstem structures[12,13]. Moreover, both the posterior and anterior cingulate cortices are key contributors to autonomic regulation, specifically modulations of heart rate and blood pressure[5,14,15]. For example, the anterior cingulate is commonly activated during maneuvers that elicit an increase in sympathetic activity such as the Valsalva maneuver, maximal inspiratory apneas and lower-body negative pressure. Furthermore, increased ACC activation has also been coupled with direct recordings of sympathetic nerve activity[16]. This is important, as improper regulation of heart rate, blood pressure and efferent sympathetic activity are cardinal features of autonomic failure. Moreover, the insula cortex (IC) has been highly investigated in autonomic regulation. Anatomically, the insula is reciprocally linked to brainstem autonomic nuclei[4,17], which provides an anatomical basis for autonomic influence. Functionally, the IC is extremely complicated. The IC has been partitioned into anterior/posterior portions as well as lateralized into left and right, each contributing separately to autonomic regulation. For example, stimulation of the rostral posterior IC in rats induced tachycardia while bradycardia was elicited via caudal stimulation[18] .

Furthermore, Zhang et al., (1998) demonstrated that damage to the left IC increased cardiac baroreceptor gain with no effect on heart rate or blood pressure, while right IC lesions resulted in increased baseline heart rate and blood pressure with no effect on gain[19]. Despite the complicated nature of the IC it is nevertheless involved in autonomic regulation. Finally, the current results extend beyond functional imaging studies, and coincides with existing functional connectivity literature in healthy controls, which also report significant brainstem connectivity with the ACC, thalamus, putamen and cerebellum[20].

A second key finding highlighted significantly less functional connectivity between the brainstem and the cerebellum/vermis during VM in patients with autonomic failure. The cerebellum is a key central structure that is commonly seen in functional imaging studies particularly studies involving blood pressure perturbations. Moreover, there is a growing amount of evidence to support a key role for the cerebellum in regulating blood pressure and limiting blood pressure extremes[21,22]. Therefore, the cerebellum may play a key role during the VM where there are large blood pressure fluctuations, and absent compensatory responses in autonomic failure. For example, patients demonstrate large blood pressure reductions during early phase II of the maneuver without an appropriate sympathetically mediated late phase II response. Similarly, following release of the maneuver, an additional burst of sympathetic activation acts to increase blood pressure back to, or above baseline levels – a response that is also absent in autonomic failure. Both responses require increased sympathetic activation to alter blood pressure and this becomes important as the cerebellum is involved, in part, with sympathetic activation through direct projections with brainstem nuclei, including the NTS and RVLM[23,24]. Evidence of reduced brainstem connectivity to the cingulate, insula and cerebellum coincide with imaging studies with concurrent recordings of sympathetic nerve activity that have correlated these regions to increased sympathetic activity[16].

Despite a patient sample consisting of autonomic failure involving post-ganglionic impairment, the current results also suggest impaired functional connectivity between the brainstem and autonomic brain structures. Recent advances in network sensitive neuroimaging techniques have begun to identify distinct patterns of functional connectivity in various diseases[25,26]. As a result, various hypotheses have emerged to explain the role neuronal networks may play in clinical

progression of various diseases. Two disease-mechanism models have been hypothesized, which may help explain the current results: 1. The Nodal stress model, and 2. Transneuronal spread. The nodal stress model postulates that certain regions, or "nodes" within the brain that are subject to heavy network trafficking may be more vulnerable to activity-related "wear and tear"[25,27]. The brainstem is the primary site for afferent input and as such sends and receives efferent and afferent information on a continuous basis to ensure proper neurovascular function. In addition to afferent feedback, efferent signals from higher cortical and sub-cortical structures also contribute to proper autonomic regulation through direct and indirect projections to the brainstem[13]. Together the brainstem is a critical hub for autonomic inputs, and the abundance of intra-network information may influence regional neurodegeneration that is activity-dependent. In a disease that fails to properly regulate autonomic responses such as blood pressure, various afferent and efferent inputs may overload brainstem networks in an attempt to rectify the failed responses. However, even if the appropriate efferent signals can be sent, the post-ganglionic lesion would interrupt the signal leading to more feedback, ultimately resulting in a viscous cycle of chronic elevated activity and possibly an eventual pathological state.

The mechanism of transneuronal spread suggests that neurodegeneration between cortical networks progresses via axonal connections[28]. Seeley et al., further suggest that disease progression starts at a primary network (i.e. the brainstem) and is more likely to extend into networks with stronger functional relationships[29]. This is important for two primary reasons. First, the results demonstrate that the cortical regions that showed significant differences between patients and controls were structures of the central autonomic network, and would therefore have a strong functional and structural relationship with the brainstem. Second, this model predicts that networks with shorter functional paths to the epicenter will be more vulnerable once the disease is present[30]. Anatomically, the cerebellum not only has direct projections with the brainstem but also demonstrates a relatively short functional pathway, and the strength of the brainstem-cerebellum connectivity was negatively correlated with autonomic severity and distribution.

4.4.1 Study limitations

Despite the promising contributions that these data may add to our understanding of autonomic failure, the current study contains the following limitations. 1) The current study applied a brainstem mask that covered the whole brainstem. Certainly, there are a number of brainstem nuclei that have different contributions to autonomic control. In the current study, we did not focus on whether the brainstem shows increased or decreased activity, simply that there was reduced connectivity to key cortical and subcortical sites. The authors believe the next logical step will be to assess functional connectivity related to specific brainstem nuclei. 2) The two proposed mechanistic models have been primarily investigated in neurodegenerative disorders such as Alzheimer's Disease, PD, Huntington's disease and Amyotrophic Lateral Sclerosis. Even though there are neurodegenerative diseases associated with autonomic failure, this work has yet to include these patient groups. Further studies are needed to investigate these models in autonomic failure. 3) Blood pressure data was not directly measured during the MRI session as this often involves the invasive insertion of an arterial line. Therefore, in the current study the heart rate change during the MRI session was compared with the heart rate changes that occurred in the laboratory during the Valsalva maneuver. Additionally, the expiratory pressure during the maneuver was monitored to ensure compliance. If there was a similar heart rate response, it was assumed that the corresponding blood pressure changes in response to the maneuver were similar. 4) The current study had a heterogeneous patient sample, including patients diagnosed with PD plus autonomic failure. To test whether the current results were related to autonomic failure and not PD, a sub-analysis of the patient group was performed. No significant differences between our autonomic failure patients with and without PD were found, suggesting the current results are related to the presence of autonomic failure and not PD pathology.

4.5 Conclusion

In the current study, patients with peripheral autonomic failure had significantly less functional connectivity between the brainstem and key central autonomic structures both at rest and during an autonomic maneuver. Patients showed reduced coupling between brainstem and regions of the central autonomic network, including the cerebellum, insula, thalamus and cingulate cortices.

These results may be attributed to two mechanistic models, including nodal stress and transneuronal spread, which may contribute, in part, to the pathophysiology of autonomic failure.

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CHAPTER 5

5 Evidence of impaired cerebellar connectivity at rest and during autonomic maneuvers in patients with autonomic failure[4]

5.1 Introduction

Neurogenic Orthostatic Hypotension (NOH) is a cardinal feature of autonomic failure. NOH is defined as a persistent and consistent drop in systolic blood pressure ≥30 mmHg upon standing, without an appropriate compensatory postural tachycardia[1]. Proper regulation of autonomic responses is mediated through several brainstem nuclei, including the nucleus tractus solitarius (NTS), rostral and caudal ventrolateral medulla (RVLM, CVLM, respectively), parabrachial nucleus (PBN), etc., that serve as regulatory sites for autonomic control. These brainstem nuclei together are part of an integrated autonomic network involved in mediating the beat-to-beat adjustments to heart rate (HR) and blood pressure (BP). For example, during a state of hypotension, reduced baroreceptor afferents to the NTS, result in disinhibition of premotor sympathetic nuclei within the RVLM. Disinhibition of the RVLM, by the CVLM, results in increased efferent signals to the intermediolateral cell column to increase efferent sympathetic outflow with subsequent vasoconstriction and tachycardia[2]. NOH occurs due to failure of the reflexive increase in efferent sympathetic outflow that would normally counteract blood pressure changes associated with standing.

In addition to afferent inputs, efferent signals from cortical and subcortical structures also converge at the level of the brainstem to further regulate autonomic responses[3]. Many studies, incorporating different experimental modalities (i.e. electrical and chemical stimulation, functional imaging, etc.), have uncovered a network of cortical and subcortical structures involved in autonomic regulation. Structures such as the insular cortex, cingulate cortex, hippocampus, prefrontal cortex, hypothalamus, thalamus and cerebellum, together with brainstem nuclei make up key components of the central autonomic network (CAN)[3–6].

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[4] A version of this chapter is currently under review for publication.
Baker J and Kimpinski K (2019). Evidence of impaired cerebellar connectivity at rest and during autonomic maneuvers in patients with autonomic failure. The Cerebellum. (under review)

Despite its consistent presence in the literature and significant contributions to cardiovascular modulation, the cerebellum has arguably received less attention as part of the CAN, and is not commonly recognized as a significant contributor to cardiovascular and autonomic control[7]. Anatomically, autoradiography along with retrograde and anterograde tracing studies, have revealed the structural network of neurons projecting from the cerebellum to the NTS, RVLM, PBN and Nucleus Ambiguus[8–10]. Functionally, cerebellar stimulation produces significant cardiovascular responses including increased cerebral blood flow, tachycardia and arterial pressor responses with measurable increases in muscle, splanchnic and renal sympathetic nerve activity[11–16]. Cerebellar lesions result in remarkable dysfunction in the compensatory responses to hypotension, suggesting an essential role in cardiovascular compensation during large blood pressure perturbations[7,17,18]. Finally, neuroimaging studies involving awake humans have demonstrated cerebellar activation in response to lower-body negative pressure[19] and cerebellar and deep cerebellar nuclei activation with concomitant increased muscle sympathetic nerve activity[20].

Our laboratory recently investigated functional central network activity in patients with NOH during standard autonomic challenges. In this study, we found that patients with NOH as a result of autonomic failure had significantly reduced cerebellar activation in response to autonomic challenge that perturbs heart rate and blood pressure (Valsalva maneuver and lower-body negative pressure)[21]. Therefore, in the current study, we sought to determine whether NOH patients also have impaired functional connectivity between the cerebellum and CAN structures.

5.2 Methods

5.2.1 Study participants

In total, 15 patients meeting the criteria for NOH participated in the current study (67±6 years). All patients underwent standard tests for detecting severity and distribution of autonomic failure, namely the Autonomic Reflex Screen[22]. All patients met the criteria for NOH demonstrating an average blood pressure reduction of 80±25 mmHg with only an average compensatory tachycardia of 9±7 bpm during Head-up Tilt. Additionally, all patients further demonstrated evidence of adrenergic failure based on qualitative evaluation of the Valsalva maneuver, which

revealed absent adrenergic phases (late phase II and phase IV). A Neurologist (KK) with specialty training in autonomic dysfunction confirmed all testing and made the final diagnoses. The composite autonomic scoring scale (CASS) was used to quantify the severity and distribution of autonomic failure based on the ARS. The CASS provides a score across three domains: sudomotor (0-3), cardiovagal (0-3) and adrenergic (0-4)[23]. On average patients scored a 2/3, 2/3 and 4/4, respectively, resulting in a total CASS of 8/10 indicative of severe and widespread autonomic dysfunction. The patient cohort was comprised of the following diagnoses: Pure Autonomic Failure, n=4; Parkinson's Disease with autonomic failure, n=6; idiopathic NOH, n=5.

Patient data were compared against fifteen healthy, age-matched controls (61±14 years; p=0.12). Healthy participants were examined to confirm the absence of any neurological conditions including autonomic dysfunction. Healthy participants were also excluded if they fell under any one of the following categories: i) pregnant or lactating females, ii) clinically significant coronary artery disease, iii) concomitant therapy with anticholinergic, alpha- and beta-adrenergic antagonists or other medications which could interfere with autonomic functioning, and iv) failure of other organ systems or systemic illness that could affect autonomic function or participants' ability to cooperate. All laboratory data were collected in the Autonomic Disorders Laboratory at University Hospital, London, Ontario. All imaging data were collected at Robart's Research Institute Centre for Functional and Metabolic Imaging at The University of Western Ontario. Ethical approval was obtained from the Health Science Research Ethics Board at Western University, and informed consent was obtained from all participants prior to testing.

5.2.2 Neuroimaging data acquisition

All imaging data were collected using a 3T imaging system with a 32-channel head coil (Magnetom Primsa, Siemens Medical Solutions, Erlangen, Germany). A high-resolution T1-weighted structural was acquired at the beginning of the scanning session (sagittal, matrix 256x240 mm, voxel resolution 1.0x1.0x1.0 mm, 1 mm slice thickness, no gap, flip angle 9 degree, TE: 2.98 ms, TI: 900 ms, TR: 2300 ms). Blood oxygen level-dependent (BOLD) signals were acquired using a T2- weighted gradient echo-echo planar imaging pulse sequence with the

following parameters: TE: 30 ms; FOV: 240x240 mm; flip angle: 40 degrees; multiband acceleration factor: 4. Forty-eight interleaved axial slices (3.0x3.0 mm in-plane voxel resolution, TR: 1000 ms) were acquired in each volume. Following the structural scan, functional brain imaging was performed during the following: Rest: The rest period consisted of 5 minutes of functional scanning during which, all participants were instructed to remain still with their eyes closed, but not to fall asleep. Lower-body negative pressure (LBNP): Following a minimum 15-minute baseline, suction was applied at -35 mmHg for 5 minutes, followed by a 5-minute rest period. All healthy participants completed LBNP at -35 mmHg. However, due to the nature of the disease and the marked blood pressure drops in the patient group, in some cases the negative pressure was lowered. On average, LBNP was completed at a suction pressure of -27 mmHg in our patient group. To help minimize head movement, all participants underwent LBNP in the lab prior to the MRI session. In addition, all participants were secured to foot plates within the box to help with stabilization during suction. Valsalva maneuver: All participants completed three Valsalva maneuvers. The Valsalva session was modeled as a blocked design switching between periods of rest and the maneuver. Following a 1-min baseline, participants were instructed to perform a Valsalva for 15-seconds, held at an expiratory pressure of 40mmHg. Real-time visual feedback regarding the expiratory pressure was provided. Each maneuver was separated by a 2-min rest in between trials. The first 10 seconds immediately following release of the maneuver was recorded as a recovery period. The remaining 110 seconds were recorded as a rest period to ensure hemodynamics returned to baseline. To minimize head movement each participant's head was placed in a cradle packed with foam padding. To minimize head movement during VM, all participants practiced performing the VM prior to scanning while being supervised. In addition, feedback from an MRI technician was provided if there was excessive movement, in which case trials were repeated. Beat-to-beat heart rate was recorded from a continuous signal derived from an MRI-compatible pulse oximeter (Nonin Medical, 8600FO MRI, Plymouth, MN) attached to the index finger of each participant's left hand when possible. In the presence of a significant tremor (i.e. in PD+NOH patients), pulse oximetry was obtained from the hand with less potential for movement. All hemodynamic recordings were collected using WR TestWorks™ software (WR Medical Electronics Co., Stillwater, MN).

5.2.3 Neuroimaging analysis

All imaging data were analyzed using the Conn toolbox (v18a) available through SPM12 (Wellcome Department of Imaging Neuroscience, London, UK). Preprocessing steps included realignment, unwarping and slice-time correction. All structural and functional images were segmented in grey matter, white matter and cerebral spinal fluid, normalized to Montreal Neurological Institute (MNI) space and smoothed with a Gaussian kernel (Full-Width Half-Max=6 mm). To detect outlier volumes with high motion, the ART-based scrubbing method was used (ART parameters: 2-mm subject motion threshold and a global signal threshold set at Z=9). Nuisance variables including: 6 realignment parameters, first 5 principle components from the white matter and CSF and the outlier volumes from the scrubbing procedure were then regressed out of the signal. The data were linearly detrended and band-pass filtered (0.008 to 0.09 Hz). Cerebellar functional connectivity was tested with an ROI-to-ROI approach during rest, LBNP and VM. All areas for connectivity were defined on a regions-of-interest (ROI) basis using the following atlases: The cerebellum was parcellated into 26 ROIs from the AAL atlas. Cortical (91 ROIs) and subcortical (15 ROIs) atlases were derived from the Harvard-Oxford Atlas.
In the first level analysis, ROI-to-ROI maps were generated for each individual during the predefined conditions. Individual connectivity maps were created using the General Linear Model convolved with a canonical hemodynamic response function. In the second-level analysis, a between-subjects contrast (controls>patients [1, -1; patients>controls [-1, 1]) was performed on the basis of a random-effects general linear model, with a seed-level correction for multiple comparisons (false-discovery rate: $p<0.05$).

5.3 Results

Rest: Controls had significantly more posterior cerebellar connectivity to various key cortical and subcortical autonomic structures, including: bilateral anterior insula, anterior cingulate cortex, bilateral putamen and bilateral thalamus. In addition, controls had significantly more intra-cerebellar connectivity, including connectivity between posterior cerebellum lobule 9 to: bilateral cerebellum lobule 6, right cerebellum lobule 7, right anterior cerebellum lobule 2 and the vermis 6. In addition, there was significant bidirectional connectivity between right

cerebellum lobule 2 and right cerebellum lobule 6 and vermis 9 with left cerebellum lobule 9 (Figure 5.1). The effect sizes at rest were small to moderate (Table 5.1).

Valsalva maneuver (VM): During VM, controls had significantly greater functional connectivity between structures of the central autonomic network and cerebellar seed sources. The left anterior cerebellum (lobule 4/5) had significant connectivity with the vermis 3, brainstem, posterior cingulate cortex and the right insular cortex and the right cerebellar lobule 4/5 (Figure 5.2A). The anterior vermis (region 4/5) showed significant connectivity with the brainstem, while vermis region 3 was significantly connected to the right anterior parahippocampus and PCC. Additionally, the anterior vermis (region 3) showed greater connectivity with other cerebellar regions including: posterior vermis (region 8) and right cerebellum lobule 6 and bilateral cerebellar lobule 4/5. (Figure 5.2B). Finally, the posterior cerebellum (lobule 6) had significantly greater connectivity to the brainstem and right hippocampus, and the bilateral posterior cerebellum (lobule 9) was significantly connected to the brainstem (Figure 5.2C). The effect sizes for the connectivities during VM were moderate to strong (Table 5.2).

LBNP: In response to an orthostatic challenge, controls showed significantly greater connectivity between the left posterior cerebellum (lobule 9) and the bilateral thalamus as well as the right posterior cerebellum (lobule 9) and the bilateral thalamus and left putamen (Figure 5.3). The effect sizes for the connectivity during LBNP were modest (Table 5.3).

Table 5.1 Regions of greater cerebellar connectivity at rest in healthy controls versus NOH patients.

	Side	**Effect Size**	**T-value**	**p-FDR value**
Anterior Cerebellum				
(R) Cerebellum Lobule 2 - Cerebellum Lobule 6	R	0.34	3.91	0.03
Posterior Cerebellum				
(R) Cerebellum Lobule 6 - Cerebellum Lobule 2	R	0.34	3.91	0.03
Vermis Lobule 6 – Cerebellum Lobule 9	L	0.31	3.80	0.04
(L) Cerebellum Lobule 9 -				
Vermis Lobule 6		0.31	3.80	<0.05
Cerebellum Lobule 7	R	0.23	3.17	<0.05
Thalamus	L	0.33	3.15	<0.05
Putamen	L	0.28	3.03	<0.05
Thalamus	R	0.28	2.92	<0.05
Cerebellum Lobule 6	R	0.23	2.92	<0.05
Cerebellum Lobule 2	R	0.25	2.89	<0.05
Anterior Insula	R	0.26	2.82	<0.05
Cerebellum Lobule 6	L	0.23	2.79	<0.05
(R) Cerebellum Lobule 9 -				
Anterior Insula	R	0.34	4.84	<0.005
Anterior Insula	L	0.31	4.51	<0.005
Thalamus	L	0.38	4.51	<0.005
Putamen	L	0.30	4.34	<0.005
Putamen	R	0.32	4.26	<0.005
Thalamus	R	0.35	3.95	<0.005
Anterior Cingulate Cortex		0.29	3.41	<0.05
Vermis Lobule 6		0.24	2.86	<0.05

Abbreviations: R, right; L, left

Table 5.2 Regions of greater cerebellar connectivity in healthy controls versus NOH patients during Valsalva maneuver.

	Side	**Effect Size**	**T-value**	**p-FDR value**
Anterior Cerebellum				
Vermis Lobule 4/5 - Brainstem		0.76	4.31	0.02
(L) Cerebellum Lobule **4/5 to:**				
Vermis Lobule 3		0.79	4.39	0.012
Insula	R	0.76	4.21	0.012
Brainstem		0.82	3.97	0.014
Posterior cingulate cortex		0.65	3.76	0.017
Cerebellum Lobule 4/5	R	0.56	3.59	0.00
Vermis Lobule 3 to:				
Anterior Para-hippocampus	R	0.53	4.63	0.008
Cerebellum Lobule 4/5	L	0.79	4.39	0.008
Posterior cingulate cortex		0.61	4.18	0.009
Cerebellum Lobule 4/5	R	0.63	3.88	0.013
Vermis Lobule 8		0.58	3.29	0.03
Cerebellum Lobule 6	R	0.57	3.28	0.03
Posterior Cerebellum				
(L) Cerebellum Lobule 6 to:				
Brainstem		0.9	4.82	0.006
Hippocampus	R	0.6	3.87	0.03
(L) Cerebellum Lobule 9 - Brainstem		0.74	4.53	0.012
(R) Cerebellum Lobule 9 - Brainstem		0.75	5.29	0.002

Abbreviations: R, right; L, left

Table 5.3 Greater posterior cerebellar connectivity in healthy controls versus NOH patients during lower-body negative pressure.

	Side	**Effect Size**	**T-value**	**p-FDR value**
Posterior Cerebellum				
(L) Cerebellum Lobule 9 to:				
Thalamus	L	0.34	4.1	0.02
Thalamus	R	0.32	3.76	0.02
(R) Cerebellum Lobule 9 to:				
Putamen	L	0.3	4.17	0.02
Thalamus	L	0.3	3.44	0.04
Thalamus	R	0.28	3.43	0.04

Abbreviations: R, right; L, left

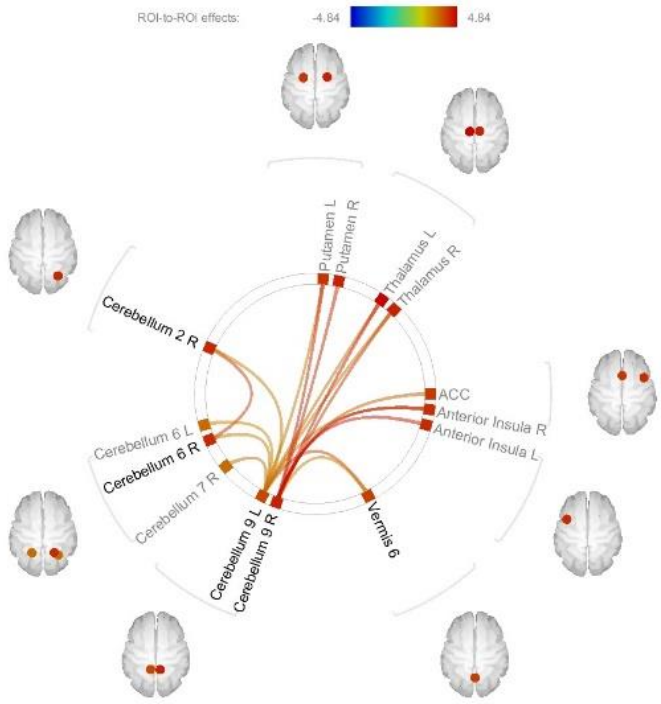

Figure 5.1. Cerebellar connectome at rest [Controls>Patients].

At rest, controls showed significantly greater intra-cerebellar connectivity, and greater cerebellar connectivity to the bilateral insula, bilateral putamen, bilateral thalamus and anterior cingulate cortex (ACC). Strength of connectivity is represented across a colour spectrum with red representing larger T-values and stronger connectivity.

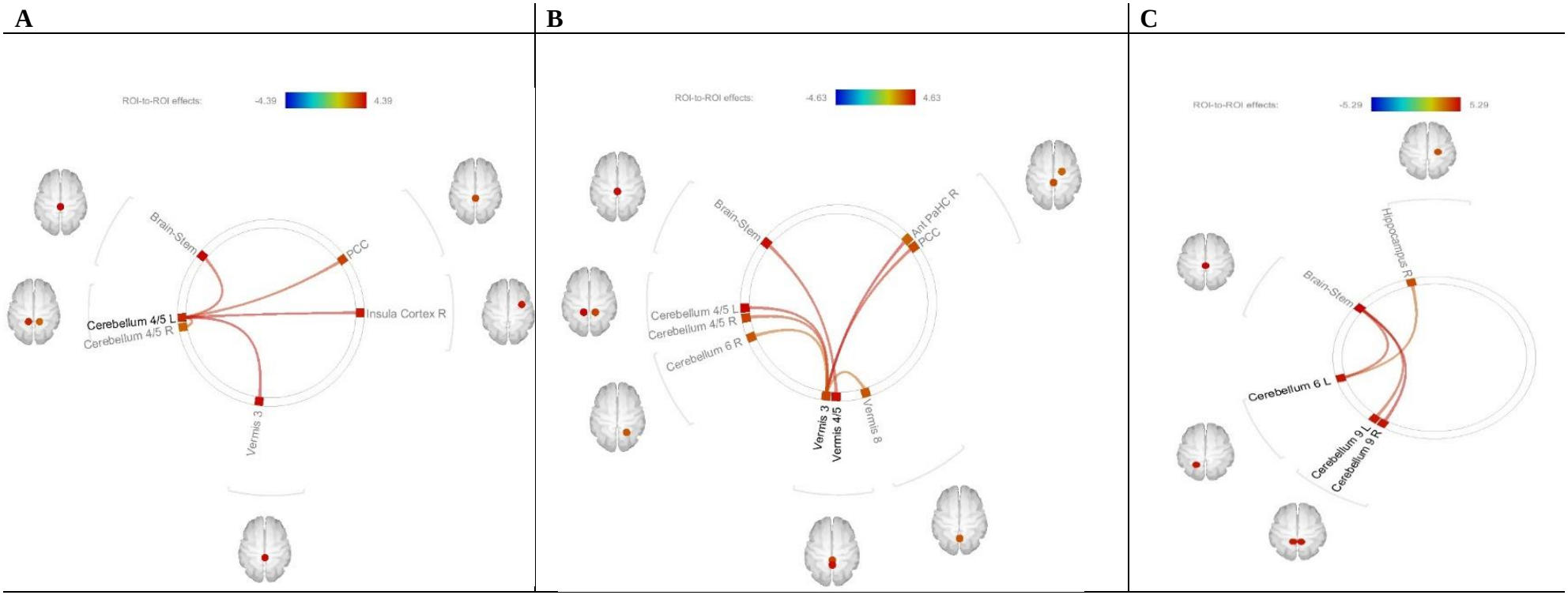

Figure 5.2 Cerebellar connectome during Valsalva maneuver [Controls>Patients].

Anterior (A), Vermis (B) and Posterior (C) cerebellar connectome during Valsalva maneuver [Controls>Patients]. Controls had significantly greater intra-cerebellar, cerebellum-cortical and cerebellar-brainstem connectivity during the Valsalva maneuver compared to NOH patients. Strength of connectivity is represented across a colour spectrum with red representing larger T-values and stronger connectivity.

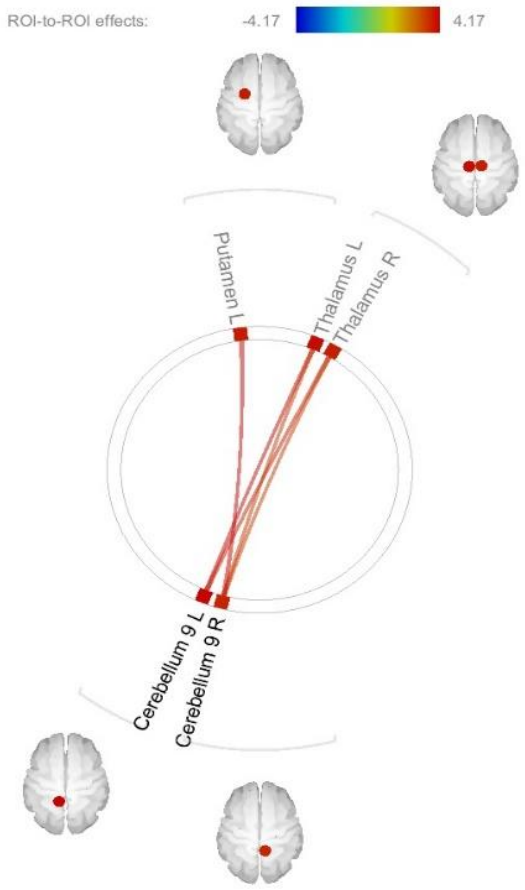

Figure 5.3. Cerebellar connectome during lower-body negative pressure [Controls>Patients].

Controls showed significantly greater connectivity between the posterior cerebellum lobule 9 and the thalamus and putamen during lower-body negative pressure. Strength of connectivity is represented across a colour spectrum with red representing larger T-values and stronger connectivity.

5.4 Discussion

Previously, we found that patients with NOH had significantly reduced cerebellar activation in response to autonomic challenges[21]. In the current study, we explored these findings further by assessing cerebellar functional connectivity and found the following three findings: 1. Patients had significantly less functional connectivity in cerebellar regions that have been highly implicated in cardiovascular autonomic control. 2. Patients had significantly less connectivity between cerebellar regions and the brainstem. 3. Patients had significantly less functional connectivity between the cerebellum and other structures of the central autonomic network.

Patients with NOH experience a significant drop in blood pressure related to upright posture. This occurs due to a failure of the autonomic nervous system to reflexively increase sympathetic nerve activity. The cerebellum is involved in the integration of vestibular information regarding postural changes to facilitate an early compensatory increase in sympathetic nerve activity via the brainstem to help maintain blood pressure upon standing. In the current study patients showed significantly less connectivity between the cerebellum, including the vermis and lobule 9, and the brainstem. Structural and functional experimental data in animal and human subjects support two major pathways between the cerebellum and brainstem through which cardiovascular autonomic reflexes are regulated. The first operates through the vestibular nucleus complex (VNC) to mediate the vestibulosympathetic reflex (VSR)[24,25] and the second through the baroreflex arc[11]. These pathways converge on several brainstem autonomic nuclei, including at the level of the RVLM. Moreover, the fastigial nucleus (FN), vermis and lobule 9 of the posterior cerebellum are key cerebellar structures involved in both pathways. Together, these structures form a cerebellar-brainstem network for cardiovascular autonomic control that may be impaired in patients with NOH.

5.4.1 Cerebellum and the baroreflex

Anatomically, regions of the cerebellum project to important brainstem nuclei involved in baroreflex control, including the NTS, PBN and RVLM[9,11,15]**.** Functionally, rostral FN stimulation results in a pronounced increase in renal sympathetic nerve activity, vasoconstriction and concomitant pressor responses in several species[12–14,16]. Following sympathectomy/Alpha

blocker, this pressor response is abolished, suggesting the response pathway is sympathetically-mediated[26,27]. Further evidence to support the FN in baroreflex control was provided by Lisander and Martner who demonstrated that FN stimulation produced similar BP, HR and blood flow patterns to that caused by carotid baroreceptor unloading[26]. Furthermore, studies reveal altered baroreflex, decreased heart rate and redistribution of cardiac output following cerebellar lesions/cerebellectomies[28,29]. The anterior vermis (AV) also has direct projections to the PBN and studies have shown that PBN stimulation also activates the anterior vermis, suggesting a vermis-PBN complex[30]. Functionally, AV stimulation inhibits sympathetic nerve activity, subsequently producing a depressor effect. In addition, when the AV is stimulated prior to FN stimulation, it effectively depresses the pressor response normally elicited by FN stimulation, suggesting an inhibitory/modulatory role of the AV[11]. Overall, the AV may mediate sympatho-inhibition that, together with the FN, provides a regulatory micro-complex for cardiovascular and baroreflex control. Finally, stimulation of lobule 9 of the posterior cerebellum induces a large increase in renal sympathetic nerve activity, with an accompanying tachycardia and pressor response. This response can be effectively abolished following sympathetic ganglion blockade, suggesting it too is sympathetically-mediated[11]. Furthermore, Paton and colleagues found that when arterial baroreceptors were activated with concomitant lobule 9 stimulation, the subsequent reflexive changes in HR and BP associated with baroreceptor activation failed to occur. The authors found that lobule 9 stimulation effectively decreased or abolished the activity of baro-sensitive neurons within the NTS, suggesting the posterior cerebellum facilitates changes in HR and BP by inhibiting baroreceptor input at the level of the NTS[31].

5.4.2 Vestibulo-sympathetic reflex

In addition to baroreflex nuclei, vestibular nuclei within the brainstem also contribute to proper cardiovascular autonomic regulation. Considerable experimental evidence shows an important role of the vestibular system in regulating sympathetic nerve activity. Together, the integration of vestibular information to generate a sympathetic response forms the physiological basis for the vestibulo-sympathetic reflex (VSR). Evidence has accumulated, which has conclusively demonstrated the contribution of the cerebellum and the vestibular system to facilitate an early burst in sympathetic nerve activity to help maintain postural control of blood pressure.

The vestibular nucleus complex (VNC) is located on either side of the brainstem in the region of the pons and medulla, and is comprised of four nuclei: the superior, inferior, medial and lateral. Each nucleus has projections to autonomic regulatory nuclei in the brainstem, including the RVLM, CVLM and NTS[24,32]. Similar to the baroreflex, the RVLM serves as the primary premotor region for mediating the sympathetic response association with the VSR. Studies to support this have shown that neurons of the RVLM not only respond to vestibular nerve stimulation[25,33], but also that the VSR is abolished following lesions to the RVLM. Furthermore, Kerman and Yates (1998) demonstrated that stimulation of the baroreceptors via a pressor response resulted in an attenuation of the VSR[34]. As both vestibular and baroreceptor inputs converge at the level of the RVLM, this may highlight a major site for modulation. The VNC also receive a wide array of input signals from the cerebellum and higher-order brain structures, thus making the VNC a prime candidate for information integration to facilitate autonomic responses.

The cerebellum projects heavily to the VNC, which, as previously discussed, sends direct inputs to the NTS, RVLM and CVLM. Together, these structures form a cerebellar vestibulo-sympathetic complex capable of integrating vestibular and cardiovascular signals to regulate autonomic responses. Similar to the cerebellum-baroreflex pathway, the cerebellar inputs to the vestibular nuclei are largely mediated through extensive projections from the fastigial nucleus and lobule 9 of the posterior cerebellum. A number of studies have demonstrated extensive bilateral projections between the FN and the VNC[11,24,32]. These fastigio-vestibular fibers appear to have excitatory actions, which may provide an additional framework for the VSR. Moreover, lobule 9 projections to the VNC have also been well described in a number of species. Specifically, projections from the lateral nodulus-uvula were observed to terminate in the lateral, superior and medial vestibular nuclei along with the PBN[11]. Purkinje cells in the uvula of lobule 9 provide monosynaptic input to the superior and medial VN, which mediated the VSR, and disynaptic inputs to the NTS[24]. Thus, lobule 9 of the posterior cerebellum appears to have the anatomical framework to support a role in regulating both the VSR and baroreflex.

5.4.3 Cerebellar-cerebral connectivity:

Finally, in the current study, patients with NOH had significantly less functional connectivity between the cerebellum and a number of higher-order structures including the insula, cingulate cortices, thalamus and hippocampus. Structures of the CAN including those previously mentioned, have been highly implicated in autonomic control. For example, the insula projects directly to a number of cortical and subcortical sites known to facilitate autonomic responses such as the hypothalamus, PBN and NTS [35–37]. However, studies have also shown direct cortico-vestibular projections suggesting an additional pathway for cortical autonomic regulation, through the VNC. In cats, retrograde tracers in the vestibular nuclei show widespread cerebral labeling[38], while studies involving non-human primates have demonstrated direct projection from a number of higher cortical structures to vestibular nuclei, including the insula and anterior cingulate cortex[39].

As most postural changes are the result of voluntary, and as such, planned and executed movements, it seems appropriate that higher brain structures would be involved to proactively regulate brainstem neurons to increase sympathetic activity. This idea of a "central command" network to facilitate autonomic responses is not new and is conclusively supported. For example, individuals who are paralyzed can elicit graded heart rate and blood pressure responses to imagined exercise[40]. Central command also contributes to adjustments to baroreflex gain and it has been suggested that higher brain regions may also adjust VSR gain, such that the response is appropriate for the ensuing postural change[24]. However, more work is needed to better understand where (i.e. VNC, RVLM, CVLM, NTS) the adjustment occurs.

5.5 Conclusion

Overall, the combination of anatomical and functional studies in animal and humans substantiate a significant role for the cerebellum in cardiovascular autonomic control. Specifically, a number of CAN structures including the cerebellum, brainstem and higher cortical structures form a complex network capable of integrating vestibular and cardiovascular information to facilitate appropriate autonomic adjustments during postural changes. In the current study, patients with neurogenic orthostatic hypotension show evidence of reduced cerebellar-brainstem and

cerebellar-cortical connectivity. Evidence of impaired cerebellar connectivity may contribute to the inability to properly regulate blood pressure during postural changes and perhaps provide further understanding of the pathophysiology of NOH.

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CHAPTER 6

6 Initial validation of symptom scores derived from the Orthostatic Discriminant and Severity Scale[5]

6.1 Introduction

Orthostatic symptoms occur when one changes position from lying or sitting to standing. Orthostatic Intolerance (OI) is associated with numerous forms of autonomic dysfunction, ranging from severe autonomic disorders (i.e. Pure Autonomic Failure, Multiple System Atrophy, Neurogenic Orthostatic Hypotension) to milder syndromes (i.e. Postural Tachycardia Syndrome, Syncope, Orthostatic Intolerance)[1–4]. Symptoms associated with OI such as lightheadedness, dizziness, faintness and heart palpitation leading to possible syncope[5] are the primary cause of morbidity in patients with dysautonomia. Additionally, these symptoms are often worsened by specific stressors including, but not limited to, exertion, high ambient temperatures, hot showers and baths, consumption of large meals and prolonged standing, making orthostatic symptoms particularly disabling and burdensome to activities of daily living[3]. However, due to the non-specific nature of orthostatic symptoms, such as lightheadedness and dizziness, other more common etiologies are often considered prior to OI and autonomic dysfunction. To add to this problem, OI can also have numerous accompanying constitutional symptoms such as: fatigue, generalized weakness and shoulder and neck pain[6]. In such cases, clinicians may focus on these symptoms, without associating them with OI. Therefore, accurate identification and distinction between orthostatic versus non-orthostatic symptomatology is important for accurate diagnoses and treatment management.

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Baker J, Paturel J, Sletten DM, Low PA and Kimpinski K (2019). Initial validation of symptom scores derived from the orthostatic discriminant and severity scale. *Clin Auton Res*. 29(1): 105-112.

Currently, there is no simple instrument, easily accessible to clinicians to help make this distinction and to discriminate symptoms as being orthostatic or non-orthostatic. Current validated questionnaires focused on orthostatic symptoms include: 1. Autonomic Symptom Profile (ASP), and 2. Orthostatic Hypotension Question (OHQ). The ASP is a comprehensive questionnaire (169 questions) with a focus on all aspects of autonomic dysfunction, with OI being a small portion of this assessment[7]. In contrast, the OHQ is short and the calculated results are easily obtainable and restricted to the assessment of the severity of orthostatic symptoms and the effects on daily living. However, the OHQ focuses on symptoms related to low blood pressure problems as opposed to generalized OI[8]. While these instruments provide important information on orthostatic symptoms, they do not address how orthostatic symptoms are differentiated from non-orthostatic symptoms.

Therefore, we developed the Orthostatic Discriminant and Severity Scale (ODSS) to help discriminate symptoms as being either orthostatic or non-orthostatic in nature. The ODSS is a short, 33-question, self-report questionnaire that provides an orthostatic score and non-orthostatic score. The ODSS implements clinical questions routinely used in practice by clinicians to identify symptoms as being either orthostatic or non-orthostatic. The objectives of the current study were to analyze the orthostatic scores and non- orthostatic symptom scores derived from the ODSS with respect to: 1. Convergent validity, 2. Clinical validity and 3. Test-retest reliability.

6.2 Methods

6.2.1 Study participants

This was a prospective study evaluating validity and reliability of the ODSS in persons with orthostatic intolerance against asymptomatic healthy controls. Patients were recruited from the Autonomic Disorder Laboratory within the Department of Clinical Neurological Sciences, University Hospital, London, Canada. All patients were seen by a neurologist to confirm the presence of orthostatic intolerance. In addition, all healthy participants were examined to confirm the absence of any neurological condition including autonomic dysfunction and symptoms related to OI. In addition, healthy participants were excluded if they fell under any one of the

following categories: i) pregnant or lactating females, ii) clinically significant coronary artery disease, iii) concomitant therapy with anticholinergic, alpha- and beta-adrenergic antagonists or other medications which could interfere with autonomic functioning, and iv) failure of other organ systems or systemic illness that could affect autonomic function or participants' ability to cooperate. All study participants completed the Autonomic Reflex Screen (ARS) and 3 self-report questionnaires (Autonomic Symptom Profile, Orthostatic Hypotension Questionnaire, Orthostatic Discriminant and Severity Scale). Study participants were asked to repeat the ODSS two weeks later to calculate test-retest reliability. Ethical approval for this study was obtained from the Health Sciences Research Ethics Board at Western University and written informed consent was obtained from each participant prior to study commencement.

6.2.2 Clinical Evaluation

6.2.2.1 Autonomic reflex screen

Standardized autonomic testing was performed as previously described[9,10]. In brief, Quantitative Sudomotor Axon Reflex Test (QSART) was used to evaluate post-ganglionic sympathetic axon integrity using a QSWEAT device (WR Medical Electronics Co., Stillwater, MN) and multi-compartmental sweat capsules. Adrenergic function was assessed using beat-to-beat blood pressure and heart rate responses to Valsalva maneuver (VM) and Head-up Tilt (HUT). Cardiovagal function was assessed using heart rate response to deep breathing (HRDB) and Valsalva ratio (VR) calculated from the VM. Heart rate and blood pressure were continuously recorded using an electrocardiograph (ECG) (Model 3000 Cardiac Trigger Monitor, IVY Biomedical Systems, Inc., Branford, CT) and Nexfin hemodynamic monitoring system (BMEYE Cardiovascular, Amsterdam, Netherlands), respectively. All data were recorded and analyzed using WR Testworks™ software. The composite autonomic scoring scale (CASS) was derived from the ARS as previously described[11]. The CASS provides a quantitative measure of the severity and distribution of autonomic dysfunction. The 10-point CASS is divided into the following 3 indices: Cardiovagal Index (0-3), Adrenergic Index (0-4) and Sudomotor Index (0-3). Qualitative assessment of the adrenergic phases associated with the Valsalva maneuver (late phase II and phase IV) were used when providing an adrenergic score. A score of 1-3 is indicative of mild autonomic dysfunction, 4-6 as moderate, and 7-10 as severe autonomic

dysfunction. An additional score of 0 was used to indicate no autonomic dysfunction. Therefore, in the context of the current study with the use of healthy control participants, an 11-point CASS was used (0-10).

6.2.2.2 Questionnaires

Orthostatic Discriminant and Severity Scale (ODSS): The ODSS was developed by clinicians experienced in autonomic dysfunction and specific orthostatic disorders, an epidemiologist with experience in questionnaire development and administration, by reviewing other validated questionnaires, and by extensive interactions with patients with orthostatic intolerance to identify symptom commonalities. The ODSS is a self-report questionnaire comprised of 33 questions. The questions are used routinely in practice to identify orthostatic intolerance, and include symptom frequency, severity, duration and recovery in addition to specific orthostatic stressors such as, prolonged standing, meal consumption and heat stress. Non-orthostatic symptoms were comprised of questions related to constitutional symptoms including, generalized weakness, fatigue and pain. In addition, symptoms of lightheadedness and dizziness unrelated to upright posture and unrelated to a change in position were included. The questions are preceded by instructions to rate each item by selecting the response that best described the symptoms one experiences on an average basis. The recall period was over the past year. This timeframe was chosen to ensure: 1. Symptoms were persistent and consistent, 2. Patients had sufficient time to experience a variety of circumstances in which their symptoms could have been affected (i.e. hot weather), 3. Symptoms that have since passed and are no longer present were not being recorded. The primary items were scored on a dichotomous scale as either “yes” or “no” questions followed by conditional questions pertaining to frequency, severity, duration, and symptom recovery. Conditional questions were used to save time for patients with few or no symptoms. Access to the questionnaire can be found in the appendix and at: https://www.surveymonkey.com/r/guestODSS

Scoring: The ODSS provides an Orthostatic Symptoms Score and a Non-Orthostatic Symptoms Score”. The Orthostatic symptoms score is calculated as the sum of 22 questions related to orthostatic intolerance, while the non-orthostatic symptoms score is calculated as the sum of 11 questions pertaining to more generalized symptoms. There were ten conditional questions

requiring a ‘yes’ or ‘no’ response. Conditional questions were given a weighted value of either 0 or 1. Questions indicative of orthostatic intolerance were given a value of 1, whereas generalized symptoms and symptoms unrelated to the upright position were given a value of 0. The following is a sample question indicative of orthostatic intolerance: “In the past year, have you experienced symptoms of faintness, dizziness, and/or lightheadedness soon after standing up from a sitting or lying position?” A positive response would be given a value of 1, whereas a negative response would receive a value of 0. In the event of a positive response, follow-up questions would ensue. Follow-up questions were assessed using a 7-point Likert scale. A 7-point Likert scale was chosen to offer more points of discrimination. Answers indicative of orthostatic intolerance were weighted more heavily. The following is an example of a follow-up question in the event the previous question had a positive response: “Please rate the amount of relief of your symptoms of faintness, dizziness and/or lightheadedness upon lying/sitting back down”. A response of ‘No relief at all’ would receive a weighted score of 0, whereas ‘Complete relief’ would receive a weighted score of 6. Similarly, if the answer for a conditional question for non-orthostatic symptoms is "No", this would warrant a score of 1, as higher scores are indicative of orthostatic intolerance. The lowest attainable Orthostatic and Non-Orthostatic scores are both a score of 0. The highest attainable Orthostatic symptoms score is 87 and 61 for a Non-Orthostatic symptoms score. In addition to the ODSS, all participants completed two other previously validated questionnaires. Additional questionnaire assessment included: the Autonomic Symptom Profile (ASP) [7] and the Orthostatic Hypotension Questionnaire (OHQ)[8]. A composite OHQ score was generated by averaging the orthostatic hypotension symptoms assessment (OHSA) and orthostatic hypotension daily activity scale (OHDAS).

6.2.3 Statistical analysis

Descriptive statistics are presented as mean ± standard deviation. All measures among persons with and without orthostatic intolerance were compared using an independent t-test. Statistical correlations were performed using Spearman’s correlation coefficient. An alpha level of 0.05 was used to denote significance. All statistical analyses were performed using SPSS® statistical software version 21 for Windows (SPSS, Inc., Chicago, IL).

6.2.3.1 Validity

Convergent validity was assessed by correlating the results of the ODSS with the Orthostatic Index of the ASP and the average OHDAS and OHSA scores calculated from the OHQ. Clinical validity was evaluated by assessing the relationship between the ODSS and a clinically validated orthostatic challenge (Head-up Tilt test), and the total CASS derived from all components of the ARS.

6.2.3.2 Reliability

Test-retest reliability was calculated using a Model 3 (two-way mixed, consistency) single measure intra-class correlation coefficient between week 1 and week 2 ODSS scores. Cronbach's alpha was determined as a measure of internal consistency for both the orthostatic and non-orthostatic symptoms scores. All items were included in the calculation of internal consistency.

6.3 Results

6.3.1 Participants

A total of 77 persons without orthostatic intolerance (age: 54±20 years) and 67 participants with confirmed orthostatic intolerance (47 Neurogenic Orthostatic Hypotension (NOH); 12 Postural Tachycardia Syndrome (POTS); 8 syncope) (age: 57±19 years) (p=0.45) completed the study. All diagnoses were confirmed by a Neurologist trained in autonomic dysfunction (KK). NOH was clinically defined as a sustained reduction in systolic blood pressure ≥30 mmHg within 3 minutes of head-up tilt (HUT) without an appropriate compensatory tachycardia [5]. The NOH cohort consisted of idiopathic NOH (n=21), Parkinson's Disease +NOH (n=12), Diabetic autonomic neuropathy (n=7), multiple system atrophy (n=4), pure autonomic failure (n=1) and autoimmune autonomic ganglionopathy (n=2). POTS was clinically defined by a heart rate increment ≥30 beats/minute within 5 minutes of HUT in the absence of orthostatic hypotension, along with orthostatic symptoms[5,12,13]. Syncope was defined as a transient loss of consciousness preceded by prodromal symptoms including, but not limited to, pallor, diaphoresis, nausea, lightheadedness, dizziness, weakness, visual disturbances etc. [14]. Table 6.1 shows the results obtained from the autonomic reflex screen. Persons with orthostatic intolerance had reduced sweat volumes at the proximal leg, distal leg and foot relative to the persons without orthostatic

intolerance. Cardiovagal tests (HRDB and VR) were also significantly lower in persons with orthostatic intolerance ($p<0.001$). Resting HR and SBP were significantly higher in the orthostatic group ($p<0.001$). Meanwhile, the absolute drop in SBP on head-up tilt was significantly larger ($p<0.001$), with a non-significant peak compensatory tachycardia ($p<0.06$). In response to Valsalva, all patients with NOH had absent adrenergic phases, which contributed to a higher adrenergic index associated with the Composite autonomic scoring scale (CASS). Lastly, the total CASS was significantly higher in the orthostatic group (4.4±3.5) versus the non-orthostatic group (0.37±0.83; $p<0.001$).

6.3.2 Questionnaires

Non-orthostatic participants had significantly lower OHDAS (0.07±0.26; $p<0.001$) and OHSA (0.20±0.54; $p<0.001$) scores calculated from the OHQ, resulting in a significantly lower composite OHQ score (0.14±0.31) and significantly lower Orthostatic Indices derived from the ASP (4.0±5.8) compared to participants with orthostatic intolerance (OHDAS: 4.87±3.05; OHSA: 4.63±2.77; Composite OHQ: 4.75±2.70; ASP: 28.25±8.8; $p<0.001$). Convergent Validity: Orthostatic (OS) and Non-orthostatic (NS) scores were significantly correlated with the Orthostatic Index derived from the ASP (OS: $r=0.903$; NS: $r=0.651$; $p<0.001$) (Figure 6.1A), and the Composite Score of the OHQ: (OS: $r=0.800$; NS: $r=0.574$; $p<0.001$) (Figure 6.1B). Clinical Validity: Persons with orthostatic intolerance obtained significantly higher orthostatic scores compared to study participants without orthostatic intolerance (66.5±18.1 vs. 17.4±12.9, respectively; $p<0.001$) (Figure 6.2A). Additionally, persons with orthostatic intolerance scored higher on the non-orthostatic symptom score compared to non-orthostatic participants (19.9±11.3 vs. 10.2±6.8, respectively; $p<0.001$) (Figure 6.2A). Orthostatic and non-orthostatic scores were significantly correlated with the total CASS score derived from the Autonomic Reflex Screen (OS: $r=0.458$; NS: $r=0.315$; $p<0.001$), and both had a significant negative correlation with the drop in systolic blood pressure on head-up tilt (OS: $r=-0.445$; NS: $r=-0.354$; $p<0.001$) (Figure 6.2B). Test-retest reliability: Test-retest reliability for orthostatic scores was strong ($r=0.96$; $p<0.001$), with an internal consistency of 0.98. The test-retest reliability for non-orthostatic scores was moderate ($r=0.57$; $p<0.001$) with an internal consistency of 0.73. On average, the

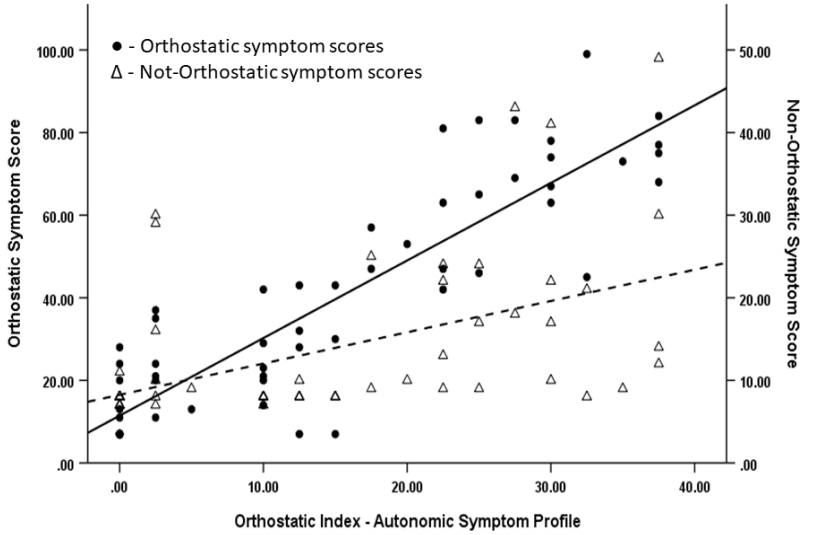
non-orthostatic study cohort completed the second ODSS 18±6 days later, and the orthostatic cohort 19±6 days later (p=0.65).

Table 6.1 Autonomic reflex screen in persons with and without orthostatic intolerance

	Orthostatic Intolerance	**Non-Orthostatic Intolerance**	
QSART (μL±SD)	Mean ± SD	Mean ± SD	p-value
Forearm	0.90±0.90	1.09±1.10	=0.30
Proximal Leg	0.69±0.91	1.18±1.20*	=0.01
Distal Leg	0.51±0.55	1.17±1.31*	<0.001
Foot	0.54±0.48	0.99±0.88*	=0.02
Deep Breathing (bpm)	10.3±11.7	17.±9.4*	<0.001
Valsalva Ratio	1.5±0.5	1.9±0.4*	<0.001
Head-Up Tilt			
Resting HR (bpm)	72.7±11.9	63.9±11.8*	<0.001
ΔHR (bpm)	18.5±15.7	23.0±11.7	=0.06
Resting SBP (mmHg)	146.2±29.3	126.7±19.9*	<0.001
ΔSBP (mmHg)	-61.9±36.5	-20.1±10.5*	<0.001

[a] Abbreviations: QSART, quantitative sudomotor axon reflex test; HR, heart rate; SBP, systolic blood pressure; Δ change from rest * - indicated significantly different values

A



B

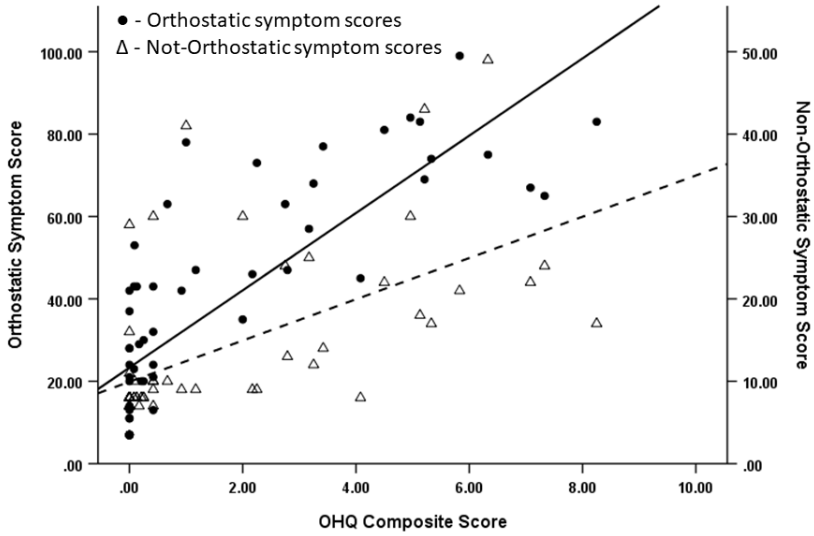


Figure 6.1 Convergent validity

Correlations between Orthostatic (OS) (solid line) and Non-Orthostatic (NS) (hashed line) Symptom scores derived from the Orthostatic Discriminant and Severity Scale and previously validated tools demonstrate strong convergent validity. A. Symptom Scores were significantly correlated with the Orthostatic Index of the Autonomic Symptom Profile (OS: $r=0.903$; NS: $r=0.651$; $p<0.001$). B. Symptom Scores were significantly correlated with the composite score of the Orthostatic Hypotension Questionnaire (OHQ) (OS: $r=0.800$; NS: $r=0.574$; $p<0.001$).

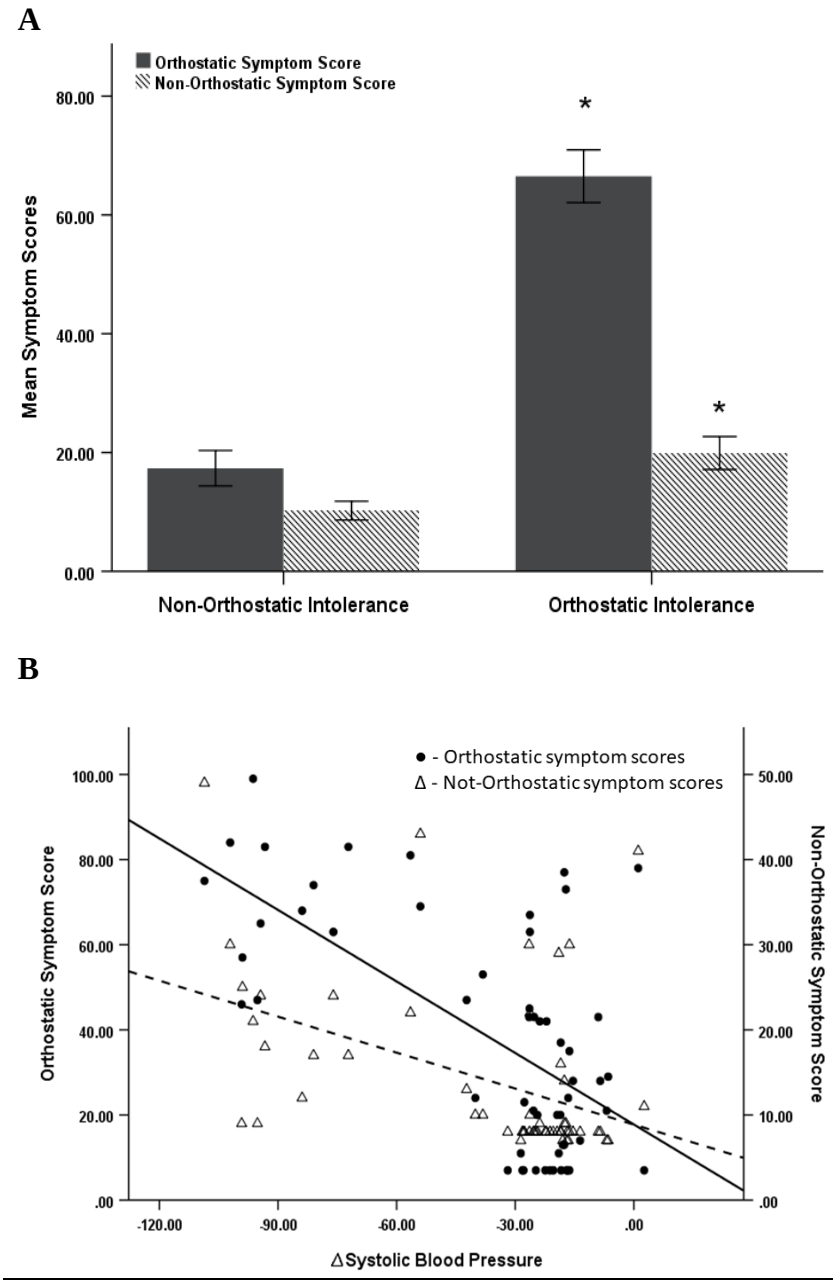


Figure 6.2 Clinical validity

A. Persons with orthostatic intolerance demonstrate significantly larger Orthostatic and Non-Orthostatic Symptom Scores compared to persons without orthostatic intolerance (*$p<0.001$). B. Orthostatic ($r=-0.445$; $p<0.001$) (solid line) and Non-Orthostatic ($r=-0.354$; $p<0.001$) (hashed line) Symptom Scores demonstrate a significant negative correlation with the change in systolic blood pressure in response to Head-up Tilt of the autonomic reflex screen.

6.4 Discussion

The objective of the present study was to demonstrate preliminary validity and reliability of the Orthostatic and Non-Orthostatic Symptom Scores derived from the Orthostatic Discriminant and Severity Score (ODSS). Our results reveal three major findings. First, the Orthostatic and Non-Orthostatic Symptom Scores demonstrated strong convergent validity as evidenced by the strong positive correlations with previously validated tools (ASP and OHQ). Second, the Orthostatic and Non-Orthostatic Symptom Scores demonstrate strong clinical validity as evidenced by: i) significant correlations with the blood pressure drop in response to an orthostatic challenge (Head-up Tilt), ii) significant correlations with the total CASS derived from tests of the ARS which are reproducible and standardized[11], and iii) patients diagnosed orthostatic intolerance produced significantly higher Orthostatic and Non-Orthostatic Symptom Scores compared to participants without orthostatic intolerance. Third, both Orthostatic and Non-Orthostatic Symptom Scores were reproducible as indicated by strong test-retest reliabilities.

Orthostatic Intolerance (OI) can produce a wide array of symptoms including lightheadedness, dizziness, and faintness. OI is important to detect because 1) it may be associated with increased morbidity, mortality, and more progressive forms of autonomic dysfunction, 2) symptoms can be improved with treatment, 3) it may reduce unnecessary tests and treatments that could further complicate a patient's orthostatic symptoms. The overall aim of the ODSS is that it will be able to address all of these important issues related to orthostatic intolerance associated with autonomic dysfunction.

The presence of OI can be indicative of more serious and progressive forms of autonomic dysfunction. Included in this group are patients with neurogenic orthostatic hypotension (NOH), pure autonomic failure, multiple system atrophy, autoimmune autonomic ganglionopathy, general neuropathies and Lewy body disorders. Typically, patients are referred to specialists for treatment and management of these diseases. However, it is not unusual for patients to experience symptoms for years prior to accurate identification of such symptoms as being related to a disorder involving orthostatic/autonomic dysfunction. Therefore, earlier symptoms assessment could lead to earlier diagnosis, more focused tests and specialized treatments.

Orthostatic symptoms can also produce non-specific symptoms such as headache, muscle and non-specific neck pain, fatigue or generalized weakness[6,15]. In such cases, patients' complaints may be dismissed due to the non-specific nature of the symptoms, or they can misguide clinicians in making a proper diagnosis. More common syndromes and disorders related to lightheadedness and dizziness, such as inner ear/vestibular issues, benign positional vertigo, migraines, hypoglycemia, anemia and even certain medications may be considered prior to autonomic dysfunction. Therefore, early and accurate identification of OI can reduce the need for unnecessary tests and avoid the use of incorrect treatments that could further complicate symptoms. For example, NOH is a form of OI characterized by a drop in systolic blood pressure ≥30mmHg upon standing[5]. However, approximately 50% of NOH patients have associated supine hypertension[16]. Traditional use of anti-hypertensives to treat hypertension greatly exacerbates the blood pressure drop upon standing, which in turn exacerbates the level of OI experienced by these patients and increases the potential for falls and more acute adverse events. Therefore, proper identification of OI helps reduce unnecessary testing and helps to focus treatment approaches

The overall aim of the ODSS is not only to identify and quantify orthostatic symptoms, but to discriminate true orthostatic intolerance from other syndromes and disorders that may present with similar symptomatology. However, prior to evaluating the ability of the ODSS in making this distinction, assessment of initial validity and reliability of the symptom score was necessary. In the current study, we demonstrated preliminary evidence that the ODSS is capable of producing scores that are both valid and reliable.

6.4.1 Study limitations

The ODSS has demonstrated preliminary evidence that it provides scores of orthostatic and non-orthostatic symptoms that are both valid and reliable. Furthermore, the ODSS is capable of accurately identifying orthostatic symptoms in patients with OI. In addition, studies including other clinical populations are ongoing with the aim of demonstrating its ability to discriminate between orthostatic and non-orthostatic symptomatology. Despite the promising results, the current study contains the following limitations: 1) The current study aimed to validate the symptom scores of the ODSS in a group of patients with known orthostatic intolerance, and 2)

the sensitivity and specificity were not assessed. To address these limitations, the next steps are to continue with recruitment of patients with and without orthostatic intolerance prior to any autonomic testing. This aspect of the study will be done in a single-blinded fashion with the researchers blinded to the results of the autonomic testing and final clinical diagnoses. In addition, we aim to describe the severity of orthostatic intolerance based on the calculated orthostatic and non-orthostatic scores. Following completion of the second part of the study, we plan to make the ODSS publicly available so clinicians have easy and global access to the scale.

6.5 Conclusions

The current study demonstrates the ability of the Orthostatic Discriminant and Severity Scale to produce Orthostatic and Non-Orthostatic Symptom Scores that are both valid and reliable. Orthostatic and Non-Orthostatic Symptom Scores were significantly larger in persons with orthostatic intolerance versus persons without, these scores demonstrated strong correlations with existing instruments, and were significantly correlated with the results of standard clinical autonomic testing, including an orthostatic challenge.

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CHAPTER 7

7 The Orthostatic Discriminant and Severity Scale (ODSS) – an assessment of orthostatic intolerance[6]

7.1 Introduction

Accurate identification and distinction between orthostatic (postural) versus non-orthostatic (non-postural) symptomatology is important for accurate diagnoses and treatment management for disorders of the autonomic nervous system. To help make the distinction and to discriminate symptoms as being either orthostatic or non-orthostatic, we developed the Orthostatic Discriminant and Severity Scale (ODSS). Previously, we presented initial validation and reliability of the symptom scores derived from the ODSS[7]. These previous data showed that the ODSS produced orthostatic and non-orthostatic symptom scores that are highly correlated with previously validated tools for assessing autonomic dysfunction and symptom severity, as well correlated highly with standard tests of autonomic function. In the current study, our objective was to evaluate specificity, sensitivity and inter-item reliability of the symptom scores derived from the ODSS. Additionally, we aimed to assess predictive power of the symptom scores in a group of patients referred for queries of autonomic dysfunction.

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Baker J, Paturel J, Sletten DM, Low PA and Kimpinski K (2019). The Orthostatic Discriminant and Severity Scale (ODSS): an assessment of orthostatic intolerance. *Clin Auton Res*. [Epub ahead of print]

7.2 Methods

7.2.1 Participants

All healthy participants were recruited from the general population via poster and newspaper advertisements, as well as in person from activity centers for aging populations, to ensure a representative sample size that would age-match with the older patient cohort (i.e. Parkinson's + NOH; Pure Autonomic Failure, etc.). All healthy participants were examined to confirm the absence of any neurological conditions including autonomic dysfunction and symptoms related to orthostatic intolerance. Additionally, due to the potential influence on the autonomic nervous system, healthy participants were excluded if they fell under any one of the following categories: i) pregnant or lactating females, ii) clinically significant coronary artery disease, iii) concomitant therapy with anticholinergic, alpha- and beta-adrenergic antagonists or other medications which could interfere with autonomic functioning, and iv) failure of other organ systems or systemic illness that could affect autonomic function or participants' ability to cooperate. The patient cohort was comprised of patients referred to the Autonomic Disorder Laboratory (Department of Clinical Neurological Science, University Hospital, LHSC, London, Canada) for consultation regarding autonomic dysfunction and/or orthostatic intolerance between September 1, 2016, through April 30, 2018. Additional exclusion criteria for completion of the questionnaires included individuals with communication difficulties, including those who require translation, are illiterate, have trouble understanding and/or producing speech. To have a representative sample size and thus improve the generalizability of the symptom scores, we collected data in a total of 132 patients and 73 healthy controls. All study participants underwent standard autonomic testing and completed three questionnaires. In addition, patients had a neurological examination and medical history interview. Assessments were performed by a neurologist (KK) who independently evaluated the presence or absence of orthostatic symptoms. Based on a comprehensive clinical evaluation, each patient was categorized as either "Orthostatic Intolerance" (OI) or "Non-orthostatic Intolerance" (Non-OI). Ethical approval for this study was obtained from the Health Sciences Research Ethics Board at Western University and written informed consent was obtained from all study participants.

7.2.2 Clinical and questionnaire evaluation

All study participants underwent standard tests of autonomic function (Autonomic Reflex Screen [ARS]) as previously described[8]. In brief, quantitative sudomotor axon reflex testing was performed to provide an assessment of post-ganglionic sympathetic fiber integrity using a QSWEAT device (WR Medical Electronics Co., Stillwater, MN). Adrenergic function was assessed using beat-to-beat blood pressure and heart rate responses to Valsalva maneuver (VM) performed to 40 mmHg, held for 15 seconds and Head-up Tilt (HUT) performed to 70° from the horizontal. Prior to HUT, all individuals were supine for a minimum of 20 minutes. Following baseline, participants were tilted for a maximum of 5 minutes, with a subsequent minimum 5-minute recovery back in the supine position. Cardiovagal function was assessed using heart rate responses to deep breathing (HRDB) at a pace of 6 cycles/min and Valsalva ratio (VR) calculated from the VM. The composite autonomic scoring scale (CASS) was derived from the ARS as previously described[9]. A total CASS score of 0 indicates no autonomic dysfunction, whereas 1-3, 4-6 and 7-10 indicates mild, moderate and severe autonomic dysfunction, respectively. Heart rate and blood pressure were continuously recorded using an electrocardiography (ECG) device (Model 3000 Cardiac Trigger Monitor, IVY Biomedical Systems, Inc., Branford, CT) and Nexfin hemodynamic monitoring system (BMEYE Cardiovascular, Amsterdam, Netherlands), respectively. All data were recorded and analyzed using WR Testworks™ software. Finally, all participants completed the ODSS[7], Autonomic Symptom Profile (ASP)[10] and the Orthostatic Hypotension Questionnaire (OHQ)[11]. An Orthostatic (sum of 22 questions) and Non-Orthostatic (sum of 11 questions) Symptom Score was generated from the ODSS. Conditional 'Yes/No' questions were given a weighted value of "1" or "0" so that an individual with no symptoms would generate a score of "0", whereas higher values would be indicative of more orthostatic and/or non-orthostatic symptomatology. The ASP was analyzed using a computer algorithm to produce a subscale score related to Orthostatic Intolerance[10]. Finally, a composite OHQ score was calculated as the average of the orthostatic hypotension symptoms assessment (6 questions) and the orthostatic hypotension daily activity scale (4 questions)[11].

7.2.3 Blinding protocol

To assess the accuracy of the ODSS in identifying patients with and without orthostatic symptoms, and to reduce any potential for bias, all clinical and questionnaire data obtained from the patient cohort were collected in a single-blinded fashion. Medical histories, neurological exams and clinical evaluation of each patient were assessed independently by a neurologist (KK). A separate researcher (JB) facilitated the completion and evaluation of each questionnaire by the patients. The two members of the research team (KK and JB) were blinded to the opposing assessment (i.e. the researcher assessing the questionnaire data was blinded to the neurologist's clinical evaluation, symptoms assessment, etc. and vice versa). The neurologist grouped each individual patient as either 'orthostatic' [OI] or 'non-orthostatic' [Non-OI] based on a comprehensive and multi-faceted patient evaluation. Clinical evaluation included symptom assessment during an orthostatic challenge (head-up tilt) as well as information gathered via thorough medical histories. For example, patient classification was determined based specifically on symptoms that were always related to a change in position, without any associated supine symptomatology. The other member of the research team (JB) performed a receiver operating characteristic (ROC) curve analysis to assess sensitivity and specificity and to determine cut-off scores. Based on a sensitivity and specificity of 80% and 86%, respectively, an orthostatic symptom score of 33.5 was denoted as the cut-off score for orthostatic symptoms. Therefore, if a patient received a score greater than 33.5, this was marked with a "1" denoting the presence of orthostatic symptoms and if patients scored less than 33.5 this was marked with a "0" denoting no orthostatic symptoms.

7.2.4 Statistical analysis

Descriptive statistics are presented as mean ± standard deviation. An ANOVA was used to compare all measures obtained from patients with and without orthostatic symptoms and healthy controls with a Bonferroni correction to correct for multiple comparisons. A paired t-test was used to compare differences between standing and lying. Statistical correlations were performed using a Spearman's correlation coefficient. Sensitivity and specificity were evaluated using a ROC curve analysis to provide an area under the curve and standard errors. Inter-item reliability was assessed using Cronbach's alpha. The positive and negative predictive power of the ODSS

to identify orthostatic versus non-orthostatic symptomatology was assessed using a chi-square crosstabulation [ODSS scores assessment X neurologist symptom assessment]. An alpha level of 0.05 was used to denote significance. All statistical analyses were performed using SPSS® statistical software version 21 for Windows (SPSS, Inc., Chicago, IL).

7.3 Results

Participant characteristics, ARS and questionnaire data in healthy controls, OI patients and Non-OI patients are presented in Table 7.1 and 7.2. Furthermore, Table 7.3 presents a breakdown of the diagnoses and male to female ratio in the OI group. In the current study, patients were categorized as idiopathic NOH if there was considerable orthostatic hypotension without an appropriate compensatory postural tachycardia, along with evidence of other questionable phenomenon (i.e. gastrointestinal issues, olfactory impairment, etc.), while not meeting criteria for other alpha-synucleinopathies. As such, the latter diagnosis may be clearer over time as patients can develop a more specific diagnosis. In contrast, patients were categorized as PAF if they had maintained a purely peripheral autonomic failure without any evidence of other pathologies for an extended period of time. A diagnosis of OI was given provided the patient had clear and specific postural symptomatology without meeting criteria for the other categories described. As is the case with idiopathic NOH, a more specific diagnosis may develop over time.

7.3.1 Autonomic reflex screen

Controls had significantly higher heart rate responses to deep breathing compared to patients with OI ($p<0.001$) and significantly larger Valsalva ratios compared to both OI ($p<0.001$) and Non-OI ($p=0.02$) patients. Similarly, controls had significantly lower resting heart rates compared to both patient groups (OI: $p<0.001$; Non-OI: $p=0.002$) and significantly lower resting systolic blood pressures compared to OI patients ($p=0.004$). On head-up tilt, both controls and Non-OI patients had significantly smaller blood pressure drops compared to patients with OI ($p<0.001$). Overall, OI patients scored significantly higher on all three indices of the CASS compared to both control and Non-OI patients ($p<0.001$) (Table 7.1).

7.3.2 Questionnaire data

All three groups produced significantly different scores on the ODSS – Orthostatic score, the composite OHQ score and the Orthostatic Index of the ASP. The general trend across all three questionnaires was that patients with OI scored the highest, followed by patients without OI, followed by healthy controls scoring the lowest. In contrast, for the ODSS – Non-orthostatic score, patients with and without orthostatic intolerance scored significantly higher than healthy controls; however, there were no significant differences between the two patient groups (Table 7.2). However, there were significant differences when the symptoms were broken down into "standing" versus "lying". In the standing position, fatigue and weakness were significantly different across all three groups ($p<0.001$), whereas pain in the standing position was not different between patient groups. Furthermore, in the lying position, there were no significant differences between orthostatic and non-orthostatic patients (pain, $p=0.5$; fatigue, $p=1.00$; weakness, $p=0.7$). Controls scored significantly lower in all three symptoms in both the lying and standing position ($p<0.001$). Subsequent paired analysis comparing symptoms in the lying versus standing position revealed that in the orthostatic intolerance group, patient reports of pain, fatigue and weakness significantly decreased from standing to lying ($p<0.001$). In the non-orthostatic patient group, fatigue and weakness showed similar significant reductions with position change ($p<0.001$). However, there were no significant change in reported pain ($p=0.8$). Controls showed no significant symptom changes associated with position (Figure 7.2). Finally, total CASS correlated significantly with both the Orthostatic ($r=0.395$; $p<0.001$) and Non-Orthostatic ($r=0.311$; $p<0.001$) symptom scores of the ODSS.

7.3.3 Sensitivity and specificity

The ROC curve analysis of the ODSS – Orthostatic symptom score showed an equivalent sensitivity and specificity to that of the ASP and the OHQ (Figure 7.1). The area under the curve (AUC) for the ODSS – Orthostatic score, Non-Orthostatic score, OHQ and ASP were: 0.89, 0.79, 0.88, and 0.91, respectively. As previously describes, a cut-off score was determined based on the data produced from the ROC curve. Based on the ANOVA data, only an orthostatic symptom score was used as a cut-off, as the non-orthostatic symptom score was not significantly

different between OI and Non-OI patients. An Orthostatic cut-off score of 33.5 was used in the sub-analysis based on a predicted yield of 80% sensitivity and 86% specificity.

7.3.4 Evaluation of predictive power

Based on the ROC curve analysis, the cut-off score of 33.5 was applied to a subset of 100 patients to calculate the positive and negative predictive powers. A subset of 100 patients was used in the blinded analysis based on the minimum recommended number for obtaining a sample size with a normal distribution. A chi-square crosstabulation between ODSS assessment and clinical outcome revealed a positive predictive power of 73% (n=43/59) and a negative predictive power of 81% (n=33/41). Additionally, there were n=16 false positives and n=8 false negative (Total patients: 100). Combined, the ODSS appropriately identified those patients with and without orthostatic symptoms with a 76% accuracy.

7.3.5 Inter-item reliability

A reliability assessment of all dichotomous (Yes/No) questions pertaining to the orthostatic and non-orthostatic symptom scores was assessed using Cronbach's alpha. Cronbach's alpha for the orthostatic and non-orthostatic symptom scores were 0.85 and 0.85, respectively. For the orthostatic score, it was determined that question pertaining to, Q: "morning symptoms" and Q: "prolonged standing" correlated the highest to the overall questionnaire score, whereas Q: "post-prandial symptoms" correlated the least. However, in an inter-item reliability assessment, all items appeared worthy of retention, resulting in a decreased alpha if deleted. For the non-orthostatic symptom score, Q: "fatigue while standing" correlated the strongest with the symptom score and Q: "light-headedness and dizziness while lying" correlated the least. However, similar to the orthostatic score, all items appeared worthy of retention, resulting in a decreased alpha if deleted. When all items were considered together the test yielded a Cronbach's alpha of 0.88, indicative of strong internal consistency. Further evaluation revealed that all items appeared worthy of retention, resulting in a decreased alpha if deleted.

Table 7.1 Participant questionnaire data

	Control mean±SD	**Orthostatic Intolerance mean±SD**	**Non-Orthostatic Intolerance mean±SD**
ODSS			
Orthostatic Score	7.8±9.7*†	47.2±17.1	25.0±21.5*
95% Confidence Interval	5.5-10.1	43.6-50.9	18.6-31.4
Non-Orthostatic Score	1.2±2.6*†	11.7±8.5	10.4±10.7
95% Confidence Interval	0.6-1.8	9.8-13.5	7.2-13.6
ASP – Orthostatic Index	3.9±5.4*†	27.8±9.6	14.0±12.1*
95% Confidence Interval	2.6-5.1	25.7-29.8	10.2-17.8
Composite OHQ score	0.13±3.0*†	4.5±2.8	1.9±2.4*
95% Confidence Interval	0.06-0.2	3.9-5.1	1.1-2.6

*significantly different from OI patients ($p<0.001$); † significantly different from Non-OI patients ($p<0.001$). Abbreviations: ODSS, orthostatic discriminant and severity score; ASP, autonomic symptom profile; OHQ, orthostatic hypotension questionnaire

Table 7.2 Summary of participant characteristics and autonomic reflex screening data

	Control mean±SD	**Orthostatic Intolerance mean±SD**	**Non-Orthostatic Intolerance mean±SD**	**P-value**
	N=73	**N=83**	**N=49**	
Age (years)	54±21	57±19	55±18	NS
Range (years)	13-88	18-88	17-88	
Sex Ratio (Male: Female)	34:39	42:41	24:25	
Autonomic Reflex Screen				
QSART (μL)				
Forearm	1.04±1.07	1.02±0.94	1.23±1.06	NS
Proximal Leg	1.19±1.21*	0.79±.91	0.91±0.73	*0.048
Distal Leg	1.17±1.32*	0.60±0.57	0.91±0.94	*0.001
Foot	0.99±0.88*	0.67±0.60	0.86±0.74	*0.027
Heart rate to Deep Breathing (bpm)	17.4±9.2*	10.8±11.5	13.6±8.8	*<0.001
Valsalva Ratio	1.9±0.4*†	1.5±0.5	1.6±0.4	*<0.001 †0.02
Head-up Tilt				
Resting Heart Rate (bpm)	63.1±9.0*†	74.0±12.5	70.6±12.6	*<0.001 †0.002
ΔHeart rate (bpm)	23.1±11.8	18.1±15.0	17.2±12.5	NS
Resting SBP (mmHg)	126.8±20.2*	140.2±30.9	132.7±23.2	*0.004
ΔSBP (mmHg)	-20.0±10.6*	-54.8±37.5	-16.9±14.3*	*<0.001
CASS				
Sudomotor Index	0.28±0.76*	0.87±1.16†	0.43±0.82	*0.001 †0.046
Cardiovagal Index	0.08±0.58*	1.17±1.05	0.38±0.66*	*<0.001
Adrenergic Index	0.14±0.12*	2.5±1.90	0.4±1.16*	*<0.001
Total CASS	0.36±0.83*	1.45±3.36	1.24±1.96*	*<0.001

*significantly different from OI patients; † significantly different from Non-OI patients.
Abbreviations: QSART, quantitative sudomotor axon reflex test; μL, microliters; bpm, beats per minute; mmHg, millimeters of mercury; SBP, systolic blood pressure; CASS; Composite autonomic scoring scale; NS, non-significant

Table 7.3 Breakdown of Orthostatic Intolerance patient group

Diagnosis	**MSA**	**PAF**	**PD+NOH**	**Idiopathic NOH**	**DAN**	**AAG**	**POTS**	**Syncope**	**OI**
Sample Size	6	3	16	21	10	2	16	4	5
M:F ratio	5:1	1:2	9:7	14:7	7:3	1:1	2:14	1:3	2:3

Abbreviations: MSA, Multiple system atrophy; PAF, Pure autonomic failure; PD+NOH, Parkinson's Disease + Neurogenic orthostatic hypotension; NOH, Neurogenic orthostatic hypotension; DAN, Diabetic autonomic neuropathy; AAG, Autoimmune autonomic ganglionopathy; POTS, Postural orthostatic tachycardia syndrome; OI, Orthostatic Intolerance; M, Male; F, Female.

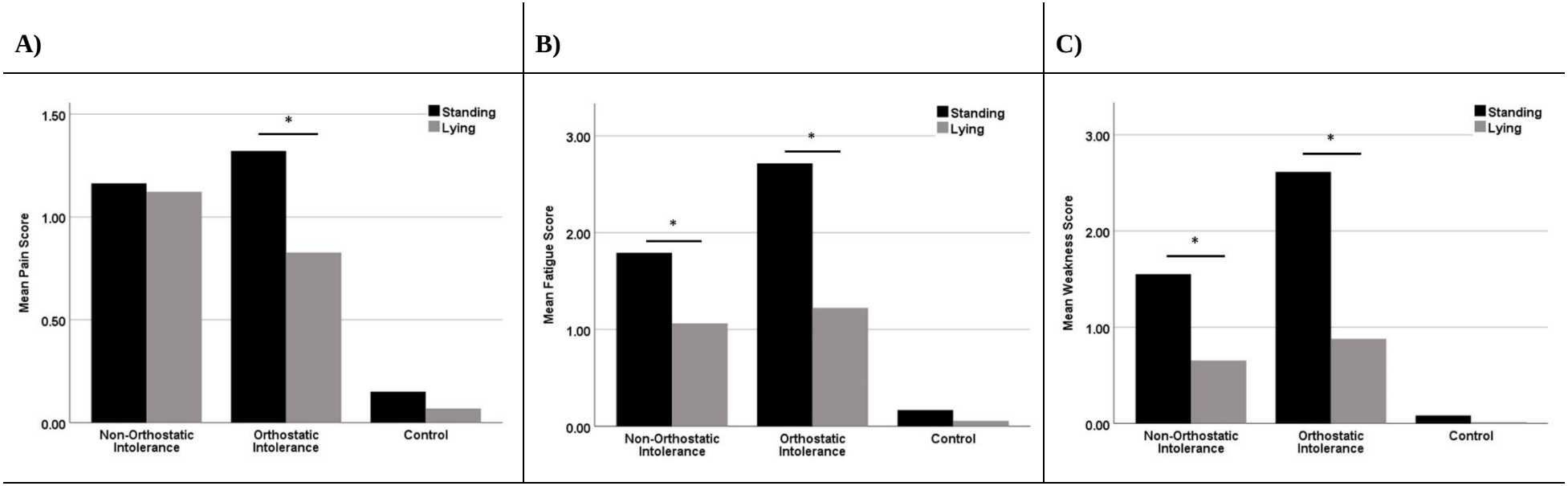


Figure 7.1 Comparison of constitutional symptoms in controls and patients, lying and standing

Pain (A), fatigue (B) and weakness (C) standing and lying in patients with and without orthostatic intolerance and healthy controls. Patients with orthostatic intolerance show a significant reduction in reported symptoms from standing to lying. Patients without orthostatic intolerance show a significant reduction in reported fatigue and weakness ($p<0.001$), but not pain ($p=0.8$). Controls show no significant changes relative to position. *significant difference between standing and lying ($p<0.001$).

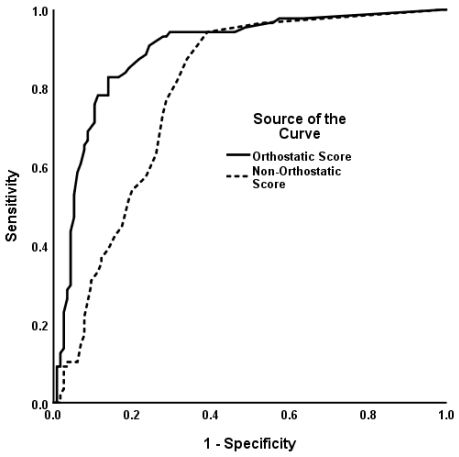
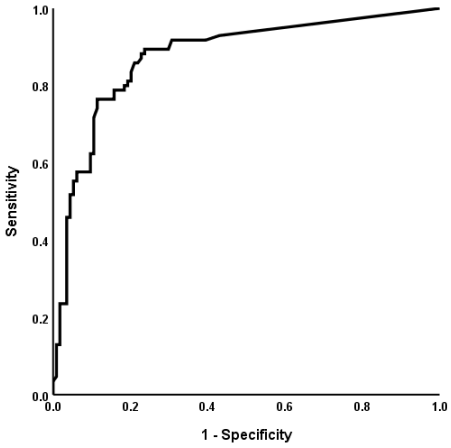
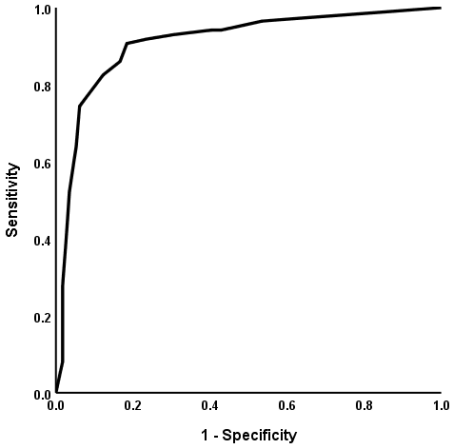
A) ODSS ROC Curve	B) OHQ ROC curve	C) ASP ROC Curve
		
AUC: Orthostatic Curve: 0.90 SE: 0.02; 95% CI: 0.85-0.94 Non-Orthostatic Curve: 0.80 SE: 0.03; 95% CI: 0.73-0.86	AUC: 0.88 SE: 0.03; 95% CI: 0.82-0.93	AUC: 0.91 SE 0.02; 95% CI: 0.87-0.95

Figure 7.2 Receiver operating characteristic (ROC) curves for the symptom scores

ROC curves for A) Orthostatic Discriminant and Severity Score (ODSS) show strong and comparable sensitivity and specificity measures to that of the B) Composite Orthostatic Hypotension Questionnaire (OHQ), and C) Autonomic Symptoms Profile (ASP) – Orthostatic Index. Abbreviations: AUC, area under the curve; SE, standard error; CI, confidence interval.

7.4 Discussion

The primary objective of the current study was to assess the ability of the Orthostatic Discriminant and Severity Scale (ODSS) to distinguish symptoms of orthostatic intolerance from non-orthostatic symptoms. Our results reveal four major findings: 1) An Orthostatic cut-off score of 33.5 provided a strong positive and negative predictive value for accurately identifying orthostatic symptoms. 2) Both Orthostatic and Non-orthostatic symptom scores were capable of distinguishing the patient cohorts. 3) Evaluation of the AUC for the ODSS – Orthostatic symptom scores yielded results similar to that of previously validated tools for symptom assessment. 4) Cronbach's alpha for the questionnaire demonstrated strong internal consistency and an inter-item reliability assessment showed that all dichotomous questions were worthy of retention.

Several questionnaires focused on diverse patient populations such as Diabetic Autonomic Neuropathies, Multiple System Atrophy, Parkinson's Disease and generalized autonomic failure do exist[12–14]. However, the majority of these questionnaires target the presence of symptoms and/or assessment of symptom burden. Additionally, few questionnaires address the non-specific symptoms, such as fatigue, weakness and pain, and most relevant, none discriminate these symptoms as being either orthostatic or non-orthostatic. The ODSS is unique in that it was designed to help identify and discriminate non-specific symptoms such as lightheadedness, dizziness, weakness, fatigue, etc., as being either orthostatic or non-orthostatic. In clinical settings, these symptoms can often overlap with other non-specific etiologies, and therefore accurate identification can be difficult. There was a significant difference in the orthostatic scores between healthy controls, patients assessed as having orthostatic symptoms and patients assessed as having non-orthostatic symptoms by a neurologist. This indicates the ODSS – orthostatic symptom score has the ability to distinguish between these three groups. Furthermore, the results of the single-blinded assessment found the ODSS – orthostatic symptom score was capable of identifying patients with orthostatic symptoms with 73% accuracy, and appropriately identified patients without orthostatic symptoms with an 81% accuracy rating.

In contrast, the assessment of non-orthostatic symptoms such as pain, fatigue and weakness did not significantly differ between patients with and without orthostatic symptoms, but rather

between patient groups and healthy controls. However, this result is not surprising as patient symptoms are not reliably distinguishable, and often have considerable overlap. For example, in the current study we evaluated pain as a non-specific, non-orthostatic symptom. However, certain types of pain (i.e. coat-hanger pain) can be orthostatically-mediated. Therefore, when evaluated separately on the basis of position, two interesting results were identified. First, all three groups were significantly different when symptoms of fatigue and weakness were assessed in the standing position. Second, patients with orthostatic intolerance reported significant symptom reduction across all three symptoms when there was a change in position from standing to lying. In contrast, in the non-orthostatic patient group, reports of pain did not significantly change with position. Finally, total CASS was significantly correlated with both orthostatic and non-orthostatic symptom scores, suggesting the presence of orthostatic and non-orthostatic symptomatology correlate with severity and distribution of autonomic dysfunction. However, these correlations are beginning to show evidence of divergence, and it will be of interest to see how these relationships evolve with the recruitment of additional clinical populations, as well when evaluated within discrete homogenous patient groups. Nonetheless, at present these findings, combined with the results of the orthostatic symptom score, may help to reliably distinguish patients with and without orthostatic intolerance.

Accurately identifying and distinguishing patients with and without orthostatic intolerance has many important clinical implications, some of which include, earlier access to specialized care, reduced risk for potential serious injury related to falls, and proper adjustments to treatments for symptom management.

Orthostatic symptoms are the primary cause of morbidity in patients with generalized autonomic dysfunction. However, application of both conservative and pharmacological measures has been shown to effectively reduce orthostatic symptoms and disease burden[15,16]. Therefore, the ability to accurately identify patients with orthostatic symptomatology would not only help to improve the streamlining of patients but would also facilitate the process by which symptom management techniques can be acquired sooner.

Additionally, accurate identification of symptoms can help reduce fall risks associated with concomitant medications. For example, patients with Neurogenic Orthostatic Hypotension

secondary to autonomic failure can also have profound hypertension while seated and/or lying flat[6]. As office blood pressure measurements are commonly not taken while a patient is standing, these results often lead to a prescribed anti-hypertensive agent that will severely exacerbate their blood pressure drop upon standing, and increase the likelihood of syncope. Therefore, accurately identifying orthostatic symptoms has the potential to expose an underlying condition related to autonomic failure and, in turn, help reduce the risk for potential serious injury related to falls.

Finally, in treating patients with orthostatic and autonomic dysfunction, treatments are often titrated to individual symptoms. For example, in patients with NOH, the use of standard anti-hypotensive agents often includes supine hypertension as a side effect. If a patient's symptoms show improvement, it is important to appropriately titrate dosages and/or schedules to reduce the risks associated with supine hypertension. Access to a simple instrument such as the ODSS provides a symptom assessment to help gauge symptoms.

7.4.1 Future studies

Overall, given the large patient sample size and the methods taken to obtain a diverse and unbiased sample, we believe the current study is representative of the patient cohort that is typically referred to a specialist clinic for questions of autonomic dysfunction. However, further investigation is warranted. In future studies we aim to collect data in another 100 patients to re-assess the positive and negative predictive powers within a larger sample size. Second, we aim to combine all data to quartile the scores to formulate a scale of symptom severity, including: No symptoms, mild, moderate and severe symptoms. Third, in a longitudinal study, we are currently collecting data to measure how symptoms change over time, and how these symptoms correlate with standard measures of autonomic function. Fourth, we aim to separate different patient groups to assess the sensitivity and specificity within smaller homogenous patient groups. This fourth aim will also include patient groups who may be more likely to have higher degrees of non-orthostatic symptoms such as chronic fatigue syndrome or fibromyalgia. Finally, with larger sample sizes, we will also be able to re-evaluate the reliability and internal consistency of each question within discrete patient cohorts, which will enable us to pars out which questions are worthy of retention within individual clinical populations. Our ultimate goal is to make the ODSS easily available in an online format with immediate score generation.

7.4.2 Study limitations

The ODSS has demonstrated a strong ability to identify patients with and without orthostatic intolerance with a relatively high accuracy. Additionally, the items of the questionnaire have yielded strong inter-item reliability and the ODSS – orthostatic score showed strong sensitivity and specificity similar to that of other validated tools. Despite these promising results the current study contains the following limitations: 1) Orthostatic intolerance as a result of autonomic dysfunction can encompass a large and diverse group of patients. In the present study, we enrolled new patients referred to the clinic without prior knowledge of the reason for referral. Due to the methodological approach, the patient sample was extremely diverse. In future assessments we aim to investigate the sensitivity of the ODSS in discrete patient cohorts. 2) In the present study, the sensitivity of the ODSS to track patients' symptoms over time and with treatment was not evaluated. Therefore, longitudinal studies, as well as implementation within a clinical trial need to be done to address this aspect of the ODSS.

7.5 Conclusion

In the current study, the ODSS demonstrated a strong ability to distinguish between patients with and without orthostatic intolerance based not only on the absolute orthostatic symptom score, but also in a blinded assessment, which yielded strong positive and negative predictive power values. Furthermore, the symptom scores of the ODSS demonstrated a sensitivity and specificity equivalent to that of other standardized measures. Finally, a reliability analysis yielded a Cronbach's alpha that showed the ODSS reached an acceptable reliability, and all items were deemed worthy of retention. Overall, the ODSS produces symptom scores that are both reliable and useful for both research and clinical practice to aid in the distinction of orthostatic versus non-orthostatic symptomatology.

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CHAPTER 8

8 General Discussion and Summary

8.1 General Discussion

In these studies, I aimed to improve our understanding and assessment of neurogenic orthostatic hypotension (NOH) related to autonomic failure. This thesis provided novel and unique findings related to this overall objective. The first portion of this thesis was designed to improve our understanding of the pathophysiology contributing to NOH. Specifically, whether patients demonstrated altered activation within the central autonomic network during autonomic challenges. The first set of results (Chapters 2 & 4) support this hypothesis as the results revealed, 1) patients had reduced cortical and sub-cortical autonomic network activation during sympathetically-mediated challenges and 2) patients had reduced brainstem functional connectivity both at rest and during autonomic maneuvers. Of interest, patients also demonstrated reduced cerebellar activity and reduced cerebellar connectivity (Chapters 3 & 5). This was of particular interest as the cerebellum integrates vestibular, spinal and brainstem afferents in response to postural changes and subsequently influences descending inputs to vestibulosympathetic pathways. For example, in response to standing and/or head-up tilt the cerebellum is involved in mediating an early sympathetic reflex to facilitate vasoconstriction, which contributes to postural control of blood pressure in the upright position. This is relevant to the clinical population as the inability to maintain adequate blood pressure in the standing position is the definition of NOH. Furthermore, studies have shown that cerebellar dysfunction/insult not only results in orthostatic hypotension but also affects postural symptoms such as lightheadedness and dizziness.

Therefore, the second part of this thesis focused to improve our ability to identify and to assess patients on the basis of **orthostatic** symptomatology, which can be indicative of autonomic dysfunction. Symptoms such as dizziness and lightheadedness are some of the most common clinical descriptions and remain an independent predictor of increased mortality after adjusting for factors such as age, race, ethnicity, sex and disease[1,2]. These symptoms can be very generalized, but they can also be very specific to postural adjustments as one changes position from lying or sitting to standing. When these symptoms are not properly assessed, accurate

identification and diagnosis can be challenging. Therefore, we devised a self-report questionnaire (the Orthostatic Discriminant and Severity Scale [ODSS]) capable of identifying and discriminating orthostatically mediated symptoms. I found that patients reported significant orthostatic symptomatology that correlated with autonomic dysfunction and orthostatic blood pressures (Chapter 6). I also found the ODSS is capable of identifying and discriminating patients with and without orthostatic symptomatology related to autonomic dysfunction (Chapter 7).

These findings (1) build upon the current understanding of the central autonomic network and the human cerebellum and how they contribute to proper autonomic control; (2) provide evidence of cerebellar impairment in autonomic failure, which may indicate a new mechanism underlying both impaired orthostatic blood pressure regulation and orthostatic symptomatology, including postural lightheadedness and dizziness and (3) provide a new validated tool for assessing postural symptomatology related to autonomic dysfunction.

The concept of a central autonomic network (CAN) involving cortical, subcortical and brainstem structures has been well established[3]. Certainly, an abundance of research has investigated regions of the human CAN and delineated specific structures to various functions. For example, in healthy individuals functional imaging studies have identified discrete neurocircuitry associated with reflex cardiovascular control during sympathetically-mediated challenges (i.e. Valsalva maneuver, handgrip, lower body negative pressure), including the insular cortex, thalamus, anterior cingulate cortex, cerebellum, amygdala and hippocampus[4–9].

Macefield and Henderson extended this work by using concurrent microneurography recordings of sympathetic nerve activity and functional imaging. This work revealed that increased activity in the anterior insular cortex, anterior cingulate cortex and cerebellum corresponded with increased sympathetic activity[10,11]. Studies have also used clinical models such as localized strokes to investigate the effect of cortical lesions on autonomic responses[12]. The results of Chapter 2 are novel in that they provide a unique clinical model of sympathetic failure that corroborates the current literature regarding functional contributions of CAN structures. In this chapter I found that patients with sympathetic dysfunction or failure have reduced activity in the

same central sites implicated in sympathetic outflow including the anterior cingulate cortex, hippocampus, thalamus and cerebellum.

Beyond basic autonomic physiology, the results contained within the first half of this thesis (Chapters 2-5) also offer new insights into the understanding of the pathophysiology of autonomic failure. The results suggest additional pathophysiology in NOH that extends beyond efferent sympathetic dysfunction. The results of Chapter 2 implicate higher forebrain involvement; meanwhile the results contained within Chapter 4 show evidence of reduced connectivity between the brainstem and cortical/subcortical structures in autonomic failure patients.

As previously discussed, several important brainstem nuclei facilitate beat-to-beat control of arterial blood pressure (BP) through the arterial baroreflex[13]. Anatomical connections between brainstem nuclei and cortical/subcortical networks have been well established, and functionally, these cortical and subcortical structures (already described herein) contribute feed-forward signals for additional regulation of cardiovascular and autonomic reflexes. Evidence of reduced functional connectivity between these two fundamental autonomic networks contributes novel information to clinical autonomic research.

Importantly, Chapters 3 & 5 build upon and provide additional support regarding an important role for the human cerebellum in autonomic functioning. While this concept is not new, the current results are novel and interesting in the context of the patient group because 1) damage to the cerebellum results in impaired postural control of blood pressure[14–16] – the same dysfunction experienced by patients with NOH, and 2) cerebellar dysfunction contributes to postural lightheadedness, dizziness and orthostatic intolerance[2,17] – the same symptomatology experienced by these patients.

Regions of the cerebellum, namely the vermis, posterior lobule 9 and the deep cerebellar nuclei, demonstrate significant functional overlap in both baroreceptor and vestibular sympathetic reflexes[18–20]. In response to postural adjustments to an upright position, both reflexes send afferent projections that converge at the level of the RVLM, with measurable increases to

peripheral sympathetic nerve activity[18,19,21–24]. Together, both reflexes facilitate vasoconstriction, blood redistribution and ultimately help maintain blood pressure while upright.

Importantly, Chapter 3 revealed that patients with NOH showed significantly reduced activation within the cerebellum during blood pressure perturbations facilitated by VM and LBNP. To extend these findings, I investigated functional connectivity specifically within the cerebellum (Chapter 5). The findings revealed that patients with NOH had reduced functional connectivity in the same regions shown to contribute to increased sympathetic outflow, including the vermis and posterior lobule 9. Interestingly, cerebellar connectivity was reduced to several important regions including the brainstem and central autonomic structures and this was evident both at rest and during an autonomic challenge (LBNP and VM).

Finally, the second half of this thesis focused on symptomatology related to NOH and autonomic dysfunction. NOH patients can often present with various orthostatic symptoms such as postural lightheadedness, dizziness, faintness, etc. These symptoms are a major cause of morbidity, reduce the ability to live independently and can greatly decrease quality of life[1,25,26]. Recognizing and appropriately discriminating the postural component of these symptoms is imperative for proper diagnosis and treatment. The Orthostatic Discriminant and Severity Scale (ODSS) was designed to help make this important distinction to identify and assess patients on the basis of their symptoms. Chapter 6 provides promising preliminary evaluation of the ODSS in the context of validity and reliability. The results of Chapter 7 showed strong sensitivity and specificity of the ODSS and importantly, demonstrated that the ODSS was capable of accurately identifying patients with and without autonomic dysfunction solely based upon a symptom assessment.

Given the degree of widespread impairment that can accompany autonomic dysfunction and the availability of both pharmacological and conservative measures to help mitigate orthostatic symptoms, early diagnosis will be pivotal to improve quality of life, extend independent living and reduce risk of falls.

8.1.1 Overall study limitations

fMRI is a widely used and accepted technique in the field of autonomic research to discern functional contributions of central structures to autonomic control. However, it is important to acknowledge that the use of functional imaging as a modality for understanding brain mechanisms is primarily limited to the underlying assumption that BOLD signal changes represent neural changes. It is important to note that the BOLD signal does not isolate nor *directly* measure neuronal activity. The BOLD signal is a surrogate signal for brain function reflecting changes in the ratio between oxygenated and deoxygenated hemoglobin.

Despite this assumption, the findings and the brain regions reported in the current set of studies are consistent with existing autonomic literature[3,6,27]. Importantly, the regions discussed including the cerebellum, cingulate, insula, hippocampus, etc. are strongly supported in autonomic and functional imaging literature, as well by studies that have used different modalities other than imaging including, clinical models (i.e. stroke), electrical and chemical stimulation, ablation, etc.

In the connectivity analysis, the use of cortical and subcortical atlases can be limiting. Specifically, application of a brainstem mask covering the whole brainstem cannot isolate discrete nuclei. Structurally and functionally, the brainstem is extremely diverse and therefore the current results would be improved if individual nuclei could be isolated. Unfortunately, the current program used to analyze the connectivity data set this limitation. The functional connectivity program currently available for a regions-of-interest analysis uses a whole brainstem mask to determine brainstem connectivity. Further investigation into discrete brainstem nuclei is certainly warranted. Despite this limitation the current results are still unique revealing that patients with autonomic failure have reduced brainstem connectivity, and importantly reduced connectivity to regions strongly supported in autonomic control.

Despite the aforementioned limitations surrounding functional imaging and functional connectivity, I remain confident in the current findings pertaining to activation of autonomic structures and their functional connections to other structures that make up the central autonomic network.

The primary limitation of the results contained in the second half of this thesis pertains to the heterogeneous sample of patients used in the early stages of developing the ODSS. This approach was primarily chosen based on methodological considerations. For example, during the blinded study all patients needed to be included as opposed to specific clinical groups (i.e. NOH) otherwise, there would in essence be no blinding. Moving forward, I believe the ODSS will be most suitable and accurate for clinical groups with NOH. However, until there is sufficient data to support this conclusion, we continue to recruit all patient groups with orthostatic intolerance related to autonomic dysfunction.

8.1.2 Future studies

The current thesis provides the foundation for a number of important and interesting directions for future research. Future studies may continue to build upon the cross-link between the cerebellum and autonomic dysfunction and the cerebellum in postural symptomatology.

An important consideration when evaluating orthostatic symptoms is that it can be quite common for patients to report no symptoms even when there is clear clinical evidence of severe OH. In a study of 105 Parkinson's patients who met the clinical criteria for NOH, only 13% reported to be symptomatic[28]. Some efforts have been made to determine which factors contribute to postural symptoms. For example, does symptomatology depend on absolute blood pressure changes or how low blood pressure goes?[28] Another hypothesis is that some disorders related to autonomic failure can progress very slowly, and as such, patients become acclimated to blood pressure falls over time. The current thesis may lend support for an alternative hypothesis regarding autonomic dysfunction and postural symptomatology related to differences in cerebellar impairment (Figure 8.1). For example, do patients with NOH and autonomic failure with evidence of cerebellar impairment report more profound postural symptoms?

In a 2016 consensus statement regarding the signs and symptoms of cerebellar dysfunction, brief occurrences (seconds-minutes) of cerebellar dizziness induced by positional changes were evident in cerebellar lesions affecting the posterior cerebellum and vermis[29]. Furthermore, one study found that 31% of patients with acute cerebellar infarctions revealed adrenergic sympathetic dysfunction evidenced by orthostatic hypotension on head-up tilt (-37mmHg drop),

absent adrenergic phases in response to Valsalva maneuver and 28% reported orthostatic dizziness on standing[17]. Furthermore, evidence shows that patients with autonomic failure have impaired baroreflex functioning. Additionally, the cerebellum has direct and indirect projections that feed into both the baroreflex and vestibulo-sympathetic reflex pathways. Therefore, an interesting avenue of future research would be to further investigate cerebellar and vestibulo-sympathetic reflexes in patients with NOH (Figure 8.1). This could be accomplished using direct transcranial magnetic stimulation (TMS) of the cerebellum or through galvanic vestibular stimulation (GVS). Either TMS or GVS could be applied at rest and during an orthostatic challenge while measuring autonomic/hemodynamic responses. Furthermore, cerebellar stimulation and/or activation of cerebellar reflexes with concurrent functional imaging would build upon the current findings. This, in conjunction with investigation into discrete brainstem nuclei including baroreceptor nuclei and vestibular nuclei in autonomic failure would be an interesting area for future research.

Regarding the ODSS, there are plenty of additional studies that would continue to improve and develop this tool. First, any questionnaire devised for clinical use, or otherwise, requires large samples sizes and continuous monitoring and adjustments to optimize its potential. Therefore, ongoing recruitment and evaluation of the ODSS to continue to assess and improve the sensitivity and specificity should be an area of future research. Moreover, the sensitivity and specificity of the ODSS should be tested within discrete clinical groups to better understand where this tool may be best applied (i.e. autonomic failure, postural tachycardia, syncope, etc.).

Second, to evaluate the ability of the ODSS to detect changes in symptoms, a longitudinal study of patients with autonomic dysfunction is required. For example, patients could be monitored over time to assess changes in reported symptoms and whether or not these changes are associated with any physiological parameter (i.e. blood pressure, distribution of autonomic dysfunction, heart rate, etc.). Additionally, longitudinal symptom assessment could be applied to monitor responsiveness to medication. This information would be extremely helpful when determining medication dosages and scheduling to best target orthostatic symptoms. Alternatively, the ODSS could be used within a clinical trial where symptoms can be assessed in response to pharmacological intervention.

Ideally, all autonomic and ODSS data obtained from various clinical cohorts, along with healthy normative individuals should be combined to generate a database containing a range of symptom severities from no orthostatic symptoms (obtained from normative database) to severe orthostasis, possibly indicative of autonomic dysfunction (obtained from clinical samples). Finally, the overall goal of the ODSS should be to have it available to clinicians to help discriminate non-specific symptoms.

8.2 Summary

The results contained in the first half of this thesis build upon the foundation of knowledge surrounding the current understanding of the central autonomic network and the contributions of discrete cortical and subcortical structures in autonomic functioning. The novelty of the current thesis lies in its potential clinical impact regarding the understanding and assessment of NOH related to autonomic failure. Specifically, reduced activation in central autonomic structures along with reduced functional connectivity between the brainstem and central autonomic structures have not been previously investigated in patients with NOH related to autonomic failure. These findings add to the current understanding of pathophysiological mechanisms contributing to autonomic dysfunction and NOH. In addition, the findings surrounding reduced cerebellar activation and reduced cerebellar connectivity to the brainstem and central autonomic structures provide novel insight into the potential role of the human cerebellum in autonomic dysfunction (Chapters 3 & 5). The latter half of this thesis (Chapters 6 & 7) will have a direct clinical contribution to early identification of patients with NOH related to autonomic dysfunction based on symptom assessment. Overall, these studies build upon current autonomic research and provide a foundation that may help direct future research geared toward improving clinical assessment and understanding of NOH related to autonomic dysfunction.

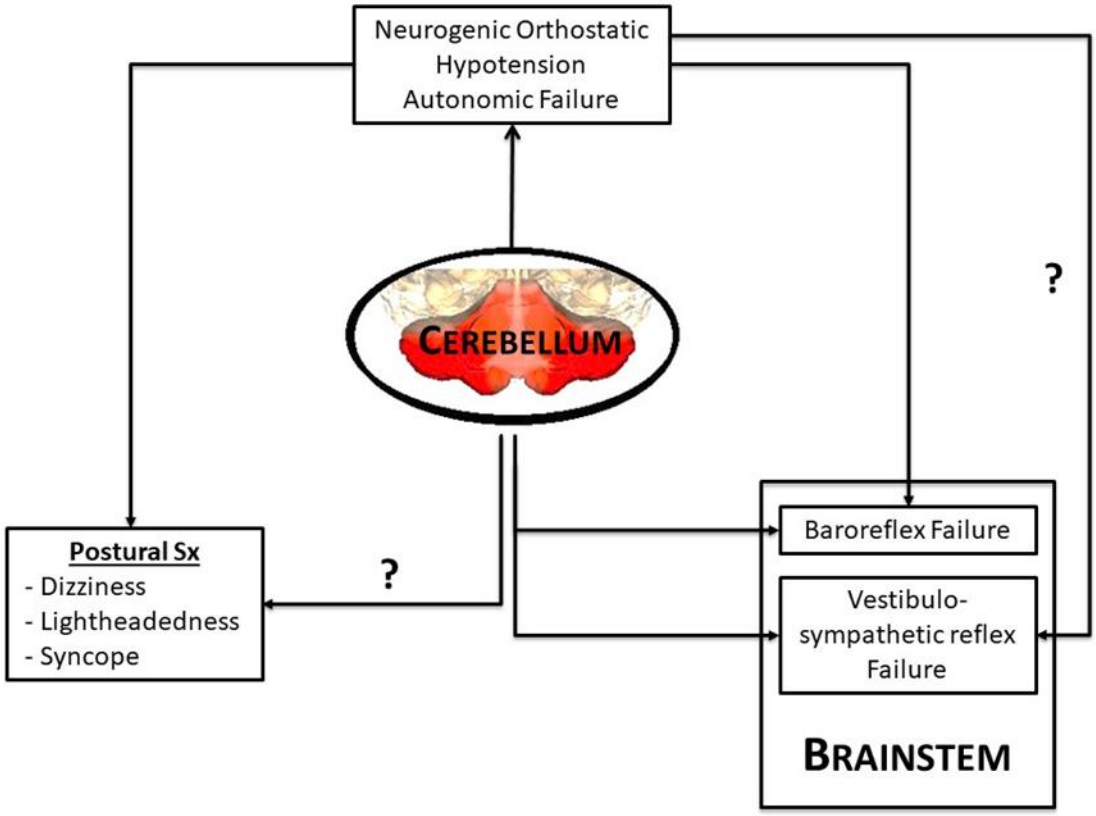

Figure 8.1 Proposed model of cerebellar integration in autonomic control, autonomic failure and postural symptomatology.

The cerebellum is established as a central component of both the vestibulo-sympathetic reflex and postural symptomatology. Therefore, future studies should investigate: 1) Whether patients with NOH and autonomic failure show impaired vestibulo-sympathetic reflexes, which may further contribute to reduced sympathetic responses while upright. 2) Whether cerebellar impairments in NOH patients correlate with the presence and/or severity of postural symptoms.

Cerebellum graphic: https://icm-institute.org/en/actualite/channels-strike-again-a-common-battle-for-axatias-and-epilepsy/

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APPENDICES

Appendix A. Ethics Approval

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Kurt Kimpinski
Department & Institution: Schulich School of Medicine and Dentistry,London Health Sciences Centre

HSREB File Number: 106008
Study Title: Investigation into the pathophysiology of neurogenic orthostatic hypotension.
Sponsor:

HSREB Initial Approval Date: January 15, 2015
HSREB Expiry Date: January 15, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Western University Protocol	Revised western protocol - clean copy	2014/11/25
Data Collection Form/Case Report Form	Questionnaire regarding the function of the autonomic nervous system for adults.(Received Nov.5, 2014)	
Recruitment Items	Email recruitment - clean copy	2014/11/25
Recruitment Items	Recruitment poster (control) - clean copy	2014/11/25
Letter of Information & Consent	Revised control Letter of Information and Consent Form - track changes	2014/11/25
Letter of Information & Consent	Revised patient Letter of Information and Consent Form - Clean copy	2014/11/25

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing and submitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

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

Ethics Officer, on behalf of , HSREB Chair

Ethics Officer to Contact for Further Information

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Appendix B. Ethics Approval

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Kurt Kimpinski
Department & Institution: Schulich School of Medicine and Dentistry,London Health Sciences Centre

Review Type: Full Board
HSREB File Number: 106652
Study Title: A questionnaire based study to determine orthostatic symptoms as they related to various degrees of dysautonomia

HSREB Initial Approval Date: July 13, 2015
HSREB Expiry Date: July 13, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Data Collection Form/Case Report Form	Orthostatic Determination and Severity Scale	2015/04/16
Data Collection Form/Case Report Form	Orthostatic Hypotension Questionnaire	2015/04/10
Data Collection Form/Case Report Form	Autonomic Symptom Profile-kids	2015/04/10
Data Collection Form/Case Report Form	Autonomic Symptom Profile-Adult	2015/04/10
Recruitment Items	email	2015/05/12
Recruitment Items	poster	2015/05/12
Western University Protocol		
Letter of Information & Consent	patients	2015/07/06
Letter of Information & Consent	controls	2015/07/06

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

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Appendix F – The Orthostatic Discriminant and Severity Scale (ODSS)

* 1. Please provide your study Identification Number (ID)

Study ID

Orthostatic Determination and Severity Scale (ODSS)

Please answer each item and, where it applies, please rate each item by selecting the response that best describes the symptoms you experience on an average basis.

2. In the past year, have you experienced symptoms of faintness, dizziness and/or light-headedness soon after standing up from a sitting or lying position?

Yes	No
○	○

3. Please rate the frequency of your symptoms of faintness, dizziness and/or light-headedness when you stand up

Never	Very Rarely	Rarely	Sometimes	Frequently	Very Frequently	Always
○	○	○	○	○	○	○

4. Please rate the severity of your symptoms of faintness, dizziness and/or light-headedness when you stand up

No Symptoms	Very Mild	Mild	Moderate	Severe	Very Severe	Cannot do due to symptoms
○	○	○	○	○	○	○

5. Please rate the duration of your symptoms of faintness, dizziness and/or light-headedness when you stand up

No Symptoms	Seconds	Minutes	Hours	Days	Weeks	>Weeks/Months or longer
○	○	○	○	○	○	○

6. Have your symptoms of faintness, dizziness and/or light-headedness caused you to sit/lie back down?

No	Yes
○	○

7. Please rate the amount of relief of your symptoms of faintness, dizziness and/or light-headedness upon lying/sitting back down

No Relief At All	Very Poor Relief	Poor Relief	Fair Relief	Good Relief	Very Good Relief	Complete Relief
○	○	○	○	○	○	○

8. Please rate the degree of weakness (if any) experienced while standing

No Weakness	Very Mild Weakness	Mild Weakness	Moderate Weakness	Severe Weakness	Very Severe Weakness	Cannot do due to Weakness
○	○	○	○	○	○	○

9. Please rate the degree of pain (if any) experienced while standing

No Pain	Very Mild Pain	Mild Pain	Moderate Pain	Severe Pain	Very Severe Pain	Cannot do due to Pain
○	○	○	○	○	○	○

10. Please rate the degree of fatigue experienced while standing

No Fatigue	Very Mild Fatigue	Mild Fatigue	Moderate Fatigue	Severe Fatigue	Very Severe Fatigue	Cannot do due to Fatigue
○	○	○	○	○	○	○

11. Do you have symptoms of faintness, dizziness and/or light-headedness affected by hot weather?

No	Yes
○	○

12. Please rate the severity of your symptoms of faintness, dizziness and/or light-headedness in hot weather

Far Better	Better	Slightly Better	Same	Slightly Worse	Worse	Far Worse
○	○	○	○	○	○	○

13. Do you have symptoms of faintness, dizziness and/or light-headedness affected by the consumption of meals?

No	Yes
○	○

14. Please rate your severity of symptoms of faintness, dizziness and/or light-headedness following a meal

Far Better	Better	Slightly Better	Same	Slightly Worse	Worse	Far Worse
○	○	○	○	○	○	○

15. Do you have symptoms of faintness, dizziness and/or light-headedness affected by hot showers/baths?

No	Yes
○	○

16. Please rate the severity of your symptoms of faintness, dizziness and/or light-headedness after a hot shower

Far Better	Better	Slightly Better	Same	Slightly Worse	Worse	Far Worse
○	○	○	○	○	○	○

17. In the past year, have you experienced symptoms of faintness, dizziness and/or light-headedness while lying down?

Yes	No
○	○

18. Please rate the frequency of your symptoms of faintness, dizziness and/or light-headedness when lying down

Never	Very Rarely	Rarely	Sometimes	Frequently	Very Frequently	Always
○	○	○	○	○	○	○

19. Please rate the severity of your symptoms of faintness, dizziness and/or light-headedness when lying down

No Symptoms	Very Mild	Mild	Moderate	Severe	Very Severe	Cannot do due to symptoms
○	○	○	○	○	○	○

20. Please rate the duration of your symptoms of faintness, dizziness and/or light-headedness when lying down

No Symptoms	Seconds	Minutes	Hours	Days	Weeks	>Weeks/Months or longer
○	○	○	○	○	○	○

21. Please rate the amount of relief of your symptoms of faintness, dizziness and/or light-headedness you experience upon standing up

No Relief At All	Very Poor Relief	Poor Relief	Fair Relief	Good Relief	Very Good Relief	Complete Relief
○	○	○	○	○	○	○

22. Please rate the degree of weakness experienced while lying down

No Weakness	Very Mild Weakness	Mild Weakness	Moderate Weakness	Severe Weakness	Very Severe Weakness	Cannot do due to Weakness
○	○	○	○	○	○	○

23. Please rate the degree of fatigue experienced while lying down

No Fatigue	Very Mild Fatigue	Mild Fatigue	Moderate Fatigue	Severe Fatigue	Very Severe Fatigue	Cannot do due to Fatigue
○	○	○	○	○	○	○

24. Please rate the degree of pain experienced while lying down

No Pain	Very Mild Pain	Mild Pain	Moderate Pain	Severe Pain	Very Severe Pain	Cannot do due to Pain
○	○	○	○	○	○	○

25. In the past year, have you experienced symptoms of faintness, dizziness and/or light-headedness upon getting out of bed, first thing in the morning?

Yes	No
○	○

26. Please rate your severity of symptoms of faintness, dizziness and/or light-headedness upon getting out of bed, first thing in the morning

No Symptoms	Very Mild	Mild	Moderate	Mildly Severe	Severe	Very Severe
○	○	○	○	○	○	○

27. Have your symptoms of faintness, dizziness and/or light-headedness caused you to lie/sit back down?

No	Yes
○	○

28. Please rate the relief of your symptoms of faintness, dizziness and/or light-headedness upon lying/sitting back down

No Relief At All	Very Poor Relief	Poor Relief	Fair Relief	Good Relief	Very Good Relief	Complete Relief
○	○	○	○	○	○	○

29. In the past year, have you ever had symptoms of faintness, dizziness and/or light-headedness while walking?

Yes	No
○	○

30. Please rate the frequency of your symptoms of faintness, dizziness and/or light-headedness while walking

Never	Very Rarely	Rarely	Sometimes	Frequently	Very Frequently	Always
○	○	○	○	○	○	○

31. Please rate the amount of relief of your symptoms of faintness, dizziness and/or light-headedness you experience when you stop walking

Complete Relief	Very Good Relief	Good Relief	Fair Relief	Poor Relief	Very Poor Relief	No Relief At All
○	○	○	○	○	○	○

32. In the past year, have you experienced symptoms of faintness, dizziness and/or light-headedness while standing for long periods?

Yes	No
○	○

33. Please rate the frequency of your symptoms of faintness, dizziness and/or light-headedness while standing for long periods

Never	Very Rarely	Rarely	Sometimes	Frequently	Very Frequently	Always
○	○	○	○	○	○	○

34. Please rate the amount of relief of your symptoms of faintness, dizziness and/or light-headedness you experience when you sit down

No Relief At All	Very Poor Relief	Poor Relief	Fair Relief	Good Relief	Very Good Relief	Complete Relief
○	○	○	○	○	○	○

Curriculum Vitae

Jacquie Baker

Post-Secondary Education and Degrees

The University of Western Ontario: 2016 - 2019
Doctorate of Philosophy – Kinesiology: Clinical Autonomic Disorders
Thesis: Improving the assessment and understanding of Neurogenic Orthostatic Hypotension
Supervisors: Dr. Kurt Kimpinski, MD, PhD; Dr. Kevin Shoemaker, PhD

The University of Western Ontario: 2011 – 2013
Master of Science – Kinesiology
Thesis: Somatosensory stimulation modulates heart rate variability changes induced by isometric handgrip exercise
Supervisor: Dr. Kevin Shoemaker, PhD

The University of Western Ontario: 2006-2010
Honors Specialization Bachelor of Science, Kinesiology

Summary of Research Contributions

Articles in Peer Reviewed Journals: **18** (1st author: 13)
Abstracts in Peer Reviewed Journals: **14** (1st author: 9)
Invited Book Chapters: **1**
Manuscripts Under Review: **1** (1st author: 1)

Honours, Scholarships and Awards	**Dates Held**
1. American Autonomic Society- Lundbeck Travel Fellowship Award	2018
2. Ontario Graduate Scholarship/Queen Elizabeth II scholarship in Science and Technology	2018-2019
3. Western Graduate Research Scholarship	2018-2019
4. American Autonomic Society- Lundbeck Travel Fellowship Award	2017
5. Ontario Graduate Scholarship/Queen Elizabeth II scholarship in Science and Technology	2017-2018
6. FHS Graduate Tri-Council Scholarship Incentive	2017-2018
7. Western Graduate Research Scholarship	2017-2018
8. Kinesiology Travel Award	2016-2017
9. Faculty of Healthy Sciences Travel Award	2016-2017
10. Western Graduate Research Scholarship	2016-2017
11. Western Graduate Research Scholarship	2011-2012 2012-2013
12. University of Western Ontario Dean's Honour List	2010
13. Western Scholarship of Distinction	2006

Teaching experience and relevant employment

1. Clinical/Research Assistant – Clinical Neurological Sciences Autonomic Disorders Clinic University Hospital, London Health Sciences Centre	2014-Present
2. Graduate Teaching Assistant: University of Western Ontario	2011-2012 2012-2013

Research Experience

1. 2018-2019; Supervisor: Dr. Kurt Kimpinski
Project Title: Identifying the pathophysiology of neurogenic supine hypertension and the effects of melatonin to reduce nocturnal hypertension in autonomic dysfunction. Identifier: NCT02963181
Contribution: Study conception and design; prepared clinical trial application to Health Canada; recruitment; data collection, analysis and interpretation; drafted two review papers for intellectual content

2. 2017-2018; Supervisor: Dr. Kurt Kimpinski
Project Title: The utility of waveform characteristics in predicting blood pressure in autonomic dysfunction
Contribution: Data collection; revised manuscript for intellectual content

3. 2017; Supervisor: Dr. Kurt Kimpinski
Project Title: Utility of heart rate variability parameters in autonomic disorders
Contribution: Data collection, analysis and interpretation; drafted two manuscripts for intellectual content

4. 2016-2019; Supervisor: Dr. Kurt Kimpinski
Project Title: A questionnaire-based study to identify orthostatic symptoms related to autonomic dysfunction
Contribution: Study design; recruitment; data collection, analysis and interpretation; drafted two manuscripts for intellectual content

5. 2016-2018; Supervisor: Dr. Kurt Kimpinski
Project Title: Investigation into the pathophysiology of neurogenic orthostatic hypotension – functional imaging study
Contribution: Study design; recruitment; data collection, analysis and interpretation; drafted two manuscripts for intellectual content

6. 2014-2015; Supervisor: Dr. Kurt Kimpinski
Project Title: A prospective 1-year study of postural tachycardia and the relation to postural versus non-postural symptoms
Contribution: Recruitment; data collection, analysis and interpretation; drafted manuscript for intellectual content

7. 2014-2015; Supervisor: Dr. Kurt Kimpinski
Project Title: Evaluation of adrenergic baroreflex in health and autonomic dysfunction
Contribution: Data collection and analysis; revised three manuscripts for intellectual content

8. 2011-2013; Supervisor: Dr. Kevin Shoemaker
Project Title: Architecture of cortical somatosensory and autonomic networks
Contribution: Recruitment; data collection, analysis and interpretation; revised two manuscripts for intellectual content

Publications, Presentations and Abstracts
Publications: Peer reviewed

1. **Baker J** and Kimpinski K. (2019). Reduced brainstem functional connectivity in patients with peripheral autonomic failure. NeuroImage: Clinical (Accepted)

2. **Baker J** and Kimpinski K. (2019). Normal versus abnormal. What normative data tells us about the utility of heart rate in postural tachycardia. Autonomic Neuroscience. Basic and Clinical (Accepted)

3. **Baker J,** Paturel J, Sletten DM, Low PA and Kimpinski K (2019). The Orthostatic Discriminant and Severity Scale (ODSS) – an assessment of orthostatic intolerance. Clin Auton Res PMID: 30604164

4. **Baker J,** Paturel J and Kimpinski K. (2019). Cerebellar impairment during an orthostatic challenge in patients with neurogenic orthostatic hypotension. Clin Neurophysiol. 130(1): 189-195 PMID: 30527385

5. **Baker J** and Kimpinski K. (2019). An updated normative data-set from the Autonomic Reflex Screen representative of Southwestern Ontario. Can J Physiol Pharmacol. 97(2): 107-111. PMID: 30517028

6. **Baker J,** Paturel J, Sletten DM, Low PA and Kimpinski K (2019). Initial validation of symptom scores derived from the orthostatic discriminant and severity scale. Clin Auton Res. 29(1): 105-112. PMID: 29492828

7. **Baker J,** Paturel J and Kimpinski K. (2018). Impaired cortical autonomic responses during sympathetic activation in Neurogenic Orthostatic Hypotension characterized by post-ganglionic autonomic dysfunction. J Appl Physiol. 125(4): 1210-1217. PMID: 30332348

8. **Baker J** and Kimpinski K (2018). Role of melatonin in blood pressure regulation: an adjunct anti-hypertensive agent. Clin Exp Pharmacol Physiol. 45(8): 755-766. PMID: 29603319

9. **Baker J** and Kimpinski K (2018). A case of Charcot-Marie-Tooth (CMT) Type 2C due to a TRPV4 gene mutation with isolated sudomotor autonomic dysfunction. J Clin Neuromuscul Dis. 19(3):144-146. PMID:29465618

10. **Baker J,** Racosta JM, Balint B and Kimpinski K (2018). The utility of time and frequency domain parameters of heart rate variability in the context of autonomic disorders characterized by orthostatic dysfunction. J Clin Neurophysiol. 35(2): 123-129. PMID:29342011

11. **Baker J**, Racosta JM, Kimpinski K (2017). A comparison of heart rate variability parameters to the autonomic reflex screen in postural orthostatic tachycardia syndrome and neurogenic orthostatic hypotension. Journal of clinical neurophysiology. J Clin Neurophysiol. 35(2): 115-122. PMID: 29210841

12. **Baker J** and Kimpinski K. (2017). Management of supine hypertension complicating neurogenic orthostatic hypotension. CNS Drugs. 31(8): 653-663 PMID:28702747

13. Palamarchuk IS, **Baker J** and Kimpinski K. (2016). The utility of Valsalva maneuver in the diagnoses of orthostatic disorders. Am J Physiol Regul Integr Comp Physiol. 310(3): R243-52. PMID:26491102

14. Palamarchuk IS, **Baker J** and Kimpinski K. (2016). Non-invasive measurement of baroreflex during Valsalva maneuver: Evaluation of alpha and beta-adrenergic components. Clin Neurophysiol. 127(2): 1645-1651. PMID:26610324

15. Palamarchuk I, **Baker J** and Kimpinski K. (2016) Non-invasive measurement of adrenergic baroreflex during Valsalva maneuver reveals three distinct patterns in healthy subjects. Clin Neurophysiol. 127(1):858-863. PMID:25953141

16. **Baker J** and Kimpinski K (2015). A prospective 1-year study of postural tachycardia and the relationship to non-postural versus orthostatic symptoms. Physiol Behav. 147:227-232. PMID:25936824

17. Shoemaker JK, Norton KN, **Baker J**, and Luchyshyn T. (2014). Forebrain organization for autonomic cardiovascular control. Auton Neurosci. 188:5-9. PMID:25458433

18. Lalande S, Sawicki CP, **Baker JR**, and Shoemaker JK. (2014). Effect of aging on the hemodynamic and sympathetic response at the onset of isometric handgrip exercise. J Appl Physiol, 116 (2): 222-227. PMID: 24336882

Articles under review

1. **Baker J** and Kimpinski K. (2019). Evidence of impaired cerebellar connectivity at rest and during autonomic maneuvers in patients with autonomic failure. The Cerebellum (Under second review)

Academic Presentations

1. **Baker J** (2019). Cortical autonomic impairment in the pathophysiology of peripheral autonomic failure. Cardiac and Circulatory Physiology Rounds. Mount Sinai Hospital, Toronto, Ontario

2. **Baker J.** (2018). Cortical autonomic impairment in the pathophysiology of neurogenic orthostatic hypotension associated with peripheral autonomic dysfunction. American Autonomic Society

3. **Baker J.** (2017). Validity and reliability of orthostatic and non-orthostatic symptom scores derived from the Orthostatic Discriminant and Severity Scale. American Autonomic Society.

4. **Baker J.** (2017). Development of a new instrument to discriminate orthostatic from non-orthostatic symptoms. Clinical Neurological Sciences Departmental Research Day

5. **Baker J.** (2016). Autonomic dysfunction in familial amyloid polyneuropathy associated with transthyretin alanine 60 variant. Clinical Neurological Sciences Departmental Research Day

6. **Baker J.** (2015). A prospective 1-year study of postural tachycardia and the relationship to non-postural versus orthostatic symptoms. Clinical Neurological Sciences Departmental Research Day

Conference Abstracts and Publications

1. **Baker J** and Kimpinski K. (2018) Evidence of cortical autonomic impairment in the pathophysiology of neurogenic orthostatic hypotension associated with peripheral autonomic dysfunction. American Autonomic Society. Newport Beach, California. October 2018. (International poster presentation) Published in: Clinical Autonomic Research

2. **Baker J** and Kimpinski K. (2018) The Orthostatic Discriminant and Severity Scale: a tool to discriminate orthostatic from non-orthostatic symptomatology. American Autonomic Society. Newport Beach, California. October 2018. (International poster presentation) Published in: Clinical Autonomic Research

3. **Baker J** and Kimpinski (2018) Cortical autonomic patterns in Neurogenic Orthostatic Hypotension. Canadian Neurological Sciences Federation. Halifax, Nova Scotia. June 2018 (National poster presentation).

4. **Baker J** and Kimpinski K. (2017) Validity and reliability of orthostatic and non-orthostatic symptoms scores derived from the Orthostatic Discriminant and Severity Scale: A new instrument to discriminate orthostatic from non-orthostatic symptoms. American Autonomic Society, Clearwater, Florida. November 2017. (International poster presentation) Published in: Clinical Autonomic Research

5. **Baker J** and Kimpinski K (2017). Development of a new instrument to discriminate orthostatic from non-orthostatic symptoms: The Orthostatic Discriminant and Severity Scale. American Academy of Neurology. Boston, Massachusetts. April 2017. (International poster presentation). Published in: Neurology

6. Racosta J, Paturel J, **Baker J** and Kimpinski k (2017). Autonomic denervation of brain vessels in patients with NOH. American Academy of Neurology. Boston, Massachusetts. April 2017. (International poster presentation). Published in: Neurology

7. **Baker J**, Racosta J and Kimpinski K (2016). Development of a new instrument to discriminate orthostatic from non-orthostatic symptoms. Canadian Neurological Sciences Federation. Quebec City, Quebec. June 2016. (National oral presentation). Published in: The Canadian Journal of Neurological Sciences.

8. **Baker J** and Kimpinski K (2016). Autonomic dysfunction in familial amyloid polyneuropathy associated with transthyretin alanine 60 variant. American Academy of Neurology. Vancouver, British Colombia. April 2016. (National poster presentation). Published in: Neurology

9. Palamarchuk IS, **Baker J** and Kimpinski K. (2016). The utility of Valsalva maneuver in the diagnoses of orthostatic disorders. American Academy of Neurology. Vancouver, British Colombia. April 2016. (National poster presentation). Published in: Neurology

10. Racosta JM, Sposato L, **Baker J** and Kimpinski K (2016). Subcutaneous vs. intravenous immunoglobulin for chronic inflammatory demyelinating polyneuropathy and multifocal motor neuropathy. Platform presentation. Canadian Neurological Sciences Federation. Quebec City, Quebec. June 2016 (National oral presentation) Published in: The Canadian Journal of Neurological Sciences.

11. **Baker J** and Kimpinski K (2015). A prospective 1-year study of postural tachycardia and the relationship to non-postural versus orthostatic symptoms. Platform presentation. Canadian Neurological Sciences Federation. Toronto, Ontario. June 2015. (National oral presentation) Published in: The Canadian Journal of Neurological Sciences.

12. Shoemaker JK, Norton KN, **Baker J**, and Heineke A. (2014). Regional cerebral cortex thickness correlates of autonomic outflow. American Autonomic Society. San Juan, Puerto Rico (International poster presentation). Published in: Clinical Autonomic Research. Clin Auto Res. 24:219.

13. **Baker J** and Shoemaker, JK. (2013). Effect of isometric handgrip exercise on heart rate variability with and without somatosensory stimulation. 27:lb833. Boston, Massachusetts. April 2013. (International poster presentation)

14. Lalande S, Sawicki CP, **Baker JR**, and Shoemaker JK. (2013). Effect of aging on the hemodynamic and sympathetic response at the onset of isometric handgrip exercise. 27:943.12. Boston, Massachusetts. April 2013. (International poster presentation)

Invited Book Chapter

1. **Baker J** and Kimpinski K. Methods of laboratory evaluation of the autonomic nervous system in wakefulness and sleep. In: *Autonomic nervous system and sleep: order and disorder*, edited by Chokroverty S, Cortelli, P. Springer, 2020.

Professional memberships

1. American Autonomic Society
2. American Academy of Neurology
3. Canadian Federation of Neurological Sciences