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Cerebellar impairment during an orthostatic challenge in patients with neurogenic orthostatic hypotension

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HIGHLIGHTS

- Autonomic failure is characterized by severe hypotension during orthostatic challenges.
- Patients have significantly less cerebellar activation during baroreflex mediated challenges.
- Failure of the vestibulo-sympathetic reflexes may contribute to the pathophysiology of dysautonomia.

ABSTRACT

Objective: Compare activation patterns within the cortical autonomic network in patients with neurogenic orthostatic hypotension (NOH) versus healthy age-matched controls during an orthostatic challenge.
Methods: Fifteen health controls and 15 NOH patients performed 3 Valsalva maneuvers, and 5-min of lower-body negative pressure (LBNP) during a functional brain MRI.
Results: Compared to controls, NOH patients had significantly less activation within the cerebellum during both LBNP and VM. Both groups had significant activation of the bilateral insula and left thalamus during LBNP. No significant differences were found during the recovery phase of LBNP.
Conclusions: The cerebellum, which plays an important role in vestibulo-sympathetic reflexes, important for blood pressure adjustments during postural changes, appear to be affected in patients with NOH. The cerebellum also appears to be affected during other baroreflex mediated stressors such as the VM.
Significance: Orthostatic reflexes mediated by the cerebellum may be impaired in patients with NOH. The results suggest an additional pathological pathway in patients with autonomic failure.

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) $\geq$30 mmHg or diastolic blood pressure of $\geq$15 mmHg within 3 min of standing or head-up tilt performed at 60° (Freeman et al., 2011). NOH is unique in that the *orthostatic* component relates to an excessive BP drop associated with an upright/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

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blood pressure (Benarroch, 2008). In addition to feedback mechanisms, feedforward or "central command" mechanisms also contribute to long- and short-term regulation of the cardiovascular system (Shoemaker and Goswami, 2015). Specifically, the cortical autonomic network (CAN) includes a network of forebrain 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 (Cechetto and Shoemaker, 2009; Shoemaker et al., 2015), including autonomic responses involved in baroreflex functioning. For example, clusters of baroreceptor cells have been identified in the insula of rats (Zhang and Oppenheimer, 1997) and monkeys (Zhang et al., 1998a), and posterior insular lesions result in altered baroreceptor gain (Zhang et al., 1998b). Similarly 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 (Kimmerly et al., 2005). Finally, in clinical models such as stroke and lesion studies, damage to these areas results in autonomic dysfunction (Norris et al., 1978; Butcher and Cechetto, 1995; Sörös and Hachinski, 2012), and in models of pure autonomic failure, reduced regional cerebral blood flow has been reported in the cingulate cortex (Hirano et al., 2009). Therefore, the purpose of the current study was to compare activation patterns within the CAN in patients with NOH as a result of autonomic dysfunction versus healthy age-matched controls during an orthostatic challenge.

2. Methods

2.1. Studied population

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 $\geq$ 30 mmHg within 3 min of head-up tilt (HUT) without an appropriate compensatory postural tachycardia as determined by the $\Delta HR/\Delta SBP$ ratio (Norcliffe-Kaufmann et al., 2018). 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. All diagnoses were confirmed by a neurologist (KK). Patients with central autonomic neurodegenerative disorders were not included in the present study in order to eliminate any potentially confounding variables associated with such central pathologies. Therefore, our NOH population 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 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. NOH 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.

2.2. Autonomic testing

All participants underwent a battery of standardized and validated tests of autonomic function, namely the autonomic reflex screen (ARS) (Low and Opfer-Gehrking, 1999; Low, 2003). 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). QSART involved transdermal iontophoresis of acetylcholine for 5 min at a constant current of 2 mA. Following 5 min of stimulation, an additional 5 min was recorded. Sweat volumes (μL) were measured from the integrated area of the entire 10-min response. Valsalva maneuver: Following a minimum 1-min baseline, participants were instructed to exhale into a mouthpiece and to maintain an expiratory pressure of 40 mmHg for 15 s. The maneuver was repeated twice, separated by a 2-min rest. Head-up Tilt (HUT): Following a 15-min baseline period in the supine position, HUT was performed at 70° from the horizontal for a maximum of 5-min, followed by a 5-min recovery period. Lower-body negative pressure (LBNP): Following a minimum baseline period of 15 min in the supine position, LBNP was conducted at a pressure of −35 mmHg for 5 min, followed by a 5-min recovery period. All healthy participants completed 5-min of LBNP at −35 mmHg. In contrast, due to the nature of the disease and the marked blood pressure drops in our patient population, in some cases the negative pressure needed to be reduced in order to ensure blood pressure did not drop below a certain threshold. On average, NOH patients completed 5-min of LBNP at a negative pressure of 27 mmHg. All patient started at −35 mmHg, 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.

2.3. Neuroimaging data acquisition

All imaging data were collected using a whole body 3 T 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 256 × 240 mm, voxel resolution 1.0 × 1.0 × 1.0 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: 240 × 240 mm; flip angle: 40°; multiband acceleration factor: (4) Forty-eight interleaved axial slices (3.0 × 3.0 mm in-plane voxel resolution, TR: 1 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 s baseline, LBNP was initiated for 5-min following by a 5-min period with LBNP off (660 volumes). Valsalva maneuver: Following a 60-s baseline, participants completed 3 VM's (15 s each), with 120 s 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 head 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 in order 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).

2.4. Neuroimaging 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-s 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 (Friston et al., 1995). 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. Cortical activation patterns during LBNP were assessed during the final 60 s, when sympathetic activation should be the greatest. Finally, the first 30-s 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.

2.5. Regions-of-interest analysis

Regions of interest (ROI) were determined based on previous work highlighting regions of the cortical 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 (Tzourio-Mazoyer et al., 2002; Maldjian et al., 2003).

2.6. 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).

3. Results

3.1. QSART and hemodynamic findings

Compared to healthy controls, NOH patients had significantly lower average sweat volumes at the proximal (1.23 ± 0.93 vs. 0.47 ± 0.47, respectively) and distal leg (1.23 ± 0.86 vs. 0.44 ± 0.40, respectively) ($p < 0.01$), and a trend toward lower sweat volumes at the forearm (1.31 ± 0.72 vs. 0.81 ± 0.69, respectively) ($p = 0.07$). Sweat volumes at the foot were not significantly different (0.91 ± 0.68 vs. 0.65 ± 0.50, respectively) ($p = 0.24$). During both HUT and LBNP in the lab session, NOH patients has significantly larger blood pressure drops with significantly smaller compensatory tachycardias versus healthy controls (Table 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 1).

3.2. Functional BOLD responses

During LBNP, healthy controls showed significant activation relative to rest in the bilateral insula (Fig. 1), bilateral thalamus, anterior cingulate cortex, and bilateral cerebellum (Table 2) ($p < 0.05$). Similarly, NOH patients had significant activation in the bilateral insula (Fig. 1) and left thalamus (Table 2) ($p < 0.05$). During LBNP, controls had significantly greater activation in the bilateral cerebellum compared to patients (Table 2; Fig. 2) ($p < 0.05$). To investi-

Table 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.

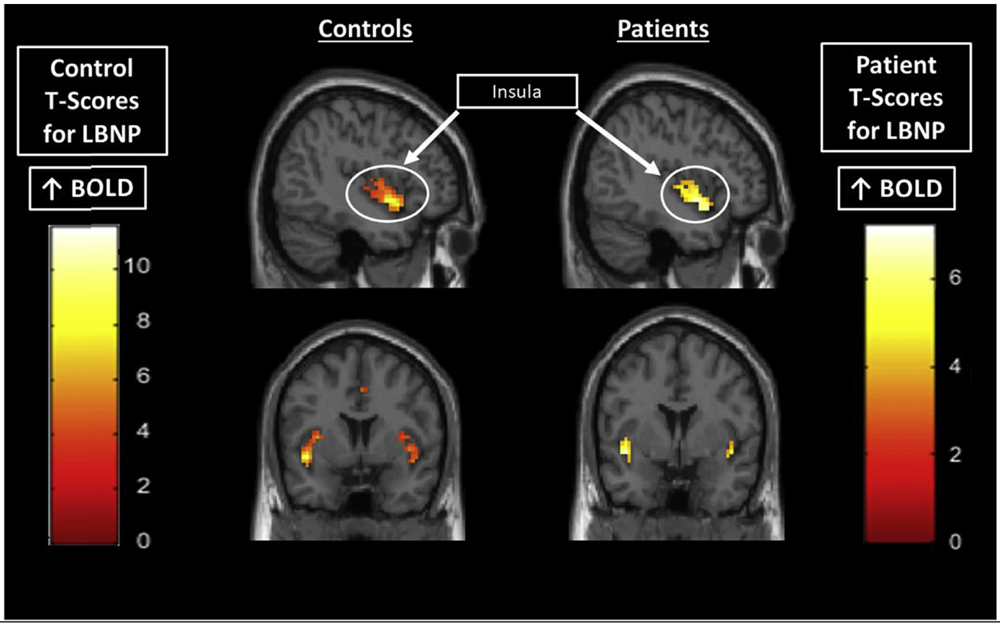

Fig. 1. Cortical activation patterns during LBNP. During LBNP both controls and patients had activation in the bilateral insula. Abbreviations: LBNP, Lower body negative pressure; BOLD, blood oxygen level dependent.

Table 2
Cortical patterns of activation during LBNP in healthy controls and NOH patients. Controls had significantly greater activation in the cerebellum relative to patients.

Region	Side	Voxel	*T*-value	*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.

gate the role of the cerebellum in a different test of baroreflex regulation, all groups completed a series of VM. Similar to LBNP, controls also had significantly greater activation in the bilateral cerebellum in response to Valsalva maneuver (Fig. 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; Fig. 3) ($p < 0.05$). Patients also had significant activation in the right anterior and midline posterior cingulate cortices (Table 3). No significant differences were found between controls and patients during the LBNP-recovery phase. Please refer to Supplementary Data for MNI coordinate information.

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. During an orthostatic stressor such as LBNP, regions within the cortical 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) Cortical 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, all groups completed a series Valsalva maneuvers. Similar to LBNP, patients had significantly less cerebellar activation during VM as 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 cortical autonomic network, the cerebellum has arguably received less attention than other cortical sites. In human and animal studies alike, a growing source of literature has emerged highlighting a number of autonomic functions that appear to involve pathways through the cerebellum (Harper et al., 1998), including postural control of blood pressure and heart rate (Nisimaru et al., 1998). 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 (Kimmerly et al., 2005; Goswami et al., 2012) 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.

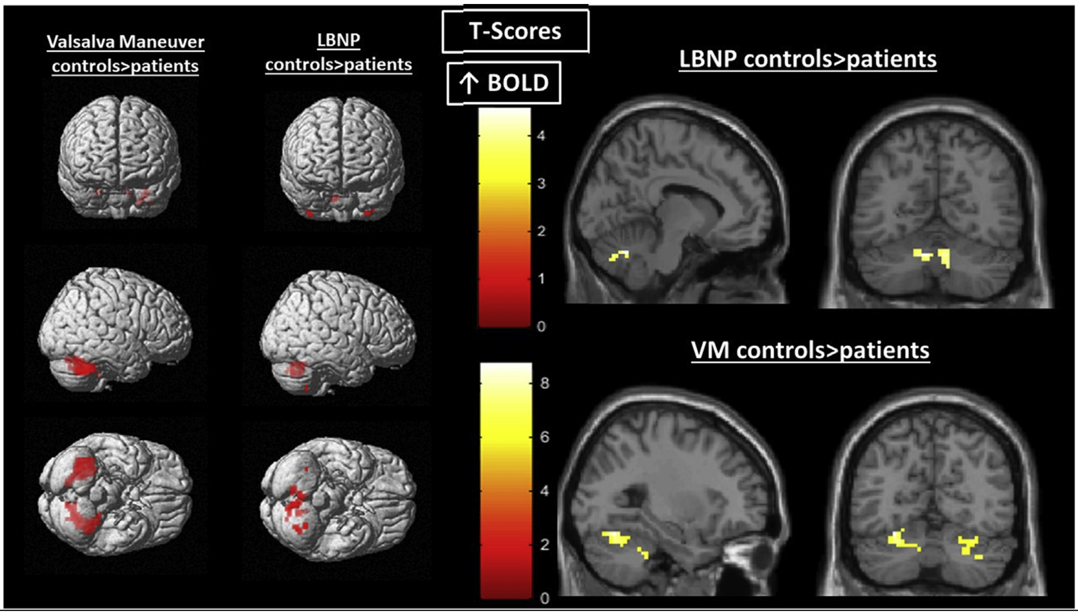

Fig. 2. Comparison of cerebellar changes during VM and LBNP. Controls had greater activation in the cerebellum during LBNP and VM relative to patients. Abbreviations: LBNP, Lower body negative pressure; BOLD, blood oxygen level dependent; VM, Valsalva maneuver.

Table 3
Cortical patterns 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*-value	*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.

Similarly, rCBF in the pons, cerebellum and right insula covaried with heart rate (Critchley et al., 2000). 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 (Paton et al., 1990) and rostral ventrolateral medulla (RVLM) (Silva-Carvalho et al., 1991), in addition to, the rostral portion of the inferior and medial vestibular nuclei (Yates and Miller, 1994; Yates et al., 1994). Together, these cortical 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 (Doba and Reis, 1974; Dormer, 1984) and posterior cerebellar cortex (Nisimaru and Yamamoto, 1977; Paton and Gilbey, 1992) 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 (Holmes et al., 2002). Despite the severe blood pressure drops that NOH patients experience, paradoxically, approximately 50% of patients also have supine hypertension (Baker and Kimpinski, 2017). The fastigial nuclei (FN) and cerebellar cortical 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 (Lutherer et al., 1983). Furthermore, in evaluating the FN neural activity during blood pressure alterations via the modified oxford method, FN neural activity increases during hypotension (Rector et al., 2006). 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, all 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 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.

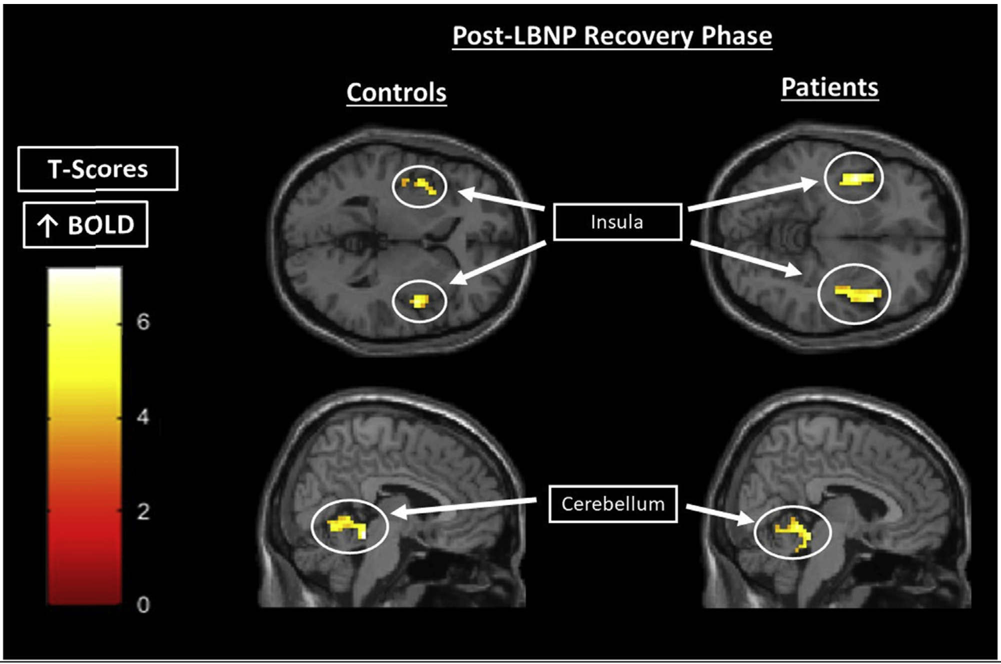

Fig. 3. Cortical activation patterns during post-LBNP recovery phase. During the post-LBNP recovery no significant differences were found between healthy controls and patients. Both controls and patients had activation in the bilateral insula and cerebellum. Abbreviations: LBNP, Lower body negative pressure; BOLD, blood oxygen level dependent.

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 our clinical population, in some cases the negative pressure that was used during LBNP was less than that in healthy controls. Despite a reduce 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 to 45 s. In the current study, we applied a single-epoch design in order 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 (Henderson et al., 2011). Furthermore, single-epoch fMRI has been previously validated against multiple epochs of stimulation, with results that yield similar activation patterns (Koyama et al., 2003). (4) The hemodynamic 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.

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 NOH patients have significantly less activation in the cerebellum during an orthostatic challenge compared to healthy controls. Furthermore, NOH 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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Conflict of interest

All authors have approved the final article and have no financial or other conflicts of interest to declare.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.clinph.2018.07.026.

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