1-1-2012

Altered cortical activation patterns associated with baroreflex unloading following 24 h of physical deconditioning

J. K. Shoemaker
Western University, kshoemak@uwo.ca

C. W. Usselman
Western University

A. Rothwell
Western University

S. W. Wong
Western University

Research Paper

Altered cortical activation patterns associated with baroreflex unloading following 24 h of physical deconditioning

J. K. Shoemaker[1,2], C. W. Usselman[1], A. Rothwell[1] and S. W. Wong[1,3]

[1] *Neurovascular Research Laboratory, Faculty of Health Sciences and School of Kinesiology and* [2] *Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario, Canada*

[3] *Department of Psychological Studies, The Hong Kong Institute of Education, Hong Kong*

Cardiovascular arousal is associated with patterned cortical activity changes. Head-down-tilt bed rest (HDBR) dimishes the baroreflex-mediated cardiac control. The present study tested the hypothesis that HDBR deconditioning would modify the forebrain organization for heart rate (HR) control during baroreflex unloading. Heart rate variability (HRV), blood pressure and plasma hormones were analysed at rest, whereas HR and cortical autonomic activation patterns (functional magnetic resonance imaging) were measured during graded and randomly assigned lower body negative pressure treatments (LBNP, −15 and −35 mmHg) both before (Pre) and after (Post) a 24 h HDBR protocol (study 1; $n = 8$). An additional group was tested before and following diuretic-induced hypovolaemia (study 2; $n = 9$; spironolactone, 100 mg day^{--1} for 3 days) that mimicked the plasma volume lost during HDBR (−15% in both studies; $P < 0.05$). Head-down bed rest with hypovolaemia did not affect baseline HR, mean arterial pressure, HRV or plasma catecholamines. Head-down bed rest augmented the LBNP-induced HR response ($P < 0.05$), and this was associated with bed-rest-induced development of the following changes: (i) enhanced activation within the genual anterior cingulate cortex and the right anterior insular cortex; and (ii) deactivation patterns within the subgenual regions of the anterior cingulate cortex. Diuretic treatment (without HDBR) did not affect baseline HR and mean arterial pressure, but did reduce resting HRV and elevated circulating noradrenaline and plasma renin activity ($P < 0.05$). The greater HR response to LBNP following diuretic ($P < 0.05$) was associated with diminished activation of the right anterior insula. Our findings indicate that 24 h of HDBR minimized the impact of diuretic treatment on baseline autonomic and cardiovascular variables. The findings also indicate that despite the similar augmentation of HR responses to LBNP and despite similar pre-intervention cortical activation patterns, HDBR and diuretic treatment produced different effects on the cortical responses, with HDBR affecting anterior cingulate cortex and right insula regions, whereas diuretic treatment affected primarily the right insula alone, but in a direction that was opposite to HDBR. The data indicate that physical deconditioning can induce rapid functional changes within the cortical circuitry associated with baroreflex unloading, changes that are distinct from diuretic-induced hypovolaemia. The results suggest that physical activity patterns exert a rapid and notable impact on the cortical circuitry associated with cardiovascular control.

(Received 23 February 2012; accepted after revision 15 May 2012; first published online 21 May 2012)

Corresponding author J. K. Shoemaker: Neurovascular Research Laboratory, Faculty of Health Sciences and School of Kinesiology, Thames Hall, Room 3110, The University of Western Ontario, London, Ontario, Canada N6A 5C1. Email: kshoemak@uwo.ca

DOI: 10.1113/expphysiol.2012.065557

Neural control of the cardiovascular system is an adaptive process that can improve with exercise training (DiCarlo & Bishop, 1990; Brum *et al.* 2000; Buch *et al.* 2002; Cooke *et al.* 2002; Mueller, 2007) and degrade with physical deconditioning (Hughson *et al.* 1994*b*; Convertino *et al.* 1997*a*; Hasser & Moffitt, 2001), reflecting disuse properties. Periods of time spent in the 6 deg head-down-tilt position produce this cardiovascular deconditioning effect, with symptoms that include a greater tachycardia and unchanged (Pawelczyk *et al.* 2001) or enhanced neurovascular regulation during orthostatic stress (Fischer *et al.* 2007). Additionally, reductions in cardiovagal baroreflex gain following prolonged deconditioning (Convertino *et al.* 1990; Eckberg & Fritsch, 1992; Hughson *et al.* 1994*a*; Pannier *et al.* 1998) indicate malleability in some component of the reflex loop between the vascular origins of the baroreceptor afferents and the cardiac response to efferent neural adjustments. These changes can develop within 4–24 h of head-down-tilt bed rest (HDBR; Butler *et al.* 1991; Pannier *et al.* 1991; Fischer *et al.* 2007).

The question of where the effects of physical deconditioning develop in the baroreflex loop was studied extensively by Hasser and colleagues (Moffitt *et al.* 1998, 1999; Hasser & Moffitt, 2001). Their results indicated that altered central nervous system processing of baroreceptor inputs involved tonic increases in $GABA_A$-mediated inhibition of the rostral ventrolateral medulla after hindlimb unloading in rats. This augmented inhibition did not arise from the caudal ventrolateral medulla (Hasser & Moffitt, 2001). Such inhibitory control may extend from supramedullary sites that modulate baroreflex function.

Extensive experimental studies in the rodent cortex suggest that the insula, thalamus and amygdala participate in reflex cardiovascular control (Cechetto & Saper, 1990). More recently, neuroimaging studies in conscious humans have exposed the collective cortical responses to physiological stressors. In particular, graded levels of lower body negative pressure (LBNP) within a functional magnetic resonance imaging (fMRI) model elicited increased activity within the posterior insula and dorsal anterior cingulate cortex (dACC) that was related to muscle sympathetic nerve activity (Kimmerly *et al.* 2005). In contrast, tachycardia in response to elevated LBNP was related to reduced activity within the ventral medial prefrontal cortex (vMPFC) and/or its anatomical neighbour, the subgenual anterior cingulate cortex (ACC). Through several studies in different laboratories, changes in heart rate (HR) are consistently related inversely to activity within this vMPFC region, be it LBNP (Kimmerly *et al.* 2005), hand-grip exercise (Wong *et al.* 2007; Goswami *et al.* 2011) or cognitive/emotional stress (Critchley *et al.* 2000; Gianaros *et al.* 2004).

The purpose of the present study was to test the hypothesis that regions of the forebrain adjust rapidly to physical deconditioning in a manner that correlates with altered cardiac responses to orthostatic stress. Our approach was to determine the activation patterns within regions of the cortical autonomic network during graded LBNP before and following 24 h of HDBR. In addition, because HDBR also induces reductions in blood volume, the cortical activation patterns during LBNP were studied in a separate group before and following diuretic-induced hypovolaemia.

Methods

Ethical approval

Each participant provided signed consent to the protocol methods that were approved by the Health Sciences Research Ethics Board at The University of Western Ontario and that conformed to the standards set by the latest revision of the Declaration of Helsinki.

Participants

Two separate protocols were performed, representing the HDBR and the diuretic studies, respectively. Thus, two groups of participants are described. Their anthropometric data and study allocation are provided in Table 1 for each of the HDBR and induced hypovolaemia (diuretic) studies. A total of 14 healthy individuals (six women) completed the HDBR ($n = 8$) and/or the diuretic studies ($n = 9$); three of the individuals completed both protocols. Overall, the participants were 26 ± 3 years old (range = 22–39 years), weighed 68 ± 12 kg and were 171 ± 9 cm tall. Notably, a total of 13 individuals were recruited into the diuretic protocol, but four failed to respond to the diuretic treatment (described in '*Diuretic methods*' below). Also, three additional participants failed to complete the post-HDBR measurements owing to back discomfort or nausea at the time of imaging. This short period of HDBR can produce transient back discomfort in some individuals, making it difficult to sustain the supine and flat posture required for neuroimaging in the LBNP device. Each participant was healthy, as determined by a medical screening.

Experimental design

Each of the HDBR and diuretic studies involved two separate experimental sessions, namely the physiological recording session and the fMRI session. The session order was randomly assigned but performed at the same time of day and separated by a minimal period of 1 week. Subjects were familiarized with the experimental procedures prior to their first test session. The pre- and postintervention studies were seprated by no more than 4 weeks.

Table 1. Characteristics of study participants

Study	Age (years)	Sample size [*n* (male/female)]	Weight (kg)	Height (cm)
Head-down bed rest	25 ± 2	8 (6/2)	68 ± 15	171 ± 11
Diuretic	28 ± 6	9 (5/4)	72 ± 12	172 ± 8

Values are mean ± SD.

Head-down bed rest methods

Each participant in this protocol completed 24 h of strict and monitored 6 deg head-down bed rest. Bed rest began at ∼11.00 h, with lights out at 23.00 h. Following the HDBR period, the participant was transported to the fMRI imaging suite in the legs-up position, maintaining the head below the feet. In the 'Pre' test, participants arrived for each of the laboratory tests and the fMRI tests on separate days. To ensure a hydrated state in the Pre test, participants were instructed to ingest 2 litres of fluid in the 24 h period prior to the test, with an additional 500 ml during the 2 h prior to the test. During HDBR, 2 litres of fluid were ingested over the 24 h period, and food intake was scheduled to satiety. With the exception of volume loading, all participants arrived at the laboratory after a 12 h fast. A granola bar and 250 ml fruit drink were provided at the laboratory approximately 30 min prior to each of the 'Pre' and 'Post' HDBR tests. All participants were asked to void their bladder before instrumentation to minimize the effects of a distended bladder on sympathetic activity and arterial blood pressure (Fagius & Karhuvaara, 1989). None of the subjects exhibited a history of autonomic dysfunction or cardiovascular disease, and none was on any medications known to affect brain function or perfusion.

Diuretic methods

Hypovolaemia was induced by the oral diuretic spironolactone (Aldactone, Pfizer Inc. New York, NY, USA), 100 mg day^{-1} for 3 days. Blood samples were assessed before and after the diuretic treatment to ensure stable ionic balance. A placebo was not used in this study because of the difficulties keeping the participants blinded to a diuretic treatment. In support of this approach, we have previously shown the effectiveness of a similar diuretic treatment (Kimmerly & Shoemaker, 2002). On each day, the participant arrived in a 12 h fasted state. In the 'Pre' diuretic test, each participant was encouraged to drink 2 litres of fluid during the 24 h period before the test, with 500 ml approximately 2 h before the test. They were allowed *ad libitum* fluid ingestion during diuretic treatment, because the major aim was to achieve hypovolaemia, which might be offset by substantial volume loading such as that required for the HDBR participants. Following the diuretic treatment, participants performed a 12 h fast from food and liquid until arrival at the laboratory, where they were provided with a granola bar and a 250 ml fruit drink 30 min prior to testing.

Protocols

Each session began with the subject in the supine position and sealed within the lower body negative pressure chamber for a minimum of 30 min. Two levels of randomly assigned LBNP (–15 and –35 mmHg) were used in the present investigation. This protocol involved a box-car model with 60 s periods of LBNP interspersed with 60–90 s periods of rest/recovery. This model and the set-up for the MRI experiment have been described in detail previously (Kimmerly *et al.* 2005). For each subject, the order of LBNP application was replicated for each of the two test sessions.

The lower (−15 mmHg) and higher (−35 mmHg) levels of LBNP were used because sympathetic activation occurs with both levels, whereas heart rate generally does not change until levels below −15 mmHg, at least in normal conditions. Sympathetic and HR responses to orthostatic stress are often greater following HDBR (Shoemaker *et al.* 1999); however, whether this enhanced response is observed at the low levels of orthostatic stress used here has not been assessed. Thus, two levels of LBNP provided a model to examine the cortical activation patterns with and without concurrent heart rate changes and whether HDBR *versus* diuretic treatment affects the HR responses differently at various levels of LBNP.

Laboratory measurements

Measures of plasma catecholamines (high-pressure liquid chromatography) and plasma renin activity (Diasorin RIA kit, Stillwater, MN, USA) were assessed from venous blood samples obtained at the beginning of each test, after 20 min of quiet supine rest. Plasma haematocrit levels were also assessed from this sample. Blood pressure was assessed from the middle digit using a finger plethysmography system (Finometer, FMS; Finapres Medical Systems BV, Amsterdam, The Netherlands). Heart rate was measured from the ECG.

Analog signals were sampled at 200 Hz for arterial blood pressure and at 1000 Hz for ECG, and collected with an online data acquisition and analysis system (PowerLab; ADInstruments, Castle Hill, NSW, Australia). The relative change in plasma volume (%ΔPV) was calculated as follows:

$$\%\Delta PV = 100/(100 - Hct_{Pre}) \times 100(Hct_{Pre} - Hct_{Post})/Hct_{Post},$$

where Hct is the haematocrit (Greenleaf *et al.* 1983).

Indices of heart rate variability (HRV) were made from time series of R–R interval data over 3–5 min at rest in the supine position. These measures were made from recordings at the onset of each of the laboratory sessions in the diuretic study and immediately prior to and following the bed rest period. These measures were made during spontaneous breathing for both HDBR and diuretic studies, as well as during paced breathing (15 breaths min^{-1}) in the HDBR study. Using Powerlab software, the various indices of time-based (SDNN, SPECNN and SDEV) and spectrally based power (high frequency power (HF), low frequency power (LF), HF/LF ratio and total power) were analysed and compared using a one-way ANOVA statistical model within each group.

Functional MRI data acquisition

During the scanning session, HR was calculated from the pulse intervals recorded on an MRI-compatible oximeter (8600FO MRI; Nonin Medical Inc., Plymouth, MN, USA) placed over the middle finger of the left hand. The absolute level of LBNP was simultaneously measured during each scanning session with the use of a pressure transducer (PX272; Edwards Lifesciences, Irvine, CA, USA) connected in series to a bridge amplifier outside of the MRI suite.

All imaging data were collected using a whole-body 4 T imaging system (Varian, Palo Alto, CA, USA; Siemans, Erlangen, Germany) with a maximal strength of 40 mT m^{-1} and a slew rate of 120 mT m^{-1} s^{-1}. A transmit–receive cylindrical hybrid birdcage radio frequency (RF) head coil (Barberi *et al.* 2000) was used for transmission and detection of the blood oxygen level dependent (BOLD) contrast signal. Prior to imaging, a global shimming procedure (RASTAMAP) using first- and second-order shims, was performed to optimize the magnetic field over the imaging volume of interest (Klassen & Menon, 2004). Nineteen or twenty-one contiguous axial slices (3.4 mm × 3.4 mm in-plane voxel resolution, time of repetition (TR) = 2.5 s) were acquired in each volume and prescribed from a series of high-resolution T_1-weighted sagittal scout images. Five steady-state volumes were acquired prior to data collection to allow for magnetization equilibrium; these were discarded prior to data analysis. Functional data were collected using a multishot T_2^*-weighted spiral imaging pulse sequence (Field of view (FOV) = 192 mm × 192 mm). A corresponding high-resolution T_1-weighted structural volume was acquired at the beginning of the same scanning session using three-dimensional Turbo FLASH (Echo time (TE) = 5.5 ms, Interval time (TI) = 600 ms, Time to repetition (TR) = 10 ms) with a voxel resolution of 0.86 mm × 0.86 mm × 1.5 mm. Each subject was immobilized during the experimental session within a head cradle and packed with foam padding and was instructed to keep their eyes closed and avoid head movements during the scanning period. The brainstem was not imaged in this study.

Functional MRI data analysis

The HR and LBNP data were averaged over 2.5 s bins and time aligned to ensure a corresponding mean value for each functional scan obtained during the fMRI collection period. All fMRI data were analysed with statistical parametric mapping (SPM2; Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks, Sherborn, MA, USA). The functional images collected were realigned across all sessions using a fourth degree B-spline reslice interpolation method. A mean functional image was created and co-registered with the participants' T_1-weighted anatomical image. The anatomical image was then transformed into a canonical stereotactic space Montreal Neurological Institute (MNI), 152 brains template) using a trilinear interpolation method. The same normalization parameters were applied to all the functional images. To correct for resultant errors from fixed deterministic drifts, a high pass filter (cut-off period = 250 s) was applied to all functional images. The scans were smoothed using a Gaussian kernel set at 8 mm full-width at half-maximum.

Statistical analysis

Physiological data. The main effects of recording session (neuroimaging *versus* physiological) or group (HDBR *versus* diuretic) and level of LBNP on HR were analysed using a mixed repeated-measures two-way ANOVA. When significant main effects were observed, Tukey's *post hoc* analysis was performed to estimate differences among means. Probability levels during multiple-pointwise comparisons were corrected using Bonferonni's approach. Statistical significance in all comparisons was set at $P < 0.05$. Values are presented as means ± SD.

Functional MRI data. Individual design matrices were constructed for the analysis of participant-by-session interactions to determine cortical areas where BOLD

Table 2. Blood variables in the head-down bed rest and diuretic studies

Study	Time point	Change in plasma volume (%)	Noradrenaline (pg ml^{-1})	Adrenaline (pg ml^{-1})	Plasma renin activity (ng l^{-1} s^{-1})
Head-down bed rest	Pre	––	206 ± 107	19 ± 8	0.23 ± 0.19
	Post	−15 ± 6	224 ± 66	24 ± 12	0.20 ± 0.07
Diuretic	Pre	––	114 ± 48	30 ± 15	0.43 ± 0.22
	Post	−15 ± 10	220 ± 142*	22 ± 10*	1.16 ± 0.58*

Values are mean ± SD. * Significantly different from Pre ($P < 0.05$).

Table 3. Impact of head-down bed rest on indices of heart rate variability before (Pre) and following (Post) hypovolaemia induced by head-down bed rest or diuretic

	Head-down bed rest		Diuretic	
Parameter	Pre	Post	Pre	Post
Heart rate (beats min^{-1})	61 ± 4	60 ± 6	59 ± 5	61 ± 3
MAP (mmHg)	79 ± 8	83 ± 8	82 ± 9	90 ± 14
Ptot (msec squared)	4349 ± 1991	6289 ± 5779	4923 ± 2667	3327 ± 2055
LF	930 ± 619	1903 ± 2223	1220 ± 800	818 ± 612
HF	2052 ± 1245	2452 ± 2882	1917 ± 1334	862 ± 567*
LF/HF	0.60 ± 0.37	1.15 ± 1.3	0.79 ± 0.47	0.96 ± 0.55
SDNN	66 ± 16	75 ± 52	65 ± 19	53 ± 19
RMSSD	66 ± 21	73 ± 41	66 ± 25	44 ± 16*
SPECNN	983 ± 68	1018 ± 115	1025 ± 96	984 ± 61

Values are mean ± SD. Abbreviations: HF, high frequency; LF, low frequency; MAP, mean arterial pressure; Ptot, total spectral power; SDNN, standard deviation of "normal-to-normal cardiac cycle length; RMSSD, square root of the mean squared difference in normal-to-normal cardiac cycles; SPECNN, mean normal-to-normal interval in the spectrum. * Significantly different from corresponding 'Pre' condition ($P < 0.05$).

activity covaried (bidirectionally) with LBNP. For each participant, Student's *t* tests were performed on these contrast images to determine the brain regions that demonstrated more or less activation during −15 and −35 mmHg LBNP. With this assurance, the regions of interest (ROIs) within the cortical autonomic network were subsequently studied separately. Based on earlier results from human stimulation (Pool & Ransohoff, 1949; Oppenheimer *et al.* 1992) and functional imaging studies (Critchley *et al.* 2003; Kimmerly *et al.* 2005; Wong *et al.* 2007), a masking procedure within SPM2 was used to localize analysis of activation patterns within the insula cortex, cingulate cortex, thalamus, amygdala and medial prefrontal cortex. Activation patterns within these ROIs were assessed using the PickAtlas toolbox with false discovery rate correction for multiple comparisons, $P < 0.05$ or an uncorrected probabililty of $P < 0.005$.

Finally, a subtraction analysis was performed to examine the ROIs that were activated to a greater or lesser extent in the Post *versus* Pre session in each of the HDBR and diuretic protocols. In each of the study levels, significant local maxima voxel are reported as Tailarach (TAL) format. In each level of analysis, significant results are reported for cluster sizes equal to or greater than 10 voxels. All fMRI figures are represented in a neurological convention (i.e. participant's left is on the left).

Results

Cardiovascular control

Blood variables are shown in Table 2. Compared with the Pre HDBR and the Pre diuretic trials, plasma volume was reduced by ∼15% ($P < 0.05$) in each of the HDBR and diuretic protocols.

In a mixed one-way ANOVA, noradrenaline values in the two groups were not different for the Pre ($P = 0.08$) or Post conditions ($P = 0.9$). A group × time interaction was observed for adrenaline ($P = 0.03$), but Pre ($P = 0.08$) and Post values ($P = 0.3$) were not different between groups in pointwise contrasts. A group × time interaction was observed for plasma renin activity levels as well ($P < 0.005$). In *post hoc* analysis, between-group plasma renin activity levels were not different Pre ($P = 0.2$) but, compared with HDBR, the diuretic group demonstrated greater Post values ($P = 0.002$).

Subsequently, the effect of HDBR or diuretic within each group was studied, as shown in Table 2. While HDBR did not affect circulating noradrenaline, adrenaline or plasma renin activity, the diuretic protocol produced

an increase in noradrenaline and plasma renin activity relative to the Pre diuretic test (Table 2; $P < 0.05$).

Heart rate and heart rate variability. The results, shown in Table 3, indicate that neither the 24 h period of HDBR nor the diuretic treatment affected baseline heart rate or mean arterial pressure. Although HDBR did not affect HRV in either spontaneous (Table 3) or paced breathing trials (data not included), acute diuretic treatment reduced both the high-frequency and RMSSD (square root of the mean squared difference in normal-to-normal cardiac cycles) variables of HRV ($P < 0.05$).

Lower body negative pressure. Based on the average HR levels at baseline and the peak HR at the end of each LBNP treatment, the heart rate response to LBNP (ΔHR) was greater in both the Post HDBR and diuretic studies compared with the corresponding Pre studies (main effect, $P < 0.05$; Fig. 1).

Blood oxygenation level dependent patterns during fMRI studies

In the group-level analysis, LBNP generally induced activation in areas such as the posterior cingulate cortex (PCC), lingual gyrus and cuneus (primary visual areas), mid-cingulate cortex (MCC), caudate, precuneus, pre- and postcentral gyri, hippocampus, bilateral insular cortex (IC), regions of the ACC, and the thalamus.

Cortical autonomic network region analysis

Table 4 summarizes the BOLD response within representative portions of the cortical autonomic network regions identified with ROI analysis and their corresponding t-test scores for both the HDBR and diuretic studies.

A three-way ANOVA (i.e. HDBR *versus* diuretic × Pre *versus* Post × LBNP −15 mmHg *versus* −35 mmHg) indicated a significant three-way interaction [$F(1,60) = 12.51$, $P = 0.001$] within the subgenual ACC. In particular, the subgenual ACC response to LBNP −35 mmHg was suppressed after HDBR but elevated after diuretic. Subsequently, two-way ANOVA *post hoc* analyses were conducted targeting specifically the individual levels of LBNP. For the −15 mmHg LBNP condition, the dACC showed an interaction effect [$F(1,60) = 10.24$, $P = 0.002$]. Likewise, for the LBNP −35 mmHg condition, the vMPFC/subgenual ACC showed an interaction effect [$F(1,60) = 12.51$, $P = 0.001$]. Subsequently, the direct hypotheses were addressed in separate ANOVAs that studied the HDBR and diuretic-treated groups separately; these results are outlined in the following subsections.

Head-down bed rest study. In the Pre HDBR session, increased cortical activation during −15 mmHg LBNP was observed within the bilateral IC, bilateral thalamus, and the left dACC. Regions activated during −35 mmHg LBNP were greater in number and included specified regions within both left and right IC, bilateral ACC, bilateral thalamus and bilateral amygdala. No reduction in activation below baseline levels (deactivation) was observed within the *a priori* ROIs in the Pre HDBR test. In the Post HDBR test, no activation patterns met the statistical criteria during −15 mmHg of suction. At

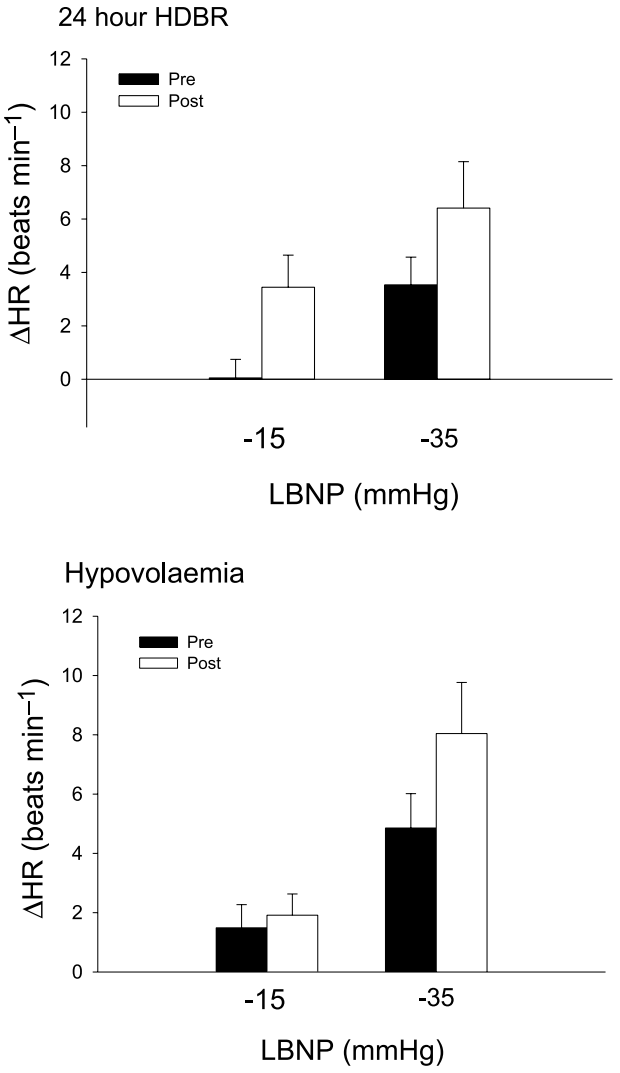

Figure 1. Mean increase in heart rate (ΔHR) during graded lower body negative pressure (LBNP) before and after 24 h of head-down-tilt bed rest (top panel) with incident hypovolaemia, or diuretic-induced hypovolaemia (bottom panel).
There was a main effect of LBNP ($P < 0.05$) and condition (Post > Pre; $P < 0.05$) for each protocol, but no LBNP × condition interaction for either study group.

Table 4. T-score values representing the magnitude of regional activation patterns in the cortical autonomic network (CAN) during −15 and −35 mmHg of lower body negative pressure before and following 24 h head-down bed rest or diuretic

			Head-down bed rest				Diuretic			
			Pre		Post		Pre		Post	
CAN region	Side	Anatomical details	−15 mmHg	−35 mmHg	−15 mmHg	−35 mmHg	−15 mmHg	−35 mmHg	−15 mmHg	−35 mmHg
			Increased activation							
IC	Left	Posterior superior	3.52*	3.58	–	2.88*	3.64	2.36	3.28	3.68
		Anterior inferior	3.62*	–	–	–	3.75	5.00	–	3.41
	Right	Posterior superior	3.58*	3.58	–	3.16*	3.78	2.72	4.08	3.97
		Anterior inferior	–	–	–	–	2.78	2.99	–	2.77
ACC	Left	Dorsal	5.68	3.59*		3.67*	3.33*	3.74	–	4.12
		Subgenual	–	3.57*	–	–	–	–	–	3.54
	Right	Dorsal	–	–	–	2.81*	–	–	3.35	3.51*
		Subgenual	–	3.57*	–	–	–	2.92	–	3.26
Amygdala	Left		–	3.93	–	4.18	–	–	–	3.59
	Right		–	3.85	–	2.77	–	–	–	2.93
Thalamus	Left		4.27	3.39	–	3.12	4.88	4.59	2.96	5.14
	Right		2.88	5.83	–	3.88	2.77	4.11	–	4.19
			Decreased activation							
ACC	Left	Subgenual	–	–	–	4.72	–	–	–	-
	Right	Subgenual	–	–	–	3.36*	–	–	–	-

Values represent t-scores of the peak voxel in the defined region using false discovery rate correction for multiple comparisons ($P < 0.05$) or, if highlighted by an asterisk (*), regions accepted at the $P < 0.005$ but uncorrected level. Masks were performed on bilateral insular cortex (IC), thalamus, amygdala and the dorsal, genual and subgenual regions of the anterior cingulate cortex (ACC). No increased activation was observed in the medial prefrontal or subgenual anterior cingulate cortical region.

−35 mmHg, the Post session was characterized generally by a similar forebrain organization and response as in the Pre −35 mmHg test. In contrast to the Pre session, where no deactivation patterns were observed, Post HDBR LBNP at −35 mmHg demonstrated reduced activation (deactivation) in the bilateral subgenual ACC region.

Diuretic study. As in the Pre HDBR tests, Pre diuretic LBNP (−15 mmHg) was associated with increased cortical activation in the bilateral IC, bilateral thalamus and left dACC. At −35 mmHg, LBNP activated bilateral IC, bilateral ACC, left amydala and bilateral thalamus. Post diuretic, at −15 mmHg, left IC activation was restricted to the posterior regions, although right IC activation was similar to the Pre test. Activation within the ACC and thalamus was also observed Post diuretic. The higher level of LBNP elicited a greater number of cortical activation patterns in the Post *versus* the Pre diuretic tests, with notable additions of detected activity within the ACC and amygdala regions. No deactivation was observed within the *a priori* regions in Pre or Post diuretic LBNP.

Subtraction analysis

Head-down bed rest study. Relative to the Post LBNP session, cortical activation was greater in the Pre LBNP session in the the dACC at −15 mmHg (Fig. 2*A*) and subgenual ACC region at −35 mmHg ($P < 0.005$; Fig. 2*B*). In contrast, the activation was greater in the Post LBNP session in the genual ACC (Fig. 2*C*) and right anterior posterior IC (Fig. 2*D*).

Diuretic study. Cortical activation was greater in the Pre *versus* Post session of LBNP in the right anterior superior IC (Fig. 3). No other differences in cortical activation patterns were observed in the subtraction analysis at either level of LBNP.

Discussion

Both HDBR and diuretic conditions produced similar reductions in plasma volume and augmented the HR responses to mild levels of LBNP. However, diuretic, but not HDBR, reduced HRV and augmented plasma noradrenaline in conditions of supine rest. Furthermore, diuretic treatment had minimal impact on the cortical activation patterns during LBNP, notwithstanding a diminished activation in the right anterior IC. In contrast, HDBR elevated the activity at bilateral genual ACC and the right anterior IC during LBNP, as well as reducing the activity of the subgenual ACC during LBNP. Therefore, the two interventions that produced similar changes in plasma volume and HR responses to LBNP were associated with different patterns of impact on baseline autonomic function, as well as the forebrain circuitry associated with baroreceptor unloading. On the basis of these

observations, it is concluded that HDBR (which includes hypovolaemia as a side effect) affects the organization of a forebrain network that is associated with baroreflex cardiovascular control in a manner that is distinct from that affected by hypovolaemia alone. Moreover, adaptation to HDBR appears to minimize the impact of diuretic alone on sympathetic regulation. The short time frame of the intervention suggests that the brain adapts rapidly to the physical deconditioning stimulus of HDBR.

Head-down bed rest, diuretic and cardiovascular control

In line with previous reports (Pannier *et al.* 1991; Hirayanagi *et al.* 2004), 24 h of bed rest had minimal impact on heart rate variability in baseline conditions. In contrast, the diuretic treatment reduced both RMSSD and HF indicators of HRV, providing time-domain and spectrally based evidence of reduced heart rate

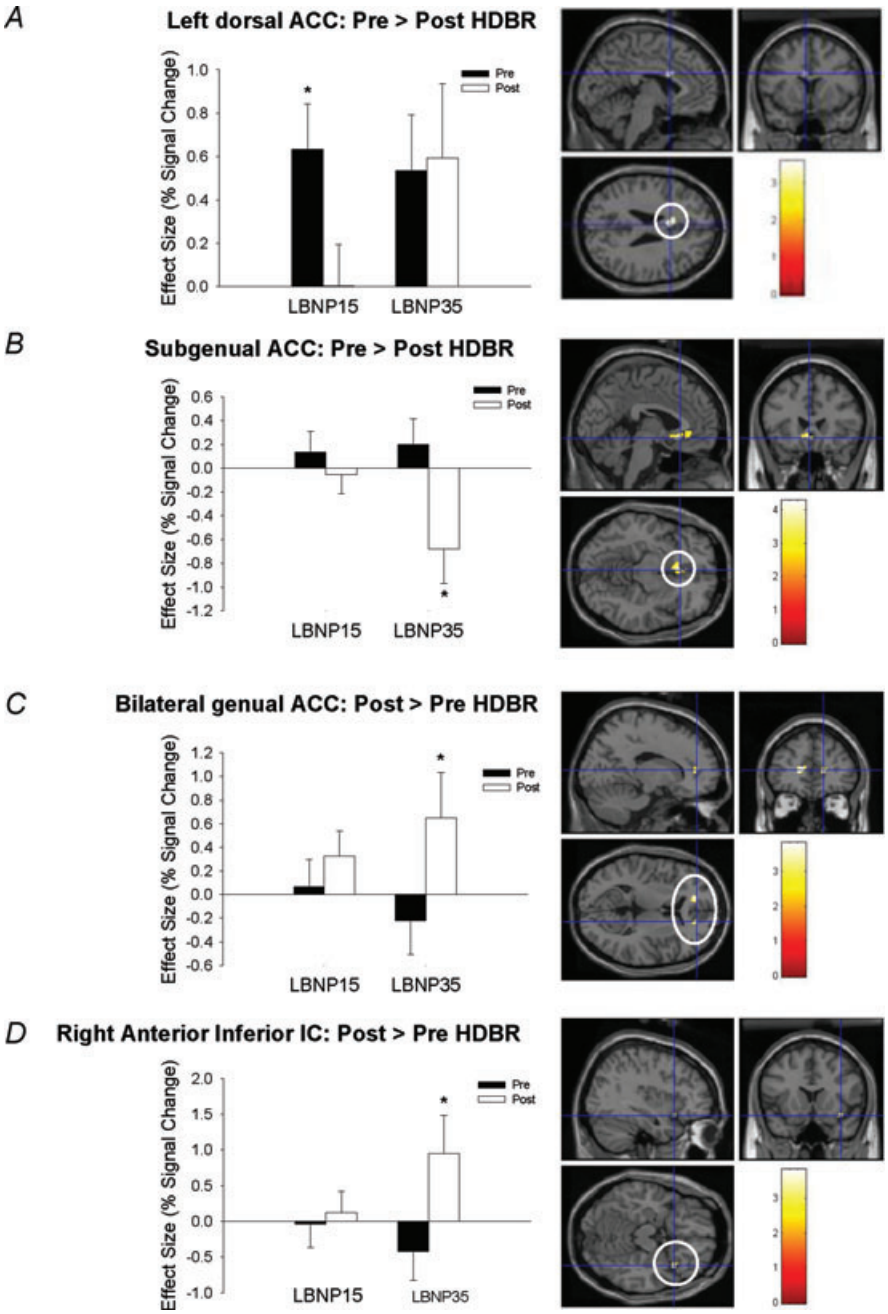

Figure 2. Subtraction analysis of blood oxygenation level dependent (BOLD) response to LBNP before (Pre) and following (Post) 24 h of head-down-tilt bed rest (HDBR).
Abbreviations: ACC, anterior cingulate cortex; dACC, dorsal ACC; and IC, insular cortex. * Significantly different from Pre ($P < 0.05$). Values represent means ± 95% confidence intervals.

variability. Although some authors express caution regarding interpretation of these indices (Pomeranz *et al.* 1985; Taylor *et al.* 2001), the RMSSD and high-frequency HRV are used as markers of cardiovagal control and, as such, suggest a primary impact of the diuretic on parasympathetic cardiac control. Furthermore, plasma catecholamine and renin levels indicated that baseline sympathetic activation was not altered by HDBR but, as with earlier studies (Kimmerly & Shoemaker, 2002), was increased following the diuretic. Thus, while the independent impact of the diuretic treatment altered autonomic balance in baseline conditions, bed rest did not. Therefore, it appears that something about adaptations to HDBR modify the independent impact of concurrent hypovolaemia on baseline autonomic outflow.

In this study, circulating adrenaline levels were not increased with diuretic treatment despite the expected large increases in sympathetic drive (Kimmerly & Shoemaker, 2002). Very little literature exists pertaining to adrenaline responses to diuretic treatment, particularly the model used in this study. Haemorrhage models of hypovolaemia in swine do lead to increased adrenaline levels if performed quickly, but not if performed slowly (Carey *et al.* 1976), despite large noradrenaline increases in both. Also, rodents that are resistant to cardiovascular collapse during haemorrhage do not demonstrate any increase in adrenal gland secretion of adrenaline (Gómez *et al.* 2012). Therefore, in the present analysis, the lack of change (or even decrease) in circulating adrenaline using a 3 day diuretic model without notable cardiovascular detriment mimics these previous studies. Regardless, the lack of direct studies on this question in humans illustrates the need for replication of our results.

In contrast to differential effects on baseline autonomic function, both HDBR and diuretic protocols produced an overall change to the heart rate response to LBNP. Yet, it appears that HDBR elicited a change in the sensitivity of cardiac reflex activation with a notable increase in HR at −15 mmHg LBNP in the Post test, a change that was not evident in the Pre HDBR test or with diuretic. The mechanism of the larger HR response to LBNP following HDBR and diuretic could include the following: (i) altered intrinsic cardiac function; (ii) altered neurogenic regulation; and/or (iii) altered β-adrenergic regulation of heart rate. Whether such short-term physical deconditioning can modify intrinsic heart rate regulation is not known. A change in the neural regulation of HR is an attractive option in this study because the HR response to LBNP is due to reduced vagal and increased sympathetic neural signals (Ferguson *et al.* 1983; Convertino & Sather, 2000). However, the heightened baseline sympathetic drive and somewhat diminished parasympathetic outflow that occurred only in the diuretic protocol cannot explain the altered HR response to LBNP in both HDBR and diuretic studies.

In the absence of intrinsic or neural contributions, the greater increase in HR for a given LBNP stimulus following both HDBR and diuretic suggests an increase in the sensitivity of cardiac function in response to a neural signal. Previous studies showed that cardiac responses to isoprenaline infusions were greater following periods of HDBR (Convertino *et al.* 1997*b*; Edgell *et al.* 2007). Although these studies were restricted to HDBR periods of 14 days or longer, such a heightened sensitivity to β-adrenergic stimulation is consistent with a greater HR response to LBNP. To our knowledge,

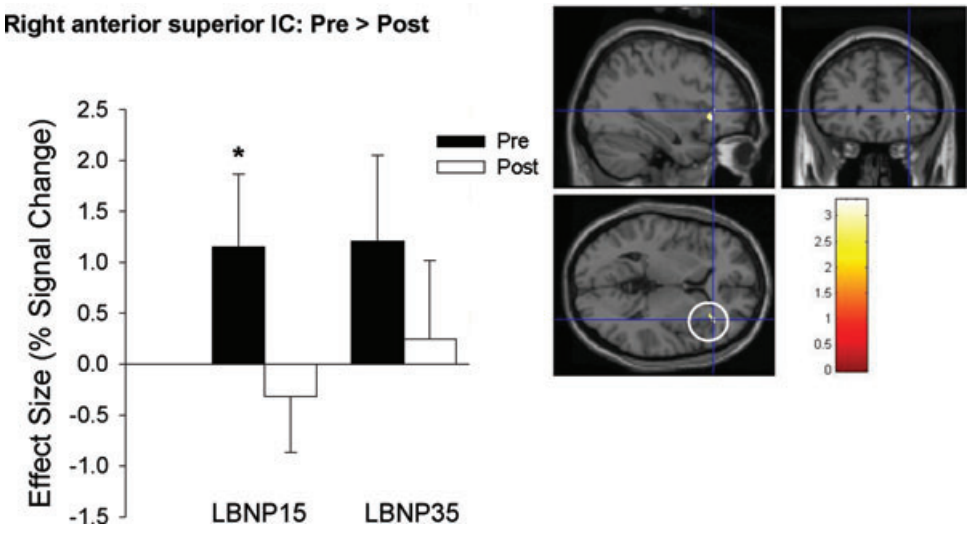

Figure 3. Subtraction analysis of BOLD response to LBNP before (Pre) and following (Post) the diuretic treatment.
Abbreviation: IC, insular cortex. * Significantly different from Pre ($P < 0.05$). Values represent means ± 95% confidence intervals.

cardiac β-adrenergic responses have not been studied following short-term HDBR or acute hypovolaemia. Previously, we showed that sympathetic nerve activity responses to graded LBNP were augmented in acute hypovolaemia (Kimmerly & Shoemaker, 2002) but not following HDBR (Arbeille *et al.* 2008*a*,*b*). Thus, as suggested above, it appears that adaptation to HDBR offsets the impact of hypovolaemia on sympathetic regulation. It remains possible, therefore, that different neural mechanisms contribute to HR responses to LBNP in the two protocols. Although MSNA data could not be obtained in the fMRI studies performed here, these previous and present findings suggest that HDBR may lead to sustained baseline autonomic function, retaining the option for enhanced parasympathetic nervous system withdrawal as a mechanism for HR control during orthostatic stress compared with the sympathetic response observed following acute hypovolaemia. This speculation of enhanced parasympathetic nervous system withdrawal following HDBR is consistent with the appearance of a deactivation in the subgenual ACC region in response to LBNP following bed rest, an issue that is discussed in the next subsection in greater detail.

Cortical regions demonstrating increased neural activation in response to baroreceptor unloading

This is the first study to examine the impact of HDBR or diuretic treatment on the cortical network associated with baroreflex cardiovascular control. Previously, we demonstrated a reproducible organization of the forebrain for cardiovascular regulation during LBNP (Kimmerly *et al.* 2005). This network included the insular cortex, anterior cingulate cortex, cerebellum and a few localized regions within the frontal and parietal cortices. The association of this network with LBNP was confirmed here. Overall, it appears that the ACC and IC regions were the dominant regions of interest undergoing modifications in response to HDBR. These two regions may act separately or, as outlined below, as an axis of control involved in integrating sensory inputs with an autonomic output. In contrast, the diuretic treatment affected anterior IC sites.

Various regions of the ACC have been emphasized in cardiovascular arousal. Functional imaging studies, combined with HRV analysis during effortful cognitive and motor behaviour, suggest that the dorsal ACC supports the increased heart rate during cardiovascular arousal (Critchley *et al.* 2003). The mechanisms of this association are not known. However, baroreceptor-related neuronal interconnections have been observed both within and between the insular cortex and anterior cingulate cortex (Verberne & Owens, 1998), and extend to the amygdala (Yasui *et al.* 1991) and the mediodorsal nucleus of the thalamus (Zhang & Oppenheimer, 2000*a*,*b*). The present findings are consistent with our earlier observations of synchronized decreases in activation of the ACC and midbrain during LBNP with higher HR (Kimmerly *et al.* 2005).

Importantly, several studies implicate the ventral medial prefrontal and subgenual ACC regions in HR control, with a consistent inverse relationship between these regions and HR (Critchley *et al.* 2004; Gianaros *et al.* 2004; Goswami *et al.* 2011; Wong *et al.* 2007), or a direct relationship to HRV (Thayer *et al.* 2012), during a variety of active and passive stimuli. Stimulus-induced tachycardia correlates with a reduction in activity (i.e. less active relative to baseline) in the subgenual ACC/vMPFC regions, producing an inverse relationship between activation in this forebrain region and HR. While the pharmacological and experimental studies remain to be performed, such consistent relationships and the collective data provoke the hypothesis that the subgenual ACC and medial prefrontal cortical regions modulate vagal control of heart rate. In this regard, the development of LBNP-induced decreases in activation of this region following HDBR and the greater and concurrent HR response to LBNP are consistent findings.

Activation within various regions of the left and right IC was observed in response to LBNP in both the Pre and Post periods of the present study, and in our previous studies with LBNP (Kimmerly *et al.* 2005). Of these, increased activation within the posterior regions of the right insula has been a common observation. The right posterior insula is tonically active (Butcher & Cechetto, 1995), contains neurons responsive to baroreceptor input and produces changes in heart rate and blood pressure upon activation (Zhang & Oppenheimer, 1997). Furthermore, electrophysiological studies during pharmacological blood pressure challenges have repeatedly shown a high percentage of sympathoexcitatory neurons within the right posterior insula (Oppenheimer & Cechetto, 1990; Zhang & Oppenheimer, 1997; Zhang *et al.* 1998). Thus, the presence of activation within this region in the present studies was expected.

While the posterior regions of the IC were consistently activated in both Pre and Post sessions, the unique observation was the change in activation pattern in the right anterior IC that differed between the HDBR and diuretic interventions. The deactivation of the anterior portions of both the left and the right IC following diuretic was observed only at -15 mmHg (not at -35 mmHg), and the statistical threshold was generous in the ROI analysis, being 0.01 for the left IC. Therefore, we hesitate to put too much emphasis on the functional implications of this region in the context of the present study. Nonetheless, stimulation of the bilateral anterior insular cortex in humans was associated more with decreases than increases in HR (Oppenheimer *et al.* 1992). In this light, the novel expression of diuretic-induced deactivation of a region

that actively affects bradycardia is consistent with a higher HR.

In contrast to diuretic, HDBR resulted in greater anterior IC activation. This region is proposed to be associated with HR regulation during emotive responses (Lane *et al.* 2009). However, it is unlikely that the anterior IC is the sole determinant of HR control. Rather, the greater IC activation following HDBR occurred concurrently with deactivation in the subgenual ACC region and increased activation within the genual ACC. It is noteworthy that the coactivation patterns of the anterior IC and subgenual ACC fit well with the recent interpretation that these regions form an axis of conjoint activity such that the anterior IC integrates sensory information and the ACC co-ordinates the preparation and execution of an autonomic response to the sensation (Medford & Critchley, 2010). Inasmuch as the subgenual and adjoining ventral medial prefrontal regions are involved in cardiac slowing, the present results implicate this anterior IC–subgenual ACC axis as an important determinant of augmented cardiovascular arousal in response to baroreceptor unloading following a brief period of deconditioning.

Limitations

It is possible that the observed patterns of neural activity measured during the LBNP protocol are related to the central processing of environmental, emotional and/or sensory stimuli rather than modulation of efferent autonomic cardiovascular control. Events related to environmental stress were reduced by studying subjects who had participated in previous functional magnetic resonance imaging experiments, and a subset of them performed a repeat neuroimaging session in the present study. None of the participants reported any adverse feelings of emotional stress or subjective arousal during the scanning periods. In addition, it is not known whether the cortical activation patterns during LBNP reflect cardiac sensory inputs rather than efferent signals that modify autonomic cardiovascular control. Our recent studies (Goswami *et al.* 2011) indicate that somatosensory signals produce activation patterns within CAN regions of interest. However, these activation patterns were in a direction that was opposite to that observed during manoeuvres that elicit cardiovascular arousal. Similar studies on cardiac sensory signals have not been reported. Furthermore, some non-specific cortical responses may be elicited by altered states of awareness or even altered visual or auditory stimuli during the LBNP trials. These may explain the observed activation patterns within the visual and lingual cortices that are not reported to be associated with autonomic or cardiovascular arousal.

Unfortunately, it was not possible to assess blood pressure or cardiac output during these fMRI scanning sessions. Also, conducting pre-fMRI laboratory-based measures was not possible in the Post HDBR studies, because such studies may act as a countermeasure, effectively removing the HDBR effect. Thus, it remains possible that the hypovolaemia would result in greater decrements in both stroke volume and pulse pressure during LBNP (Pawelczyk *et al.* 2001; Kimmerly & Shoemaker, 2002) in the Post *versus* Pre tests, producing a larger baroreflex stimulus for the same level of LBNP. Nonetheless, the change in HR with −15 mmHg LBNP appeared to be affected more with HDBR than with diuretic alone, and adaptations in the cortical regions of interest were affected differently by the HDBR and diuretic interventions. These changes are not consistent with greater baroreceptor unloading in the Post diuretic condition compared with Post HDBR.

In addition, reductions in plasma volume may affect the distribution of blood volume within the brain, and in turn, the BOLD response reflecting neural activity. The BOLD signal reflects, in large part, the relative concentration of deoxyhaemoglobin in the postcapillary venuoles. Reductions in cerebral venous volume are observed with levels of LBNP used in this study, with little change in arterial inflow (Wilson *et al.* 2005). Inasmuch as the BOLD response represents a change from some baseline level of regional activation, a generalized change in baseline blood flow or volume distribution could affect the ability to detect a change in BOLD. However, such an effect should manifest itself as global BOLD signal change throughout the cerebral cortex. The finding of specific, lateralized and reproducible cortical activation patterns that were correlated with the magnitude of baroreceptor unloading imply that the BOLD signal changes reported in this study result from the recruitment of central autonomic centres related to the baroreflex and not from overall changes in cerebral haemodynamics. Moreover, if plasma volume change exerted an independent and dominant impact on the BOLD signal, then the impact of HDBR and diuretic on cortical activation patterns during LBNP should have been the same; this was not the case.

Finally, this study required repeated measures in the fMRI environment, raising the question about reproducibility of BOLD responses to LBNP. Previously, we demonstrated that responses within the regions of the cortical autonomic network are reproducible (Kimmerly *et al.* 2005). In fact, it is only the reproducible regions that were considered in our masking procedure. This approach, the consistent demonstration of these regions in whole-brain analysis approaches, and the scaling of the same network regions with autonomic responses in the same individuals (Kimmerly & Shoemaker, 2002; Kimmerly *et al.* 2005, 2007*a*,*b*) provide confidence that the changes observed here reflect acute malleability of the cortical autonomic network.

Perspectives

The mechanism(s) mediating neurological changes with physical activity patterns have become an emerging focus of neuroscientists, particularly in the context of an ageing population with enhanced disease burden. Ageing and cardiovascular disease are associated with impaired reflex cardiovascular control that can be reversed with exercise training (Monahan *et al.* 2000). Exercise training also improves cortical activation patterns related to cognitive tasks such as memory (Colcombe *et al.* 2004). To date, inference has been made to the potential role of supramedullary sites in the alterations to baroreflex cardiovascular control that attend physical deconditioning, but the studies have not been performed. The present observations provide new evidence that parts of the brain known to be involved in cardiovascular control are sensitive to physical activity patterns, namely the insula–ACC axis. In this regard, they offer explanatory detail to earlier observations of rapid impairment of baroreflex blood pressure control with merely 4 h of head-down tilt bed rest (Butler *et al.* 1991) and address earlier work by Hasser & Moffitt (2001), who inferred an impact of hindlimb-unweighted deconditioning on supramedullary modulation of brainstem autonomic pathways. Therefore, the collective outcomes suggest that physical activity patterns exert a rapid and robust impact on the cortical circuitry associated with cardiovascular control.

Summary

The present study indicates that the cortical autonomic network can undergo a rapid functional change in response to 24 h of head-down bed rest, with consequences for cardiovascular control during orthostatic stress. Both HDBR and hypovolaemia modified heart rate responses to mild and moderate levels of orthostatic stress. Moreover, these changes in HR regulation with HDBR, but not diuretic treatment, were associated with alterations in the ACC and insular cortex regions. Thus, the results support the idea that functionally relevant changes in the cortical network associated with baroreflex-mediated cardiovascular control can occur within a 24 h time frame. These modifications in cortical patterns appear to contribute to adaptive changes in cardiovascular regulation following deconditioning in a manner that is specific to HDBR and separate from hypovolaemia.

References

Arbeille P, Kerbeci P, Mattar L, Shoemaker JK & Hughson R (2008*a*). Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest. *Am J Physiol Heart Circ Physiol* **295**, H1846–H1854.

Arbeille P, Kerbeci P, Mattar L, Shoemaker JK & Hughson RL (2008*b*). WISE-2005: tibial and gastrocnemius vein and calf tissue response to LBNP after a 60-day bed rest with and without countermeasures. *J Appl Physiol* **104**, 938–943.

Barberi EA, Gati JS, Rutt BK & Menon RS (2000). A transmit-only/receive-only (TORO) RF system for high-field MRI/MRS applications. *Magn Reson Med* **43**, 284–289.

Brum PC, Da Silva GJ, Moreira ED, Ida F, Negrão CE & Krieger EM (2000). Exercise training increases baroreceptor gain sensitivity in normal and hypertensive rats. *Hypertension* **36**, 1018–1022.

Buch AN, Coote JH & Townend JN (2002). Mortality, cardiac vagal control and physical training – what's the link? *Exp Physiol* **87**, 423–435.

Butcher KS & Cechetto DF (1995). Insular lesion evokes autonomic effects of stroke in normotensive and hypertensive rats. *Stroke* **26**, 459–465.

Butler GC, Xing H, Northey DR & Hughson RL (1991). Reduced orthostatic tolerance following 4 h head-down tilt. *Eur J Appl Physiol* **62**, 26–30.

Carey LC, Curtin R & Sapira JD (1976). Influence of hemorrhage on adrenal secretion, blood glucose and serum insulin in the awake pig. *Ann Surg* **183**, 185–192.

Cechetto DF & Saper CB (1990). Role of the cerebral cortex in autonomic function. In *Central Regulation of Autonomic Functions*, ed. Loewy AD & Spyer KM, pp. 208–223. Oxford University Press, New York.

Colcombe SJ, Kramer AF, Erickson KI, Scalf P, McAuley E, Cohen NJ, Webb A, Jerome GJ, Marquez DX & Elavsky S (2004). Cardiovascular fitness, cortical plasticity, and aging. *Proc Natl Acad Sci USA* **101**, 3316–3321.

Convertino VA, Bloomfield SA & Greenleaf JE (1997*a*). An overview of the issues: physiological effects of bed rest and restricted physical activity. *Med Sci Sports Exerc* **29**, 187–190.

Convertino VA, Polet JL, Engelke KA, Hoffler GW, Lane LD & Blomqvist CG (1997*b*). Evidence for increased β-adrenoreceptor responsiveness induced by 14 days of simulated microgravity in humans. *Am J Physiol* **273**, R39–R99.

Convertino VA & Sather TM (2000). Effects of cholinergic and beta-adrenergic blockade on orthostatic tolerance in healthy subjects. *Clin Auton Res* **10**, 327–336.

Convertino VA, Thompson CA, Eckberg DL, Fritsch JM, Mack GW & Nadel ER (1990). Baroreflex responses and LBNP tolerance following exercise training. *Physiologist* **33**, S40–S41.

Cooke WH, Reynolds BV, Yandl MG, Carter JR, Tahvanainen KU & Kuusela TA (2002). Effects of exercise training on cardiovagal and sympathetic responses to Valsalva's maneuver. *Med Sci Sports Exerc* **34**, 928–935.

Critchley HD, Corfield DR, Chandler MP, Mathias CJ & Dolan RJ (2000). Cerebral correlates of autonomic cardiovascular arousal: a functional neuroimaging investigation in humans. *J Physiol* **523**, 259–270.

Critchley HD, Mathias CJ, Josephs O, O'Doherty J, Zanini S, Dewar BK, Cipolotti L, Shallice T & Dolan RJ (2003). Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain* **126**, 2139–2152.

Critchley HD, Wiens S, Rotshtein P, Ohman A & Dolan RJ (2004). Neural systems supporting interoceptive awareness. *Nat Neurosci* **7**, 189–195.

DiCarlo SE & Bishop VS (1990). Exercise training enhances cardiac afferent inhibition of baroreflex function. *Am J Physiol* **258**, H212–H220.

Eckberg DL & Fritsch JM (1992). Influence of ten-day head-down bedrest on human carotid baroreceptor-cardiac reflex function. *Acta Physiol Scand Suppl* **144**, 69–76.

Edgell H, Zuj KA, Greaves DK, Shoemaker JK, Custaud MA, Kerbeci P, Arbeille P & Hughson RL (2007). WISE-2005: adrenergic responses of women following 56-days, 6 degrees head-down bed rest with or without exercise countermeasures. *Am J Physiol Regul Integr Comp Physiol* **293**, R2343–R2352.

Fagius J & Karhuvaara S (1989). Sympathetic activity and blood pressure increases with bladder distension in humans. *Hypertension* **14**, 511–517.

Ferguson DW, Thames MD & Mark AL (1983). Effects of propranolol on reflex vascular responses to orthostatic stress in humans: role of ventricular baroreceptors. *Circulation* **67**, 802–807.

Fischer D, Arbeille P, Shoemaker JK, O'Leary DD & Hughson RL (2007). Altered hormonal regulation and blood flow distribution with cardiovascular deconditioning after short-duration head down bed rest. *J Appl Physiol* **103**, 2018–2025.

Gianaros PJ, van derVeen FM & Jennings JR (2004). Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during working-memory tasks: implications for the cortical and subcortical regulation of cardiac autonomic activity. *Psychophysiology* **41**, 521–530.

Gómez H, Mesquida J, Hermus L, Polanco P, Kim HK, Zenker S, Torres A, Namas R, Vodovotz Y, Clermont G, Puyana JC & Pinsky MR (2012). Physiologic responses to severe hemorrhagic shock and the genesis of cardiovascular collapse: can irreversibility be anticipated? *J Surg Res*. doi:10.1016/j.jss.2011.12.015

Goswami R, Frances MF & Shoemaker JK (2011). Representation of somatosensory inputs within the cortical autonomic network. *Neuroimage* **54**, 1211–1220.

Greenleaf JE, Morse JT, Barnes PR, Silver J & Keil LC (1983). Hypervolemia and plasma vasopressin response during water immersion in men. *J Appl Physiol* **55**, 1688–1693.

Hasser EM & Moffitt JA (2001). Regulation of sympathetic nervous system function after cardiovascular deconditioning. *Ann N Y Acad Sci* **940**, 454–468.

Hirayanagi K, Kamiya A, Iwase S, Mano T, Sasaki T, Oinuma M & Yajima K (2004). Autonomic cardiovascular changes during and after 14 days of head-down bed rest. *Auton Neurosci* **110**, 121–128.

Hughson RL, Maillet A, Gharib C, Fortrat JO, Yamamoto Y, Pavy-Letraon A, Riviere D & Guell A (1994*a*). Reduced spontaneous baroreflex response slope during lower body negative pressure after 28 days of head-down bed rest. *J Appl Physiol* **17**, 69–77.

Hughson RL, Yamamoto Y, Maillet A, Fortrat JO, Traon AP-L, Butler GC, Güell A & Gharib C (1994*b*). Altered autonomic regulation of cardiac function during head-up tilt after 28-day head-down bed-rest with counter-measures. *Clin Physiol* **14**, 291–304.

Kimmerly DS, O'Leary DD, Menon RS, Gati JS & Shoemaker JK (2005). Cortical regions associated with autonomic cardiovascular regulation during lower body negative pressure in humans. *J Physiol* **569**, 331–345.

Kimmerly DS & Shoemaker JK (2002). Hypovolemia and neurovascular control during orthostatic stress. *Am J Physiol Heart Circ Physiol* **282**, H645–H655.

Kimmerly DS, Wong S, Menon R & Shoemaker JK (2007*a*). Forebrain neural patterns associated with sex differences in autonomic and cardiovascular function during baroreceptor unloading. *Am J Physiol Regul Integr Comp Physiol* **292**, R715–R722.

Kimmerly DS, Wong SW, Salzer D, Menon R & Shoemaker JK (2007*b*). Forebrain regions associated with postexercise differences in autonomic and cardiovascular function during baroreceptor unloading. *Am J Physiol Heart Circ Physiol* **293**, H299–H306.

Klassen LM & Menon RS (2004). Robust automated shimming technique using arbitrary mapping acquisition parameters (RASTAMAP). *Magn Reson Med* **51**, 881–887.

Lane RD, McRae K, Reiman EM, Chen K, Ahern GL & Thayer JF (2009). Neural correlates of heart rate variability during emotion. *Neuroimage* **44**, 213–222.

Medford N & Critchley HD (2010). Conjoint activity of anterior insular and anterior cingulate cortex: awareness and response. *Brain Struct Funct* **214**, 535–549.

Moffitt JA, Foley CM, Schadt JC, Laughlin MH & Hasser EM (1998). Attenuated baroreflex control of sympathetic nerve activity after cardiovascular deconditioning in rats. *Am J Physiol* **274**, R1397–R1405.

Moffitt JA, Schadt JC & Hasser EM (1999). Altered central nervous system processing of baroreceptor input following hindlimb unloading in rats. *Am J Physiol* **277**, H2272–H2279.

Monahan KD, Dinenno FA, Tanaka H, Clevenger CM, DeSouza CA & Seals DR (2000). Regular aerobic exercise modulates age-associated declines in cardiovagal baroreflex sensitivity in healthy men. *J Physiol* **529**, 263–271.

Mueller PJ (2007). Exercise training and sympathetic nervous system activity: evidence for physical activity dependent neural plasticity. *Clin Exp Pharmacol Physiol* **34**, 377–384.

Oppenheimer SM & Cechetto DF (1990). Cardiac chronotropic organization of the rat insular cortex. *Brain Res* **533**, 66–72.

Oppenheimer SM, Gelb A, Girvin JP & Hachinski VC (1992). Cardiovascular effects of human insular cortex stimulation. *Neurology* **42**, 1727–1732.

Pannier B, Slama M, Guérin A, Martel E, London G, Safar M & Cuche JL (1998). Further study on the carotid baroreflex system in the cardiovascular deconditioning induced by head-down tilt. *Aviat Space Environ Med* **69**, 904–910.

Pannier BM, Lacolley PJ, Gharib C, London GM, Cuche JL, Duchier JL, Levy BI & Safar ME (1991). Twenty-four hours of bed rest with head-down tilt: venous and arteriolar changes of limbs. *Am J Physiol* **260**, H1043–H1050.

Pawelczyk JA, Zuckerman JH, Blomqvist CG & Levine BD (2001). Regulation of muscle sympathetic nerve activity after bed rest deconditioning. *Am J Physiol Heart Circ Physiol* **280**, H2230–H2239.

Pomeranz B, Macaulay JB, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ & Benson H (1985). Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* **248**, H151–H153.

Pool JL & Ransohoff J (1949). Autonomic effects on stimulating rostral portion of cingulate gyri in man. *J Neurophysiol* **12**, 385–392.

Shoemaker JK, Hogeman CS & Sinoway LI (1999). Contributions of MSNA and stroke volume to orthostatic intolerance following bed rest. *Am J Physiol* **277**, R1084–R1090.

Taylor JA, Myers CW, Halliwill JR, Seidel H & Eckberg DL (2001). Sympathetic restraint of respiratory sinus arrhythmia: implications for vagal-cardiac tone assessment in humans. *Am J Physiol Heart Circ Physiol* **280**, H2804–H2814.

Thayer JF, Ahs F, Fredrikson M, Sollers JJ 3rd & Wager TD (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci Biobehav Rev* **36**, 747–756.

Verberne AJ & Owens NC (1998). Cortical modulation of the cardiovascular system. *Prog Neurobiol* **54**, 149–168.

Wilson TD, Shoemaker JK, Kozak R, Lee TY & Gelb AW (2005). Reflex-mediated reduction in human cerebral blood volume. *J Cereb Blood Flow Metab* **25**, 136–143.

Wong SW, Masse N, Kimmerly DS, Menon RS & Shoemaker JK (2007). Ventral medial prefrontal cortex and cardiovagal control in conscious humans. *Neuroimage* **35**, 698–708.

Yasui Y, Breder CD, Saper CB & Cechetto DF (1991). Autonomic responses and efferent pathways from the insular cortex in the rat. *J Comp Neurol* **303**, 355–374.

Zhang Z & Oppenheimer SM (1997). Characterization, distribution and lateralization of baroreceptor-related neurons in the rat insular cortex. *Brain Res* **760**, 243–250.

Zhang Z & Oppenheimer SM (2000*a*). Electrophysiological evidence for reciprocal insulo-insular connectivity of baroreceptor-related neurons. *Brain Res* **863**, 25–41.

Zhang ZH, Dougherty PM & Oppenheimer SM (1998). Characterization of baroreceptor-related neurons in the monkey insular cortex. *Brain Res* **796**, 303–306.

Zhang ZH & Oppenheimer SM (2000*b*). Baroreceptive and somatosensory convergent thalamic neurons project to the posterior insular cortex in the rat. *Brain Res* **861**, 241–256.

Acknowledgements

This study was supported by the Canadian Space Agency (contract no. 9F007-046020/001/ST).