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Forebrain neural patterns associated with sex differences in autonomic and cardiovascular function during baroreceptor unloading

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Kimmerly DS, Wong S, Menon R, Shoemaker JK. 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, 2007. First published July 6, 2006; doi:10.1152/ajpregu.00366.2006.—Generally, women demonstrate smaller autonomic and cardiovascular reactions to stress, compared with men. The mechanism of this sex-dependent difference is unknown, although reduced baroreflex sensitivity may be involved. Recently, we identified a cortical network associated with autonomic cardiovascular responses to baroreceptor unloading in men. The current investigation examined whether differences in the neural activity patterns within this network were related to sex-related physiological responses to lower body negative pressure (LBNP, 5, 15, and 35 mmHg). Forebrain activity in healthy men and women (n = 8 each) was measured using functional magnetic resonance imaging with blood oxygen level-dependent (BOLD) contrast. Stroke volume (SV), heart rate (HR), and muscle sympathetic nerve activity (MSNA) were collected on a separate day. Men had larger decreases in SV than women (P < 0.01) during 35 mmHg LBNP only. At 35 mmHg LBNP, HR increased more in males than females (9 ± 1 beats/min vs. 4 ± 1 beats/min, P < 0.05). Compared with women, increases in total MSNA were similar at 15 mmHg LBNP but greater during 35 mmHg LBNP in men [1,067 ± 123 vs. 658 ± 103 arbitrary units (au), P < 0.05]. BOLD signal changes (P < 0.005, uncorrected) were identified within discrete forebrain regions associated with these sex-specific HR and MSNA responses. Men had larger increases in BOLD signal within the right insula and dorsal anterior cingulate cortex than women. Furthermore, men demonstrated greater BOLD signal reductions in the right amygdala, left insula, ventral anterior cingulate, and ventral medial prefrontal cortex vs. women. The greater changes in forebrain activity in men vs. women may have contributed to the elevated HR and sympathetic responses observed in men during 35 mmHg LBNP.

autonomic nervous system; baroreflex; microneurography; functional neuroimaging

The physiological responses to a reduction in central blood volume involve baroreflex-mediated increases in heart rate (HR) and vascular resistance to adequately maintain arterial pressure and cerebral perfusion. Compared with men, reduced orthostatic tolerance has been observed in women (7, 18), and this has been associated with differential (14, 36) autonomic and cardiovascular responses to baroreceptor unloading. In general, women respond to an orthostatic challenge with a greater HR increase, whereas men have larger increases in sympathetic vasconstrictor activity and vascular resistance. The underlying mechanisms responsible for these varied responses are unclear but may be influenced by differences in the central processing and modulation of baroreceptor afferent information.

Although the medulla oblongata remains the primary regulatory site of cardiovascular function, there is mounting evidence to suggest that higher cortical regions modulate efferent autonomic outflow (6, 20–23, 33). Nonetheless, the central neural network responsible for the regulation of baroreflex-mediated autonomic and cardiovascular function in humans is relatively unknown. However, the rapid advancements in functional magnetic resonance imaging (fMRI) techniques have provided an opportunity to explore the cortical structures associated with cardiovascular regulation in conscious humans. Using this approach, we have recently characterized a discrete cortical autonomic network associated with cardiovascular regulation during lower body negative pressure (LBNP) in young healthy men (22). The specific forebrain regions associated with the magnitude of baroreceptor unloading and the resultant reflex adaptations included the bilateral insular cortex, anterior cingulate cortex (ACC), medial prefrontal cortex (MPFC), and the amygdala. These forebrain structures have also been implicated in the modulation of cardiovascular reactions to volitional tasks including exercise (9, 42), the Valsalva maneuver (20), and mental stress (9).

The present study tested the hypothesis that physiological differences in the response to baroreceptor unloading between men and women are related to corresponding changes in the activity patterns within these forebrain regions associated with autonomic control of cardiovascular regulation. To examine this hypothesis, functional neuroimaging procedures were used in combination with separately recorded HR and microneurographic measures of muscle sympathetic nerve activity (MSNA) during LBNP to elucidate the forebrain regions associated with the modulation of these efferent responses.

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METHODS

Subjects. Eight women and eight men, matched for age, provided voluntary signed consent for the current investigation that was approved by the University of Western Ontario Health Sciences Ethics Review Board. Previously, physiological and neuroimaging data were reported from the eight men (22). All subjects were normotensive and none were endurance-trained athletes. The physical characteristics of each group are presented in Table 1. Each subject completed a magnetic resonance imaging readiness questionnaire to ensure safe comparability within a high magnetic field environment. No subjects smoked, exhibited a history of autonomic dysfunction, or were taking any cardiovascular-altering medications. None of the women were pregnant during the study, and all used oral contraceptives. To minimize potential variability in responses associated with the menstrual cycle, women were tested within the first 6 days after the start of menses (i.e., follicular phase).

Experimental protocol. Each subject participated in two experimental sessions that were randomly assigned and separated by a minimum of 7 days. One session was dedicated to the collection of the functional neuroimaging data and the other to physiological measurements. Each experiment was performed at the same time of day ≥3 h after a light meal and ≥24 h after exercise or consumption of caffeinated products and alcoholic beverages. The LBNP protocol used for both test sessions consisted of three randomly assigned levels of decompression at 5, 15, and 35 mmHg. Subjects were positioned within the LBNP chamber for a minimum of 30 min before the collection of baseline measures. A sequence of repeated (2–4) LBNP exposures (45–60 s duration) were used separated by intervening rest periods (30–90 s). For each subject, the same LBNP protocol was used in both neuroimaging and physiological tests. For the neuroimaging sessions, a specifically constructed LBNP chamber and an antishock trouser (David Clark, Worcester, MA, USA) system was used to minimize subject head movement during scanning (22).

Physiological data collection and analyses. Peripheral venous pressure estimates of central venous pressure (CVP) were determined using the method of Gauer and Sieker (15). Subjects were placed into a modified right lateral decubitus position with the arm ~15 cm below the heart. In this position, a 20-gauge catheter was inserted into an antecubital vein of the right arm and connected to a pressure transducer (Edwards Lifesciences, Irvine, CA) for assessment of peripheral venous pressure. The pressure transducer was positioned at heart level and kept patent with isotonic saline. HR was determined by standard three-lead electrocardiogram methods. Cardiac stroke volume (SV) velocity and aortic dimensions were obtained to calculate cardiac output (CO). Aortic diameters were determined using two-dimensional B-mode echo Doppler images (2.5-MHz probe, GE/Vingmed System Five) with a parasternal long-axis view of the aorta. SV velocity was measured from the suprasternal notch using a hand-held 2-MHz pulsed wave probe (Vingmed CM750). Beat-by-beat measures of arterial blood pressure (ABP) were obtained using finger photoplethysmographic techniques (Finapres, Ohmeda Medical, Columbia, MD) with the hand held at heart level. These blood pressure measures were corrected against sphygmomanometrically collected systolic and diastolic pressures intermittently throughout the experiment.

Multifrequency recordings of postganglionic MSNA were recorded in 14 subjects (7 men and 7 women). Tungsten microelectrodes were inserted percutaneously into muscle fascicles of the right common peroneal nerve with a reference electrode positioned subcutaneously 1–3 cm from the recording site (19). Neural activity was amplified 1,000 times by a preamplifier and an additional 75 times through a variable-gain isolated amplifier. The signal was band-pass filtered (0.7–2.0 kHz), full-wave rectified and integrated with a resistance-capacitance circuit (0.1-s time constant). Criteria for an acceptable MSNA recording included pulse-synchrony with the cardiac cycle, increased activity to a voluntary apnea but not to emotional arousal (i.e., a loud noise).

Analog signals for CVP, ABP, and MSNA were sampled at 200 Hz, and ECG was sampled at 400 Hz for online data acquisition and analysis (PowerLab, AD Instruments, Castle Hill, NSW, Australia). Mean arterial pressure (MAP) was calculated as diastolic pressure + 1/3 pulse pressure. HR was determined from the beat-to-beat intervals between successive R-waves of the ECG. Stroke volume was calculated as the product of SV velocity and the aortic cross-sectional area for the mean cardiac interval. To compensate for sex differences in body mass and blood volume (7), SV was normalized to body surface area (SVi) for all subjects. Normalized cardiac output (Qi) was calculated as HR × SVi. Total peripheral resistance (TPRi) was calculated as the quotient of MAP and Qi. The normalization procedure should not affect interpretation of the LBNP-mediated reductions in stroke volume or cardiac output as the absolute changes in these parameters predominately influence the normalized responses.

Only MSNA bursts with characteristic rising and falling slopes and amplitudes with a 2:1 or greater signal-to-noise ratio were measured for amplitude/burst and frequency/min. The largest burst recorded during each session was assigned an amplitude value of “100”, and all remaining amplitudes were normalized as a relative percentage of this maximum amplitude. Total MSNA was calculated as the sum of relative burst amplitudes/min. Baseline data were averaged over the 2- to 5-min period before the first applied level of LBNP. Peak HR changes within each repetition of lower body suction were determined, and the mean of these responses was used for statistical analysis. LBNP-mediated changes in CVP and MSNA were determined over the first 45-s period of suction and the average over all repetitions was calculated.

Neuroimaging data collection and analyses. All imaging was conducted using a 4 Tesla Varian Unity Inova (Palo Alto, CA) whole-body MRI system equipped with a Siemens Sonata Gradient chain (Siemens, Erlangen, Germany). Subjects lay supine on the

Table 1. Group physical characteristics and resting hemodynamic and sympathetic data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
<th>T Value</th>
<th>df</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24 (2)</td>
<td>26 (2)</td>
<td>−1.04</td>
<td>14</td>
<td>0.27</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 (6)</td>
<td>166 (7)</td>
<td>3.54</td>
<td>14</td>
<td>0.004</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75 (6)</td>
<td>64 (6)</td>
<td>3.65</td>
<td>14</td>
<td>0.003</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>91 (3)</td>
<td>84 (5)</td>
<td>3.28</td>
<td>14</td>
<td>0.006</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>55 (4)</td>
<td>57 (6)</td>
<td>−0.35</td>
<td>14</td>
<td>0.35</td>
</tr>
<tr>
<td>SVi, ml/m²</td>
<td>55 (7)</td>
<td>50 (6)</td>
<td>0.62</td>
<td>14</td>
<td>0.51</td>
</tr>
<tr>
<td>COi, min⁻¹·m⁻²</td>
<td>3.0 (0.5)</td>
<td>2.8 (0.5)</td>
<td>1.70</td>
<td>14</td>
<td>0.12</td>
</tr>
<tr>
<td>TPRi, mmHg·min⁻¹·m⁻²</td>
<td>30 (2)</td>
<td>30 (4)</td>
<td>0.36</td>
<td>14</td>
<td>0.74</td>
</tr>
<tr>
<td>MSNA burst frequency, bursts/min</td>
<td>16 (7)</td>
<td>14 (8)</td>
<td>0.59</td>
<td>12</td>
<td>0.57</td>
</tr>
<tr>
<td>Total MSNA, a.u.</td>
<td>64 (25)</td>
<td>572 (285)</td>
<td>0.45</td>
<td>12</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Values are means and (SD). MAP, mean arterial pressure; HR, heart rate; SVi, normalized stroke volume; COi, normalized cardiac output; TPRi, normalized total peripheral resistance; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units; df, degrees of freedom.
scanning bed in the LBNP chamber, and foam pads were placed on either side of the head to minimize movement during scanning. A total of 133 brain image volumes were collected for each LBNP session using a gradient echo planar imaging pulse sequence (21 interleaved contiguous axial slices, 64 × 64 pixel resolution, 19.2 × 19.2 cm in-plane field of view (FOV), echo time (TE) of 12 ms, flip angle of 45°, time to repetition (TR) of 0.625 s and 4 segments per slice, thus achieving a total volume acquisition time of 2.5 s and voxel sizes of 3 × 3 × 5 mm thick). A high-resolution T1-weighted anatomical image covering the entire brain was also acquired (64 slices, 256 × 256 pixels, FOV = 19.2 × 19.2 cm, TE = 5.5 ms, inversion time of 600 ms and TR = 10 ms for a voxel size of 0.75 × 0.75 × 2.5 mm thick).

All neuroimaging data were processed using statistical parametric mapping software (SPM2, Wellcome Department of Cognitive Neurology). Complete details of the functional imaging analysis have previously been reported (22). Briefly, all functional image volumes were realigned to the first volume collected. A mean functional image was created using the realigned volumes and coregistered to the anatomical image. For optimal coregistration, these images were bias corrected, skull and scalp stripped (37). The functional images were spatially normalized into a fixed stereotactic space using the Montreal Neurological Institute-152 template. To suppress noise and effects due to intersubject differences in functional and cortical anatomy, these image volumes were spatially normalized with a Gaussian kernel set at 8 mm full width at half maximum. Furthermore, all functional images were high-pass filtered, corrected for serial correlations [autoregressive model, AR(1)] and normalized for global activations before statistical inference.

Statistical analyses. Unless otherwise stated, all data are expressed as means (SD). Differences in subject characteristics were evaluated using unpaired t-tests. All physiological variables were analyzed using a repeated-measures 2-way (group × LBNP) ANOVA. Tukey’s post hoc analysis was performed to estimate differences among means. Probability levels during multiple pointwise comparisons were corrected using Bonferroni’s approach. Statistical analyses for all cardiovascular MSNA data were performed using a computer-based software program (SAS, Cary, NC). A critical significance level of P < 0.05 was set for all cardiovascular and sympathetic comparisons.

A two-level statistical paradigm was used for all functional neuroimaging data. First, a within-subject analysis was performed to identify differences in signal intensity between baseline and LBNP periods. The change in BOLD signal over repeated LBNP exposures was modeled using a canonical hemodynamic response function. This resulted in subject-specific contrast images containing whole brain information related to both sites of increased and decreased activation patterns during LBNP relative to baseline. At the second level of analysis, these contrast images were included in a between-subjects mixed effects ANOVA. A combination of global conjunction and inclusive masking procedures were used to identify significant (P < 0.005, uncorrected for multiple comparisons) changes in signal intensity that corresponded with sex-specific HR and MSNA responses measured during LBNP (i.e., group × LBNP interaction effects). To reduce the risk of reporting false-positive results, a minimum cluster threshold of 10 voxels was included. Significant voxels were color-coded for t-score and overlaid onto a spatially normalized anatomical template. The effect size, representing the mean ± SE percent change in BOLD signal from baseline was calculated for each significant cluster. All fMRI data are represented in a neurological convention (i.e., subject’s left appears on the left).

On the basis of our first fMRI investigation in men (22) and previous data highlighting the role of discrete cortical structures associated with central cardiovascular control (6, 9, 10, 21, 23), we focused on signal intensity changes within specific forebrain regions. These a priori anatomical regions of interest (ROI) included the bilateral insular cortex, ACC, MPFC, and the amygdala. All ROI normalized masks were generated using the WFU_PickAtlas software program (ver. 1.04) (26).

RESULTS

Subject characteristics and resting supine physiology. A summary of the descriptive data for men and women are presented in Table 1. Women had lower (P < 0.01; Table 1) height, weight, and MAP values at rest. Also, compared with males, the lower (P < 0.02) systolic [119 mmHg (5) vs. 129 mmHg (6)] and diastolic blood pressures [65 mmHg (3) vs. 71 mmHg (3)] contributed to the lower MAP in females. The two groups did not differ in age, normalized SV, CO, or total peripheral resistance (all, P > 0.12; Table 1). However, absolute measures of SV [82 ml/beat (11) vs. 109 ml/beat (10)] and cardiac output [4.70 l/min (0.7) vs. 5.96 l/min (0.6)] were less (P < 0.003) in women than in men. Baseline MSNA burst frequency and total MSNA values were similar between the two groups (P > 0.57; Table 1).

Hemodynamic responses to LBNP. Compared with baseline, CVP, SVi, HR, TPRi, or MAP did not change during 5 mmHg LBNP (P > 0.14). Increases in TPRi and reductions in CVP, SVi, and Q, occurred in both groups during 15 and 35 mmHg LBNP (all parameters, P < 0.03). For all above comparisons, changes during 35 mmHg LBNP were greater than 15 mmHg LBNP (P < 0.04). Steady-state MAP values during 15 and 35 mmHg LBNP were not different (P > 0.23) from supine rest in either group. Importantly, group × LBNP interactions were observed for LBNP-mediated changes in CVP, SVi, and HR (all, P < 0.03). Specifically, women had a greater decrease in CVP than men during 35 LBNP (P = 0.04), whereas SVi decreased (P = 0.004; Fig. 1A) and HR increased (P = 0.001; Fig. 1B) more in men than women during this moderate level of suction.

MSNA responses to LBNP. There was a significant group × LBNP interaction effect (P = 0.04) observed for total MSNA. During 5 mmHg LBNP, neither group increased total MSNA above supine rest values. Men and women increased total MSNA similarly during 15 mmHg LBNP (both P < 0.01). However, the increase in Total MSNA during 35 mmHg LBNP was greater in men than women (P = 0.03; Fig. 1C). This sex-specific difference in the total MSNA response during 35 mmHg LBNP was related to an attenuated increase in both burst frequency [14 bursts/min (6) vs. 18 bursts/min (4); P = 0.08] and burst amplitude [1 au (2) vs. 10 au (3); P < 0.04] in women.

Forebrain BOLD signal responses to LBNP. To expose forebrain structures involved in the modulation of efferent autonomic processes during LBNP, BOLD signal changes associated with the observed group × LBNP interactions for both heart rate and total MSNA were identified. The anatomical locations with BOLD signal changes that corresponded with sex-specific heart rate responses are shown in Table 2 and Fig. 2. During 35 mmHg LBNP, both groups demonstrated increased BOLD signal (from baseline) within the dorsal anterior cingulate cortex (dACC) (Fig. 2A) that was greater in the men (1.43 ± 0.35% than women (0.87 ± 0.33%) (P < 0.002). Conversely, men showed a larger decrease in BOLD signal during 35 mmHg LBNP (P < 0.005) within the ventral anterior cingulate cortex (vACC) (−1.34 ± 0.40% vs. −0.67 ± 0.40%) and the right amygdala (−1.20 ± 0.30% vs. −0.65 ± 0.27%) (Fig. 2, B and C).
Fig. 3. Patterns of increased activation (P) observed for total MSNA changes are shown in Table 3 and changes that matched the group LBNP interaction for all variables at 35 mmHg LBNP. * in both men and women only during 35 mmHg LBNP. There was a group MSNA increased in both groups during 15 and 35 mmHg LBNP. HR increased 35 mmHg lower body negative pressure (LBNP). SV decreased and total Table 2. Significant changes in BOLD signal consistent with sex-specific heart rate changes in forebrain response to LBNP.

Sex differences in forebrain neural activity patterns during LBNP. The comparable physiological adjustments to 15 mmHg LBNP between men and women support previous observations (13), highlighting the lack of sex-based differences in the autonomic and cardiovascular responses to mild levels of orthostatic stress. However, at the higher level of LBNP, the increases in heart rate and MSNA were greater in men than in women. Although a premise of the study protocol was that LBNP induces a similar magnitude of baroreceptor unloading in the two groups, relating these augmented responses in men to a particular baroreflex stimulus variable is multifaceted. During 35 mmHg, women with LBNP had a larger decrease in CVP, whereas men demonstrated a greater drop in SV. Although the mechanisms of these varied responses were not studied, the CVP responses support previous findings that women pool more blood than men during orthostasis (41). Also, the augmented heart rate and sympathetic nerve responses in men could be the result of greater integrated baroreceptor unloading, as suggested by the larger drop in stroke volume. Nonetheless, the current data support earlier conclusions about attenuated cardiogal baroreflex gain (1, 38) and reduced heart rate responses to carotid baroreceptor stimulation (7) in women vs. men. Although cardiogal and sympathetic baroreflex control may be affected by menstrual cycle (8, 28), it is unclear whether this effect can overwhelm the overall difference between male and female orthostatic responses (36).

**DISCUSSION**

With respect to sex differences in the integration of peripheral and central autonomic cardiovascular control during baroreceptor unloading, the primary new findings of this study were as follows: 1) the augmented heart rate response in men vs. women was associated with greater activation within the dorsal anterior cingulate cortex and deactivation of the ventral anterior cingulate cortex and right amygdala, and 2) the elevated sympathetic neural response in men vs. women corresponded with greater BOLD signal increases within the right posterior and anterior insula and decreased neural activity in the ventral medial prefrontal cortex and left anterior insula. These data suggest that sex differences in the sympathetic and cardiovascular reactions to baroreceptor unloading are associated with altered central processing and cortical modulation of baroreceptor afferent information between men and women. To our knowledge, this is the first observation in humans to indicate such close associations between graded responses in autonomic cardiovascular outflow and discrete changes in forebrain activation.

**Sex differences in the physiological response to LBNP.** The comparable physiological adjustments to 15 mmHg LBNP between men and women support previous observations (13), highlighting the lack of sex-based differences in the autonomic and cardiovascular responses to mild levels of orthostatic stress. However, at the higher level of LBNP, the increases in heart rate and MSNA were greater in men than in women. Although a premise of the study protocol was that LBNP induces a similar magnitude of baroreceptor unloading in the two groups, relating these augmented responses in men to a particular baroreflex stimulus variable is multifaceted. During 35 mmHg, women with LBNP had a larger decrease in CVP, whereas men demonstrated a greater drop in SV. Although the mechanisms of these varied responses were not studied, the CVP responses support previous findings that women pool more blood than men during orthostasis (41). Also, the augmented heart rate and sympathetic nerve responses in men could be the result of greater integrated baroreceptor unloading, as suggested by the larger drop in stroke volume. Nonetheless, the current data support earlier conclusions about attenuated cardiogal baroreflex gain (1, 38) and reduced heart rate responses to carotid baroreceptor stimulation (7) in women vs. men. Although cardiogal and sympathetic baroreflex control may be affected by menstrual cycle (8, 28), it is unclear whether this effect can overwhelm the overall difference between male and female orthostatic responses (36).

**Sex differences in forebrain neural activity patterns during LBNP.** In contrast to the mild orthostatic stress (i.e., 15 mmHg LBNP), in which only MSNA increased, the response to 35

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### Table 2. Significant changes in BOLD signal consistent with sex-specific heart rate responses to lower body negative pressure

<table>
<thead>
<tr>
<th>Anatomical Structure</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>T Value</th>
<th>Z Value</th>
<th>Cluster Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal anterior cingulate cortex</td>
<td>4</td>
<td>18</td>
<td>26</td>
<td>3.61</td>
<td>3.35</td>
<td>43</td>
</tr>
<tr>
<td>Decreased activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right amygdala</td>
<td>24</td>
<td>0</td>
<td>-22</td>
<td>3.11</td>
<td>2.94</td>
<td>75</td>
</tr>
<tr>
<td>Ventral anterior cingulate cortex</td>
<td>-2</td>
<td>46</td>
<td>-2</td>
<td>2.84</td>
<td>2.70</td>
<td>90</td>
</tr>
</tbody>
</table>

The x, y, and z values represent the Montreal Neurological Institute coordinates for the voxel with the greatest activation within each cluster.
mmHg LBNP represented a stress that required coordinated parasympathetic and sympathetic nervous system activity. By comparing these two levels of LBNP, it may be possible to link focal cortical regions with discrete parasympathetic vs. sympathetic function. For example, sites of cortical activation that change at 15 mmHg LBNP are likely related specifically to the sympathetic nervous system. These regions were restricted to increased activity in the right dorsal posterior insula and right ventral anterior insula, as well as decreased activity in the left ventral anterior insula and ventral medial prefrontal cortex. At 35 mmHg LBNP, responses within these forebrain structures were enhanced and accompanied by increased activity within the dorsal ACC and decreased activity of the ventral ACC and right amygdala. Therefore, the latter triad appears to be associated primarily with heart rate control, whereas the insular and ventral medial prefrontal regions have more complex involvement with parasympathetic and sympathetic pathways. Given the likelihood that these forebrain regions encompass multiple baroreflex-mediated efferent autonomic processes, the putative regulatory influence of these cortical structures on cardiac function and sympathetic vasomotor outflow are highlighted below.

Studies in rodents have identified the MPFC as a cardiovascular depressor area (31, 39) involved in the regulation of autonomic responses associated with baroreflex activation (32, 40). Depressor responses evoked by stimulation of the MPFC are accompanied by a reduction in the discharge of sympathoexcitatory barosensitive neurons within the rostral ventrolateral medulla (RVLM) (39). In addition, blockade of synaptic transmission within the MPFC attenuates baroreflex-mediated parasympathetic outflow to the heart (32). In the current study, men

Table 3. Significant changes in BOLD signal consistent with sex-specific total muscle sympathetic nerve activity responses to lower body negative pressure

<table>
<thead>
<tr>
<th>Anatomical Structure</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>T Value</th>
<th>Z Value</th>
<th>Cluster Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventral anterior insula</td>
<td>40</td>
<td>14</td>
<td>−2</td>
<td>3.19</td>
<td>3.00</td>
<td>62</td>
</tr>
<tr>
<td>Right dorsal posterior insula</td>
<td>32</td>
<td>−36</td>
<td>18</td>
<td>3.21</td>
<td>3.01</td>
<td>155</td>
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<td>Decreased activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventral anterior insula</td>
<td>−36</td>
<td>8</td>
<td>−6</td>
<td>3.00</td>
<td>2.84</td>
<td>64</td>
</tr>
<tr>
<td>Ventral medial prefrontal cortex</td>
<td>−4</td>
<td>46</td>
<td>−14</td>
<td>3.62</td>
<td>3.36</td>
<td>32</td>
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</tbody>
</table>

The x, y, and z values represent the Montreal Neurological Institute coordinates for the voxel with the greatest activation within each cluster.
decreased activity within the ventral MPFC more than women during 35 mmHg LBNP. The greater deactivation in men was associated with larger heart rate and MSNA responses. These observations are consistent with a depressor role of the ventral MPFC and suggest that this effect is well expressed at baseline. Furthermore, this observation implies that women had an attenuated removal of this sympathoinhibitory or parasympathetic control over cardiac function during LBNP.

The anterior cingulate cortex is considered part of the MPFC and contains separate functional subdivisions. Both the vACC and dorsal ACC (dACC) divisions of the anterior cingulate have been implicated with the modulation of autonomic processes (2, 12). Both stimulation and neuroimaging studies indicate differential roles of these ACC subdivisions in the control of cardiac function (22). Recently, we observed decreased activation within the ventral ACC associated with higher heart rates, whereas Critchley et al. (10) demonstrated increased activity within the dorsal ACC related to sympathetic control of the heart. Furthermore, these results can be extended to nonbaroreflex control of cardiac function, as elevations in heart rate elicited by stressful cognitive tasks have also been associated with increased dorsal ACC and decreased ventral

Fig. 3. BOLD signal activity patterns within the right dorsal posterior insula (A), right ventral anterior insula (B), left ventral anterior insula (C), and ventral medial prefrontal cortex (MPFC) (D), consistent with sex-specific changes in total MSNA during LBNP. The bar chart on the right of each figure represents the effect size (i.e., percentage of signal change) at the particular region specified by the crosshair in the figure. *P < 0.005 vs. 5 LBNP, †P < 0.005 vs. 15 LBNP, §P < 0.005 group × LBNP interaction. For specific MNI coordinates, please refer to Table 3.
ACC activity (17, 27). The larger BOLD signal increase in the dorsal ACC and greater deactivation of the ventral ACC in males vs. females during 35 mmHg LBNP associated with an augmented heart rate response in men supports these previous observations. Taken together, these results suggest that sympathetic vs. parasympathetic control of the heart is modulated by synaptic relays through the dorsal and ventral subdivisions of the ACC, respectively.

The amygdala has anatomical projections to autonomic cardiovascular control sites, including the parabrachial nucleus, nucleus of the solitary tract, RVLM, and dorsal motor nucleus of the vagus (4, 11, 34, 35) and has been shown to receive baroreceptor input (3). Previously, electrical stimulation of the amygdala in rats produced hypotension associated with the inhibition of presympathetic neurons in the RVLM (16). This pathway was supported by our previous study (22), in which decreased neural activity within the amygdala during baroreceptor unloading was associated with increases in both heart rate and MSNA (i.e., potential disinhibition of RVLM neurons). An example of how the gender effect may help identify a functional role of this region is highlighted by the current observation that men deactivated the amygdala more than women but had a greater heart rate increase during LBNP. This highlights the possibility that this forebrain structure influences parasympathetic cardiac control, a possibility that is supported by identified projections from the amygdala to autonomic-related nuclei within the brainstem, including the dorsal vagal complex (11). Assuming this pathway exists in humans, our data indicate that the amygdala might be involved with the removal of tonic inhibitory parasympathetic influence over the heart during baroreceptor unloading. Therefore, the larger deactivation of the amygdala in men vs. women may have resulted in a further removal of tonic inhibitory input to the RVLM and/or vagal control centers of the heart, thereby contributing to the augmented HR and MSNA responses observed during 35 mmHg LBNP in men.

Among the forebrain structures responsible for the modulation of baroreceptor reflex action, the insular cortex has received the most focus (5, 33, 43). The role of the insular cortex in autonomic regulation appears to be lateralized, with the left side predominantly mediating depressor responses and the right insula regulating pressor actions (29, 30). During 35 mmHg LBNP, men responded with augmented MSNA and HR increases than women. These autonomic adjustments were associated with greater deactivation of the left insula and activation of the anterior and posterior right insula. These data, together with our previous findings that right insular activation increased during baroreflex-mediated elevations in heart rate (22), supports a generalized sympathoexcitatory role for the right insular cortex.

Stimulation of the left insular cortex in humans is associated with bradycardia and depressor responses (29). Moreover, stroke damage to the left insula has been linked with increased basal sympathetic tone (30). In the current study, progressive reductions in left insular activity were observed in both groups during 15 and 35 mmHg LBNP. The greater deactivation within the left insula during 35 mmHg LBNP, together with greater HR and MSNA responses in men vs. women, is consistent with these previous reports. Taken together, these data suggest that the left insula modulates the physiological responses to baroreceptor unloading via inhibition of parasympathetic cardiac tone or by disinhibition of sympathetic outflow directed to the heart or peripheral blood vessels.

Limitations. A common limitation inherent in functional neuroimaging studies is the inability to determine excitatory vs. inhibitory synaptic inputs. BOLD functional images are sensitive to the concentration of deoxyhemoglobin in the blood. Neural activation is followed by an inflow of oxygenated blood, a decrease in deoxyhemoglobin concentration, and a subsequent increase in BOLD signal intensity. Likewise, a decrease in BOLD signal is interpreted as a decline in synaptic activity. Therefore, BOLD-sensitive fMRI measures neurovascular changes rather than direct neural activity. However, work in animals has demonstrated that changes in BOLD signal correlate with local field potentials and reflect intracortical processing (24, 25). Therefore, the terms “activation” and “deactivation” used in the present study reflect changes in overall synaptic activity and are not related to specific excitatory or inhibitory processes. The interpretation of our functional neuroimaging results would be enhanced with electrophysiological, stimulation, and/or neuroanatomical tracing experiments in animals to assist with the identification of specific excitatory vs. inhibitory and sympathetic vs. parasympathetic-mediated effects on cardiovascular function.

The possibility exists that the observed BOLD signal differences between men in women measured during LBNP are related to the central processing of sensory or emotional stimuli rather than baroreflex-mediated events. In an attempt to control for the somatosensory experiences associated with lower body negative pressure all fMRI comparisons during 15 and 35 mmHg LBNP were compared against the BOLD signal changes recorded during the 5 mmHg level of suction. Although this low level of LBNP may not perfectly match the stimulus intensity produced at the higher levels of suction, these confounding influences should have been minimized by the statistical comparison between men and women at these higher levels of LBNP.

In summary, in the present study, we extended our previous observations (22) and examined forebrain regions associated with sex differences in the central modulation of baroreflex function. The varied physiological reactions in HR and MSNA in response to different levels of LBNP corresponded with sex-specific differences in the activation patterns of discrete forebrain structures. The larger heart rate increase in men was associated with greater activation of the dACC and deactivation of the vACC and amygdala. The augmented rise in sympathetic nerve activity in men was matched by greater activity within the right insula and greater deactivation of the left insula and ventral medial prefrontal cortex. These data provide a functional cortical basis for sex-dependent differences in baroreflex cardiovascular control. Furthermore, these responses paralleled greater SV reductions in the men and may, therefore, reflect the magnitude of baroreceptor input to this network. Thus it remains to be determined whether these male-female variations are due to true sex differences or, rather, to differences in baroreceptor afferent input to cortical regulatory sites. Finally, characterization of the specific functions of this network in normal cardiovascular function requires further assessment.
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