Forebrain organization representing baroreceptor gating of somatosensory afferents within the cortical autonomic network

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Goswami R, Frances MF, Steinback CD, Shoemaker JK. Forebrain organization representing baroreceptor gating of somatosensory afferents within the cortical autonomic network. J Neurophysiol 108: 453–466, 2012. First published April 18, 2012; doi:10.1152/jn.00764.2011.—Somatosensory afferents are represented within the cortical autonomic network (CAN). However, the representation of somatosensory afferents, and the consequent cardiovascular effects, may be modified by levels of baroreceptor input. Thus, we examined the cortical regions involved with processing somatosensory inputs during baroreceptor unloading. Neuroimaging sessions (functional magnetic resonance imaging [fMRI]) recorded brain activity during 30 mmHg lower-body negative pressure (LBNP) alone and combined with somatosensory stimulation (LBNP+SS) of the forearm (n = 14). Somatosensory processing was also assessed during increased sympathetic outflow via end-expiratory apnea. Heart rate (HR), blood pressure (BP), cardiac output (Q), and muscle sympathetic nerve activity (MSNA) were recorded during the same protocols in a separate laboratory session. SS alone had no effect on any cardiovascular or MSNA variable at rest. Measures of HR, BP, and Q during LBNP were not different compared with LBNP+SS. The rise in MSNA burst frequency was attenuated during LBNP+SS versus LBNP alone (8 vs. 12 bursts/min, respectively, P < 0.05). SS did not affect the change in MSNA during apnea. Activations within the insula and dorsal anterior cingulate cortex (ACC) observed during LBNP were not seen during LBNP+SS. Anterior insula and ACC activations occurring during apnea were not modified by SS. Thus, the absence of insular and dorsal ACC activity during LBNP+SS along with an attenuation of MSNA burst frequency suggest sympathoinhibitory effects of sensory stimulation during decreased baroreceptor input by a mechanism that includes conjoint insula-dorsal ACC regulation. These findings reveal that the level of baroreceptor input influences the forebrain organization of somatosensory afferents.

sensory gating; lower-body negative pressure; muscle sympathetic nerve activity; insular cortex; anterior cingulate cortex

CENTRAL PROCESSING OF AFFERENT feedback from baroreceptors modulates autonomic cardiovascular function, with decreased baroreceptor firing augmenting muscle sympathetic nerve activity (MSNA) (Delius et al. 1972). Baroreflex activity affects the regulation of various components of autonomic function, including a role for carotid sinus baroreceptors in pulmonary modulation of the autonomic nervous system (Eckberg and Orshan 1977). Baroreceptor activity also has an influence on the processing of somatosensory stimuli and subsequent effects on autonomic function. Specifically, afferent input from arterial baroreceptors is a powerful modulator of the excitatory cardiovascular response evoked by skeletal muscle afferents during muscle contractions (Potts and Li 1998). Furthermore, somatosensory stimuli delivered 200–400 ms after the R wave of the cardiac cycle inhibits MSNA, whereas no change in MSNA is observed with delivery synchronous with the electrocardiogram (ECG)-R wave (Donadio et al. 2002). Thus, ongoing feedback from baroreflex activity plays an important role in the integration of somatosensory stimuli and the resultant effects on autonomic control of cardiovascular function.

Although activation of somesthetic afferents in groups III and IV have been shown to interact with baroreflex activity (Potts et al. 2003; Quest and Gebber 1972), low-threshold electrical stimulation of large-diameter type I and type II fibers may also engage somatosensory afferents that influence cardiovascular responses mediated by changes in autonomic outflow (Hollman and Morgan 1997; Owens et al. 1979). For instance, the sympathoexcitatory effect of static handgrip exercise was attenuated by concurrent ipsilateral submotor threshold electrical stimulation of type I and type II afferents over the forearm (Hollman and Morgan 1997). These authors postulated that the depressor effect on MSNA depends on an interaction of type III and type IV afferents that are active during handgrip exercise with type I and type II afferents at the spinal level (Hollman and Morgan 1997). However, no human studies to date have investigated the autonomic response and cortical processing of low-level somatosensory stimulation during sympathoexcitatory conditions induced by reduced baroreceptor afferent input.

The first human evidence of cortical involvement of baroreflex-mediated changes in autonomic function during baroreceptor unloading via an orthostatic stressor (lower-body negative pressure [LBNP]) was demonstrated by Kimmerly et al. (2005). Functional magnetic resonance imaging (fMRI) revealed activity in the insular cortex, anterior cingulate cortex (ACC), medial prefrontal cortex (MPFC), cerebellum, and amygdala (Kimmerly et al. 2005, 2006), supporting an established cortical and subcortical network responsible for regulating visceromotor activity (Benarroch 1997; Cechetto and Saper 1990; Dampney et al. 2002; Verberne and Owens 1998). In addition, we recently identified forebrain centers within the cortical autonomic network (CAN) involved with sensory level type I/II afferent stimulation, including the insula and ventral MPFC (vMPFC), during supine conditions at basal levels of baroreceptor loading (Goswami et al. 2011). As such, the cortical organization of somatosensory inputs during episodes of baroreflex-mediated sympathoexcititation in humans requires investigation.

There is evidence that both somatosensory and baroreceptor afferent neural integration occurs at brain stem and supramed-
ular sites associated with autonomic cardiovascular control. In particular, afferent projections from both muscle and baroreceptor sensory afferents synapse in discrete regions of the brain stem, such as the nucleus tractus solitarii (NTS) (Kalita et al. 1981; Potts et al. 2002). Sites of somatosensory and baroreceptor integration within the forebrain have also been observed. Specifically, in nonhuman primates, somatosensory and baroreceptive inputs converge on the same neurons within the insula (Zhang et al. 1999). Furthermore, Gray and colleagues (2009) have demonstrated an involvement of the insula, amygdala, and brain stem nuclei in the integration of somatosensory stimuli presented either before or during early cardiac systole in humans, showing that baroreceptor activity across the cardiac cycle influences the cortical processing of somatosensory stimuli (Gray et al. 2009). The concept of baroreceptor modulation also extends to the gating of other sensory responses including nociception (Edwards et al. 2008; Gray et al. 2010). Thus, we sought to examine the sympathetic response and cortical representation of baroreflex gating of somatosensory afferent inputs. Specifically, the purpose of the present study was to examine the hypothesis of a sympathoinhibitory effect of type I and type II sensory stimulation exhibited during reductions in baroreceptor afferent activity that would be associated with alterations in activation patterns within the CAN, particularly the insula, ACC, and/or MPFC.

MATERIALS AND METHODS

Ethical Approval

Experimental procedures were conducted with approval by the Health Sciences Research Ethics Board at Western University. All participants provided informed written consent to the study, which was in accordance with the standards set by the Declaration of Helsinki.

Participants

Fifteen healthy participants [6 females, 9 males; age range 18–31 years; 25(3) years; height 175(8) cm; weight 74(12) kg; mean(SD)] volunteered for this study. All subjects were nonsmokers, not taking any medication, and had no prior history of cardiovascular, neurological, or musculoskeletal disorders. In addition, each participant was classified as right-handed according to the Edinburgh Handedness Inventory (Oldfield 1971). Prior to the experimental day, each participant was familiarized with transcutaneous electrical nerve stimulation (TENS) at submotor threshold levels and the parameters for the stimulus intensities were determined (see the following text). As well, each participant was assessed for a tolerance to perform 20-s end-expiratory apneas.

Experimental Approach

Our approach was to assess cortical activation patterns and autonomic responses to somatosensory stimulation while baroreceptor loading was varied. Second, we studied the autonomic and brain responses to end-expiratory apneas, a significant stimulus known to activate the sympathetic nervous system (Fagius and Sundlöf 1986) in the absence of orthostasis. Baroreceptor unloading was achieved using 30 mmHg LBNP (Sundlöf and Wallin 1978). TENS at submotor threshold stimulation was used to activate the type I and type II afferent nerve fibers (Hollman and Morgan 1997; Radhakrishnan and Sluka 2005).

The participants were tested on two separate experimental days to obtain: 1) the cardiovascular and neural recordings (PHYS session) and 2) the neuroimaging data (fMRI session). The PHYS session was always performed first to assess tolerance of LBNP and establish forearm electrical stimulation levels.

Experimental Stimuli and Procedures

The somatosensory stimuli were generated electrically by a neurostimulator (Digitimer DS7A, Hertfordshire, UK) with a symmetrical and biphasic stimulation waveform delivered at a frequency of 100 Hz and pulse width duration of 50 μs. Two self-adhesive electrodes with modified wiring for MRI compatibility (4 × 4 cm; StimCare, Empi, St. Paul, MN) were placed over the right forearm flexors, identified by palpation during resisted flexion. The location for the electrodes and stimulation intensity were each individually adjusted to attain submotor threshold at an intensity just below motor threshold. On fMRI testing days, levels of stimulation for each participant were determined outside the scanner and verified once inside the scanner just prior to the imaging session. The average intensity at submotor threshold was achieved at 15 ± 3 mA.

Minimization of head movement during LBNP was achieved in the fMRI session through the use of military antishock trousers (MAST) (David Clark, Worcester, MA), as well as an adjustable bicycle seat and footplate within the LBNP chamber. The suction within the lower body chamber was applied continuously while the MAST controlled the venous pooling. Specifically, during LBNP, inflation of the antishock trousers countered venous pooling and reflected baseline, supine conditions, whereas deflation of the trousers induced venous pooling and orthostatic stress. The viability and reproducibility of the use of antishock trousers with LBNP has previously been demonstrated by Kimmerly et al. (2006), who showed that measures of central venous pressure were not different between LBNP sessions with and without antishock trousers (Kimmerly et al. 2006). During the fMRI sessions, the participants’ heads were immobilized within a head cradle with foam padding and were instructed to refrain from performing any active tasks and to avoid head movements.

Experimental Protocol

Participants were asked to fast for a minimum of 3 h and to refrain from caffeine, nicotine, alcohol, and physical activity for at least 12 h prior to testing. Participants were also asked to arrive to the laboratory 2 h prior to the experimental testing to determine the stimulation parameters and perform cutaneous anesthetization. To achieve cutaneous afferent blockade, the forearm was treated with EMLA cream (Astra Pharmaceuticals, Wayne, PA) at the location of optimal electrode placement. The experimental session began by having subjects lay in the supine position, sealed in the LBNP chamber at the level of the iliac crest.

The somatosensory stimulation (SS) session was a block design consisting of four repetitions of stimulation lasting 30 s with 15-s rest provided between. Two runs were performed, with a total of 8 SS trials.

The LBNP session was a block design that exposed participants to three 60-s bouts of LBNP with 30-s rest in between. Two runs were performed, producing a total of 6 LBNP trials. The LBNP + somatosensory stimulation (LBNP+SS) session was a similar block design consisting of three 60-s trials of LBNP. Submotor stimulation was applied during the last 30 s of LBNP to allow for steady-state conditions. Like the LBNP session, the LBNP+SS session was repeated twice for a total of 6 LBNP+SS trials.

Apnea sessions consisted of two conditions, including Apnea and Apnea + somatosensory stimulation (Apnea+SS). The apneas were performed as maximal end-expiratory breath holds, each lasting 20 s with 45-s rest provided between trials to ensure that MSNA, heart rate (HR), and blood pressure (BP) returned to baseline values after the transient sympathetic withdrawal that occurs after breath holds (Steinback et al. 2010; Watenpaugh et al. 1999). Apnea and Apnea+SS
were each repeated three times within a single run in randomized order. Two runs were performed, producing a total of 6 Apnea and 6 Apnea+SS trials.

The PHYS and fMRI sessions were identical except for a 2-min baseline allotted at the beginning of each run during the PHYS sessions to allow for calculation of baseline MSNA.

**Physiologic Recording Session (PHYS)**

**Data acquisition.** Heart rate was acquired from a standard lead II ECG (Pilot 9200; Colin Medical Instruments, San Antonio, TX). Arterial BP was measured continuously on a beat-by-beat basis from the left middle finger with a photoplethysmograph finger cuff (Finometer; Finapres Medical Systems BV, Amsterdam, The Netherlands), from which mean arterial pressure (MAP) was obtained. Cardiac output (Q) was acquired using the Finometer Modelflow algorithm (Wesseling et al. 1993). Pneumotrace recordings were used to measure rate and depth of breathing (Siemens, Pi-Products, Amberg, Germany). Multi-fiber MSNA was recorded from the right fibular (peroneal) nerve using microneurography (Hagbarth and Vallbo 1968). A tungsten microelectrode (length = 35 mm, diameter = 200 μm) tapered to a 1- to 5-μm uninsulated tip was inserted percutaneously into the fibular nerve posterior to the fibular head. A reference electrode was placed subcutaneously 1–3 cm from the recording site. Confirmation of a suitable MSNA site was determined by bursts exhibiting pulse synchrony, as well as a burst pattern that did not produce skin paresthesias and increased in response to voluntary apnea but not during arousal to a loud noise (Hagbarth and Vallbo 1968).

**Data analysis.** ECG, BP, Q, and rectified and integrated neurogram signals were collected at a sampling rate of 1 kHz, the amplified and filtered neurogram signals were sampled at 10 kHz, and stored offline for further analysis (Powerlab software, ADInstruments, Colorado Springs, CO).

MSNA bursts exhibiting pulse synchrony, with characteristic rising and falling slopes and having a signal-to-noise ratio of at least 2:1 (i.e., the ratio of the amplitude of the burst and baseline), were included in the analysis. MSNA was quantified as: burst frequency (number of bursts per minute), burst amplitude (amplitude of the largest burst occurring during the sampling period was set to 100%, with the rest of the burst amplitudes expressed as a percentage of the maximal burst, and the mean burst amplitude computed from this distribution), total MSNA activity (product of mean burst amplitude and burst frequency), and burst incidence (bursts per 100 heartbeats).

**Statistical analysis.** The effect of each condition on each hemodynamic and MSNA variable versus their respective baseline was assessed using a two-tailed Student’s t-test. Statistical analyses were performed using SAS (SAS Institute, Inc., Cary, NC). Statistical significance was set at \( P < 0.05 \). Data are reported as mean (SD).

**fMRI Session**

**Data acquisition.** Heart rate was calculated from pulse intervals obtained from an MRI-compatible pulse oximeter (Nonin Medical, 8600FO MRI, Plymouth, MN) secured over the left middle finger. Absolute levels of LBNP were acquired from a pressure transducer (Edwards Lifesciences, PX272, Irvine, CA) that was connected to a bridge amplifier outside the MRI suite. Analog signals of the pulse oximeter and LBNP levels were sampled at 1 kHz and stored for analysis (Powerlab, ADInstruments).

Imaging data were collected on a 3-Tesla scanner (Magnetom TRIO TIM, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil. A high resolution T1-weighted structural volume was acquired with a 3D MPRAGE sequence at the beginning of the scanning session (sagittal, matrix 256 × 240 mm, voxel size 1 × 1 × 1 mm, 1 mm slice thickness, no gap, flip angle 9°, repetition time [TR] = 2,300 ms, echo time [TE] = 2.98 ms). Whole brain blood oxygenation level–dependent (BOLD) contrast fMRI data were acquired by T1-weighted gradient echo planar imaging (EPI) pulse sequence (TE, 30 ms; flip angle, 90°; field of view [FOV], 240 × 240 mm; in-plane voxel resolution, 3 × 3 mm). Functional volumes consisted of 45 interleaved axial slices (TR 2,500 ms, 3-mm slice thickness, no gap). For each of the runs in the SS session, 78 volumes were acquired, 120 volumes were acquired during each of the LBNP and LBNP+SS runs, and 174 volumes were acquired in the Apnea paradigm. The first two images of each run were automatically discarded to allow for analysis of an equilibrated MRI signal.

**Data analysis.** Raw fMRI data were analyzed by SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). The EPI images were motion corrected using a fourth-degree B-spline interpolation to create a mean EPI image, which was coregistered to the same space as each individual’s structural image. Images were then segmented into gray matter, white matter, and cerebrospinal fluid (Ashburner and Friston 1997), normalized to the Montreal Neurological Institute (MNI) coordinate system and resampled to 2 × 2 × 2 mm. The scans were spatially smoothed using a full-width-half-maximum (FWHM) Gaussian kernel of 8 mm, and normalized for global activations. To reduce low-frequency noise due to scanner drift, a high-pass filter with 250-s cutoff was applied to the data set (autoregressive model).

**Statistical analysis.** Two levels of analysis were performed. First, within-subject analyses were constructed to form a General Linear Model (GLM) modeled with a conventional block design using a canonical hemodynamic response function. Second, each individual’s contrast images reflecting differences in signal intensity, between the conditions of interest and baseline, were entered into a random-effects group analysis. Subtraction analyses were also performed to compare between conditions. BOLD responses containing information of increased and decreased activation patterns were thresholded at \( P < 0.005 \) (uncorrected) and a minimum cluster size of 10 voxels was used. The effect size (mean ± SE percentage change) of the BOLD signal was obtained from particular clusters.

A region of interest (ROI) analysis was performed based on previous literature pertaining to baroreflex-mediated changes in autonomic activity (Henderson et al. 2004; Kimmerly et al. 2005), as well as somatosensory processing (Goswami et al. 2011; Gray et al. 2009). These ROIs include the insula, ACC, and MPFC. Identification of anatomic locations was obtained using Talairach Daemon software (Lancaster et al. 2000). All figures are represented in neurologic convention (i.e., left is on the left).

**RESULTS**

**Physiological Responses**

Due to a loss of somatosensory stimuli from one participant during the fMRI recording session, neuroimaging data are based on 14 participants. Similarly, MSNA recordings with acceptable signal-to-noise ratios for all the conditions were obtained in 11 participants upon which analysis of the recordings was performed.

Baseline measures were similar among all runs. Baseline HRs were not different between SS, LBNP, LBNP+SS, and Apnea/Apnea+SS runs in both the PHYS and fMRI sessions (Tables 1, 2, and 3). Baseline MAP and Q were not different between SS, LBNP, LBNP+SS, and Apnea/Apnea+SS in PHYS sessions (Tables 1, 2, and 3). Baseline MSNA burst frequency, burst amplitude, total MSNA, and burst incidence were similar between SS, LBNP, LBNP+SS, and Apnea/Apnea+SS sessions (Tables 2 and 3).

**Somatosensory stimulation (SS).** Individual physiological responses to SS are shown in Fig. 1A. Somatosensory stimu-
lation alone did not affect HR, MAP, or Q (Table 1). As well, SS did not have a significant impact on MSNA burst frequency \[21 \text{ (9.66) vs. 21 \text{ (9.16) bursts/min; baseline vs. SS}}\], burst amplitude \[43 \text{ (10.24) vs. 43 \text{ (8.81)%; baseline vs. SS}}\], total MSNA \[907 \text{ (524.32) vs. 891 \text{ (480.50) a.u., baseline vs. SS}}\], or burst incidence \[38 \text{ (17.11) vs. 38 \text{ (16.18) bursts/100 heart beats; baseline vs. SS}}\]. The group-level responses in MSNA during SS are shown in Fig. 2.

**Table 1. Hemodynamic measures during baseline and somatosensory stimulation**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (MRI) (beats/min)</td>
<td>59 (9)</td>
<td>59 (9)</td>
</tr>
<tr>
<td>HR (PHYS) (beats/min)</td>
<td>57 (9)</td>
<td>56 (9)</td>
</tr>
<tr>
<td>MAP (PHYS) (mmHg)</td>
<td>93 (6)</td>
<td>93 (7)</td>
</tr>
<tr>
<td>Q (PHYS) (L/min)</td>
<td>5.3 (1.4)</td>
<td>5.3 (1.4)</td>
</tr>
</tbody>
</table>

All values are expressed as means (SD). MRI indicates measures collected during the neuroimaging session. PHYS indicates physiological measures collected during the laboratory session. SS, somatosensory stimulation; HR, heart rate; MAP, mean arterial pressure; Q, cardiac output.

In response to LBNP, all indices of MSNA were increased above baseline levels, including burst frequency \(P < 0.05\), burst amplitude \(P < 0.05\), total MSNA \(P < 0.05\), and burst incidence \(P < 0.05\) (Table 2). Although, compared with baseline, LBNP+SS elicited elevations in burst frequency \(P < 0.05\), burst amplitude \(P < 0.05\), total MSNA \(P < 0.05\), and burst incidence \(P < 0.05\) (Table 2), the absolute increase in MSNA burst frequency \(\Delta\text{frequency}\) was smaller during LBNP+SS compared with that of LBNP \(8 \text{ vs. 12 bursts/min, respectively,} \ P = 0.05\) (Fig. 3A). No differences between LBNP and LBNP+SS were observed with respect to changes in burst amplitude, total MSNA, and burst incidence compared with baseline (Fig. 3, B–D).

![Fig. 1. Individual responses in heart rate (HR), mean arterial pressure (MAP), and cardiac output (Q) during somatosensory stimulation (SS; A, top), lower-body negative pressure (LBNP; B, middle), lower-body negative pressure + somatosensory stimulation (LBNP+SS; B, middle), and apnea and apnea + somatosensory stimulation (Apnea+SS; C, bottom). *Significant difference from Baseline 1 \(P < 0.05\); †significant difference from Baseline 2 \(P < 0.05\).](http://jn.physiology.org/doi/10.1152/jn.00764.2011)
Apnea and Apnea+SS. Individual physiologic responses to Apnea and Apnea+SS are shown in Fig. 1C. Heart rate was not different from baseline during Apnea and Apnea+SS, in either the PHYS or fMRI sessions (P > 0.05; Table 3). As well, MAP and Q during Apnea and Apnea+SS were not altered compared with baseline in the PHYS session (P > 0.05; Table 3).

Apnea elicited increases in MSNA burst frequency (P < 0.05), burst amplitude (P < 0.05), total MSNA (P < 0.05), as well as burst incidence (P < 0.05) above baseline (Table 3). There were no differences in the absolute increase of any of the MSNA measures during Apnea compared with Apnea+SS (Fig. 4, A–D).

Neuroimaging Responses

Somatosensory stimulation. Regions associated with increased activity during SS included the left insula, prefrontal cortex, posterior cingulate cortex (PCC), supplementary motor area (SMA), bilateral precentral, and postcentral gyri as well as superior temporal gyrus (Fig. 5, Table 4). Decreased activity was observed in the cerebellum and midbrain (Table 4).

LBNP and LBNP+SS. LBNP was associated with increased activity in the right insula, dorsal ACC, amygdala, bilateral cerebellum, and ventral pons (Fig. 6, Table 5). In contrast to LBNP, subtraction analysis showed the absence of activity in the right insula and dorsal ACC during LBNP+SS (Fig. 7). LBNP+SS was associated with increased activity in the dorsal pons and left postcentral gyrus (Table 5). Regions that increased signal intensity similarly during LBNP and LBNP+SS as shown by conjunction, included the right amygdala and bilateral cerebellum (Fig. 6, Table 5). Decreased activity during LBNP occurred in the bilateral subgenual ACC, PCC, and left mediodorsal thalamus (Fig. 6, Table 5). Similarly, decreased signal intensity during LBNP+SS was observed in the bilateral subgenual ACC, PCC, and mediodorsal thalamus (Fig. 6, Table 5). In addition, de-

Table 2. Hemodynamic and sympathetic nerve activity measures during baseline, lower-body negative pressure, and lower-body negative pressure + somatosensory stimulation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline 1</th>
<th>LBNP</th>
<th>Baseline 2</th>
<th>LBNP+SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (fMRI) (beats/min)</td>
<td>58 (8)</td>
<td>65 (10)*</td>
<td>59 (8)</td>
<td>64 (10)†</td>
</tr>
<tr>
<td>HR (PHYS) (beats/min)</td>
<td>58 (10)</td>
<td>61 (10)*</td>
<td>58 (9)</td>
<td>60 (10)†</td>
</tr>
<tr>
<td>MAP (PHYS) (mmHg)</td>
<td>98 (11)</td>
<td>98 (11)</td>
<td>97 (10)</td>
<td>96 (11)</td>
</tr>
<tr>
<td>Q (PHYS) (L/min)</td>
<td>5.3 (1.3)</td>
<td>5.0 (1.2)</td>
<td>5.3 (1.3)</td>
<td>5.0 (1.0)†</td>
</tr>
<tr>
<td>MSNA (PHYS) (bursts/min)</td>
<td>19 (7)</td>
<td>31 (8)*</td>
<td>20 (8)</td>
<td>28 (7)†</td>
</tr>
<tr>
<td>MSNA (PHYS) (amplitude, %)</td>
<td>43 (7)</td>
<td>50 (7)*</td>
<td>43 (9)</td>
<td>50 (7)†</td>
</tr>
<tr>
<td>MSNA (PHYS) (total activity, a.u.)</td>
<td>822 (314)</td>
<td>1,507 (404)*</td>
<td>856 (405)</td>
<td>1,387 (449)†</td>
</tr>
<tr>
<td>MSNA (PHYS) (bursts/100 heart beats)</td>
<td>33 (13)</td>
<td>54 (14)*</td>
<td>35 (14)</td>
<td>49 (14)†</td>
</tr>
</tbody>
</table>

All values are expressed as means (SD). fMRI indicates measures collected during the neuroimaging session. PHYS indicates physiological measures collected during the laboratory session. LBNP, lower-body negative pressure; SS, somatosensory stimulation; HR, heart rate; MAP, mean arterial pressure; Q, cardiac output; MSNA, muscle sympathetic nerve activity; a.u., arbitrary units. *Significant difference from Baseline 1 (P < 0.05). †Significant difference from Baseline 2 (P < 0.05).
creased BOLD activity observed during LBNP+SS and not during LBNP occurred in the right thalamus and posterior insula (Fig. 7, Table 5).

Apnea and Apnea+SS. Brain regions showing increased BOLD activity during Apnea included the bilateral anterior insula, dorsal ACC, cerebellum, thalamus, putamen, SMA, precentral gyr, and right postcentral gyrus (Fig. 8, Table 6). Apnea+SS was associated with activity in the right insula, dorsal ACC, left cerebellum, bilateral thalamus, putamen, SMA, precentral gyrus, and right postcentral gyrus (Fig. 8, Table 6). In a conjunction analysis, common activity to Apnea and Apnea+SS was observed in the right insula, dorsal ACC, left cerebellum, bilateral thalamus, putamen, SMA, precentral gyr, and right postcentral gyrus. In further analysis, the effect size (percentage signal change) in the right dorsal ACC was greater during Apnea compared with that during Apnea+SS (Fig. 7). The effect size in the right anterior insula was greater during Apnea+SS compared with Apnea alone (Fig. 7).

Decreased activity during Apnea was observed in the right posterior insula, right cerebellum, bilateral hippocampus, superior temporal gyrus, left middle occipital gyrus, and right inferior occipital gyrus (Fig. 8, Table 6). Decreased signal intensity during Apnea+SS occurred in the right posterior insula, right cerebellum, bilateral hippocampus, left superior temporal gyrus, bilateral middle occipital gyrus, and right inferior occipital gyrus (Fig. 8, Table 6).

DISCUSSION

This study presents new observations of the forebrain organization associated with interactions between baroreceptor and somatosensory afferents with outcome measures of sympathetic nerve activity. Specifically, we have shown the interactive effects of type I and type II somatosensory afferents with baroreceptor activity on autonomic control in humans. In the present findings, SS by itself did not affect baseline MSNA and was associated with increased activity in the left insula and prefrontal cortex. Baroreceptor unloading during LBNP was associated with increased activity in the right insula and dorsal ACC, as well as decreased activity in the subgenual ACC, consistent with increased MSNA and HR during LBNP. However, SS during baroreceptor unloading attenuated the LBNP-induced increase in MSNA burst frequency and reversed the activation of the right insula and dorsal ACC. In contrast, SS did not affect the LBNP-induced deactivation in the subgenual ACC. However, SS during expiratory apnea did not affect the...
rise in MSNA nor abolish the activity in the right insula and dorsal ACC. These findings suggest that 1) somatosensory afferent input has an inhibitory effect on sympathetic outflow through regions of the brain associated with LBNP-induced sympathetic activation, and 2) the effect of somatosensory input on neural patterns of activity and on sympathetic outflow is dependent on, and specific to, the levels of baroreceptor afferent input and not just a state of elevated sympathetic outflow. Thus, baroreceptor activity may operate as a gating mechanism for the distribution of somatosensory afferents.

**Physiologic Responses**

We used sensory level stimulation to recruit the large-diameter type I and type II afferents during low baroreceptor input and observed an inhibitory effect on MSNA burst frequency. Given that LBNP generally elicits robust increases in burst frequency as opposed to burst amplitude (Kimmerly et al. 2004; Shoemaker et al. 2001) and that different mechanisms of control may exist for burst incidence and strength (Kienbaum et al. 2001), the finding of decreased burst frequency and not amplitude was not unexpected. Previously, a reduction in systolic BP as well as a smaller increase in MSNA burst frequency and total activity was reported during concurrent handgrip exercise and TENS (Hollman and Morgan 1997). This inhibition was attributed to an interaction between type III

**Table 4. Brain regions associated with somatosensory stimulation**

<table>
<thead>
<tr>
<th>Location</th>
<th>Side</th>
<th>MNI (x, y, z)</th>
<th>T-score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Increased Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insula</td>
<td>L</td>
<td>−38, −20, 18</td>
<td>4.33</td>
</tr>
<tr>
<td>Insula</td>
<td>L</td>
<td>−38, 0, 14</td>
<td>4.69</td>
</tr>
<tr>
<td>Prefrontal Cortex</td>
<td>L</td>
<td>−4, 48, −12</td>
<td>3.26</td>
</tr>
<tr>
<td>Posterior Cingulate Cortex</td>
<td>L</td>
<td>−12, −46, 22</td>
<td>3.25</td>
</tr>
<tr>
<td>Supplementary Motor Area</td>
<td>L</td>
<td>−8, 10, 62</td>
<td>3.80</td>
</tr>
<tr>
<td>Postcentral Gyrus</td>
<td>L</td>
<td>−44, −34, 46</td>
<td>3.57</td>
</tr>
<tr>
<td>Precentral Gyrus</td>
<td>R</td>
<td>38, −30, 42</td>
<td>5.47</td>
</tr>
<tr>
<td>Precentral Gyrus</td>
<td>L</td>
<td>−28, −6, 54</td>
<td>5.04</td>
</tr>
<tr>
<td>Precentral Gyrus</td>
<td>R</td>
<td>30, −8, 50</td>
<td>6.52</td>
</tr>
<tr>
<td>Superior Temporal Gyrus</td>
<td>L</td>
<td>−54, −20, 0</td>
<td>4.31</td>
</tr>
<tr>
<td>Superior Temporal Gyrus</td>
<td>R</td>
<td>54, −20, −4</td>
<td>3.57</td>
</tr>
<tr>
<td><strong>B. Decreased Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>R</td>
<td>8, −44, −46</td>
<td>3.28</td>
</tr>
<tr>
<td>Midbrain</td>
<td>R</td>
<td>12, −20, −20</td>
<td>4.10</td>
</tr>
</tbody>
</table>

MNI, Montreal Neurologic Institute coordinates; L, left; R, right.
and type IV afferent fibers activated during exercise with type I and type II fibers at the spinal cord. Although that interpretation may apply for fatiguing muscular contractions, the present observations suggest that the type I and type II afferents interact with baroreceptor afferents in higher cortical centers. In addition, it has been found that the increase in diastolic BP is lower during a 2-min moderate intensity handgrip test during TENS at a nonpainful intensity (Sanderson et al. 1995). However, no changes in HR or BP were observed when TENS was applied during Valsalva’s maneuver (Sanderson et al. 1995). Inasmuch as LBNP and Valsalva’s maneuver engage baroreflex mechanisms, the present data support the idea that somatosensory inputs do not affect HR or BP per se, but rather focus on a modest attenuation of sympathetic outflow. We found no change in arterial pressure or MSNA during apnea combined with SS compared with apnea alone. These

Table 5. Brain regions associated with lower-body negative pressure, and lower-body negative pressure + somatosensory stimulation

<table>
<thead>
<tr>
<th>Location</th>
<th>LBNP</th>
<th>LBNP+SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insula</td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>Dorsal ACC</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Amygdala</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Pons</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Postcentral gyrus</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Subgenual ACC</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>Subgenual ACC</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>PCC</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>PCC</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Thalamus</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Thalamus</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Insula</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

MNI, Montreal Neurologic Institute coordinates; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; L, left; R, right.

Fig. 6. BOLD SI changes in brain regions associated with LBNP, and LBNP+SS. ACC, anterior cingulate cortex; sACC, subgenual anterior cingulate cortex; PCC, posterior cingulate cortex.
observations suggest that the impact on sympathetic tone depends on baroreceptor activity and not simply a state of heightened sympathetic outflow.

The anatomic evidence for this suggestion is presented from earlier studies in animals. Specifically, baroreceptor afferents synapse in the NTS as shown by antidromic mapping techniques and in vivo in the cat (Mifflin et al. 1988; Spyer 1994), and tracer techniques and electrophysiological recordings in the cat reveal monosynaptic projections of type I and type II skeletal muscle afferents to the NTS (Nyberg and Blomqvist 1984; Person 1989). In addition, horseradish peroxidase tracer studies in rodents have shown retrograde and anterograde projections between the insula and NTS (Ruggiero et al. 1987), including anterograde tracings to the parasympathetic motor nuclei in the NTS (Shipley 1982). Thus, relays between medullary circuits and higher cortical centers including the insula may be functionally involved in modulating the convergent somatosensory and baroreflex inputs.

Cortical Responses

Lower-body negative pressure. Insula. The cortical responses to LBNP observed in the present study support the baroreceptor-mediated autonomic network established during mild and moderate levels of LBNP including the right superior insula, dorsal ACC, cerebellum, amygdala, and prefrontal cortex (Kimmerly et al. 2005, 2006). These reports in humans, which suggest a lateralization of insula effects on autonomic cardiovascular control, are consistent with evidence provided from anesthetized rodents. Specifically, HR and BP increase upon stimulation of the right insula, and left insular stimulation usually produces depressed cardiac and pressor responses (Oppenheimer et al. 1992). Experimental studies indicate further that electrical stimulation of the posterior insula increases HR and BP in anesthetized rats (Ruggiero et al. 1987), and a large predominance of sympathoexcitatory neurons exists in the right posterior insula (Oppenheimer and Cechetto 1990; Zhang et al. 1998). During direct stimulation of the rodent brain, the superior insula is associated with tachycardia, whereas inferior portions produce bradycardia (Oppenheimer and Cechetto 1990).

The present finding of enhanced right superior insular activation during LBNP supports these earlier observations of the involvement of this region with sympathetic outflow. The lack of activation in the insula during LBNP+SS, combined with a reduction in the rise in MSNA burst frequency, suggest a
sympathoinhibitory effect of SS, supporting the interpretation that this insular region is involved in sympathetic modulation. In addition, deactivation was observed in the right posterior insula during LBNP/H11001 SS, a region that contains sympathoexcitatory neurons as well as neurons responsive to convergent baroreceptor and muscle receptor input (Zhang et al. 1998, 1999). Studies in rodents suggest that the right posterior insula is tonically active (Butcher and Cechetto 1995) and our results suggest that the right posterior insula is involved in basal sympathetic regulation and is inhibited with type I and type II muscle afferent input during baroreceptor unloading.

Medial prefrontal cortex and anterior cingulate cortex. The MPFC is involved in a range of visceromotor autonomic functions (Neafsey 1990), including hypotensive (Hardy and Holmes 1988), sympathoinhibitory (Verberne 1996), and parasympathetic responses (Wong et al. 2007). The dorsal ACC is engaged during heightened levels of sympathetic drive during baroreceptor unloading (Kimmerly et al. 2005), whereas the ventral/subgenual ACC is implicated in parasympathetic activity/sympathoinhibition (Critchley et al. 2003; Kimmerly et al. 2005; Verberne 1996) and bradycardia (Buchanan et al. 1994). Consistent with increases in MSNA during LBNP, we observed increased activity in the dorsal ACC, and decreased activity in the subgenual ACC. With LBNP+SS, dorsal ACC activation was absent, whereas deactivation was maintained in the subgenual ACC. The lack of dorsal ACC involvement with LBNP+SS may reflect a sympathoinhibitory effect, whereas the persistent deactivation patterns in the subgenual ACC may be related to a state of increased sympathetic activity and/or, as shown in other models (Gianaros et al. 2004; Goswami et al. 2011; Wong et al. 2007), decreased parasympathetic activity with LBNP. This latter interpretation is supported by similar changes in HR during LBNP with or without SS. Thus, subgenual ACC/prefrontal cortex may continue to modulate the HR and sympathetic response to LBNP with and without concurrent sensory stimulation.

End-expiratory apnea

End-expiratory apnea evoked an increase in MSNA that was associated with activity patterns in the anterior insula, dorsal ACC, and cerebellar nuclei, similar to patterns evoked by inspiratory apnea reported earlier (Macefield et al. 2006). Increases in BOLD activity in the anterior insula have been reported during respiratory challenges, including Valsalva’s maneuver and loaded breathing (Henderson et al. 2003; Macey et al. 2003). In addition, inspiratory loading has been associated with increased signal intensity in the deep cerebellar nuclei (Harper et al. 1998). The ACC has also been associated with autonomic function in HR and BP control in obstructive sleep apnea as well as with an intention role of upper airway muscle control (Henderson et al. 2003).

Somatosensory stimulation did not change apnea-induced activation in the right anterior insula, right dorsal ACC, and left cerebellum. These comparable central responses during Apnea and Apnea+SS combined with similar increases in MSNA suggest that SS did not influence sympathetic outflow during apnea. Thus, we suggest that integrating somatosensory stimuli during expiratory breath hold does not depress sympathetic autonomic function. The interpretation that somatosensory afferents interact
with baroreflex but not chemoreflex pathways is supported by evidence from studies in rats whereby skeletal muscle afferent stimulation attenuated the reflex bradycardia induced by ramp increases in arterial pressure but not during chemoreflex activation (Potts et al. 2003). In light of this, we observed stronger activity in the right anterior insula during Apnea+SS compared with Apnea, whereas stronger activity in the dorsal ACC occurred during Apnea compared with Apnea+SS. The time courses of activity were similar between the insula and dorsal ACC; thus, both regions may be involved in the initiation of MSNA with apnea. In addition, the activation patterns present in the anterior insula and dorsal ACC support the conjoint action of these two regions as a system involved in the generation of autonomic responses (Medford and Critchley 2010). The basis for the connections between the two regions are supported by the presence of von Economo neurons in the anterior insula and ACC (Craig 2009), by structural connections observed between the dorsal ACC and the insula in nonhuman primates (Mesulam and Mufson 1982; Vogt and Pandya 1987), as well as by functional connectivity of the insula and ACC in the resting state of humans (Taylor et al. 2009).

Implications

The present results point to two important implications for human physiology. First, we have provided functional evidence that type I and type II somatosensory afferents project to the forebrain and integrate with regions known to affect cardiovascular control through the autonomous nervous system. Second, as observed previously in smaller animal models, the present data provide evidence of measurable but reflex-specific baroreceptor gating of somatosensory inputs in humans. As such, these observations add to previous observations with respect to the interactions of baroreflex inputs on nociception and small-diameter type III and type IV somatosensory afferents (Edwards et al. 2008; Gray et al. 2010; Potts et al. 2003; Quest and Gebber 1972).

The benefits or purpose of this gating process is not clear but may be part of the complex processing involved in BP regulation during volitional exercise and postural changes, events that occur rapidly and commonly in conscious humans. In addition, the impact of sensory stimulation on parasympathetic modulation (Gademan et al. 2011; Haker et al. 2000), together with the importance of parasympathetic outflow on cardiac health (Eckberg et al. 1971; Langewitz et al. 1994; Townend and Littler 1995), raises the possibility for therapeutic benefits, similar to those provided by vagal stimulation (Groves and Brown 2005; Sabbah 2011).

Methodological Considerations

Both LBNP and SS are complex maneuvers that, in addition to the observed cortical activation effects, must engage neural pathways associated with awareness, cognition, and/or sensory inputs. Along with the insula and ACC as key substrates involved with interoceptive awareness, skin somatosensory afferents are involved in the network representing the visceral state of the body (Khalsa et al. 2009). Although these extraneous stimuli may have affected the cortical activation patterns in the present study, we expect such impacts to be minimal because of the following: 1) LBNP, apneic, and SS procedures were not novel experiences for these participants in the fMRI

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**Table 6. Brain regions associated with apnea and apnea + somatosensory stimulation**

<table>
<thead>
<tr>
<th>Location</th>
<th>Side</th>
<th>APNEA (MNI (x, y, z), T-score)</th>
<th>APNEA+SS (MNI (x, y, z), T-score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insula (anterior)</td>
<td>L</td>
<td>−38, 16, −4 3.40</td>
<td>38, 20, −10 6.25</td>
</tr>
<tr>
<td>Insula (anterior)</td>
<td>R</td>
<td>40, 20, −6 5.26</td>
<td></td>
</tr>
<tr>
<td>Dorsal ACC</td>
<td>L</td>
<td>−4, 30, 34 4.11</td>
<td>6, 30, 30 3.24</td>
</tr>
<tr>
<td>Dorsal ACC</td>
<td>R</td>
<td>−4, 32, 32 4.78</td>
<td>−16, −52, −32 3.13</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>L</td>
<td>−22, −78, −50 3.49</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>R</td>
<td>−10, −16, 6 5.73</td>
<td>−10, −16, 6 5.99</td>
</tr>
<tr>
<td>Thalamus</td>
<td>L</td>
<td>12, −16, 6 4.89</td>
<td>10, −16, 8 5.12</td>
</tr>
<tr>
<td>Thalamus</td>
<td>R</td>
<td>−26, −10, 4 7.46</td>
<td>−26, −16, 10 6.51</td>
</tr>
<tr>
<td>Putamen</td>
<td>R</td>
<td>4, −22, 54 4.41</td>
<td>6, 12, 50 3.12</td>
</tr>
<tr>
<td>SMA</td>
<td>L</td>
<td>−44, 4, 50 3.65</td>
<td>−42, 2, 50 3.07</td>
</tr>
<tr>
<td>Precentral Gyrus</td>
<td>R</td>
<td>48, 8, 40 5.53</td>
<td>46, 2, 46 4.30</td>
</tr>
<tr>
<td>Postcentral Gyrus</td>
<td>R</td>
<td>58, −24, 46 3.19</td>
<td>58, −24, 44 4.41</td>
</tr>
</tbody>
</table>

MNI, Montreal Neurologic Institute coordinates; ACC, anterior cingulate cortex; SMA, supplementary motor area; L, left; R, right.
sessions; 2) cortical patterns associated with the LBNP procedure are graded with the levels of orthostatic and cardiovascular response (Kimmerly et al. 2005); 3) the skin was anesthetized to minimize cutaneous inputs so that the participants did not experience any level of pain nor report unpleasant sensations during SS (but were aware of a light tingling sensation over the forearm); and 4) the same SS stimulus was applied during the LBNP and apnea sessions, but was associated with altered MSNA and cortical responses only during the LBNP trials. Thus, the effect of SS on autonomic activity does not appear to be related importantly to cognitive, emotive, or other sensory inputs associated with the study protocols.

The apnea maneuver requires a volitional component that will also induce cortical activation patterns. Also, the urge to breathe begins after approximately 10 s after apnea commencement (Bloch-Salisbury et al. 2003), potentially initiating an emotional and mechanical response as well as global changes in end-tidal carbon dioxide levels and venous pressures. The ability to perform 20-s expiratory breath holds was tolerable for the participants both with and without SS. In the apnea sessions, prior rebreathing was not performed so that similar basal levels of end-tidal carbon dioxide would be achieved between all participants. However, MSNA was increased comparably during Apnea compared with LBNP, with similar increases in burst frequency. Importantly, arterial pressure was not significantly higher than baseline during apnea; thus, the effect ofafferent BP information to the central autonomic regions via the baroreceptors was minimized. Overall, SS did not affect these nonbaroreflex patterns, supporting the conclusions that the SS impact was associated with baroreceptor inputs and not sympathoexcitation alone.

Finally, the precise nature and type of sensory fibers recruited with submotor electrical stimulation is difficult to discern in humans (Swett and Bourassa 1981). At the median nerve, the response to type I fibers can be recorded at threshold for perception of the stimulus (Vallbo et al. 1979); thus, submotor threshold stimulation was expected to recruit the large-diameter I and II afferents.

Summary and Conclusions

We have identified central and autonomic responses to the interactive effects of baroreceptor unloading and type I and type II somatosensory afferent input. We have shown that low threshold sensory stimulation during baroreflex-mediated sympathoexcitation alters autonomic function in humans and engages discrete cortical responses. The sympathoinhibition occurring with somatosensory input during baroreceptor unloading is suggested to be modulated cortically, and appears to be a reflex-specific mechanism because SS applied during an expiratory apnea did not alter the sympathetic or cortical responses to chemoreflex activation. These findings indicate a discrete network of sites involved in convergent baroreceptive and somatosensory processing that modulate autonomic control.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


