Neural correlates of voluntary breathing in humans

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McKay, L. C., K. C. Evans, R. S. J. Frackowiak, and D. R. Corfield. Neural correlates of voluntary breathing in humans. J Appl Physiol 95: 1170–1178, 2003.—To investigate the functional neuroanatomy of voluntary respiratory control, blood O₂ level-dependent functional magnetic resonance imaging was performed in six healthy right-handed individuals during voluntary hyperpnea. Functional images of the whole brain were acquired during 30-s periods of spontaneous breathing alternated with 30-s periods of isocapnic hyperpnea (spontaneous vs. voluntary: tidal volume = 0.5 ± 0.01 vs. 1.3 ± 0.1 (SE) liters and breath duration = 4.0 ± 0.4 vs. 3.2 ± 0.4 (SE) s). For the group, voluntary hyperpnea was associated with significant (P < 0.05, corrected for multiple comparisons) neural activity bilaterally in the primary sensory and motor cortices, supplementary motor area, cerebellum, thalamus, caudate nucleus, and globus pallidum. Significant increases in activity were also identified in the medulla (corrected for multiple comparisons on the basis of a small volume correction for a priori region of interest) in a superior dorsal position (P = 0.012). Activity within the medulla suggests that the brain stem respiratory centers may have a role in mediating the voluntary control of breathing in humans.

respiratory control; brain stem; cortex; functional magnetic resonance imaging

IN HUMANS, breathing is a unique motor act, inasmuch as it can be controlled reflexly, with output to the respiratory muscles originating in the respiratory centers of the brain stem, and behaviorally, with output originating in supra-brain stem structures (26). Reflex motor output requires no conscious input and can be clearly observed during sleep or anesthesia, when environmental stimuli and behavioral factors are minimized. In contrast, breathing can be modulated behaviorally, for example, during speech and emotional events. Volitional regulation of breathing is another aspect of behavioral control, evident when deliberately taking a deep breath or when holding one’s breath. Skilled breathing control is also required for tasks such as singing and wind instrument playing.

The voluntary control of breathing is presumed to be of cortical origin. The neural basis for the corticomotor control of respiratory muscles in humans was first investigated during neurosurgery; electrical stimulation of the cortical surface induced a twitch of the diaphragm (20), indicating representation of the respiratory muscles within the primary motor cortex. Subsequently, studies using percutaneous electrical (24) and transcranial magnetic stimulation of the brain (36) have identified a fast-conducting pathway from the motor cortex to the diaphragm. Each hemisphere of the diaphragm is represented predominantly in the contralateral motor cortex (34). In addition, studies of brain activity using positron emission tomography (PET) have highlighted areas of the motor cortex, thalamus, and cerebellum that are associated with volitional breathing (7, 18, 43). These structures may also mediate the control of other behavioral breathing acts, for example, breathing for speech (35) and exercise (17), behavioral tasks in which breathing control may be modified by learning and experience (33).

Voluntary and behavioral respiratory motor tasks must be performed in the context of ongoing reflex demands on breathing. Even small increases in reflex chemical drive can perturb the accuracy of voluntary respiratory movements (11). However, the neural basis for the integration of reflex and behavioral breathing is unclear. Behavioral control of the respiratory spinal motor neurons may be mediated “directly” via corticospinal neurons or “indirectly” via the brain stem respiratory centers and their associated bulbospinal neurons. In animals, neurophysiological studies have demonstrated a pathway from the motor cortex to the spinal motor neurons innervating the respiratory muscles (1). Clinical observations in humans with damage to the upper spinal cord indicate two anatomically distinct pathways: a corticospinal pathway from the cortex to the respiratory motoneurons and a bulbospinal pathway from the medulla descending in the ventrolateral quadrant of the spinal cord to the respiratory motoneurons. Damage to the ventrolateral spinal cord during neurosurgery can result in the loss of reflex
breathing, while the control of voluntary breathing is preserved (37, 44), presumably by the corticospinal tract. Observations from transcranial magnetic stimulation also suggest a direct functionally distinct corticospinal pathway in humans (9). However, the firing patterns of respiratory neurons within the medulla are closely correlated with breathing patterns modulated by respiratory behaviors in the cat (40) and mouse (5). These observations support a model in which the behavioral control of breathing is mediated by the respiratory centers in the medulla and by bulbospinal motoneurons (39).

Because of limited spatial resolution and a limited field of view, our previous respiratory-related PET studies provided an incomplete picture of the voluntary motor control of respiration. In particular, the ability to resolve separate sensorimotor foci in the cortex was limited, and we were unable to determine respiratory-related activity in the brain stem. More recently, we demonstrated the suitability of blood O2 level-dependent (BOLD) functional magnetic resonance imaging (fMRI) to determine cortical and brain stem motor-related activity associated with voluntary tongue contraction (10). The aim of the present study was to use the enhanced sensitivity and spatial resolution of BOLD fMRI to describe suprapontine activity associated with voluntary breathing, specifically voluntary hyperpnea. In addition, we wished to determine whether BOLD fMRI is a sensitive tool with which to identify activity within the medulla that may have a potential role in the behavioral control of breathing. We hypothesized that volitional hyperpnea would be associated with increased BOLD signal within sensorimotor areas of the cortex, subcortical nuclei, and medulla.

METHODS

Subjects

Six healthy, right-handed individuals (20–35 yr of age, 5 men and 1 woman) were studied. All subjects gave informed consent according to the Declaration of Helsinki (1991) and were studied with ethical approval from the Riverside Ethics Committee and the Joint Ethical Committees of the Institute of Neurology (Imperial College London) and the National Hospital for Neurology and Neurosurgery (University College London).

Physiological Monitoring

Subjects breathed through a circuit designed to maintain end-tidal PCO2 (PETCO2) to ±2 Torr of normocapnia (3). Inspiratory gas was kept mildly hyperoxic (30% inspired O2). Respired gases were sampled via a probe inserted into the mouthpiece, and PETCO2 and end-tidal PO2 (PETO2) were determined by a quadrupole respiratory mass spectrometer (model MGA 2000, Case Medical). Changes in respiratory frequency and tidal volume (VT) were measured by a pneumotachograph (Collins) positioned in the expiratory line of the circuit and connected to a differential pressure transducer (Validyne). Airway pressure was determined via a probe inserted into the mouthpiece, which was connected to a differential pressure transducer (Validyne). All signals were recorded on a personal computer (Optiplex GX, Dell) via an analog-to-digital interface (model 1401 Plus, Cambridge Electronic Design, Cambridge, UK). All subjects wore a nose clip throughout the experiment. Monitoring apparatus taken into the scanner room contained no ferrous components.

Imaging

Brain images were acquired by using a Siemens Vision MRI scanner operating at 2 T at the Wellcome Department of Imaging Neuroscience, Institute of Neurology. All subjects underwent a positioning scan and then a T1 weighted "structural" scan to determine brain morphology. Subsequently, the functional images were acquired by using BOLD echo planar imaging. The T2* weighted functional sequences, consisting of 156 brain volumes, were acquired continuously throughout the breathing tasks. Each brain volume from cortex to upper spinal cord was collected in 5.2 s and consisted of 56 transverse slices with an isotropic voxel resolution of 3 mm and a matrix size of 64 × 64 pixels. Subjects lay in a supine position on the scanner bed. To minimize head movement artifacts, the head was held in place by firm padding, and subjects were attached to the breathing circuit via a personalized mouthpiece, which was securely attached to the head coil by using customized support. To minimize scanner noise, the subjects wore earplugs, which were connected to a microphone in the control room, enabling the subjects to hear verbal instructions.

Breathing Paradigm

Subjects carried out 12 periods of 31 s (i.e., 6 whole brain images per period) of isocapnic hyperpnea alternating with 31-s periods of spontaneous breathing. A verbal cue was given at the beginning and end of each hyperpnea period. The hyperpnea-breathing pattern was designed to increase ventilation by approximately three times resting breathing, predominantly through an increase in Vr. Subjects underwent training to perform the hyperpnea without feedback on a previous occasion at the National Heart and Lung Institute, Charing Cross Campus, Imperial College School of Medicine.

Data Analysis

Ventilatory data were analyzed on a breath-by-breath basis (Spike 2, Cambridge Electronic Design). Functional imaging data were analyzed by using SPM99 software (Wellcome Department of Cognitive Neurology; http://www.fil.ion.ucl.ac.uk/spm). Before statistical analysis, the data underwent a number of preprocessing stages. To account for small head movements, images from each subject were realigned to the first image in the data series. To enable group analysis, the images were normalized into standard stereotaxic Montreal Neurological Institute space, resampled at a resolution of 2 × 2 × 2 mm, and spatially smoothed [filter size, full-width half-maximum = 6 mm (21)]. After the preprocessing, statistical tests were performed for the group and for each individual to determine BOLD signal changes that were significantly related to the breathing task. The data underwent a multiple linear regression time series analysis performed voxel by voxel to produce statistical maps of significant neural activity. For this, the data were fitted to a binary "boxcar" function, chosen to represent the signal changes occurring with the on-off periods of voluntary hyperpnea. This boxcar model was then convolved with a hemodynamic response function to represent the relationship between neural activity and cerebral blood flow changes (22). The data were then temporally smoothed by application of a high-pass filter.
tory effort associated with hyperpnea. PETCO2 remained breathing condition, indicating the increased respira-

of the pressure changes associated with the voluntary

jects. There is a threefold increase in VT (c cutoff set at 124 s, twice the experimental period) to remove

low-frequency signal changes, such as signal drift, and a

low-pass filter (4 s Gaussian) to remove high-frequency noise.

To account for changes in BOLD signal intensity that might be due to changes in “global” signal intensity (e.g., changes in global cerebral blood flow), the average global signal intensity was included as a regressor of no interest. For the whole brain, a statistical threshold of P < 0.05 (corrected for multiple comparisons, T > 5) was set to determine statistical significance voxel by voxel. Our a priori hypothesis was that voluntary hyperpnea would be associated with increased neural activity within the medulla; consequently, we corrected for multiple comparisons on the basis of a small volume of interest [10-mm radius centered in the medulla; SPM99 software (47)]. A conjunction analysis was performed at a statistical threshold of P < 0.001 to determine activity common to all individuals. The positions of the local maxima are reported, in Montreal Neurological Institute space (see above), in terms of the x, y, and z coordinates relative to the anterior commissure, with the horizontal plane running through the anterior and posterior commissures (46). To determine the anatomic location of each maximum, the statistical maps were superimposed onto a group-mean structural image and onto the appropriate individual’s structural image and identified with reference to standard anatomic atlases (14, 15, 27, 46).

RESULTS

Ventilatory Data

The ventilatory data for one subject collected during the scanning experiment are illustrated in Fig. 1. The 12 periods of spontaneous breathing alternating with 13 periods of isocapnic hyperpnea are clearly visible on traces. VT, tidal volume; Pmouth, pressure at the mouth.

(cutoff set at 124 s, twice the experimental period) to remove low-frequency signal changes, such as signal drift, and a low-pass filter (4 s Gaussian) to remove high-frequency noise. There was also a clear increase in the amplitude of the pressure changes associated with the voluntary breathing are clearly visible on traces. VT increased from 0.5 to ~1.5 liters. There was also a clear increase in the amplitude of the pressure changes associated with the voluntary breathing condition, indicating the increased respiratory effort associated with hyperpnea. PETCO2 remained constant throughout the scanning period.

Table 1 summarizes the mean data for the six sub-

jects. There is a threefold increase in VT (P < 0.001) from spontaneous to voluntary breathing and also a slight increase in breathing frequency (P > 0.05); voluntary breathing was not associated with any change in PETCO2 (P > 0.05) or PETCO2 (P > 0.05).

Neural Activity Associated With Voluntary Hyperpnea

Group analysis. An overall view of the neural activity is illustrated as a maximum intensity projection within a “glass brain” in Fig. 2. The coordinates and statistical values of the most significant maxima for each anatomic location are listed in Table 2 and illustrated in Fig. 3.

The most significant activity identified in this study was within the supplementary motor cortex (SMA). Activity in the SMA was observed bilaterally close to the midline; the clusters extended laterally and deep into the sulcus.

In the superior precentral gyrus, significant bilateral activity was identified, with a cluster of local maxima located posteriorly, extending deep into the gyrus (Fig. 3A) and a second smaller discrete cluster of activity located in an anterior position in the gyrus (Fig. 3B). Bilateral activity was also present in the inferior precentral gyrus (Fig. 3C).

Within the superior postcentral gyrus, bilateral activations extended deep into the cortex. The identified increases in activity within the sensorimotor cortices described above were observed bilaterally, with the most significant maxima located on the left.

Table 1. Summary of mean ventilatory data

<table>
<thead>
<tr>
<th></th>
<th>VT, liters</th>
<th>Tr, s</th>
<th>PETCO2, Torr</th>
<th>PETCO2, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous breathing</td>
<td>0.5 ± 0.0</td>
<td>4.0 ± 0.4</td>
<td>41.3 ± 0.8</td>
<td>192.8 ± 2.5</td>
</tr>
<tr>
<td>Voluntary breathing</td>
<td>1.3 ± 0.1</td>
<td>3.2 ± 0.4</td>
<td>40.1 ± 0.9</td>
<td>193.2 ± 3.2</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. VT, total volume; Tr, Total respiratory cycle duration; PETCO2 and PETCO2, end-tidal PCO2 and PO2. P value for each set of variables was calculated by using paired t-tests. There is a significant increase in VT (P < 0.001) from spontaneous to voluntary breathing. Changes in Tr, PETCO2, and PETCO2 were not significant.

J Appl Physiol • VOL 95 • SEPTEMBER 2003 • www.jap.org
Fig. 2. Overall view of neural activity during the voluntary breathing task (corrected for multiple comparisons, \( P < 0.05 \)) for the group (\( n = 6 \)) shown within a “glass brain,” represented according to the convention of Talairach and Tournoux (46). For an overall appreciation of the location of maxima, all 3 planes must be considered. The sagittal plane is a view through the brain from the side, the coronal plane is a view through the brain in a posterior-to-anterior orientation, and the transverse plane is a view through the brain from above. For clarity, not all structures are labeled. A, anterior; R, right; t, thalamus; c, cerebellum.

Smaller discrete clusters were also observed bilaterally within the anterior thalamic nuclei, globus pallidum, caudate nuclei, and inferior parietal gyri. On the right, discrete clusters were identified within the premotor cortex, midcingulate gyrus, superior frontal gyrus, anterior middle frontal gyrus, and superior temporal gyrus.

Significant signal change was identified inferior to the posterior cingulate gyrus, in the vicinity of the internal cerebral vein. Within the cerebellum, activity was dispersed bilaterally over the cerebellar cortices, with clusters observed deep within the cerebellum.

Individual analyses. In all individuals, when corrected for multiple comparisons (\( P < 0.05 \)), increased neural activity was observed bilaterally within the superior sensorimotor cortices. In four of the six individuals, the anatomic spatial extent of activity within the superior sensorimotor cortices was almost identical. Although the other two individuals likely contributed to the main effects observed for the group, their activity in this region was limited to small discrete bilateral maxima. In all six subjects, activity was observed bilaterally in the cerebellum, but the location of the maxima varied between subjects. Activity within the cingulate gyrus was identified in four of the six individuals, and, in the thalamus, discrete clusters of activity were identified in three of the six individuals. However, activity was observed in these areas in all subjects at a lower statistical threshold (\( P < 0.001 \)) uncorrected for multiple comparisons. A conjunction analysis uncorrected for multiple comparisons (\( P < 0.001 \)) showed similar activations common to all individuals within the sensorimotor cortices, thalamic nuclei, and cerebellum.

Medullary activity. Our a priori hypothesis, that voluntary breathing would be associated with activity within the medulla, allowed a small volume correction to be performed on an area centered in the medulla (0, −37, −56) with a 10-mm radius. For the group, a discrete cluster of significant activity was observed within the superior dorsal medulla (\( P = 0.012; \) Fig. 3D) extending bilaterally from the midline.

Table 2. Coordinates of local maxima of significant signal increases associated with voluntary isocapnic hyperpnea

<table>
<thead>
<tr>
<th>Activated Brain Areas</th>
<th>Left Side</th>
<th>Right Side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( x )</td>
<td>( y )</td>
</tr>
<tr>
<td>Supplementary motor area</td>
<td>−2</td>
<td>−12</td>
</tr>
<tr>
<td>Superior postcentral gyrus</td>
<td>−22</td>
<td>−36</td>
</tr>
<tr>
<td>Superior precentral gyrus</td>
<td>−22</td>
<td>−26</td>
</tr>
<tr>
<td>Inferior precentral gyrus</td>
<td>−52</td>
<td>−8</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>−64</td>
<td>−34</td>
</tr>
<tr>
<td>Premotor cortex</td>
<td>−10</td>
<td>−12</td>
</tr>
<tr>
<td>Anterior thalamic nucleus</td>
<td>−44</td>
<td>−46</td>
</tr>
<tr>
<td>Inferior parietal cortex</td>
<td>−22</td>
<td>−6</td>
</tr>
<tr>
<td>Globus pallidum</td>
<td>−22</td>
<td>−64</td>
</tr>
<tr>
<td>Cingulate</td>
<td>−16</td>
<td>−64</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>−26</td>
<td>−36</td>
</tr>
<tr>
<td>Medulla</td>
<td>0</td>
<td>−38</td>
</tr>
</tbody>
</table>

Only the most significant maximum is listed for each anatomic location; in the superior precentral gyrus, a distinction is made between anterior (+) and posterior clusters. Coordinates are in mm. \( x \), Distance right (+) or left (−) from midsagittal line; \( y \), distance anterior (+) or posterior (−) to a vertical plane through the anterior commissure; \( z \), distance above (+) or below (−) the intercommissural (anterior-posterior) plane. \( T \) score is the significance statistic; \( n = 6 \).

J Appl Physiol • VOL 95 • SEPTEMBER 2003 • www.jap.org
In four of the six individual analyses, activity within the medulla survived the small volume correction. In three of the individuals, the clusters appeared to have an anatomic location similar to the identified cluster in the group analysis. In another individual, the cluster was located laterally in the caudal medulla.

**Time course of signal changes.** The time course of the signal changes, associated with voluntary hyperpnea within the motor cortex and medulla, was similar. However, the magnitude of the signal change within the motor cortex was −20% larger than that within the medulla. This is illustrated for a single subject in Fig. 4.

**DISCUSSION**

This study has identified increases in BOLD signal that are associated with voluntary isocapnic hyperpnea; in common with other BOLD fMRI studies, we interpret these signal increases as a marker of increased neural activity that may be of pre- or postsynaptic origin (2, 32). The identified activity within the cortex, specifically the identification of distinct clusters within the precentral gyrus and other suprapontine structures, extends the observation of previous imaging studies of voluntary breathing (7, 16, 18, 43). A further novel observation of this study is that increased activity was identified within a discrete region of the medulla.

**Primary Sensorimotor Cortices**

One of our main findings with respect to supra-brain stem structures is the identification of two distinct clusters of activity within the primary motor cortex. It is known that the motor cortex is involved in the generation of voluntary movements; an early imaging example of this with the use of PET is activation of the motor cortex during a simple, repetitive arm movement (6). With respect to breathing, Foerster (20) first determined a site of thoracic muscle representation within the superior motor cortex and anterior to this...
location, a site representing the diaphragm. More recently, significant foci within the superior motor cortex have been identified in respiratory-related PET studies from our laboratories (7, 18, 43) and in an fMRI study (16). However, none of these imaging studies report more than one distinct cluster within the superior motor cortex. From Foerster’s stimulation studies, we hypothesize that, in our study, diaphragmatic movements are associated with the anterior cluster of activity identified within the superior motor cortex and thoracic muscle activity is associated with the significant foci in the posterior location.

In the present study, respiratory-related activity was also identified inferiorly within the motor cortex, at a site that Ramsay et al. (43) showed to be associated with volitional expiration. A further study has shown this area to be active during vocalization (35), which is normally associated with active expiration. Our hyperpnea task involves the recruitment of predominantly inspiratory muscles, but it is probable that expiratory muscles were recruited during the task, which would explain the activity we have identified within the inferior motor cortex.

In the present study, significant activity was also identified bilaterally in the sensory cortex, immediately adjacent to the motor cortical foci. These were not identified in the respiratory-related PET studies (7, 43), presumably because the PET experimental design controlled for afferent feedback from the lung and chest wall by matching the subject’s breathing pattern (frequency and volume) with positive-pressure ventilation (see Study Design).

SMA and Premotor Cortex

In our study, we identified extensive activity bilaterally within the SMA. The SMA is functionally divided into an anterior portion, involved in the planning and selection of motor action (19, 42), and a posterior portion, associated with execution and imagination of movements (42, 45). The respiratory-related task performed by the subjects in this study was a learned task requiring precision and control in planning and executing the movement, thereby explaining activation of the SMA. Also, in common with the previous imaging studies (7, 16, 43), voluntary hyperpnea in this study was associated with a lateralization of premotor activity to the right. This activity may be attributed to a greater degree of attention (41) associated with volitional inspiration or volitional expiration.

Other Cortical Areas

Other cortical areas activated in this study were the cingulate, inferior parietal gyri, right superior temporal gyrus, and right superior and middle frontal gyri. The anterior cingulate is known to play a crucial role in the initiation and motivation of movements with a specific goal (13). Recently, a PET study reported that the anterior cingulate might be important in integrating peripheral cardiovascular changes with cognitive effort, motor preparedness, and emotional states (12). The peripheral cardiovascular changes during hyperpnea in this study were not measured; however, subjects were cognitively aware that they were increasing the effort of breathing while performing the hyperpnea task, and this may be accompanied by a change in emotional state. Furthermore, a previous PET study from our laboratory (8) identified significant bilateral activity within the cingulate associated with CO₂ simulated breathing. Activity within the frontal and parietal cortices may represent components of the frontoparietal network associated with motor planning and attention (42), required by the subjects to attend to the learned hyperpnea task.

Subcortical Activity

Subcortical activity was identified in the thalamus, globus pallidum, caudate, and cerebellum. These areas are typically associated with the voluntary control of movement (29, 42) and in a previous PET study were
shown to be active when associated with hyperpnea during and immediately after exercise (17). In addition, previous volitional breathing studies have also identified activity within the thalamus, cerebellum (7, 43), and globus pallidus (16) associated with the movement of respiratory musculature. Findings of the aforementioned studies (7, 16, 17, 43) and the more extensive results of this study enable us to reasonably conclude that the voluntary control of respiration is similar to the control of other general voluntary movements requiring activity throughout an integrated network of cortical and subcortical structures.

Brain Stem Activity

Anatomic localization. In this study, we report activity in the superior dorsal medulla, spreading bilaterally from the midline. Previous studies of behavioral breathing (7, 16–18, 35, 43) have had a more limited field of view that has not included the medulla; we believe that our study is the first report of behavioral respiratory-related activity within the human medulla. Because the BOLD technique detects signal changes in the venules and small veins draining a region of activation, signal changes may not be anatomically superimposed directly on the activated nuclei. Corfield et al. (10) used voluntary tongue contraction to identify activity specifically within the hypoglossal nucleus; the BOLD signal change occurred within a region of the dorsal medulla similar to that in the present study but was located more dorsally on either side of the midline and extended in a rostrocaudal direction of up to ~2 cm. With reference to the sections in the brain atlases of Haines (27) and Duvernoy (14), the increase in BOLD signal seen here is most likely due to increased activity in respiratory neurons associated with the nucleus of the solitary tract or the nucleus ambiguus.

Functional significance. The presence of medullary activity in the present study suggests that the medullary respiratory centers play some part in the mediation of volitional breathing. Although evidence indicates that the corticospinal pathway can functionally bypass the brain stem respiratory centers (see the introduction), these results suggest that the corticospinal pathway does not solely mediate the cortical control of breathing. If this were the case, we would have predicted no increase in activity within the medulla. The present findings would be consistent with a corticomotor-related excitation or inhibition at the medullary respiratory centers.

However, an alternative explanation for our medullary activity may be that of sensory input from the lungs and chest wall. Inflation of the lungs excites sensory vagal afferents of the lungs, airways, and muscles of the chest wall. The vagal fibers project to and terminate onto the respiratory neurons of the nucleus of the solitary tract, where they have an excitatory or inhibitory effect on these neurons (4, 30). Evidence has shown that afferents of intercostal nerves project to the cerebral cortex (23); however, it is not known whether this projection is relayed via the brain stem. We did not control for proprioceptive input in this study (see Study Design); therefore, the increased activity may be explained by sensory afferents having an excitatory or inhibitory synaptic effect on cells within the respiratory centers.

A previous respiratory-related fMRI study has reported activity in the pons (a region of the brain stem also containing respiratory-related neurons) in response to inspiratory resistive loading (25). In our study, we did not identify pontine activity, perhaps because the load of breathing in this study was not sufficient to induce activity within the pons.

Study Design

The activation-related signal changes associated with voluntary hyperpnea reflect a differential increase in activity, i.e., the increase in neural activity produced by the hyperpnea compared with the neural activity present in the control state of spontaneous eupneic breathing. Consequently, the pattern of reported activity reflects the overall sensorimotor activity associated with voluntary hyperpnea; it will include the increased motor-related activity producing the hyperpnea and the increased sensory-related activity produced by increased afferent activity arising from the lungs and chest wall. Additionally, signals in motor structures may reflect increased motor-related output and/or increased input from activity in sensory-related areas.

This control state differs from that used in previous imaging studies of voluntary hyperpnea in which the level of hyperpnea was matched by a control state of “passive” positive-pressure ventilation at an elevated VT (7, 16, 18, 43). This latter control state was used because it accounts for differences in lung inflation; therefore, the reported activations are predominantly motor related. However, such a control is not without problems; although the pump muscles may be relaxed during the passive ventilation, this relaxation is a motor-related task, achieved behaviorally, by undefined cortical structures. Additionally, mechanical ventilation produces a positive pressure in the mouth and upper airways, which requires active stabilization of these structures; mechanical ventilation is therefore associated with sensory and motor activations that are not directly related to the intended control state. As a result, it is likely that some breathing-related neural activity that is common to both conditions will not be observed.

In practice, the transition from voluntary hyperpnea to mechanical ventilation, and vice versa, requires data to be acquired discontinuously, in discrete sessions. This is a suitable protocol for activation-related studies using PET, but it is a less ideal design for BOLD fMRI studies, where low-frequency noise and signal drift, occurring between sessions, may predominate over activation-related signal changes. The on-off block design with a repeat time of ~1 min, as used in the present study, is a robust approach for fMRI studies, which results in increased sensitivity. Evans et al. (16) car-
ried out an fMRI study of voluntary hyperpnea, in a session-by-session way, using mechanical ventilation to diminish sensory feedback; although they did report activity associated with volitional breathing, the activations are weaker than those reported here.

In summary, no one study design is optimal; each has strengths and weaknesses. The present protocol was optimized to have good signal-to-noise characteristics, and it lacks the confounds associated with mechanical ventilation. However, it retains sensory-related signals associated with the voluntary hyperpnea that prevent the reported activations from being interpreted as exclusively motor related.

Other Methodological Issues

An increase in the BOLD fMRI signal will occur as a result of increases in global or local cerebral perfusion that result in a decrease in deoxyhemoglobin concentration (31, 38). Changes in breathing pattern, such as hyperpnea, alter the partial pressure of gases within the blood. Because CO₂ is a vasodilator, any changes in PCO₂ will ultimately have an effect on global cerebral blood flow and will therefore affect global signal changes. The global signal changes may confound the detection of local signal changes associated with the task if the time courses of the two signals correlate. To minimize such an effect in this study, PETCO₂ levels were clamped to ±2 Torr. To further exclude any nonspecific changes related to the global signal, the time course of the mean global signal intensity was included within the model as an effect of no interest.

BOLD fMRI is highly sensitive but is susceptible to motion-induced signal changes. Motion artifacts may be caused by head movements or may be related to tissue movements correlated to the cardiac and respiratory cycles. If movements correlate with the task, they may produce an artifactual activation-related signal (28). Imaging activity within the brain stem is potentially more problematic than imaging cortical activity, because the anatomic structures within the brain stem are smaller and tissue movements and signal variance are greater. However, our previous work indicates that robust motor-related signal changes can be determined in the medulla by using fMRI (10). In the present study, we corrected for head-related movement by realigning all the functional brain images for each subject to a reference image. To further correct for the potential effects of head movement, we constructed a second statistical model that included the parameters used for realignment as effects of no interest. This model is highly conservative, inasmuch as it would remove signal changes related to head movement that correlate with the hyperpnea task; therefore, it may remove “real” signal changes related to neural activity. This approach gave an anatomically identical pattern of activation to the statistical model without the realignment parameters, suggesting little effect of movement on our study. We have therefore reported the results of the first model only.

The characteristics (Fig. 4) of the signal changes within the medulla are similar to the signal changes within the motor cortex; however, signal changes within the medulla related to voluntary hyperpnea are ~20% smaller. This might result from a larger tissue inhomogeneity in the medulla than in the motor cortex with a different ratio of gray to white matter. The weaker signal within the brain stem, together with an increased signal variance, may explain why medullary activity reaches significance in only half of the individuals.

Conclusion

We have identified distinct cortical and subcortical structures that are associated with voluntary hyperpnea. In addition, we have successfully imaged activation-related signal changes in the medulla, highlighting a potential role of the medullary respiratory centers in behavioral respiratory control in humans. We have not identified the precise role of the medullary respiratory centers in mediating this control, and this remains the objective of further study.

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DISCLOSURES

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