fMRI responses to cold pressor challenges in control and obstructive sleep apnea subjects

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OBSTRUCTIVE SLEEP APNEA (OSA) patients exhibit repetitive periods of upper airway muscle atonia in the presence of enhanced diaphragmatic efforts during sleep. The resultant cessation of airflow from a collapsed airway induces enhanced venous return, large transient elevations in arterial pressure, and a sequence of tachycardia and bradycardia associated with significant alterations in sympathetic nervous system outflow (30). Anatomic characteristics of patients, including restricted airway dimensions resulting from deviated septa, macroglossia, micrognathia, hypertrophied tonsils, or obesity, play a significant role in the genesis of the syndrome (32). However, several features of OSA implicate a role for central neural dysfunction in initiating, maintaining, or worsening the physiological characteristics. The maintenance of upper airway atonia while diaphragmatic musculature receives enhanced drive suggests a centrally mediated loss of integration of airway or cardiovascular afferent activity with efferent flow to the oral musculature. The findings that OSA prevalence increases with age (39), an effect not related to obesity, and that moderate sleep apnea often progresses to severe apnea without significant changes in anatomic features (41) suggest that neural, perhaps including degenerative, changes play a role in OSA. Furthermore, atrial pacing reduces obstructed events (11); the mechanisms presumably involve central integration of cardiovascular afferent signals with upper airway motor control. Patients with OSA show significant gray matter loss in cortical regions mediating sensory and motor control of the upper airway, as well as areas involved in respiratory patterning and blood pressure control, such as limbic sites and the cerebellum (23). It is unclear whether the gray matter loss found in OSA subjects preceded the onset of symptoms, or whether damage was a consequence of repetitive hypoxic episodes associated with apneic events. In either case, the neural morphological changes have the potential to exacerbate characteristics of OSA and to result in further deterioration of symptoms. Examination of functional responses of neural sites mediating integration of sensory, motor, and cardiovascular integration in OSA may reveal the nature of aberrant function.

The reflexive interactions between the cardiovascular and respiratory systems provide a potential means for dysfunctional cardiovascular control to induce upper airway atonia or alter appropriate timing of muscle action. Transient blood pressure elevation normally diminishes respiratory muscle activity and slows breathing (15), an effect that is preferentially en-
hanced in upper airway musculature (25). The occurrence of enhanced rather than diminished diaphragmatic effort that is out of synchrony with oral muscle activity in OSA suggests disorders of neural reflexive timing or recruitment mechanisms for both blood pressure and somatic breathing actions in afflicted patients. Responses to other autonomic challenges that significantly modify blood pressure, including the Valsalva maneuver, are deficient in patients with OSA (14, 47). The presence of aberrant autonomic reflex expression has the potential to contribute to the disordered action of upper airway musculature in the syndrome.

An autonomic reflex that exerts considerable influence on breathing is the cold pressor "dive reflex," elicited by application of a cold stimulus to the forehead. Such stimulation exerts profound involuntary effects on blood pressure and breathing, including slowed breathing rate, marked blood pressure elevation, and bradycardia. The challenge has been used to examine the integrity of autonomic nervous system components in a number of disorders, including OSA (45); data from this latter study suggest an altered bradycardic response to the cold application in a subset of patients. Using functional MRI, which allows noninvasive visualization of neural responses, we examined neural responses to a cold stimulus to the forehead in OSA and control subjects. The objective was to outline the normal physiological and neural responses to a cold pressor challenge, to determine whether neural responses to this involuntary autonomic challenge differed in OSA patients, and whether any brain sites showing differences overlapped those previously reported as showing gray matter loss in OSA (23).

METHODS

Subjects. Twenty-one male patients with a confirmed sleep laboratory diagnosis of OSA and 21 healthy control men participated. Subjects were matched by age. Scanner size limitations or claustrophobia precluded inclusion of some OSA patients, including several women. An earlier analysis of all cases showed that pharmacological agents contaminated both the neural and physiological responses. Thus only data from drug-free cases are described here. Subjects were excluded who were currently treated with cardiovascular-related medications (angiotension conversion enzyme inhibitors, diuretics, β-blockers, α-blockers, angiotension blockers) or mood-altering drugs (e.g., serotonin reuptake inhibitors). Data from subjects in whom significant artifactual signals rising from tissue-cerebrospinal fluid or tissue-bony interfaces that were beyond the maximal 6% change expected by normal blood oxygen level-dependent processes were also excluded.

Of the initial 21 OSA subjects, 10 drug-free cases remained (mean age (range): 46 ± 12 (SD) yr (28–64 yr); and mean body mass index: 31 ± 6 kg/m² (24–41 kg/m²)], and, of the 21 control subjects, 16 were used for further analysis [mean age: 47 ± 10 yr (30–60 yr); body mass index: 27 ± 3 kg/m² (20–33 kg/m²)]. OSA severity was diagnosed according to standard practices (1) as mild (n = 1), mild-moderate (n = 1), moderate (n = 2), moderate-severe (n = 2), or severe (n = 4). Mean apnea/hypopnea index was 38 ± 27 (range: 8–95), and the mean respiratory disturbance index was 38 ± 24 (range: 19–95). The interval between OSA diagnosis and the study ranged from 0 to 6 yr (mean: 16 ± 21 mo). Three controls and two OSA subjects were left-handed. The research protocol was approved by the Institutional Review Board. All subjects gave their written, informed consent.

Physiological recording. Electrophysiological recordings were continuously collected during scanning. An ECG was obtained by using magnetic resonance (MR)-compatible surface electrodes, and oxygen saturation was collected by using a Nonin oximeter with a finger transducer (Nonin Medical, Plymouth, MN). Low-noise amplifiers, constructed to operate in high-magnetic field environments, were used to provide signal gain and initial filtering with optical transfer of signals outside the scanner room (38). A measure of respiration was provided by pressure changes from thoracic wall movement by using an air-filled bag placed over the thoracic wall. These pressure signals were conveyed by low-compliance tubing to a transducer outside the scanner room. Arterial pressure readings were obtained immediately before and immediately after each scanning period by using an automated sphygmomanometer. All physiological signals were acquired at 1 kHz on a portable computer with a Quatech analog-to-digital converter (Quatech, Akron, OH). Breathing and heart rates were determined from breath-to-breath and cardiac interbeat intervals, which were calculated from output of a peak-searching algorithm applied to thoracic wall movement and ECG signals, respectively. The heart and breathing rates were filtered by using a median filter with window lengths of 30 and 60 s, respectively. Thresholds for breathing rate changes were determined from the first significant change in rate, as determined by a repeated-measures analysis of variance, and values for the nadir were similarly calculated.

Subsequent to the imaging studies, beat-by-beat blood pressure was collected from a subset of control (n = 6) and OSA (n = 8) subjects by using an identical challenge outside the scanner with a noninvasive blood pressure monitor (Colin 7000; Colin Medical Instruments). Subjects lay in a comparable position, with scanner sounds played through earphones. Data were recorded in a similar fashion as in imaging trials.

MR imaging. Images were collected with a 1.5-T MR scanner (General Electric Signa EchoSpeed System, Milwaukee, WI). Foam was placed on either side of the head to minimize head movement during scans, and masking tape was applied to the forehead and to the headrest to further minimize motion. To explore rapid signal changes, the gradient-echo, echo-planar procedure, with blood oxygen level-dependent contrast (31), was used for image acquisition. Two series of 25 echo-planar image volumes, comprising 20 oblique sections, were acquired during 150-s time periods. The first series was a baseline with no challenge. The second series consisted of an initial 60-s baseline (10 volumes) followed without pause by a 90-s challenge period (15 volumes). Image parameters were as follows: repetition time (TR) = 6 s per image set, echo time = 60 ms, flip angle = 90°, field of view = 30 × 30 cm, no interslice gap, voxel size = 2.3 × 2.3 × 5.0 mm thick. A series of T1-weighted spin echo images (TR = 500 ms, echo time = 9 ms, field of view = 30 × 30 cm, no interslice gap, voxel size = 1.2 × 1.2 × 5.0 mm thick) was then collected at the same slice locations as the echo-planar images to assist anatomic identification.

The challenge consisted of application of a cold (4°C) bag of deuterium oxide (D2O, heavy water) to the forehead. D2O, rather than water (H2O), was used as a cold medium to
reduce the MRI signal artifact produced by liquid water in the bag.

Early in the study, in a subset of subjects, the bag was inadvertently dropped on a forehead area covered with a bandage, which insulated the subject from the cold stimulus. These subjects showed no cardiovascular or respiratory responses and none of the neural signal changes to the challenge. The data were excluded from analysis of cold effects, but served as a partial control for stimulus effects of bag application.

Data analysis. The first scan (6 s) of each series was excluded from analysis due to signal saturation. The scan representing the transition from baseline to application of the cold challenge was also removed to avoid artifactual contamination with the rapid introduction of the D2O bag near the brain. The remaining 23 image challenge and 24 image baseline sets were evaluated, in part by using SPM99 software (8). SPM99 was used for all of the preprocessing steps, except for intensity normalization (see below). Image sets were initially corrected for slice timing and motion (52) and were then spatially normalized so that each image volume in the time series was in the standardized three-dimensional Montreal Neurological Institute (MNI) reference space (26).

Spatial normalization was performed in two steps. Before normalization, the echo-planar images and T1 images were manually coregistered. In the first step, echo-planar images were normalized to an echo-planar imaging template provided by the MNI. The resulting normalization parameters were applied to the registered T1 image. In a second step, the T1 images from the first normalization step were normalized to the MNI template, and the resulting parameters were applied to the echo-planar images obtained in the first step. This process was necessary due to signal drop-out in the dorsal pons, dorsal and ventral midbrain, dentate and fastigial nuclei (13, 40, 53). A second "cluster" analysis compared baseline and cold pressor conditions on a voxel-by-voxel basis, with changes in signal intensity modeled with the average heart rate trends. SPM was used for the cluster analysis. A second-level population analysis was used, as this procedure was most effective in finding regions of consistent responses across all subjects compared with a single-level analysis. Extraction of the time trends was performed with custom software. The baseline included the baseline series and the first 60 s of the challenge series. Averaged signal intensity was determined from clusters showing significant differences on the statistical maps at each time point. Time courses of these averaged values were then plotted by using custom software. This second analysis was designed to evaluate differences in the group responses to the challenge while considering a principal physiological accompaniment of the challenge, the bradycardia associated with cold application. After review for potential warping of images relative to anatomic slices, significant voxel changes were mapped onto spatially normalized mean T1-weighted anatomic images. Particular attention was paid to distortion characteristics with the potential to introduce artifacts from inhomogeneous fields or other error sources, such as the vasculature, bone-tissue interfaces, or tissue-cerebrospinal fluid junctures. Maximum temporal resolution was limited by the TR of 6 s.

RESULTS

Physiology. Application of the cold pressor challenge resulted in heart rate slowing in both control (nadir 4.8 ± 0.9%) and OSA subjects (nadir = 4.0 ± 1.1%); heart rate returned toward baseline earlier in the OSA group (Fig. 1A). Breathing rates declined in controls (nadir = 8.3 ± 3.3%); in OSA patients, breathing rates also declined (nadir = 4.9 ± 12.4%), but with

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Fig. 1. Mean (± SD) percent change of median heart rate (A) and median breathing rate (B) for 16 control (shaded trace) and 10 obstructive sleep apnea (OSA; solid trace) subjects, plotted over time. Time 0 represents onset of the cold pressor application. *Significant group heart rate differences (P < 0.05); †significant group variability differences (P < 0.05).
significantly increased intersubject variability (Fig. 1B). The onset time for the decline and nadir of the breathing rate was later in the OSA group (P < 0.05). Baseline breathing rates for controls were 14 ± 5 (SD) breaths/min and for OSA were 16 ± 5 breaths/min; baseline heart rates for controls were 64 ± 12 beats/min and for OSA were 73 ± 8 beats/min. Beat-by-beat blood pressure measures, acquired from a subset of subjects outside the scanner, showed higher baseline blood pressures in OSA cases (mean baseline blood pressure: controls, 87 ± 5 mmHg; OSA, 95 ± 12 mmHg) and increased elevation to the challenge in the last 30 s of the challenge (mean rise: controls, 19.3 ± 4.4%; OSA, 26.9 ± 6.6%; P < 0.05, repeated-measures ANOVA).

Imaging: normalization for overall signal changes. Overall perfusion changes induced by the challenge or by scanner drift caused global-intensity trends averaging 0.2% difference in gray matter relative to baseline over the course of the scan, and corrections for these trends were incorporated by intensity, normalizing all images before analysis or extraction of time trends.

Control subject responses. Eight of fourteen anatomically defined regions of interest showed altered signal responses in control subjects (Fig. 2; on these and following images, the neurological presentation convention is adopted, with the right side of images representing the right side of the brain). These areas included the dentate nucleus of the cerebellum; dorsal, medial, and ventral medulla; insula; lenticular nuclei; head of caudate; and ventral midbrain (head of caudate and medial medulla not shown). The hippocampus showed significant signal decrease. Clusters of voxels that increased in a pattern inverse to that of heart rate for control subjects are shown in Fig. 3 and Table 1. These regions included portions of the cerebellar cortex; thalamus; insula; hippocampus; amygdala and superior temporal cortex; and ventral, frontal, and cingulate cortices.

Similar responses in OSA and control subjects: cluster analysis. Significant increases in signal, modeled to the inverse of heart rate, occurred in both groups in the superior and anterior insula, superior temporal cortex, quadrangular lobe of the cerebellum, and the anterior and left posterior cingulate (Fig. 4, Table 1). The OSA group showed significantly higher signals than controls in the cerebellar quadrangular cortex and lower signals in the superior (dorsal) insula late in the challenge.

OSA signals different from control subject responses: anatomically defined areas. Eleven of fourteen anatomically defined regions showed response pattern differences between the OSA and control subjects; 10 of those patterns in which signal intensities were greater in control subjects over OSA cases are shown in Fig. 2. The cerebellar fastigial nucleus (not shown in Fig. 2) was the only one of anatomically defined structures in which OSA signal changes were larger than in control subjects; the remaining three areas (dorsal pons, head of the caudate, medial medulla) showed no group differences. Although the insula signals increased significantly in both groups, the increase in the OSA group was transient, whereas the increase in the control group was sustained. Similarly, the hippocampus in both groups showed decreased signal intensity; however, the change was more sustained in the OSA group.

Cluster analysis: OSA signal values less than control responses. With modeling to the heart rate, signals in the ventral thalamus, ventral anterior insula, and regions within the hippocampus modestly increased in controls, but decreased in OSA cases (Fig. 5; Table 1).

Cluster analysis: OSA greater than control subject responses. The cluster analysis, modeling to inverse heart rate, demonstrated signal intensity increases in OSA cases within the quadrangular lobe of the cerebellar cortex, anterior insula and medial frontal cortex, and anterior and posterior cingulate gyri, with declines or no change in the control subjects (Fig. 6; Table 1). The responses were, on occasion, expressed unilaterally or were greater on one side over the other, e.g., left posterior cingulate, right insula.

DISCUSSION

Several generalizations emerged from this study of a trigeminal mediated cold pressor challenge. The classic physiological response of bradycardia and respiratory rate slowing appeared in control subjects, whereas, in OSA cases, onset of respiratory slowing was delayed, the decreased breathing rate was not as sustained and was substantially more variable, and heart rate did not decline as precipitously. Blood pressure rises to the challenge in a subset of OSA cases outside the scanner were higher. The control neural responses to the challenge included increased signals in thalamic, medullary, and cerebellar cortex; dentate nucleus; and insular, cingulate, and frontal cortex areas. Cerebellar, insular, cingulate, and frontal cortex areas have previously been implicated in cardiovascular activation triggered by cognitive tasks and isometric exercises in normal subjects by using positron emission tomography procedures (5a). The principal response pattern differences between groups emerged.
in the cerebellar cortex, the insular and posterior cingulate cortex, thalamus, and the hippocampus. The response patterns differed inversely in certain areas. Cerebellar and posterior cingulate structures responded to the challenge with increased signal in controls, but increased signal in OSA; signals in insular, thalamic, and hippocampal areas decreased in OSA cases, but remained largely unchanged in controls. Some of these regions have earlier been shown to exhibit diminished gray matter volume in OSA (23) or in heart failure patients with sleep-disordered breathing (51). The findings suggest multiple impaired neural sites in OSA patients, with cerebellar and limbic structures particularly implicated as deficient in the pressor challenge.

**Cold pressor challenge.** The cardiovascular and breathing effects elicited by cold facial stimulation (the dive reflex) originate from primitive processes that, under unusual circumstances, serve to override homeostatic control mechanisms. The processes include induction and maintenance of apnea necessary for underwater motility in lower animals, despite increasing CO₂ and reduced O₂ availability, and increases in sympathetic outflow, despite an expected decrease after blood pressure elevation accompanying dives (27). The sudden drop in forehead temperature activates trigeminal sensory afferents, which synapse in the spinal trigeminal nucleus of the medulla (19, 27). Neurons within the spinal trigeminal nucleus project to a number of brain stem cardiorespiratory regions, including the nucleus of the solitary tract, parabrachial regions of the dorsal pons, and, indirectly, to A1, A5, and C1 cells of the medulla (7, 28, 37). Neurons within the spinal trigeminal nucleus project directly to the medial ventroposterior region of the thalamus, which in turn projects to the lateral aspect of the precentral gyrus for perception of temperature. The challenge elicited less of a rate slowing for both breathing and heart rate and

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**Table 1. MNI coordinates of significant clusters for 6 response comparisons**

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MNI, Montreal Neurological Institute; OSA, obstructive sleep apnea.
a delayed onset for the respiratory response in OSA. An enhanced blood pressure response over controls was found in a subset of OSA cases, suggesting that reactions to blood pressure in the cold pressor challenge were also altered. However, those values were obtained subsequent to the imaging task (necessary because a MR-compatible noninvasive blood pressure monitor was not available), did not include all of the subjects, and were obtained in an environment similar, but not identical, to the scanning circumstance. Thus correlations of signal changes were taken with heart rate measures.

**Thalamic structures.** The ventroposterior thalamic region showed increased signal intensities in controls, but not in OSA subjects, when signal changes were modeled to heart rate. Thus the differences in responses between the two groups may depend partially on a loss of primary sensory input to thalamic areas, a possibility that is supported by findings of diminished responses to oral airway stimulation in OSA subjects (24).

**Medullary and midbrain structures.** The absence, in OSA cases, of normal responses within the dorsal medulla may also reflect a diminished recruitment of the nucleus of the solitary tract by reduced trigeminal nucleus input or diminished participation of the solitary tract nucleus. The ventral medullary areas showed an early decline in the OSA cases; this region receives projections from the solitary tract nucleus and plays a significant role in respiratory pattern generation. The early decline in signal in the ventral medulla may contribute to the late onset of respiratory slowing in the OSA group. Similarly, diminished signal changes, relative to controls, in the dorsal and ventral midbrain likely represent reduced participation of periaqueductal gray regions. These areas project to the

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**Fig. 3.** Top: regions showing signal increases that correlated significantly ($P < 0.01$) with inverse heart rate in control subjects evoked by the cold pressor challenge. Regions showing significant increases included the cerebellum (1–2), thalamus (5–7; A, C), insular cortex (3–8; A–F), hippocampus, amygdala, and superior temporal cortex (2–4; C–D), frontal cortex (3–4; G–H), and cingulate regions (8–10; G–H). The regions are overlaid onto the mean of the 16 control subjects’ anatomic images. The color bar codes $t$ statistic. Bottom: slice levels for 1–10 in the axial plane and A–H in the coronal plane are shown in the outline drawings.
Fig. 4. Regions showing significant ($P < 0.05$) signal increases that correlated with inverse heart rate in major brain structures. Significance levels ($t$ statistic) are conveyed in the color bar and overlaid onto a mean of a set of anatomic images (16 control, 10 OSA) in sagittal, coronal, and axial views. The signal intensity changes, plotted over time, for each cluster at the center of the cross hairs are shown on the right. Time 0 represents onset of challenge. *Significant between-group differences are shown at the relevant time points ($P < 0.05$).
nucleus of the solitary tract and to the ventral medulla, modify respiratory patterning and cardiovascular control (13, 17), and have the potential to influence the pattern differences found here.

Cerebellar structures. Localized regions within the quadrangular lobe of the cerebellar cortex showed a signal intensity rise to the challenge in OSA subjects, as opposed to little change in signal intensity in the control group. Other localized regions within the cerebellar cortex showed comparable responses in both groups. The cerebellar cortical areas of aberrant responses overlapped regions of gray matter loss in OSA patients (23), as well as patients with heart failure showing disordered breathing during sleep (51). Cerebellar structures classically have been associated with roles in coordinating somatic afferent with efferent information in the performance of “error correction” on a moment-to-moment basis. A role in breathing control is frequently overlooked, despite recognition that respiratory muscles represent a substantial proportion of all skeletal musculature and that respiratory muscle modulation is heavily dependent on afferent information from thoracic wall stretch receptors, chemoreceptors, and arterial pressure receptors. Experimental evidence from animal models emphasizes the role of the deep cerebellar nuclei in regulating phrenic outflow and regulation of inspiratory onset after apnea (22). Moreover, neurons in the fastigial nucleus respond to CO₂ and mechanical manipulation (54), and interpositus nuclei are involved in cough responses, which are associated with transient elevations of blood pressure (54). Damage to cerebellar areas is accompanied by a high incidence of sleep-disordered breathing, including OSA, both in human adults and infants (4, 50).

The breathing response in OSA subjects during the cold pressure challenge was characterized by extreme variability, with some subjects exhibiting increased, and others decreased, breathing rates. These varied responses appear to reflect a “loss of modulatory control” by relevant neural structures to the cold pressure challenge. The increase in signal intensity in the cerebellar cortex of OSA subjects may reflect efforts accounting for this loss of normal modulatory control.

Insula. The insula signal intensity changes differed significantly in OSA cases compared with controls. The insular cortex plays substantial roles in autonomic control in humans (34). The pattern of responses in the insular cortex paralleled the heart rate responses in both groups. In OSA subjects, both heart rate and insula signal intensity returned to baseline levels significantly earlier than in controls. Discrete portions of the insula also markedly differed in responses to the challenge in OSA over controls, with significantly greater declines in signal in OSA compared with controls in the right ventral insula. The enhanced laterality of the response difference to the right side is of particular interest, because the nature of autonomic influence exerted unilaterally by the insula differs, with parasympathetic outflow modified by the left insula and sympathetic outflow modulated by the right (33, 35, 36). The larger neural signal response difference on the right (sympathetic-related) side may underlie the higher basal heart rates found in the OSA group. Between the insula show substantial gray matter loss in heart failure patients with sleep-disordered breathing, a group that also shows high sympathetic tone (51); a similar gray matter decline was not detected in OSA patients (23), possibly because of limited resolution of the anatomic technique. It remains to be determined whether enhanced-resolution MR procedures will reveal gray matter loss in OSA; however, the extent of alterations in neural response indicates...
Fig. 6. Statistically significant voxels, overlaid on anatomic images from various brain areas, together with averaged trends of signal changes in OSA and control subjects during the course of the cold pressor challenge, where signals increased in OSA and remained the same or declined in control subjects. Time 0 represents onset of the challenge. Clusters are color-coded for significance according to the color bar for $t$ statistic.
significant functional impairments in a brain structure shown by others to contribute substantially to autonomic control.

Hippocampus. The tissue loss found earlier in OSA also included regions of the hippocampus that are damaged by intermittent hypoxia in animals (12) and an area preferentially susceptible to injury as a consequence of seizure activity triggered by excitation of the principal afferent pathway, the perforant pathway, or other seizure-related hypoxia-ischemia (46). In addition to cognitive roles apparently affected in both OSA and epilepsy (2, 55), hippocampal structures participate in cardiovascular and respiratory regulation. Direct chemical stimulation of the hippocampus evokes marked decreases in arterial pressure and heart rate (44). Furthermore, hippocampal structures contain neurons that discharge with the respiratory and cardiac cycle (10), show significant patterns of activity change before sigh-apnea sequences in animals (42), and, on stimulation, alter breathing patterns (44). The hippocampus may play a modulatory role, adjusting the gain of the cardiorespiratory reflex in the cold pressor challenge, because resting arterial pressure and heart rate are unaffected by hippocampal blockade (49). The diminished hippocampal response in the OSA group may represent a reduced modulatory role, resulting in the significant differences in heart rate and respiratory responses in OSA subjects in response to the cold application.

Cingulate gyrus. The posterior cingulate also showed significant response differences from those of controls. A motor role for both anterior and posterior portions of the cingulate has been recognized by several stimulation studies (e.g., Ref. 6). The anterior cingulate contains neurons that discharge with both the respiratory and cardiovascular cycles (9) and is involved in vocalization, a task requiring intricate control of the fine musculature of the upper airway (48). The role in vocalization has been especially related to affective components of expressive sounds. Dyspnea activates the right posterior cingulate (40), and this activation may derive from affective contributions to the breathing task. However, the posterior cingulate functions may be even more complex. A growing body of evidence suggests that the structure integrates information of internal movement cues for spatial navigation (16). It is possible that the “internal movement” cues extend to appropriate motor behaviors of a wider range, including respiratory muscle motions in these OSA subjects.

More rostral portions of the cingulate gyrus show gray matter loss in OSA subjects (23). The rat posterior cingulate shows a region-specific sensitivity to excitotoxicity apparently mediated through N-methyl-D-aspartate-related disinhibition (20), a finding that suggests that the area may be at particular risk in humans during the intermittent hypoxia of OSA. Cingulate areas near sites showing significantly different response patterns from controls project to the anterior insula and parahippocampal regions in primates (29). Thus deficient functional and anatomic characteristics appear in regions with anatomic projections to other areas showing gray matter loss. These areas include the insula of heart failure patients with sleep-disordered breathing and the cingulate and hippocampus of OSA (23) and heart failure cases (51). It is unclear whether the commonality of axonal connections and response deficiencies relate to aspects of hypoxic or other damage in sleep-related breathing pathology.

 Portions of the cingulate gyrus play a role in mediating responses to blood pressure challenges, as shown by functional MRI evidence from animal and human studies (13, 18, 56). Stimulation results in large changes in blood pressure and heart rate (3). It may be the case that the posterior cingulate is involved in the heart rate slowing found here; however, the history of its role in motor control suggests that participation in the respiratory pattern changes is more likely.

Lateralization of responses. The cold pressor challenge applied to the entire forehead should elicit bilateral recruitment of primary sensory neurons and, therefore, activity of bilateral central neural structures. The accompanying blood pressure elevation and slowing of heart and respiratory rates would presumably be mediated by areas on both sides of the brain. Instead, pronounced lateralization of signal changes was observed in multiple regions, including the right insula, right cingulate, and cerebellar cortex. The laterality finding was similar to that observed previously with multiple blood pressure elevation stimuli (13) and also noted in response to pronounced inspiratory loading challenges (40). As in the earlier studies, no relationship between lateralization of the signal and handedness of the subjects was observed.

The lateralization of neural responses in some structures in response to autonomic challenges, a finding also shown elsewhere (5a, 13), raises significant theoretical and practical issues. The finding places aspects of breathing and cardiovascular control within the framework of a variety of other behavioral acts, including language expression and voluntary motor behaviors, both of which are highly lateralized. Unilateral neural damage from OSA may interact with ipsilateral breathing control mechanisms and may be especially important during sleep, in which affected areas may be less capable of an adequate response, lacking the parallel redundancy of respiratory control typically available in waking states.

Overall perfusion changes. Any manipulation that exerts significant blood pressure effects, including cold pressor challenges, can alter overall neural signal properties through gross perfusion changes. Autoregulatory processes normally operate to protect against major or sustained perfusion changes, including those from hypotension (57). However, autoregulatory processes may not operate normally in the OSA population, by reason of repetitive hypoxic, hypercarbic, and transient hypertensive damage associated with obstructed breathing and momentary global perfusion changes that can occur with autonomic manipulations (14). Overall perfusion changes induced by the dive reflex are of particular concern, because the primitive reflex response partially serves to conserve metabolic.
resources for underwater survival. Manipulations such as increased inspired CO₂ can elicit overall signal changes from altered cerebral perfusion. However, determination of regional neural changes in primary visual cortex to photic stimulation can be readily evaluated in such conditions, because the changes are superimposed on the hypercapnia-induced global signal intensity alterations (5, 21), and the global changes can readily be partitioned by intensity normalization procedures. We found an average 0.2% global change in gray matter in both groups and used intensity normalization of gray matter signals to control for the contribution of global changes from regional patterns. Separation of global trends in gray matter from those in fibers precluded differential global effect contributions in the normalization process. Comparable procedures minimized effects of other autonomic manipulations in normal and OSA subjects (14). The use of normalization procedures, together with findings that areas immediately adjacent to recruited sites showed different patterns of signal intensity response, argue that signal changes reported in this study result from differential recruitment of brain sites and not from overall changes in cerebral blood flow.

In summary, cerebellar, insular, and cingulate cortex areas, as well as hippocampal, medullary, midbrain, and thalamic sites, showed significant response pattern differences to the cold pressor challenge in OSA cases. Relative to control subjects, cerebellar and limbic areas appeared to be particularly affected when signal changes modeled to heart rate were considered. The affected limbic structures have been associated with fine control of the upper airway musculature and autonomic outflow. The cerebellar dysfunction may have contributed to a mismatch of afferent input with coordinated motor output. The inappropriate neural responses to the cold pressor challenge suggest that specific neural structures involved in affect and motor control contribute to the respiratory dysfunctions of OSA.

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REFERENCES


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