Hypercapnic Exposure in Congenital Central Hypoventilation Syndrome Reveals CNS Respiratory Control Mechanisms

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1Department of Neurobiology, 2Department of Radiology, 3School of Nursing, and the 4Brain Research Institute, University of California at Los Angeles and 5Childrens Hospital Los Angeles, Los Angeles, California; and 6Kosair Children’s Hospital Research Institute, Department of Pediatrics, University of Louisville School of Medicine, Louisville, Kentucky

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Harper, R. M., P. M. Macey, M. A. Woo, K. E. Macey, T. G. Keens, D. Gozal, and J. R. Alger. Hypercapnic exposure in congenital central hypoventilation syndrome reveals CNS respiratory control mechanisms. J Neurophysiol 93: 1647–1658, 2005; doi:10.1152/jn.00863.2004. Congenital central hypoventilation syndrome (CCHS) patients show impaired ventilatory responses and loss of breathlessness to hypercapnia, yet arouse from sleep to high CO2, suggesting intact chemoreceptor afferents. The syndrome provides a means to differentiate brain areas controlling aspects of breathing. We used functional magnetic resonance imaging to determine brain structures responding to inspired 5% CO2-95% O2 in 14 CCHS patients and 14 controls. Global signal changes induced by the challenge were removed on a voxel-by-voxel basis. A priori–defined volume-of-interest time trends (assessed with repeated measures ANOVA) and cluster analysis based on modeling each subject to a step function (individual model parameter estimates evaluated with t-test, corrected for multiple comparisons) revealed three large response clusters to hypercapnia distinguishing the two groups, extending from the (1) posterior thalamus through the medial midbrain to the dorsolateral pons, (2) right caudate nucleus, ventrolaterally through the putamen and ventral insula to the mid-hippocampus, and (3) deep cerebellar nuclei to the dorsolateral cerebellar cortex bilaterally. Smaller clusters and defined areas of group signal differences in the midline dorsal medulla, amygdala bilaterally, right dorsal-posterior temporal cortex, and left anterior insula also emerged. In most sites, early transient or sustained responses developed in controls, with little, or inverse change in CCHS subjects. Limbic and medullary structures regulating responses to hypercapnia differed from those previously shown to mediate loaded breathing ventilatory response processing. The findings show the significant roles of cerebellar and basal ganglia sites in responding to hypercapnia and the thalamic and midbrain participation in breathing control.

INTRODUCTION

Congenital central hypoventilation syndrome (CCHS) provides an “experiment of nature” that can differentiate brain mechanisms controlling breathing. Affected children exhibit deficient ventilatory responses to hypercapnia and hyponxia and chronic respiratory insufficiency in the absence of major pulmonary, cardiac, neuromuscular, or chest wall disease, and show a diminished drive to breathe during sleep (Commare et al. 1993; Haddad et al. 1978; Oren et al. 1987; Paton et al. 1989). Deficiencies also include a loss of dyspnea, i.e., the perception of air hunger or discomfort for the need to breathe to either hypoxic or hypercapnic exposures (Paton et al. 1989; Shea et al. 1993). Furthermore, CCHS patients display reduced influences of breathing on cardiac rate variation (Woo et al. 1992) and a range of both sympathetic and parasympathetic nervous system control deficits, including profuse sweating and a proclivity for syncope (Vanderlaan et al. 2004; Weese-Mayer et al. 1992). Despite reduced ventilatory responses to hypercapnia or hypoxia, peripheral chemoreceptor responses are partially preserved, particularly in those children who are able to sustain near-adequate ventilatory output during wakefulness (Gozal et al. 1993). Affected patients increase ventilation to exercise and to passive motion of the extremities, even during sleep (Gozal and Simakajornboon 2000; Gozal et al. 1996), indicating that integration of respiratory motor output with cyclic locomotor pattern systems (Dejoures 1959) are preserved, even with central chemoreceptor integrative failure. Although ventilatory responses to chemoreceptor stimulation are impaired, affected children do arouse from sleep to high CO2 (Marcus et al. 1991), suggesting that centralafferent processes are largely intact and that breathing deficits in CCHS result from diminished integration of respiratory motor processes with sensory input.

The combination of intact aspects of breathing control with selective deficiencies in CCHS provides a unique opportunity to examine central control of discrete components of breathing regulation. Despite the range of respiratory deficiencies, affected patients apparently possess a relatively unimpaired afferent system for central chemoreception (since arousal to hypercapnia is preserved), functioning motor output systems, and integrity of respiratory motor/locomotor patterning organization. We used functional MRI (fMRI) procedures to evaluate responses to hypercapnia throughout the brain in CCHS patients and control subjects. We hypothesized that brain structures implicated in mediating chemoreception and responsive to air hunger, the latter including the cingulum, insula, and amygdala (Banzett et al. 2000; Evans et al. 2002; Peiffer et al. 2001), would be less responsive in CCHS, and that motor coordination areas of the cerebellum underlying rapid adjustment of breathing muscle action and compensatory cardiovascular responses would react abnormally in the syndrome.

METHODS

Fourteen children with a CCHS diagnosis, based on standard criteria (American Thoracic Society 1999) (7 males and 7 females) and 14 controls (8 males and 6 females) participated. The groups were
Subjects lay supine in a 1.5-T MRI scanner (General Electric Signa, Milwaukee, WI) and breathed through a two-way, nonrebreather valve. Tracheostomy openings were closed throughout the studies, and subjects wore noseclips. Head movement was minimized by application of masking tape over the forehead and foam pads on either side of the head. Airflow and the ECG (lead I) were recorded together with fMRI images (Macey et al. 2004c). End-tidal CO₂ (ETCO₂) and O₂ saturation were measured in a subset of 10 control and 7 CCHS subjects, respectively. Each subject underwent two scanning periods, separated by ≥8 min: the first consisting of a 150-s baseline breathing room air, and the second period with a 30-s baseline followed without pause by a 120-s challenge. The gas mixture (5% CO₂-95% O₂) was room air, and the second period with a 30-s baseline followed without pause by a 120-s challenge. The gas mixture (5% CO₂-95% O₂) was delivered via the inspiratory arm of the two-way valve throughout the 120-s challenge period. The high level of O₂ was used to suppress peripheral chemoreceptor afferent activity.

Each 150-s scanning period consisted of 25 volumes of 20 oblique image slices, collected using a gradient-echo echo-planar imaging (EPI) protocol [repetition time (TR) = 6 s, time to echo (TE) = 60 ms, flip angle = 90°, field of view (FOV) = 30 × 30 cm, no interslice gap, and voxel size = 2.3 × 2.3 × 5 mm]. Blood oxygen level–dependent (BOLD) intrinsic contrast was used to evaluate neural responses to the challenge. Spin-echo T1-weighted images (TR = 500 ms, TE = 9 ms, FOV = 30 × 30 cm, no interslice gap, voxel size = 1.2 × 1.2 × 5 mm) were collected at comparable orientation and positioned to assist anatomical identification.

The images were evaluated with a statistical parametric mapping package, SPM (Friston et al. 1995), and custom software. Preprocessing included correction for slice timing and motion, and volumes were spatially normalized. Gray matter was segmented from white matter and cerebrospinal fluid (CSF) to create a mask of regions where the probability of gray matter was >0.5 (Ashburner and Friston 1997); after application of the mask to the spatially normalized images, the resultant images were smoothed. Global signal changes, induced by overall perfusion changes from the challenges or other sources, were removed (Macey et al. 2004b), and significant regional changes in signals were assessed.

Two analytic approaches were used: a cluster analysis and a volume of interest (VOI) procedure. Cluster analysis provided an overall whole brain search on a voxel-by-voxel basis to determine what brain areas were activated by hypercapnia, assuming a step function of OFF to ON response pattern (termed “boxcar”), of generally increased or decreased signal to the challenge. The time course of each voxel was matched using a linear model of a boxcar pattern convolved with a standard hemodynamic response function, and the resulting parameter estimates for each subject were saved as one “volume.” These estimates were compared within the control group using a one-sample t-test (a population, or random effects procedure; $P < 0.05$, false discovery rate correction for multiple comparisons) and across groups using a two-sample t-test, and “clusters” of adjacent significant voxels were mapped to show an overview of responsive sites. Time courses of all significant voxels within selected clusters were extracted, averaged across all voxels to avoid multiple comparisons issues, plotted for the two groups, and verified using repeated measures ANOVA (RMANOVA; $P < 0.05$). The RMANOVA procedure is specifically designed to account for multiple comparisons across time, and although the technique can be considered an extension of standard ANOVA, it is implemented using a linear model (Littell et al. 1996). Even though clusters often encompass more than one structure, the time course of the cluster response is representative of the response time course of all areas within that cluster. Physiological changes to hypercapnia described earlier (Macey et al. 2004c) showed unique time trends, including early, transient patterns, which did not necessarily follow an ON-OFF sequence. For that reason, we implemented VOI analysis using custom routines that evaluated participation of a priori–defined structures without assumptions about the response pattern and were unaffected by potential variation in spatial normalization, since each VOI was outlined on a subject-by-subject basis. Voxel intensities were averaged for each subject at each time-point, and trends of these VOI analyses were plotted. RMANOVA evaluated differences from baseline for each group and response differences between groups (Littell et al. 1996). The VOI analysis involved averaging all voxel values within the VOI prior to statistical analysis, and therefore there were no multiple comparisons across voxels. Additionally, the Tukey-Fisher criterion for multiple comparisons was applied, namely the overall effect of the model was tested for significance ($P < 0.05$) prior to testing individual time-points for within- or between-group effects.

The study was approved by the Institutional Review Board of the University of California at Los Angeles. The procedures were conducted with the understanding and written consent of the subjects and parents or guardians.

RESULTS

Physiology

Respiratory and cardiac rate and variability responses to hypercapnia have been described earlier (Macey et al. 2004c). Briefly, breathing and heart rates rose during the hypercapnic challenge in both control and CCHS groups, but a transient decline to baseline breathing rates after 60 s in control subjects did not occur in CCHS patients. A remarkable augmentation of heart rate variability, contributed largely by respiratory-related variation, increased only transiently and to a much lesser extent in CCHS cases. ETCO₂, measured in a subset of subjects, was higher in CCHS compared with control subjects during baseline and challenge periods, and increased in both groups during hypercapnia.

Global BOLD signal

The global BOLD signal increased in both groups 20 s after challenge onset (Macey et al. 2003), but the increase was greater in control subjects from 30 s onward. Detrending removed all global effects (Macey et al. 2004b).

Regional response patterns

Figure 1 shows regions of significant response in the control group based on the cluster analysis. Most patterns appeared early and transiently, with rapid increases bilaterally in the dorsal cerebellar cortex and deep nuclei (Fig. 1, A and E, see FN) and in the posterior right insula (Fig. 1B). Rapid, but largely transient, signal decreases occurred in a region encompassing the dorsal pons, including the parabrachial region, midbrain, the posterior thalamus, and hypothalamus (Fig. 1, A and C, time trend), a portion of the right hippocampus and overlying cortex (Fig. 1D), the caudate nuclei bilaterally (Fig. 1, E, time trend, and F), and left putamen (Fig. 1F).

Several clusters of response differences were apparent in CCHS patients. These clusters included a region extending from the caudate nucleus through the putamen and ventral insula and extended to the amygdala and dentate and CA3 areas of the hippocampus and surrounding cortex on the superior-lateral surface of the hippocampus (Fig. 2A, I, 2, 4,
and 5); signal changes in CCHS patients were larger in this cluster (blue-coded areas), with values rising in CCHS, but showing an initial transient decline in control subjects (Fig. 2B). The pattern was primarily unilateral, with the largest cluster on the right side, although a smaller cluster in the left basal ganglia was also apparent (Fig. 2A3).

A second extensive cluster of enhanced signals in CCHS patients ranged from the posterior-dorsal, medial, and ventral...
thalamus (Fig. 3A, 1 and 2) through the medial midbrain (Fig. 3A, 1–3) and, with a short interruption, terminated in the dorsolateral pons (Fig. 3A4). As in the more rostral cluster of Fig. 2, an initial transient decline emerged in control subjects, with a gradual return to baseline, but CCHS patients showed only a late rise (Fig. 3B).

A third large cluster extended from the deep cerebellar nuclei bilaterally to the dorsal cerebellar cortex (Fig. 4A, 1–4) in a defined columnar arrangement (Fig. 4A, 2 and 3); in this cerebellar cluster, signals were greater in control subjects (red-orange-white coding), with pattern differences emerging early in the challenge (Fig. 4B).

Increased signals in control over CCHS subjects were also apparent in a region encompassing the dorsal medulla, within which lies in the solitary tract nucleus (NTS; Fig. 5A, 1–3). The corresponding trend plot (Fig. 5A, right) shows an early and sustained rise in control subjects but an initial transient decline in CCHS cases. A bilateral cluster in the amygdala, extending to nearby cortex (Fig. 5B, 1–3) showed initial, transient divergent responses in the two groups, a decline in CCHS, and an increase in control subjects (Fig. 5B, right). In contrast to the transient decline in the control response in the right insula, a sustained rise in a cluster in the left anterior insula appeared, with no change in CCHS (Fig. 5C). Signals in CCHS patients were larger in a cluster over the posterior-superior temporal cortex, on the right side only (Fig. 5D, 1–3); an early transient decline in the control group appeared (Fig. 5D, right).

The response time trends of defined VOI are outlined in Fig. 6, and Table 1 represents a summary of those changes. The large, early, transient responses found in Figs. 2–5 for multiple structures also appeared in VOI for the dentate nucleus, hippocampus, caudate head, left anterior insula, medial medulla,
DISCUSSION

The most remarkable aspect of the regional fMRI signal responses to hypercapnia, assessed after correction for global changes, consisted of pronounced differences expressed in regions not classically associated with breathing control, but traditionally related to affect, autonomic regulation, or motor coordination. A second noteworthy finding in control subjects was the early transient nature of the responses, especially in motor regions. The early reactions were muted or absent in cerebellar, thalamic, and basal ganglia sites in CCHS, whereas limbic sites, principally the hippocampus and nearby cortex, amygdala, insula, and dorsal and ventral midbrain showed responses that were delayed or were in the opposite direction compared with control subjects. A column of deficient responses extending from the dorsal, medial, and ventral thalamus through the midbrain to the dorsolateral pons was unex-
expected. Aberrant responses also appeared in some, but not all, sites presumably targeted by PHOX2B expression, mutations of which have been found in a high proportion of CCHS patients (Amiel et al. 2003; Weese-Mayer et al. 2003); these areas included the dorsal medulla and other sites that should play a role in the modulation of the hypercapnia challenge (Dauger et al. 2003).

fMRI signal interpretation

Images collected during fMRI procedures are sensitive to levels of deoxyhemoglobin in the blood, with higher levels of deoxyhemoglobin leading to lower signal intensities; these signal intensities are termed the BOLD signal. Neural activation results in an inflow of oxygenated blood that decreases deoxyhemoglobin concentration and increases the BOLD signal (Ogawa et al. 1990). There is a strong correlation between changes in BOLD signal and local field potentials, and therefore changes in the BOLD signal reflect input and intracortical processing (Logothetis et al. 2001).

Control responses

The findings confirm earlier fMRI studies in the adult that noted cerebellar and more rostral involvement in mediating hypercapnia (Brannan et al. 2001; Corfield et al. 1995; Gozal et al. 1994; Harper et al. 1998; Kastrup et al. 1999; Parsons et al. 2001), as well as animal studies, indicating a significant role for deep cerebellar nuclei in such challenges (Xu and Frazier 1997, 2002). Several of these studies showed more substantial changes within multiple sites in the adult than appeared here in children. However, we adopted very conservative correction
procedures to avoid global signal contributions from CO₂ effects on the vasculature, and these procedures may have reduced the extent of signal change in several areas (Macey et al. 2004b). Specifically, we partitioned gray matter and only examined effects therein, since gray and white matter have differing patterns of global signal change, and we removed all signal patterns matching the overall global pattern within gray matter (to view global signal effects, see Macey et al. 2003). It is useful to extend the adult findings to children, but of perhaps greater importance are the time courses of responses in affected
structures. The most common response was an early transient rise or fall in signal in cerebellar and basal ganglia structures, with an intermediate-duration response in thalamic-midbrain and hippocampal sites, and a gradual onset and more-prolonged response in the insula. The longer-duration reactions in limbic structures likely represent autonomic regulatory control aspects, whereas the transient cerebellar and basal ganglia patterns presumably reflect faster motor components of the hypercapnic challenge, i.e., respiratory efforts required to increase tidal volume.

**Overall reactivity in CCHS**

We earlier found that cerebral vascular reactivity seems to be altered in CCHS (Macey et al. 2003); this alteration may reduce BOLD signal changes assumed to represent neural activity variation between the two groups, given equivalent levels of neural activity change. Many of the group differences here seemed to be of an attenuated nature, with a missing early transient or muted late response to hypercapnia. The extent of overall reduction of early and late responses could reflect global vascular reactivity deficiencies. This possibility, however, does not explain all the abnormal patterns in CCHS subjects, given that other group differences were very robust, with signals that differentiated the two groups moving in opposite directions. These latter patterns appeared in the dorsal and ventral midbrain, the amygdala, and the hippocampus (Fig. 6). The different response patterns included both transient (dorsal and ventral midbrain) and more prolonged (amygdala, hippocampus) signals. The presence of several regions with responses in the opposite direction is an unlikely scenario for overall muting by impaired cerebral reactivity processes.

**FIG. 6.** Time trends of volume of interest (VOI) from control and CCHS subjects during baseline and challenge periods. *Time-points of group difference (RMAVONA, $P < 0.05$); time-points of significant signal increase or decrease relative to baseline within each group are indicated by bars above or below plots (key at bottom).
Cerebellar Purkinje cells normally inhibit the deep cerebellar nuclei (Ohtsuka 1988), which showed response deficits in CCHS. Purkinje cells of the cerebellum are exceptionally sensitive to ischemia-induced damage (O’Hearn and Molliver 1997; Schadé and McMenemey 1963); a portion of this damage seems to be mediated via excitotoxic processes through fibers from the inferior olive, a major afferent input to Purkinje cells (Welsch et al. 2002). CCHS patients are often exposed to hypoxia by virtue of inadequate assisted ventilation during sleep or during waking periods associated with infection or rest. Thus some portion of the cerebellar damage may be secondary to repetitive hypoxic exposure, although intrinsic damage from the syndrome cannot be excluded.

An exaggerated increase in respiratory-related heart rate variation accompanied hypercapnia in control subjects; this rise was not found in CCHS patients, who showed minimal variation from respiratory sources even during baseline (Macey et al. 2004c). The reduced respiratory-related arrhythmia points to a loss of integration of rapid response respiratory and cardiac interactive elements, a role the cerebellum serves, among other motor coordination tasks (Lutherer and Williams 1986; Lutherer et al. 1989; Xu and Frazier 1994, 1997, 2002).

Limbic structures

The control subjects showed recruitment of several limbic structures, and the CCHS patients showed temporal, magnitude, and directional differences in these responses. The limbic areas likely serve multiple functions in mediation of CO₂ responses, as suggested by animal and human studies, including chemoreception, perception of air hunger, and autonomic aspects of the breathing challenge.

The perception of respiratory discomfort to hypoxia or hypercapnia is a powerful drive to inspiratory effort (Moosavi et al. 2003; Simon et al. 1989) and is lacking in CCHS (Paton et al. 1989; Shea et al. 1993). The level of CO₂ used in this study (5%), together with delivery of a hyperoxic (95% O₂) balance (a combination that minimizes air hunger; Moosavi et al. 2003), was unlikely to induce breathlessness in the control subjects. Nevertheless, fMRI differences in limbic structures in this study may relate to the neural systems that incorporate sensation of high CO₂ to elicit air hunger, because limbic regions mediate affective responses to high CO₂ or hypoxia. Limbic sites, such as the amygdala and insula, have long been implicated in distress processing (Amaral 2003) and are recruited in mediating restricted-breathing tasks that induce dyspnea (Evans et al. 2002). Several limbic sites, particularly the insula and amygdala, revealed responses to hypercapnia in control subjects that differed from CCHS patients, but not the cingulate gyrus, a structure that is recruited during extreme loaded and restricted breathing in adults (Evans et al. 2002; Peiffer et al. 2001) and shows substantial response differences in CCHS from control subjects in expiratory loading sufficient to induce respiratory discomfort (Macey et al. 2004a). The cingulate gyrus showed increased responses to hypercapnia, but the increase was comparable in both groups. At least part of the perception of breathlessness from excessive exertion is

### Table 1. Summary of VOI analysis

<table>
<thead>
<tr>
<th>Area Name of VOI</th>
<th>Coordinates of Mean Center of VOI</th>
<th>Control</th>
<th>CCHS</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal ganglia</td>
<td>Head of caudate [0 16 7]</td>
<td>++</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Lentiform [1 5 4]</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Limbic regions</td>
<td>Amygdala (left) [−21 −6 −18]</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amygdala (right) [22 −6 −18]</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hippocampus [0 −20 −17]</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insula (left anterior) [−22 −3 −18]</td>
<td>+</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insula (right anterior) [22 −2 −18]</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insula (left posterior) [−21 −10 −19]</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insula (right posterior) [22 −9 −18]</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Brainstem regions</td>
<td>Dorsal midbrain [0 −33 −15]</td>
<td>−</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Ventral midbrain [0 −25 −15]</td>
<td>−</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Dorsal pons [0 −37 −35]</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ventral pons [0 −28 −35]</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dorsal medulla [0 −46 −54]</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Medial medulla [0 −40 −53]</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Ventral medulla [0 −41 −54]</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>Cerebellar regions</td>
<td>Dentate nucleus [0 −61 −38]</td>
<td>++</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Fastigial nucleus [−1 −39 −22]</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vermis [0 −63 −25]</td>
<td>+</td>
<td>+</td>
<td></td>
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</tbody>
</table>

Significance was tested using RMANOVA (P < 0.05). Significant signal increase (+), decrease (−), or no significant difference over > 1 time-point (0) is indicated for each group, relative to baseline. Group differences are indicated by an asterisk (*). Where both groups increased and a group effect was noted, the group with the greater extent of signal change is indicated by “++.” Where a significant decrease was followed by a significant increase, “−−++” represents an initial decrease followed by an increase and a later decrease. The coordinates in MNI space (X Y Z, in mm) of the average center across subjects of each VOI are shown. VOI, volume of interest; RMANOVA, repeated measures ANOVA; MNI, Montreal Neurological Institute; CCHS, congenital central hypoventilation syndrome.

Moreover, CCHS patients, although showing specialized cognitive and affective deficits, perform adequately in a number of other motoric and cognitive tasks (Chen and Keens 2004; Vanderlaan et al. 2004), an unexpected outcome for a generalized insufficiency in vascular reactivity. It may be the case that deficits in vascular reactivity contribute to some of the muted responses found here, but other regions most likely responded with inappropriate neural activation or deactivation, either from syndrome-specific dysfunction or by “release” from other muted sites.

Cerebellar structures

The cerebellar cortex and deep nuclei showed large bilateral areas of response to hypercapnia in control infants, as has been shown earlier in adults (Gozal et al. 1994); these areas exhibited marked deficits in CCHS patients. The normal cerebellar response is an early, short-lasting increase, suggestive of a “signaling” role. The signal is likely that of chemoreception, a role well-shown by others in animal studies of the fastigial nucleus (Xu and Frazier 1997). The fastigial nucleus also assists regulation of large blood pressure changes (Lutherer et al. 1989). Deep cerebellar nuclei project to multiple reticular and rostral sites, including the basal ganglia, with the fastigial nucleus projecting to ventrolateral and posterior thalamic areas, in addition to pontine reticular regions (for review, see Carpenter and Batton 1982; Person et al. 1986). The findings of altered responses in CCHS, particularly in dorsal thalamic, caudate, and lentiform nuclei, may be secondary to the cerebellar deficiencies.

Cerebellar Purkinje cells normally inhibit the deep cerebellar nuclei (Ohtsuka 1988), which showed response deficits in CCHS. Purkinje cells of the cerebellum are exceptionally sensitive to ischemia-induced damage (O’Hearn and Molliver 1997; Schadé and McMenemey 1963); a portion of this damage seems to be mediated via excitotoxic processes through fibers from the inferior olive, a major afferent input to Purkinje cells (Welsch et al. 2002). CCHS patients are often exposed to hypoxia by virtue of inadequate assisted ventilation during sleep or during waking periods associated with infection or rest. Thus some portion of the cerebellar damage may be secondary to repetitive hypoxic exposure, although intrinsic damage from the syndrome cannot be excluded.

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responses to low O2 in fetal sheep are dependent on dorsal
tonation, a mutation found in a high proportion of CCHS patients
be expected to be affected by mutations in PHOX2B transcrip-
tion with insular and amygdala structures, while chemorecep-
tor-related affective components are mediated primarily by
insular and amygdala sites.

The deficient responses in the amygdala may contribute to
the loss of air hunger in CCHS and the reduction of respiratory
drive. The amygdala has direct projections to the rostral vent-
rolateral medullary respiratory group of neurons (Gaytan and
Pasaro 1998) and contains chemosensitive neurons (Teppema
and Dahan 2005).

Both the left and right insula showed group response differ-
ences: the right ventral insula as part of a larger response
center, with CCHS signals larger and missing an early tran-
sient decline found in control subjects, and the left as an
anterior cluster showing a sustained increase in the control
subjects, but no change in CCHS patients. The insula shows
lateralized autonomic properties, with the right insula prin-
cipally modulating sympathetic characteristics, and the left side
controlling parasympathetic aspects (Cechetto and Chen 1990;
Oppenheimer et al. 1992). Since the enhanced respiratory-
related influences on heart rate resulting from hypercapnia are
dependent on vagal outflow (to rapidly correct heart rate), a
component of the diminished heart rate variation to the chal-
lenge in CCHS may derive from reduced parasympathetic
control mediated by the left anterior insula. The right insula
response characteristics provide a basis for the exaggerated
sympathetic responses encountered in CCHS (Weese-Mayer
et al. 1992), since the right insula often provides an inhibitory or
disfacilitatory action on sympathetic action (Henderson et al.
2003).

Thalamic and midbrain areas

The cluster of deficient responses from the posterior dorsal,
ventral, and medial thalamus through the medial midbrain and
extending to an area of the dorsal lateral pons in which the
locus coeruleus is sited likely relates to chemoresponse roles.
The locus coeruleus contains chemosensitive neurons (Andr-
zewski et al. 2001; Oyamada et al. 1998). Although the role
in CO2 modulation is unclear, appropriate respiratory re-
sponses to low O2 in fetal sheep are dependent on dorsal
thalamic and midbrain structures (Koos et al. 1998), and the
posterior thalamus shows c-fos expression to hypoxia (Sica
et al. 2000). Deficient responses to hypoxia also emerged in the
posterior thalamus, midbrain, and dorsal pons of CCHS cases
(Macey et al. 2005b). Mediation of both low oxygen and
hypercapnia apparently are affected in diencephalic and mid-
brain areas in the syndrome.

PHOX2B targets

The dorsal medulla, which contains among other structures
the NTS, showed deficient responses to hypercapnia and would
be expected to be affected by mutations in PHOX2B transcrip-
tion, a mutation found in a high proportion of CCHS patients
(Amiel et al. 2003; Weese-Mayer et al. 2003). The dorsal
medulla, however, showed no change from a control pattern to
a cold pressor challenge (Macey et al. 2005a), which elicited
substantial cardiovascular and breathing responses, both of
which are deficient in CCHS (Kim et al. 2002). Collectively,
the findings suggest that the syndrome may result from multi-
ple failures in genetic expression; other genetic processes in
central breathing control are currently being explored (Rhee
et al. 2004).

Temporal patterns

Among physiological responses, an increase in respiratory-
related heart rate variation began immediately, in temporal
association with the altered difference in signal in the cerebel-
rum and associated motor structures. An early onset was also
noted in the dorsal medulla, insula, and amygdala, the latter
structure presumably related to affective components of the
signal. A 60-s nadir in response in the hippocampus for control
over CCHS subjects overlapped a decline in respiratory rate
found in controls, but not found in CCHS patients. The hip-
locampus has been previously implicated in aspects of respi-
atory timing and may contribute to conditions for inspiratory
drive (Poe et al. 1996).

Limitations

When these data were collected, the sample period (~2 min)
was the maximum available due to scanner constraints. Ideally,
longer scanning times, with multiple stimulation periods,
would be used. Additionally, the results showed early and
transient responses, and therefore higher temporal resolution
would have allowed for detailed examination of timing pat-
terns. Recently developed scanners have the capabilities to
implement improved protocols.

The analyses revealed several regions where early, but
transient responses occurred, patterns consistent with rapid
chemoreceptor responses. The cluster analysis was performed
using a pattern (step function) which highlighted regions dif-
fering between the groups either throughout the challenge or at
specific periods. Ideally, further cluster analyses would be
performed using other models, e.g., an initial rapid but tran-
sient response to more specifically highlight areas responding
in such a pattern. However, the difficulties associated with
specifying a complex model or of using several separate
models were such that we opted for the simpler step function
pattern, with the additional aspect of presenting time trends
from clusters to allow for determination of response timing.

Global signal changes

Hypercapnia, by inducing substantial cerebral vasodilation
(Kety and Schmidt 1948), results in large global BOLD signal
changes, on which regional changes are superimposed. Others
have evaluated regional responses to large global changes,
including primary visual cortex responses to photic stimulation
after hypercapnic challenges (Corfield et al. 2001; Li et al.
2000) and corrected for such overall effects by intensity nor-
malization procedures. The global signal here increased in both
groups 20 s after challenge onset and increased further in
controls later in the challenge (Macey et al. 2003). Differences
appeared between gray and white matter levels of response, so
global trends in gray matter were separated from those in fibers to preclude differential overall effect contributions for the later detrending analysis. The overall signals were removed to examine regional responses using a voxel-by-voxel detrending procedure (Macey et al. 2004b), a more conservative method than intensity normalization. The use of detrending procedures, together with response trends that showed closely adjacent areas with differing patterns of response, argue that the findings result from differential recruitment of brain areas and not from global changes.

Summary

The control responses and the fMRI response deficits in CCHS revealed neural processes for hypocapnia that included brain regions not usually considered to mediate respiratory drive. Since chemoreceptor signals reach at least some central sites in CCHS, and components of motor output are intact, major breathing deficits must lie in integrating input and output processes. That integration loss may derive from failure of cerebellar and diencephalic sites to adequately mediate chemosensory signals and likely includes several deficient processes: 1) inadequate reception or translation of signals from chemosensitive regions in cerebellar deep nuclei to appropriate motoric control structures in more rostral areas, 2) disruption of posterior thalamic, midbrain, and dorsal pontine areas in integrating sensory information to pontine arousal sites, 3) failure to recruit the discomfort of breathlessness in limbic sites (the discomfort in controls may be below the level of conscious awareness), and 4) failure of limbic (insula, amygdala) and cerebellar sites to appropriately regulate autonomic outflow. These neural deficits may result from specific syndrome-related damage to limbic sites whose development may have been affected by the defective targeting resulting from altered PHOX2B or other gene expression, and a portion of the deficits may arise from syndrome-related pathology in overall vascular reactivity in the brain.

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