Hypoxia reveals posterior thalamic, cerebellar, midbrain, and limbic deficits in congenital central hypoventilation syndrome

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Macey, P. M., M. A. Woo, K. E. Macey, T. G. Keens, M. M. Saeed, J. R. Alger, and R. M. Harper. Hypoxia reveals posterior thalamic, cerebellar, midbrain, and limbic deficits in congenital central hypoventilation syndrome. J Appl Physiol 98: 958–969, 2005. First published November 5, 2004; doi:10.1152/japplphysiol.00969.2004.—Congenital central hypoventilation syndrome (CCHS) patients show deficient respiratory and cardiac responses to hypoxia and hypercapnia, despite apparently intact arousal responses to hypercapnia and adequate respiratory motor mechanisms, thus providing a model to evaluate functioning of particular brain mechanisms underlying breathing. We used functional magnetic resonance imaging to assess blood oxygen level-dependent signals, corrected for global signal changes, and evaluated them with cluster and volume-of-interest procedures, during a baseline and 2-min hypoxic (15% O2, 85% N2) challenge in 14 CCHS and 14 age- and gender-matched control subjects. Hypoxia elicited significant (P < 0.05) differences in magnitude and timing of responses between groups in cerebellar cortex and deep nuclei, posterior thalamic structures, limbic areas (including the insula, amygdala, ventral anterior thalamus, and right hippocampus), dorsal and ventral midbrain, caudate, claustrum, and putamen. Deficient responses to hypoxia included no, or late, changes in CCHS patients with declining signals in control subjects, a falling signal in CCHS patients with no change in controls, or absent early transient responses in CCHS. Hypoxia resulted in signal declines but no group differences in hypothalamic and dorsal medullary areas, the latter being a target for PHOX2B, mutations of which occur in the syndrome. The findings extend previously identified posterior thalamic, midbrain, and cerebellar roles for normal mediation of hypoxia found in animal fetal and adult preparations and suggest significant participation of limbic structures in responding to hypoxic challenges, which likely include cardiovascular and air-hunger components. Failing structures in CCHS include areas additional to those associated with PHOX2B expression and chemoreceptor sites.

magnetic resonance imaging; cardiovascular; chemoreceptor; blood oxygen level dependent; PHOX2B

METHODS

The study examined fMRI responses over the brain in 14 children with a diagnosis of CCHS (7 boys, 7 girls) and 14 age- and gender-matched controls (age in years for each group: mean, 11; range, 8–15; SD 2). Diagnosis was based on standard criteria, which included ventilatory dependency during sleep but not during waking, diminished ventilatory responses to hypoxia and hypercapnia, and absence of Hirschsprung’s disease or other cardiac, pulmonary, or neuromuscular disorders (2). The study was approved by the Institutional Review Board of the University

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of California at Los Angeles, and the procedures were conducted with the written consent of the subjects and their guardians.

The subjects spontaneously breathed room air through a two-way non-rebreather valve while lying supine in an MRI scanner, with their tracheostomy openings closed. Potential head motion was minimized by use of masking tape over the forehead and foam pads on either side of the head. Airflow and the ECG were recorded together with the fMRI signal (40). A baseline scanning period during which the subject breathed room air was collected over 150 s. A second scanning period was performed, consisting of a 30-s baseline (room air) followed without pause by a 120-s hypoxic (15% O2 in 85% N2) challenge. The hypoxic challenge was administered through the inspiratory arm of the valve throughout the 120-s period.

A General Electric Signa (GE Medical Systems, Milwaukee, WI) 1.5-T scanner was used; 25 volumes of 20 oblique image slices were collected using a gradient-echo echo planar imaging protocol (repetition time 6 s, time to echo = 60 ms, flip angle = 90°, field of view = 30 × 30 cm, no interslice gap, and voxel size collected using a gradient-echo echo planar imaging protocol (repetition time 1.5-T scanner was used; 25 volumes of 20 oblique image slices were collected using a gradient-echo echo planar imaging protocol (repetition time 6 s, time to echo = 60 ms, flip angle = 90°, field of view = 30 × 30 cm, no interslice gap, and voxel size = 2.3 × 2.3 × 5 mm) during each scanning period. The first volume of each series was excluded from analysis to avoid transient effects. Conventional spin-echo T1-weighted images (time to repetition = 500 ms, time to echo = 9 ms, field of view = 30 × 30 cm, no interslice gap, voxel size = 1.2 × 1.2 × 5 mm) were also acquired to aid in anatomical identification.

Both custom software and an fMRI statistical parametric mapping analysis package, SPM (21), were used to process the images. The functional volumes were subjected to correction for slice timing and motion and were spatially normalized. Gray matter was segmented from white matter on these spatially normalized images (5), and the resulting images.

Regions of overlap

<table>
<thead>
<tr>
<th>Area Name of Cluster</th>
<th>MNI Coordinates (t-Statistic Maximum)</th>
<th>Cluster Size (No. of Voxels)</th>
<th>Significance (t-Statistic Maximum)</th>
<th>Control vs. CCHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insula/lentiform (right)</td>
<td>24 -12 -2</td>
<td>124</td>
<td>2.8</td>
<td>&gt;</td>
</tr>
<tr>
<td>Insula (left)</td>
<td>-36 -2</td>
<td>-4</td>
<td>2.1</td>
<td>&gt;</td>
</tr>
<tr>
<td>Middle temporal gyrus (right)</td>
<td>52 -66 -2</td>
<td>59</td>
<td>2.1</td>
<td>&gt;</td>
</tr>
<tr>
<td>Caudate nucleus (right)</td>
<td>18 12</td>
<td>10</td>
<td>2.1</td>
<td>&gt;</td>
</tr>
<tr>
<td>Hippocampus (right)</td>
<td>-30 -18 -22</td>
<td>28</td>
<td>2.0</td>
<td>&lt;</td>
</tr>
<tr>
<td>Fusiform gyrus (right)</td>
<td>32 -56</td>
<td>-12</td>
<td>6</td>
<td>1.8</td>
</tr>
<tr>
<td>Cerebellar cortex (quadrangular lobule)</td>
<td>-14 -42 -16</td>
<td>268</td>
<td>3.6</td>
<td>&lt;</td>
</tr>
<tr>
<td>Temporal gyrus (left)</td>
<td>-64 -18 -2</td>
<td>8</td>
<td>3.2</td>
<td>&lt;</td>
</tr>
<tr>
<td>Cerebellar cortex (quadrangular lobule)/vermis</td>
<td>-4 -54 -10</td>
<td>440</td>
<td>3.1</td>
<td>&lt;</td>
</tr>
<tr>
<td>Ventral posterior lateral thalamus (left)</td>
<td>-16 -32</td>
<td>8</td>
<td>2.7</td>
<td>&lt;</td>
</tr>
<tr>
<td>Middle temporal gyrus (left)</td>
<td>-54 -60</td>
<td>4</td>
<td>2.3</td>
<td>&lt;</td>
</tr>
<tr>
<td>Medial dorsal thalamus (left)</td>
<td>-2 -8</td>
<td>8</td>
<td>2.0</td>
<td>&lt;</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>48 -4</td>
<td>8</td>
<td>1.8</td>
<td>&lt;</td>
</tr>
<tr>
<td>Posterior medial thalamus</td>
<td>6 -30</td>
<td>0</td>
<td>1.8</td>
<td>&lt;</td>
</tr>
<tr>
<td>Dorsal pons</td>
<td>6 -36</td>
<td>-26</td>
<td>1.8</td>
<td>&lt;</td>
</tr>
</tbody>
</table>

P < 0.05, false discovery rate correction for multiple comparisons. MNI, Montreal Neurological Institute.
Cluster analyses. Figures 1–5 and Table 1 show regions of signal differences between groups as determined by cluster procedures \((P < 0.05)\). A prominent cluster emerged in the deep cerebellar nuclei overlapping the fastigial and dentate nuclei, extending to the left dorsolateral cerebellar cortex (Fig. 1A), where CCHS signals remained unchanged to hypoxia, but signals in control subjects declined (Fig. 1B). A large cluster in the right putamen and claustrum, extending to the ventral insula (Fig. 2A, 1–3), showed no change in control subjects but a signal decline in CCHS patients (Fig. 2B). A similar response pattern was present in a more restricted cluster, principally sited in the left claustrum, and extending to the deep left insula (Fig. 2A, 4, and Fig. 5D). An extensive area of response difference in the left dorsal thalamus, extending across the midline, was also apparent (Fig. 3A); the area on the right side was more limited in size (Fig. 3A, 2). Signals declined late in the dorsal thalamic area in CCHS patients, but an earlier, more substantial decline occurred in control subjects (Fig. 3B). A portion of the right midhippocampus showed a decline in CCHS patients (Fig. 4A), where control subjects showed highly variable patterns late in the challenge (Fig. 4B). Discrete sites of signal differences appeared in the anterior thalamus, with the control values lower than CCHS (Fig. 5A), and right dorsolateral pons (Fig. 5B), which showed less pronounced signal changes in CCHS. The posterior temporal cortex showed a large, transient, increased response in the syndrome.

Fig. 1. A: sagittal, coronal, and axial views of significant response differences between congenital central hypventilation syndrome (CCHS) and control (Con) groups in a cluster extending from the deep cerebellar nuclei (1) to the dorsal cerebellar cortex (1–3), on the left side. The cluster is overlaid onto a single high-resolution anatomical image derived from one mid-age range control subject. [Overlays onto a mean anatomical image, derived from scans of all subjects at the same slice locations as their functional images, demonstrate variability of structures due to the spatial normalization and lower resolution of functional magnetic resonance imaging (fMRI) images and are placed in the web-based supplementary data files: http://jap.physiology.org/cgi/content/full/00969.2004/DC1]. Regions of greater signal in control than CCHS subjects are color coded in yellow-red scale, and regions of reduced signal in control relative to CCHS subjects are color coded in blue-green \((P < 0.05)\). B: time trends of the average of all voxels within the cluster for CCHS and control groups, plotted as group means with between-subject standard error bars.
VOI analysis. Table 2 and Fig. 6 show the significant responses in VOI of defined brain areas ($P < 0.05$). Notable by the absence of a group difference were the dorsal, medial, and ventral medulla, hypothalamus, and head of caudate. These structures showed signal declines to hypoxia, but both groups acted similarly. Significant and early group differences emerged in the dorsal and ventral pons and the dorsal and ventral midbrain. Other regions showing differences included the left amygdala, with a transient early decline in the control group and an opposite response in CCHS, the left and right lentiform nuclei, which showed late differences, and the vermis, which showed a more sustained separation.

DISCUSSION

The findings were remarkable in the large number of brain areas responding differently to modest levels of hypoxia (15% $O_2$, comparable to 3,000-m altitude). The areas of response differences ranged from limbic sites to basal ganglia, midbrain, and cerebellar structures. Several areas, such as the posterior thalamus, have been previously implicated in suppressing the ventilatory response to hypoxia in fetal sheep (28, 29); other areas, such as the posterior thalamus and the midbrain, includ-
ing the periaqueductal gray, are involved in postnatal mediation of hypoxia in animals (51). The disparate responses from control subjects in CCHS emerged at different times in the challenge, with some patterns emerging early and transiently, whereas others developed later or were of a sustained nature. Although the dorsal pons showed aberrant reactions, the dorsal medulla, a target of PHOX2B gene expression, mutations of which are found in a high proportion of CCHS cases (3, 16, 60), did not.

The data suggest that more than mutations of PHOX2B expression contribute to the characteristics of CCHS. The findings also indicate that processes underlying air hunger may be specific to unique sensory processes related to that negative perception, with sensations related to hypoxia not as dependent on cingulate cortex as expiratory loading and perhaps more related to insular and amygdala structures. The divergent temporal patterns reflect the complex mediation of hypoxic responses and suggest that multiple components are impaired in CCHS.

**BOLD signal interpretation and limitations.** The brain images collected in fMRI are sensitive to levels of deoxyhemoglobin in the blood, and differences in signal intensities between two images collected successively correspond to changes in blood oxygenation (44) and are referred to as the BOLD signal. Neural activation is followed by an inflow of oxygenated blood and a consequent decrease in deoxyhemo-

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**Fig. 3.** A: axial, sagittal, and coronal views of significant response differences between CCHS and control groups in a cluster extending from the left posterior thalamus (1, 3) across the midline (2). B: trends of values for the cluster. Trace, color coding, and labeling conventions are the same as in Fig. 1.
globin and thus increased BOLD signal intensity. Therefore, BOLD fMRI detects neurovascular changes rather than electrophysiological neural activation. However, recent animal work has demonstrated a strong correlation between the two, indicating that changes in BOLD signal correlate with local field potentials and, therefore, reflect input and intracortical processing that occur within a given area (33). The effect is diffuse throughout adjacent tissue and has a latency in the order of 6 s. The long latency poses temporal resolution limitations, but the technique has allowed for studies of the whole brain with a spatial resolution of typically 1 cm$^3$. A decline in the BOLD signal is interpreted as a decline in synaptic activity (33). In addition to spatial and temporal resolution, signal variability remains a limitation, requiring data from multiple subjects and, on occasion, multiple trials to obtain stable values.

The CCHS children exhibited higher CO$_2$ and lower blood O$_2$ levels than the controls (40), and these factors will influence the overall fMRI signal. However, the influence of different blood CO$_2$ and O$_2$ levels is spread throughout the brain, resulting in an overall increased or decreased BOLD signal, and such global influences are removed using a detrending procedure (39). Because fMRI is based on relative measures, the question arises as to whether changes in the BOLD signal are influenced by different resting CO$_2$ and O$_2$ levels. Differences in changes in the BOLD signal between groups are

Fig. 4. A: axial, sagittal, and coronal views of significant response differences between CCHS and control groups in a cluster in the right mid-hippocampus (1–3). B: trends of values for the cluster. Trace, color coding, and labeling conventions are the same as in Fig. 1.
present (37), but these changes must be present throughout the brain and, therefore, were removed using detrending. The detrending method used was conservative, with the MRI data only retaining local changes, which were not associated with the pattern of global changes.

Together with other autonomic deficits, nighttime blood pressure patterns are abnormal in CCHS subjects (59), and evoked blood pressure changes to hypoxia could also be aberrant and potentially result in different global influences on the BOLD signal. However, because these influences would be reflected in global signal patterns, they would be removed by the detrending procedure.

Another issue is whether localized BOLD signal responses, assumed to correspond to neural activity changes, would be similar, given equivalent levels of neural activity increase, but different resting CO$_2$ and O$_2$ levels. In normal subjects, the magnitude of the BOLD signal response is approximately equivalent for differing resting blood CO$_2$ levels (14), but whether the magnitudes of regional BOLD responses in CCHS would match those in controls is unclear. Given the abnormal cerebral vascular reactivity (37) and the reduced responsiveness at challenge onset, it is possible that CCHS subjects would show a muted hemodynamic response. However, as shown in the time trends, the majority of findings highlighted areas of differing direction of signal change, which likely represent differing types of neuronal responses (e.g., activation vs. deactivation, deactivation vs. no change). Those responses that alter direction between groups presumably reflect some other process in CCHS patients rather than a simple inability to respond to the same extent.

To summarize, changes in the BOLD signal represent an indirect measure of changes in neural activation. The results should be viewed in the context that the technique has limited

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**Fig. 5.** Left: axial, sagittal, and coronal views of 4 regions of significant response differences between CCHS and control groups. Right: trends of values for the adjacent clusters. A: anterior thalamus; B: dorsolateral pons; C: left posterior temporal cortex; D: left insula. Trace, color coding, and labeling conventions are the same as in Fig. 1.
spatial and temporal resolution, and the acquired signal is variable. However, the statistical procedures ensured that only robust group differences emerged, and the technique did allow for measuring regional responses across the entire brain in awake children.

The 2-min period of the hypoxia challenge allowed development of initial respiratory and cardiac responses (for respiratory and cardiac outcomes, see Ref. 40), but not longer term patterns, which is a limitation of storage capacity in the magnetic resonance scanner used in this study. Other longer term characteristics of the hypoxic response are of interest, especially the biphasic nature of the ventilatory response, with a return to baseline (the “roll-off” effect) (7, 34). The CCHS model may be especially valuable in examining neural areas responding to late-developing ventilatory pattern responses and targets of neuromodulator action that can mediate late-term ventilatory reaction (34). These issues can be examined with newly available scanners.

Comparable control and CCHS group responses. The finding of comparable declines in signal in the two groups in the dorsal, ventral, and medial medulla was surprising for this ventilatory challenge, because hypoxia induces significant respiratory rate differences between groups (40). The dorsal medulla, including the NTS, plays a significant role in mediating input from carotid body chemoreceptors and relaying that information to ventral medullary sites for appropriate modulation of final common paths for blood pressure and respiratory muscle outputs. The data reinforce earlier suggestions that principal deficits in CCHS lie not with central reception of CO$_2$ or O$_2$, but relate to integration of afferent chemoreceptor information with output (23, 41) or neural influences on respiratory drive from the perception of breathlessness (47, 53, 55).

Consequences of PHOX2B mutations would be expected to be manifested in sites regulating autonomic nervous system control and in structures involved in detecting CO$_2$ and O$_2$ levels, such as the NTS. Aberrant responses in the NTS to hypercapnia in CCHS have been noted (R. M. Harper, unpublished observation); however, those deficits appear to be specific to CO$_2$. The implication is that neural structures other than those targeted by PHOX2B expression are responsible for at least part of the deficiencies found in the syndrome. The PHOX2B effects may relate more to autonomic aspects of CCHS (16, 48, 60); genetic mechanisms that affect responses to hypoxia (20) may be exerted through different structures.

**Thalamic and midbrain responses.** The posterior thalamus and portions of the periaqueductal gray have previously been implicated in mediating “breathing” movements to hypoxia in fetal sheep (28–30) and show c-fos expression in rats after hypoxia (54). The normal fetal response to hypoxia is inhibition of breathing movements (9); however, lesioning the posterior thalamus abolishes that suppression, and stimulation studies suggest the most effective thalamic area to be a region near the parafascicular nucleus (28, 29). In the fetal brain preparation, other sites that are associated with modified breathing patterns upon stimulation include the thalamic dorsal medial nucleus, rostral periaqueductal gray, zona incerta, and ventral tegmental area (30). Stimulation of dorsomedial thalamic structures in adult cats also modifies breathing (4). The thalamic posterior group, including the parafascicular and subparafascicular thalamic nuclei, as well as the periaqueductal gray, show prominent anatomical relationships with the ventrolateral medullary respiratory area of the rat (22). The anatomical data suggest a direct means for thalamic areas to modify respiratory patterning.

The CCHS responses in the posterior thalamus were muted over those of controls, were primarily on the left side, and showed a difference from baseline only very late in the challenge, unlike control subject responses where the signals declined early. Thalamic structures have classically served sensory selection and output integration roles for other stimuli; it is unclear whether such roles are being mediated here. The response pattern is suggestive of delayed sensory input, but that possibility is speculative. FMRI studies of animal preparations with genotypic differences may assist determination of specific thalamic roles in hypoxia (20).

**Cerebellar responses.** A pronounced difference in group responses emerged in the fastigial nuclei, extending to portions of the dentate nuclei and the lateral cerebellar cortex. That region did not change from baseline to the end of the challenge in CCHS, whereas the control signals declined to hypoxia. The finding is consistent with evidence from the animal literature indicating that the fastigial nucleus plays a major role in modulating breathing patterns, especially hypoxia-related aspects. Fastigial neurons modify activity following cyanide (35), hypoxic or hypercapnic exposure (66), and an essential role for fastigial nuclei to mediate ventilatory responses to hypoxia or cyanide exposure has been demonstrated (67), with profound reduction in respiratory frequency and tidal volume to hypoxia in cats following bilateral lesions of those nuclei. As in the case of posterior thalamic structures, cerebellar deep nuclei and cortex receive substantial projections from the ventral respiratory area (22).

It is unclear whether the aberrant CCHS responses in cerebellar structures result from maldevelopment as a consequence of the syndrome, or as an outcome of successive hypoxic exposures that afflicted patients’ encounter with inadvertent incidents of insuffi-
cient ventilation during sleep or other circumstances. Cerebellar structures, and particularly Purkinje cells, are preferentially tar-
goted during chronic hypoxic exposure (11, 12), with hypoxic damage possibly induced by excitotoxic mechanisms (6, 63). However, children unaffected by CCHS who are exposed to chronic hypoxia typically show cerebral cortex damage and related behavioral dysfunctions associated with such damage (8). The absence of these characteristics in CCHS patients, together
with the limited spatial organization of the cerebellar deficits shown here, argue for a developmental origin.

**Time sequences.** The principal difference in respiratory response was a transient increase in rate in controls emerging 60 s into the challenge (40). No simple change in signal pattern within the control or CCHS group emerged that was temporally coincident with that transient increase, except for a nadir in the left insula and lentiform nuclei and dorsal and ventral midbrain at that time. The most sustained physiological response was that of heart rate, where differences in rate continued throughout the challenge.

**Amygdala, hippocampus, and anterior thalamus.** The range of limbic structures that showed response differences to hypoxia was of particular interest. Amygdala and insular structures likely contribute to air-hunger aspects of the challenge (19, 49), reflecting their well-described roles in mediating affective perceptions (1), whereas the hippocampus may be involved in triggering of inspiratory onset (50). The amygdala in CCHS patients did not show the normal initial transient decline to hypoxia, suggesting that the signaling for air hunger is rapid, if that mechanism indeed underlies that structure’s role. c-Fos evidence from adult rats demonstrates that amygdala central nucleus neurons are recruited to hypoxic, as well as hypercapnic, challenges (56, 57). Other areas in CCHS subjects showed sustained differences, including the right insula. The insula may contribute to the long-lasting changes in cardiac rate in response to low O2 (40); an insular role for cardiovascular control has been outlined by others (10, 45). The anterior thalamus showed an inverse response in CCHS, increasing in that group, but declining in control subjects. The role for this structure in hypoxia is unclear.

**Hypothalamus.** A large body of evidence indicates that hypoxia triggers a range of responses in the hypothalamus (26). The VOI encompassing the hypothalamus showed a significant decline in signal, but no group differences. Because the hypothalamus is likely to modulate the output path of the cardiovascular response to hypoxia by influencing ventral medullary final paths, the finding of no group difference suggests that the deficits in CCHS occur at an integration level before that output site. Thus the influences from other limbic regions, or from cerebellar areas, may be exerting more of a role in the differential cardiovascular response.

**Cingulate gyrus.** The absence of a group difference in the cingulate gyrus is of interest, because that structure shows substantially disparate responses in CCHS to other respiratory challenges, such as loaded breathing (36); moreover, both CCHS and control subjects reacted similarly to hypercapnia in this structure as well (R. M. Harper, unpublished observation). Areas within the cingulate gyrus are recruited to loaded or restricted breathing challenges sufficient to induce dyspnea (19, 49), as are insular and amygdala sites. The cingulate gyrus shows significant tissue loss in syndromes accompanied by sleep-disordered breathing, including obstructive sleep apnea and heart failure (38, 64). The modest level of hypoxia used in this study may have triggered air-hunger processes, perhaps below the level of conscious awareness. The absence of a group response difference to the hypoxic challenge in the cingulate gyrus adds further support that the structure is principally involved in mediating aspects of respiratory load rather than aspects of chemoreceptor stimulation. This mediation may involve “urge to breathe” perception; however, it is now apparent that at least two forms of “need to breathe” have been revealed by studies of CCHS cases: one generated by exercise, and the other by chemoreceptor stimulation (52, 53). The cingulate area may be particularly important for air hunger triggered by breathing loads.

**Summary.** Hypoxia elicited comparable responses in CCHS and control subjects in medullary and hypothalamic structures and significantly different patterns in cerebellar, dorsolateral pontine, thalamic, basal ganglia, limbic, and midbrain areas. The response differences emerged both early and late in the challenge, depending on the site, and in regions previously implicated in responding to hypoxia in fetal and adult animal studies. The cingulate gyrus, which exhibits loaded breathing deficits in CCHS, showed no group differences, and neither did the dorsal medulla, a region targeted by PHOX2B, mutations of which are frequently found in a high proportion of CCHS patients. In several sites, including the cerebellar cortex and amygdala, the response to hypoxia in CCHS patients was no change from baseline, whereas signals declined in control subjects. In other areas, such as the right insula, the inverse pattern emerged. The genetic mechanisms that result in maldevelopment of breathing control in CCHS affect specific structures shown earlier to mediate responses to hypoxia and include areas other than those dependent on PHOX2B processes. The contribution of each area toward the cardiovascular, respiratory, and temperature responses to hypoxia have yet to be determined.

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