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

R. M. Harper, P. M. Macey, M. A. Woo, K. E. Macey, T. G. Keens, D. Gozal, and J. R. Alger

Department of Neurobiology, Department of Radiology, School of Nursing, and the Brain Research Institute, University of California at Los Angeles and Children's Hospital Los Angeles, Los Angeles, California; and Kosair Children's Hospital Research Institute, Department of Pediatrics, University of Louisville School of Medicine, Louisville, Kentucky

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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 hypoxia 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 responses to either hypoxic or hypercapnic exposures (Paton et al. 1989; Haddad et al. 1978; Oren et al. 1987; Paton et al. 1989). 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 matched on age, sex, and body mass index.

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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. ET\textsubscript{CO\textsubscript{2}}, 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,
and amygdala (Fig. 6), with more modest changes in CCHS or changes in the opposite direction (dorsal and ventral midbrain, hippocampus, amygdala). A nadir emerged in control subjects in the hippocampus 60 s after onset of the challenge, which temporally corresponded to a respiratory rate difference between groups (Macey et al. 2004c). The VOI for the lentiform nuclei, ventral medulla, and ventral pons showed significant responses to the challenge but no group differences.

A data supplement includes the same clusters and time trends as Figs. 2–5, but with the clusters overlaid onto a mean of all subjects’ T1-weighted anatomical images collected at the same slice locations as the fMRI data. The supplementary figures are provided to give a visual indication of the data variation due to spatial registration procedures.1

**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-

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1 The Supplementary Material for this article (four figures) is available online at http://jn.physiology.org/cgi/content/full/00863.2004/DC1.
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.

![Figure 4](image-url)
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.
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-known 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. 2001), was unresponsive to ischemia-induced damage (O'Hearn and Molliver 1997; Schade 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.

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 cerebellum includes the deep cerebellar nuclei and the cerebellar cortex. The deep cerebellar nuclei contribute to a variety of 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.
responds to low O2 in fetal sheep are dependent on dorsal
medialization, a mutation found in a high proportion of CCHS patients
be expected to be affected by mutations in PHOX2B transcrip-
tion. 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-
um 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
case 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

**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 chemoresponsive roles.
The locus coeruleus contains chemosensitive neurons (And-
ziejewski 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
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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 hypercapnia 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 cerebellum 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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