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Congenital Central Hypoventilation Syndrome and the *PHOX2B* Gene: A Model of Respiratory and Autonomic Dysregulation

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Abstract

The paired-like homeobox 2B gene (*PHOX2B*) is the disease-defining gene for congenital central hypoventilation syndrome (CCHS). Individuals with CCHS typically present in the newborn period with alveolar hypoventilation during sleep and often during wakefulness, altered respiratory control including reduced or absent ventilatory responses to hypercarbia and hypoxemia, and autonomic nervous system (ANS) dysregulation; however, a subset of individuals present well into adulthood. Thermoregulation is altered and perception of shortness of breath is absent, but voluntary breathing is retained. Structural and functional magnetic resonance imaging (MRI) and limited post-mortem studies in subjects with CCHS reveal abnormalities in both forebrain and brainstem. MRI changes appear in the hypothalamus (responsible for thermal drive to breathing), posterior thalamus and midbrain (mediating O₂ and oscillatory motor patterns), caudal raphe and locus coeruleus (regulating serotonergic and noradrenergic systems), the lateral medulla, parabrachial pons, and cerebellum (coordinating chemoreceptor and somatic afferent activity with

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breathing), and insular and cingulate cortices (mediating shortness of breath perception). Structural and functional alterations in these sites may result from *PHOX2B* mutations or be secondary to hypoxia/perfusion alterations from suboptimal management/compliance. The study of CCHS, with collaboration between physician-scientists and basic scientists, offers a rare opportunity to investigate control of breathing within the complex physiological network of the ANS.

Keywords

Congenital Central Hypoventilation Syndrome (CCHS); Control of breathing disorders; *PHOX2B* gene; Autonomic dysregulation

1. Introduction

Congenital Central Hypoventilation Syndrome (CCHS), first described in 1970 (Mellins et al., 1970), is a prototypical example of respiratory control disorders occurring with related autonomic nervous system dysregulation (ANS). This unique and growing group of rare diseases termed respiratory and autonomic disorders of infancy, childhood, and adulthood (RADICA; Weese-Mayer et al., 2009), has served to broaden our understanding of respiratory and autonomic function and developmental changes from infancy through adulthood. Improved non-invasive monitoring technologies, advances in providing respiratory support, and increased awareness of autonomic medicine have improved care for and study of individuals with RADICA; this includes the ability to perform end tidal CO₂ monitoring, portable pocket-sized pulse oximetry, beat-to-beat blood pressure measures, continuous temperature recording, smaller home ventilators, and even minimally invasive thoroscopic diaphragm pacer implantation. Collectively, these disorders share the risk of sudden death, altered neurocognitive outcomes, and varied long-term quality of life. The study of RADICA, with collaboration between physician-scientists and basic scientists, offers a rare opportunity to investigate control of breathing within the complex physiological network of the autonomic nervous system (ANS).

The CCHS phenotype offers a unique model for elucidation of a variety of crucial processes, including interactions between behavioral states and control of breathing, anatomic sites of central chemoreception, and integration of ancillary drives to breathing processes. Specifically, the study of individuals with CCHS offers a means to determine if the observed chemosensory impairment results from a deficit in sensory input processes, integration of sensory input with respiratory motor output, brain processes that mediate influences of quiet versus active sleep on brainstem respiratory rhythm generators, or a combination of all these processes. Research in this disorder may also guide identification of anatomic sites contributing to affective drive in shortness of breath. Identifying deficient autonomic regulatory sites and understanding dysfunctional interactive processes binding respiratory and autonomic regulatory areas would also help expose essential mechanisms underlying integration of cardiovascular and respiratory functions. The multiple processes that contribute to breathing, combined with the widespread nature of involved brain sites responsive to the principal drive of CO₂ (Nattie and Li, 2009; Teppema and Dahan, 2005; Williams et al., 2007), indicate that no single brain locus is responsible for respiratory dysfunction. Collectively, CCHS patients who have been diagnosed early and optimally managed offer a singular opportunity to understand crucial elements of respiratory and autonomic regulation.

2. Background

CCHS, first characterized by Mellins et al. (1970), was described primarily as case reports as CCHS-focused care at major medical centers did not become prominent until decades later. Familial cases of CCHS, association with disorders of anatomic ANS development (aganglioneosis of the distal intestine, i.e., Hirschsprung disease, and neural crest tumors), and consideration of candidate genes in animal models helped refine the search for genetic causes (Weese-Mayer et al., 2009). In 2003, the paired-like homeobox 2B gene (*PHOX2B*), which encodes a homeodomain transcription factor with an important role in murine ANS reflex circuit development (Pattyn et al., 1997, 1999), was identified as the disease-defining gene for CCHS (Amiel et al., 2003; Matera et al., 2004; Sasaki et al., 2003; Weese-Mayer et al., 2003). *PHOX2B* has been shown to determine eventual cell fate as a sympathetic, parasympathetic, or enteric neuron. Located at 4p12, *PHOX2B* normally contains a 20 alanine repeat sequence on exon 3; so an unaffected individual would have the *PHOX2B* genotype denoted as 20/20, reflecting the normal number of alanines on each allele. However, individuals with CCHS are heterozygous for a *PHOX2B* mutation. The specificity of the association between mutations in the *PHOX2B* gene and the CCHS phenotype is supported by Hung et al. (2007), who failed to identify *PHOX2B* mutations in ~2000 Taiwanese controls. The majority (90-92%) of individuals with CCHS have a polyalanine repeat expansion mutation (PARM) in the *PHOX2B* gene (Weese-Mayer et al., 2009). For those CCHS patients with PARMs, the number of polyalanine repeats on the affected allele range from 24 to 33 repeats. In addition, *PHOX2B* alterations including missense, nonsense, and frameshift mutations, termed non-polyalanine repeat mutations (NPARM), make up the remaining 8-10% of CCHS cases. The new American Thoracic Society Statement on CCHS provides additional details regarding CCHS, with focus on the past decade of advances (Weese-Mayer et al., 2010).

Internationally, nearly 1000 cases with *PHOX2B* mutation-confirmed CCHS have been diagnosed as of early 2010 (Weese-Mayer et al., 2009, 2010). Along with confirmation of a *PHOX2B* mutation, CCHS is diagnosed in the absence of primary lung, cardiac, neuromuscular disease, or an identifiable brainstem lesion accounting for the central hypoventilation and overall phenotype. CCHS is a life-long disease in which an individual is unable to appropriately adapt her/his ventilation to environmental demands nor arouse from sleep in response to physiologic compromise. These patients will also have altered or absent perception of shortness of breath when awake (Shea et al., 1993c). Consequently, they have diminished tidal volumes and monotonous respiratory rates in both behavior states (Weese-Mayer et al., 2010), with more profound and life-threatening hypoventilation during sleep. Accordingly, these individuals require a tracheostomy with mechanical ventilation during sleep and, in severe cases, additional mechanical ventilation or diaphragm pacing while awake. Along with respiratory control abnormalities, CCHS is associated with both anatomic and physiologic symptoms of ANSD. Anatomic symptoms of ANSD can include presence of Hirschsprung disease (HSCR) and neural crest tumors (such as neuroblastoma). Physiologic symptoms can include diminished pupillary response, esophageal dysmotility, profound constipation even in the absence of HSCR, breath-holding spells, reduced basal body temperature control, sporadic profuse diaphoresis, lack of perception to dyspnea, altered perception of anxiety and pain, and lack of physiologic responsiveness to the challenges of exercise and environmental stressors (Faure et al., 2002; Goldberg and Ludwig, 1996; Marazita et al., 2001; O'Brien et al., 2005; Paton et al., 1993; Pine et al., 1994; Shea et al., 1993a, 1993b, 1993c; Silvestri et al., 1995; Spengler et al., 1998; Trang et al., 2005; Weese-Mayer et al., 1992, 2001, 2010). Thus, a constellation of features is emerging that defines the *PHOX2B*-genotype/CCHS-phenotype correlation, including severity of facial dysmorphology, ventilatory dependence, symptoms of autonomic dysregulation, cardiac asystoles, relation to age at presentation, HSCR, and neural crest

tumors (Berry-Kravis et al., 2006; Gronli et al., 2008; Matera et al., 2004; Repetto et al., 2009; Todd et al., 2006; Trang et al., 2004; Trochet et al., 2005; Weese-Mayer et al., 2003, 2005).

A subgroup of *PHOX2B* mutation-confirmed CCHS cases have recently been identified outside the newborn period (Weese-Mayer et al., 2009, 2010). Many of these cases (termed later-onset congenital central hypoventilation syndrome, LO-CCHS) have a more subtle phenotypic profile and, genotypically, have the shorter PARMs (heterozygous with genotypes 20/24 and 20/25) or a variant of NPARM that minimally alters the protein product (Parodi et al., 2008; Trochet et al., 2005). While these individuals are often diagnosed in infancy and childhood, they can remain undiagnosed even to adulthood. In these individuals, discovery of hypoventilation may, at times, require an environmental cofactor such as sedation, anesthesia, anticonvulsants, severe respiratory illness, treated obstructive sleep apnea (Antic et al., 2006; Weese-Mayer et al., 2005) or the homozygous condition (Trochet et al., 2008) to elicit overt clinical events like hypoventilation and/or respiratory arrest. Thus far, fewer than 50 LO-CCHS cases have been reported, but considering the subtlety of the clinical profile in those cases, the prevalence in the general population is likely to be significantly higher than suggested by these few cases.

3. Mosaicism, mode of inheritance, and clinical testing

While *de novo* germline mutations cause the majority of CCHS cases, the existence of mosaicism has been identified in a subset (5-10%) of parents of CCHS probands (Trochet et al., 2005; Weese-Mayer et al., 2003). An autosomal-dominant inheritance from these mosaic parents (Weese-Mayer et al., 2003), as well as from probands (Trochet et al., 2005; Weese-Mayer et al., 2003), has also been established. This knowledge has led to improved educational efforts and genetic counseling in CCHS families regarding reproductive risks. The *PHOX2B* Screening Test (Weese-Mayer et al., 2003) (also known as fragment analysis) is a clinically available method using targeted mutation analysis for detection and sizing of the repeat sequence associated with the polyalanine tract expansion (patented; proceeds support CCHS research). This test is now widely used for prenatal diagnosis, family testing to ascertain mosaicism and disease, and diagnosis of individuals with relevant symptoms. With a negative *PHOX2B* Screening Test and a strong clinical suspicion of CCHS, the ATS Statement on CCHS recommends the whole-gene *PHOX2B* Sequencing Test as a subsequent test (Weese-Mayer et al., 2010). Both methods of clinical testing are available at www.genetests.org.

4. Carbon dioxide chemoreception and CCHS

The defining feature of CCHS is reduced physiological response to elevated CO₂, especially during quiet or non-rapid eye movement (NREM) sleep. Table 1 summarizes relevant publications describing reports of individuals with CCHS who underwent hypercarbic challenges, either from exogenous manipulation of breathing gases or from spontaneously arising endogenous hypercarbia events during wakefulness, sleep, or ventilator withdrawal, excluding case studies with insufficiently detailed challenges (reproduced with permission; Carroll et al., 2010). Several limitations of this body of research cited in Table 1 are immediately apparent. These include the following: small sample size, variable experimental and analytic methods, infrequent age, gender and ethnicity-matched control subjects, and inconsistent documentation of a *PHOX2B* mutation. Most of the cited studies report cases with (likely) the mildest phenotypes judging by the complexity of the protocols and the need for adequate awake spontaneous breathing, and paucity of analyses stratified by *PHOX2B* genotype. Despite identification of *PHOX2B* as the disease-defining gene for CCHS in 2003, authors have continued to publish research absent *PHOX2B* confirmation (Harper et

al., 2005; Kumar et al., 2005, 2008b; Macey et al., 2003, 2004a, 2004b, 2005a), to include patients with non-CCHS diagnoses in grouped data analysis (Huang et al., 2008), and to analyze phenotype data without stratification by *PHOX2B* genotype. With the aim to better understand the role of specific *PHOX2B* genotypes/mutations in the development and function of CO₂ chemoreception, autonomic dysregulation, and CCHS phenotype, it is essential that future studies include *PHOX2B* genotype information and that the cohorts be of ample size to analyze the results by genotype.

5. Carbon dioxide chemoreception and *PHOX2B* in the animal model

Early mouse studies from homozygous *phox2b*^{-/-} knock-out models showed dramatic circuit specific anatomic deficits along the chemosensory reflex arc, including peripheral afferent ganglia and central integration sites (Dauger et al., 2003). While these mice were non-viable, heterozygous knockouts survived and showed a transient neonatal physiological phenotype, consisting of a reduced ventilation response to hypercapnia and hypoxia with recovery of wild-type responses by postnatal day 10, thus highlighting the discordance between this model and the clinical presentation in humans (Dauger et al., 2003). These concerns led to the development of knock-in models which inserted the most common CCHS-associated human variant, the PARM 20/27 *phox2b* genotype (Dubreuil et al., 2008). These mice may more accurately reproduce the toxic gain-of-function suggested by human genotype-phenotype association and inheritance patterns, in contrast to the loss-of-function generated by the heterozygous knock-out models (Dubreuil et al., 2008). In fact, these mice show site-specific loss of a group of medullary neurons known alternately as the retrotrapezoid nucleus (RTN; Guyenet et al., 2008) or the parafacial respiratory group (pFRG; Dubreuil et al., 2008; Onimaru et al., 2009). These neurons have been shown to be important for integration and/or relay of chemosensory drive to respiratory rhythm and pattern generating circuits (Stornetta et al., 2006). The difficulty with this model, however, is that it is short-lived and, as true for the human with the *PHOX2B* 20/27 genotype, would require artificial respiratory support to survive beyond the neonatal period. If longer survival becomes possible, it would be useful to evaluate these mice for the presence of several human-CCHS-phenotypic features, such as HSCR, cardiac asystoles, or symptoms of physiologic autonomic dysregulation, which are all associated with the *PHOX2B* 20/27 genotype in humans. As is clear, animal studies have improved our understanding of the importance of *phox2b* in CO₂ chemoreception (Abbott et al., 2009; Takakura et al., 2008), but are limited in direct translation to the human CCHS clinical phenotype. A set of schematic diagrams outlining current concepts of brainstem sites expressing *phox2b* and the role of maturation in those sites found in basic animal studies is shown in Figure 1. Recent findings of *phox2b* expression in cerebellar and forebrain sites, which likely contribute to respiratory/autonomic deficits, and overlap the MRI structural and functional findings of altered tissue, described in the Allen (Lein et al., 2007), and GenSat (Gong et al., 2003) atlases, are not outlined in Figure 1.

6. Waking and sleep influences on chemoreception

As noted recently by Carroll et al. (2010), description of the precise nature of altered central chemoreception in CCHS in the published literature is limited, because the studied subjects likely have the “mildest” of the *PHOX2B* genotypes. Specifically, to be physiologically able to complete the described protocols safely, it is likely that the subjects in most studies showing ventilatory facilitation with exercise, limb movement, or mental exercise (Gozal et al., 1993; Paton et al., 1993; Shea et al., 1993c) had the 20/25 genotype. Such facilitation of breathing indicates that other, non-chemoreceptor influences enhance the rhythmogenesis of breathing. However, we do not know if similar compensatory mechanisms exist in patients with the more “severe” genotypes who require 24 hour/day artificial ventilation. In addition

to CO₂ insensitivity, individuals with CCHS have an absent perception of shortness of breath in response to heavy exercise (Spengler et al., 1998), intercurrent illness, such as pneumonia (Weese-Mayer et al., 2010), and hypoxia. The loss leads to an absence of enhanced respiration (inspiratory effort or respiratory rate) with an apparent failure to sense the affective associations of shortness of breath.

Severity of hypoventilation in CCHS differs between wakefulness and sleep, and even within sleep, with more severe hypoventilation appearing during quiet (NREM) sleep, and more moderate breathing deficits during REM sleep (Fleming et al., 1980). Brain structures that induce and maintain quiet sleep are principally located in the basal forebrain (Suntsova et al., 2007; Szymusiak et al., 2007; Szymusiak and McGinty, 2008), while structures that generate REM sleep lie below the forebrain, in pontine areas (Jouvet, 1967). The state-dependent hypoventilation relationship in CCHS suggests inhibitory or loss of excitatory influences on breathing stemming from basal forebrain mechanisms on brainstem rhythmogenesis or neurotransmitter sites. Furthermore, other facilitatory or disinhibitory neural influences on breathing operate during REM sleep. Sleep-state changes in neuromodulatory tone, such as the loss of dorsal raphe serotonergic discharge during REM sleep (McGinty and Harper, 1976), may exert direct effects on medullary respiratory rhythm generators (Doi and Ramirez, 2008). The CCHS sleep-state breathing relationships, however, may be largely related to the specific *PHOX2B* mutation, potentially impacting normal CO₂ influences on breathing that are known to be reduced during NREM sleep compared to wakefulness (Skatrud and Dempsey, 1983). These state-related breathing patterns pose several issues for consideration in interactions with CO₂ processing: although multiple forebrain sites, including cortical regions, can act to suppress respiratory muscle action (Marks et al., 1987), other forebrain areas, such as the prefrontal and cingulate cortex involved in vocalization, interact with midbrain periaqueductal gray and medullary regions to regulate breathing patterns (Holstege, 1992; Subramanian et al., 2008; Subramanian and Holstege, 2009, 2010). Moreover, the caudal hypothalamus participates in hypoxic influences on breathing (Horn and Waldrop, 1997). The basal forebrain, prefrontal and cingulate cortex, hypothalamus, and midbrain periaqueductal gray areas are all affected in children with CCHS (Kumar et al., 2005, 2008b, 2010), and may contribute to multiple CCHS respiratory deficits, or be the sequelae of inadequately treated hypoventilation and resultant physiologic compromise.

7. Autonomic dysregulation: relevance to breathing

Although the immediate concern for patients with CCHS is the loss of respiratory drive, pronounced dysfunction in global autonomic regulation is also a major characteristic of the disorder (Marazita et al., 2001; Weese-Mayer et al., 2001). This characteristic is likely a consequence of mutations in the *PHOX2B* gene, which principally targets progenitors of neurons in autonomic ganglia (Amiel et al., 2003; Pattyn et al., 1999, 2000). Although not yet prospectively studied in a large cohort of children with *PHOX2B* mutation-confirmed CCHS, we do have a basic understanding of the interrelationship in non-CCHS subjects between breathing and systems affected by the ANS. Deficiencies in autonomic regulation can interact with impaired respiratory regulatory sites, partially by pharmacologic alteration of blood pressure/somatic respiratory interactions. These interactions have been illustrated in the adult cat, where transient elevation of arterial pressure elicits apnea (Trelease et al., 1985), and in the adult dog, where transient hypotension enhances ventilation (Ohtake and Jennings, 1992). The autonomic/respiratory interactions are especially important during sleep, with the exaggerated sympathetic bursts and blood pressure swings in REM sleep, and the enhanced inspiratory efforts during NREM sleep, which can modify vagal outflow as demonstrated in both dogs and infants (Verrier and Harper, 2009). Since both sympathetic

and parasympathetic systems are affected in CCHS, studies must consider sites affecting both control of breathing and integration of autonomic regulatory systems.

8. Brain structural and functional deficits in CCHS

This section must be prefaced by recognition that the cohort studied thus far with structural and functional magnetic resonance imaging is small (13-16 subjects in any given study), of variable age (7-21 years), until very recently of unknown *PHOX2B* genotype, and without partitioning by specific *PHOX2B* variant. Although the results are compelling, they must be interpreted with caution, and cannot be applied to all children with CCHS. Nevertheless, the affected structures underlying control of breathing in CCHS can be assessed non-invasively by magnetic resonance imaging (MRI) techniques. MRI procedures can evaluate individual brain structures (Kumar et al., 2009a, 2009b), and whole-brain regional tissue injury through local changes in free water content, indicative of primary cell and white matter damage (Kumar et al., 2005); and through assessment of directional diffusion properties of water in axons that can indicate integrity of myelin or axons (Kumar et al., 2008b, 2010). The functional integrity of regional structures can be determined by evaluating local changes in arterial deoxyhemoglobin relative to oxyhemoglobin, by using differences in their magnetic properties as contrast; this is referred to as the blood-oxygen-level-dependent (BOLD) procedure for functional MRI (fMRI). All of these structural and functional procedures have been used to supplement the very limited data available from autopsy material (Cutz et al., 1997; Folgering et al., 1979; Liu et al., 1978; Tomycz et al., 2010).

9. Structural brain findings in CCHS

Early MRI studies of structural injury showed no anatomic findings sufficient to account for the respiratory and autonomic characteristics of CCHS (Weese-Mayer et al., 1988). Examination of autopsy material from a few available cases (Cutz et al., 1997; Folgering et al., 1979; Liu et al., 1978; Tomycz et al., 2010), showed insufficient or inconsistent tissue changes that could potentially underlie many features of the CCHS phenotype. A recent autopsy case study of an infant with alveolar hypoventilation and HSCR (although without confirmation by *PHOX2B* testing), reported findings in the locus coeruleus, retrotrapezoid nucleus, temporal and frontal cortex, as well as in other areas (Tomycz et al., 2010); the first two structures have been implicated in CO₂ sensitivity, and the latter cortical areas assist cognitive and autonomic regulation (among many other functions).

MRI studies that evaluate the total amount and distribution of water and its interaction with the surrounding microstructural environment (T2-relaxometry procedures), diffusion of water in the tissue (mean-diffusivity measures), and volumetric procedures that assess precise regional tissue changes, show relatively consistent, although still variable abnormalities in multiple brain structures in a cohort of CCHS subjects, aged 7 to 21, breathing spontaneously during a waking study, with inclusion of 13-16 subjects in each study. Moreover, the advent of more-sophisticated diffusion tensor imaging (DTI) procedures, with variants of analysis allowing evaluation of axons to reveal myelin or axonal integrity, showed substantial axonal injury or depletion of myelin in locus coeruleus and parabrachial pons, and caudal raphe, lateral medulla, posterior thalamus and midbrain (Kumar et al., 2008b, 2010). Using T2-relaxometry measures (Figure 2; Kumar et al., 2005), structural changes appear in multiple sites, including the cerebellum, cingulate and insular cortex, anterior hypothalamus, and hippocampus. These structures serve a great many functions, including the ability to respond to hypercapnia (cerebellum), mediate the sensation (and thus, drive) to breathe from shortness of breath (cingulate, insular cortex), and mediate temperature drive to breathing (anterior hypothalamus). However, the T2-relaxometry techniques used in the early MRI studies provided only relatively low spatial

resolution. More-recently developed DTI techniques show alterations in the parabrachial pons (a principal respiratory phase-switching area, i.e., an area responsible for switching from inspiration to expiration, a pattern essential for respiratory cycling), and also show maldevelopment/injury in the nearby locus coeruleus, an area affected in a CCHS autopsy case report (Tomycz et al., 2010). The locus coeruleus provides noradrenergic innervation to brain structures, and may play a significant role in state-related breathing deficiencies in CCHS. The higher-resolution DTI procedures also showed alterations in a portion of the lateral medulla, which has significant cardiovascular regulatory roles, and the caudal midline raphe (Figure 3). A region extending from the posterior thalamus, important for oxygen processing in the lamb (Koos et al., 1998), through the ventral midbrain and mid pons, and portions of the hippocampus, also showed anatomical differences in CCHS. A loss of tissue in CCHS within regional areas of the hippocampus has been outlined with manual tracing and surface morphometric techniques using T1-weighted structural images (providing much higher-resolution) (Macey et al., 2009), together with loss of fibers in the fornix and volume of the mammillary bodies (Kumar et al., 2009b).

10. Normal brain responses to CO₂

To determine inappropriate brain responses to chemoreceptor stimulation, response patterns to CO₂ need to be evaluated in healthy controls from infancy to adulthood. Normal responses of the brainstem and cerebellum to 5% CO₂-95% O₂ challenges using fMRI have been described over multiple sites in the brain in healthy adolescents (Harper et al., 2005) and adults (Gozal et al., 1994; McKay et al., 2010). Responses in normal adolescents appear in multiple brain areas, extending from the cerebellum to diencephalic structures (Harper et al., 2005). The brain responses to CO₂ vary substantially from site-to-site; signal increases appear in some areas, and decline in others, with responses in some sites being transient, occurring only at the onset of the challenge, and other signals maintained during the entire course of CO₂ exposure. A subset of the responses is shown in Figure 4. In healthy adolescent subjects, as in the case for adults, 5% CO₂-95% O₂ challenges induced a substantial signal rise in the cerebellar cortex and deep nuclei. The responses were transient in the cerebellum, rapidly increasing after onset of CO₂ exposure, and rapidly declining with continued CO₂ exposure. Other regions showed declining signals at onset of exposure to increased CO₂, which typically remained lower during continued exposure-- particularly in the head of the caudate nucleus, putamen, and hippocampus, posterior thalamus, parabrachial pons, and midbrain tegmentum. The dorsal medulla, amygdala, and right insular cortex showed signal increases. High levels of CO₂ elicit the intense sensation of shortness of breath in normal subjects, and thus recruit limbic sites, e.g., cingulate, insula, hippocampus, amygdala, serving that perception (Evans et al., 2002; Peiffer et al., 2001); the emotions could also be considered stressful, and activate structures involved in mediating such stimuli (Schneider et al., 2009).

The patterns of CO₂ responses in the healthy adolescent have several implications for understanding central processing of chemoreception. Integration of the afferent CO₂ information is served by multiple structures in widespread areas of the brain. The integration involves sites serving comparison of motor output with sensory input roles, important for comparing responses of chemosensitivity with respiratory muscle action within the cerebellum and basal ganglia, and a number of limbic sites often associated with autonomic regulation and the perception of shortness of breath, including the insular cortex (Evans et al., 2002; Peiffer et al., 2001). The 5% CO₂-95% O₂ challenge in the healthy adolescents also evoked a response in the posterior thalamus, a region remarkable for roles in hypoxic responses in neonatal sheep (Koos et al., 1998).

11. Impaired responses to CO₂ in CCHS

In a cohort of CCHS subjects (n=14), 5% CO₂-95% O₂ challenges revealed enhanced participation of the cerebellum and dorsal medulla (Figures 3 and 4) compared to healthy controls, and even greater declines in the caudate, insular cortex, hippocampus, posterior thalamus, and caudate (Figure 5) over healthy controls.

A region of deficient responses to CO₂ extended from the posterior thalamus through the ventral midbrain and medial pons (Figure 5). A notable aspect of the differences in CCHS responses emerged in a region extending from the mid insular cortex to, and including, the mid hippocampus (Figure 6).

The fMRI studies suggest relatively consistent major changes, including phase shifts and pattern reversals in responses to multiple challenges in CCHS, and appear after matching for age and gender. Substantial developmental changes occur normally in brain structural organization over the first 20 years of life (Gogtay et al., 2004), matching for age an essential component of analysis. However, the cohort sizes were insufficient to partition effects by extent of *PHOX2B* mutation, and adequacy of ventilator management was unknown. Thus, distinguishing between maldevelopmental effects of a specific *PHOX2B* mutation and injury from recurrent hypercarbia/hypoxia consequent to suboptimal ventilatory management is not achievable. Moreover, fMRI signals derived from BOLD procedures provide relatively low temporal and spatial resolution of signal change, and T2-relaxometry procedures also do not provide fine resolution. The specialized DTI procedures outlined earlier, however, allow higher-resolution fiber structure analysis and the nature of such changes. Measures of water diffusion perpendicular to axons (radial diffusivity) versus parallel to axons (axial diffusivity), can demonstrate myelin vs. axonal integrity, respectively. When areas of structural changes in CCHS, assessed by axial and radial diffusivity techniques, are compared to sites showing functional deficits in response to acute hypercapnic, hypoxic, and cold pressor challenges (with fMRI), a remarkable overlap between the structural and functional deficits appear (Figure 7). Taken together, the findings suggest that the structural deficits may elicit functional consequences in the condition.

12. Overlap of brain areas showing deficits and *PHOX2B* expression

A portion of the fMRI findings in CCHS may develop from consequences of the innate *PHOX2B* mutations in addition to direct changes in cell differentiation resulting from altered action by the gene. Further dysfunction in CCHS likely results from age at diagnosis and intervention, and variability in ventilatory support and compliance with clinical recommendations. Determination of brain areas potentially affected by mutations in *PHOX2B* and consequences of gene dysfunction are both of interest, since hypoventilation, the hallmark of CCHS, leads to injury from physiological compromise if not adequately supported with artificial ventilation. The extent of injury consequent to suboptimally managed hypoventilation may be severe in brain areas that serve essential coordination roles for respiratory musculature or blood pressure regulation. For example, injury from intermittent hypoxia can develop within a very short period of time, within 5 hours in the rodent cerebellum (Pae et al., 2005). The extent of recurrent hypercarbic and hypoxic exposure in CCHS varies, depending on initial age at diagnosis, attention to appropriate intervention, nature of the *PHOX2B* mutation, and duration of exposure to physiologic compromise—with variations from the healthy age-, gender-, and ethnicity-matched control. The consequences of hypercarbic/hypoxic exposure may also be enhanced in CCHS by alterations in autonomic regulatory nuclei resulting from *PHOX2B* mutations, changes which may include impaired sympathetic control of the vasculature in the child with CCHS. Impaired vascular control can result in pathological changes in regional central nervous

system tissue. An example of that potential is found in the vasculature in CCHS patients, with the basilar artery showing substantial dilation in a small cohort of patients (Kumar et al., 2009c), an outcome that can significantly alter perfusion to supplied tissue.

Based on absence of *phox2b*-expressing neurons in early *phox2b* knock-out rodent models, the presence of structural deficits in the cerebellum and in many of the forebrain structures on MRI in humans with CCHS was initially believed to develop solely from hypoxic consequences of the disorder. Similarly, alterations in more rostral structures in CCHS were assumed to result from either hypoxia or from failure of vascularization or perfusion as a consequence of embryonic *PHOX2B* effects on autonomic ganglia development. However, in addition to medullary sites, recent findings in the Allen Brain (Lein et al., 2007) and GenSat (Gong et al., 2003) projects indicate the presence of *phox2b* neurons in the mouse in widespread regions at some developmental stages. These regions include areas that overlap sites of maldevelopment/injury in the human MRI studies in CCHS, including the cerebellum (<http://mouse.brain-map.org/viewImage.do?imageId=125550> and <http://www.gensat.org/imagenavigator.jsp?imageID=69648>), locus coeruleus (<http://www.gensat.org/imagenavigator.jsp?imageID=72012>), midbrain, and many of the cortical, limbic, thalamic and basal ganglia structures. Nevertheless, evidence from the GenePaint Atlas (Visel et al., 2004) ([http://134.76.20.6/cgi-bin/mgrqcg94?APPNAME=genepaint&PRGNAME=analysis_viewer&ARGUMENTS=-AQ70603276209201,-AMH,-A1217,-Asetstart,-A10:\)](http://134.76.20.6/cgi-bin/mgrqcg94?APPNAME=genepaint&PRGNAME=analysis_viewer&ARGUMENTS=-AQ70603276209201,-AMH,-A1217,-Asetstart,-A10:)) fails to support the presence of *phox2b* expression in E14.5 and P7 mice in the rostral regions identified in the previously-cited atlases. Further experiments are necessary to resolve the disparities between these data sets as well as to define possible developmental dependencies in expression. Results from these studies should help resolve whether the MRI findings in CCHS result from primary or secondary effects of the *PHOX2B* mutation.

Despite some similarities in rodent *phox2b* expression and human CCHS MRI alterations, the distribution of *PHOX2B* expression is unknown in the human; moreover, *phox2b* expression in murine models may vary significantly with age. Comparison of the fMRI and structural MRI findings in CCHS in the child/young adult with the animal *phox2b* expression data (Figure 1; also gene atlas data) may allow for improved understanding of the human condition. Comparison aside, three factors hinder understanding of the human fMRI findings in relation to the anticipated sites of *PHOX2B* expression in the human: 1) the specific sites of *PHOX2B* expression based on neuropathological studies in the adolescent human with and without CCHS are unknown, 2) the role of recurrent hypercarbia/hypoxemia as might occur in the less-than-optimally managed patient with CCHS may have incalculable effects on the brain, and 3) the limitations of MRI technology may hinder precise delineation of regions of *PHOX2B* expression, as demonstrated in animal studies.

13. Ancillary drives to breathing

A remarkable aspect of regulatory control of breathing in CCHS is the absence of the perception of shortness of breath. Brain structures mediating that perception are described in adults (Evans et al., 2002; Peiffer et al., 2001), and include the insular and cingulate cortices, hippocampus, and portions of the cerebellum; all of these structures are affected in CCHS (Kumar et al., 2005, 2006, 2008b). Those structures are in the forebrain and diencephalon, except for the cerebellum; presumably, the cerebellar effects are mediated through cortical projections, since only cortical areas can mediate conscious perception. The perception of shortness of breath is a powerful drive to inspiratory effort, and the absence of that drive in CCHS suggests a significant role for cortical areas in influencing respiratory effort.

Contributions from limbic and cerebellar structures to breathing may not be limited to periods of shortness of breath. A potential state-dependent influence on breathing can develop from limbic projections to respiratory phase switching areas, such as the amygdala projections to the parabrachial pons. Single pulse stimulation of the amygdala in animals will entrain breathing, a relationship that disappears with onset of sleep (Harper et al., 1984). The cingulate cortex, hippocampus, and cerebellum all are recruited to inspiratory efforts following apneic periods in Cheyne-Stokes breathing (Henderson et al., 2006). The Cheyne-Stokes pattern can result from a failure of coordination of central vs. peripheral CO₂ afferent information, potentially triggered by alterations in a number of drives, including descending hypothalamic temperature drive or delayed stimulation of peripheral chemoreceptors by lengthening of circulation times. Injury in those areas would have the potential to modify resumption of breathing after breathing cessation, indicating that the concept of modifying breathing by chemoreceptor drive must consider integrative effects from cortical, diencephalic, and cerebellar systems used to initiate breathing.

Alteration of hypothalamic areas in CCHS (Kumar et al., 2005, 2010) is of particular interest because of the lower basal body temperature in CCHS, coupled with recognition that temperature is a major drive to breathing. In addition, return of chemosensitivity appeared in two female adult CCHS patients following administration of the contraceptive desogestrel, which has a 3-fold binding affinity over progesterone receptors (Straus et al., 2010). Recognizing that progesterone can induce increased ventilation, an outcome dependent on the structural integrity of the hypothalamus (Bayliss and Millhorn, 1992), the facilitation of breathing in these two women with CCHS may be consequent to stimulation of residual CO₂-sensitive neurons. These new observations should be considered with caution, as changes in normal levels of progesterone, e.g., with pregnancy, have little effect on breathing in CCHS (Sritippayawan et al., 2002); the effect may depend on the enhanced binding of desogestrel to progesterone receptors. The findings do emphasize the potential contributions to chemosensitivity from unexpected sources.

14. Relationship to other sleep-disordered breathing conditions

To understand the processes underlying neural alterations in CCHS, it is useful to examine changes in brain tissue in other sleep-disordered conditions that might result in recurrent hypoxemia and hypercarbia, such as obstructive sleep apnea (OSA), though the age and severity of such physiologic compromise will not be directly comparable to the individual with CCHS whose symptoms began at birth. Presumably, the largest contributions to brain alterations in OSA develop from consequences of successive exposure to intermittent hypoxia or extreme changes in perfusion from arterial pressure swings accompanying apnea in the syndrome, although sleep deprivation and inflammatory processes likely play additional roles (Gozal, 2009; Vecsey et al., 2009). Neural alterations in adult OSA subjects studied by structural and functional MRI techniques share sites with those reported in adolescent subjects with CCHS, and include ventromedial frontal, cingulate, and insular cortex, cerebellar cortex and deep nuclei, hippocampus, fornix, and mammillary bodies (Kumar et al., 2008a; Macey et al., 2002, 2009). However, significant differences emerge; the midbrain, hypothalamic and caudal raphé findings are much more extensive in CCHS than OSA, and the lateral medullary alterations appear thus far only in CCHS (Kumar et al., 2008b, 2010; Macey et al., 2002, 2009). The extreme dilation of the brain vasculature found in CCHS (Kumar et al., 2009c) was not apparent in the adult subjects with OSA. Since the CCHS-specific findings, occurring outside OSA-specific regions, appear in sites that are normally well-perfused, e.g., hypothalamus, and less subject to hypoxic damage, one might conjecture that those sites are more likely to have been affected by *PHOX2B* mutations rather than sequelae of recurrent hypoxemia/hypercarbia.

15. Conclusions

Multiple impaired processes appear to underlie the breathing and autonomic dysregulation characteristics of CCHS. Deficiencies appear in awake ventilatory sensitivity to CO₂ and O₂, rhythmogenesis during sleep (preferentially in quiet, NREM sleep over REM sleep), affective excitatory influences from shortness of breath on inspiration, influences of reduced body temperature on breathing, and respiratory-cardiovascular interactions. Other influences on breathing are retained, including voluntary breathing efforts, and in a small subset of cases, accentuated breathing with exercise or peripheral limb motion, even during sleep. A unitary neural lesion underlying all of these processes would be unlikely. Evaluation of structural tissue loss or injury in the brain using functional or structural MRI procedures reveals alterations in multiple sites mediating cardiovascular, breathing and emotive functions, and areas that would interfere with prominent neuromodulators that modify breathing patterns, including the pontine and caudal raphé (serotonin), the locus coeruleus (noradrenalin), the ventral midbrain (dopamine), the basal forebrain (acetylcholine) and the hypothalamus (orexin, progesterone). In addition, areas that coordinate respiratory musculature (cerebellum), mediate emotional drive for inspiration from shortness of breath (insula, cingulate, and hippocampus), or axons that project from these structures (cerebellar peduncle, fornix) are significantly altered. The Allen atlas shows *phox2b*-expressing neurons in these areas, including both cortical and limbic structures in the rostral brain, as well as locus coeruleus, midbrain and cerebellar areas in mice. However, this expression is not fully supported by the GenePaint, and only partially supported by the GenSat atlases. Thus, the findings, although affecting multiple processes, may develop largely from failed neurogenesis consequent to *PHOX2B* mutations in the human embryo or *PHOX2B* effects on glia. Alternatively, findings may be secondary to hypoxic, hypercarbic, or perfusion damage confounded by known *PHOX2B* effects on autonomic ganglia, potentially resulting in impaired vascularization and functional deficits in blood pressure regulation.

CCHS offers a unique opportunity to assess the neural processing underlying CO₂ chemoreception. Continued study of CCHS will undoubtedly lead to a more clear understanding of mechanisms and manifestations of CO₂ chemoreception, central and peripheral segregation of function, the importance of feedback and feedforward effects, and the role of human analogs of affected medullary sites, such as the RTN/pFRG. Animal models that reproduce as much of the CCHS clinical profile as possible will improve the therapeutic relevance of basic research in this disease, and help decipher such issues as the differences between neonatal and adult age-at-diagnosis, or the role of neural plasticity in the presentation of the mildest phenotypes (and 20/24 and 20/25 genotypes).

The future of understanding neural substrates of CO₂ chemoreception in RADICA lies with the continued study of these diseases and mechanisms both in clinical and laboratory settings, and collaborative efforts between both. CCHS can be seen as a model for such a cooperative effort, elucidating essential mechanisms of respiratory control while improving patient care. The study of RADICA in general will allow us to learn about control of breathing in health as well as disease, thereby providing for these special needs patients while furthering an understanding of the basic science underlying CO₂ chemoreception and its relationship to the ANS. However, such success depends upon completion of careful protocols, targeting specific questions, and application in large cohorts of *PHOX2B* mutation-confirmed cases of CCHS and matched controls--with a clear hypothesis driving the analysis. Respiratory and autonomic disorders can be life-threatening if left undiagnosed or inadequately monitored and treated. Therefore, it behooves clinicians and scientists to work in unison to address fundamental research questions with application to the human condition; ultimately, leading to progress in the fields of respiratory neurobiology, autonomic medicine, and more.

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Figure redrawn and adapted from Alheid et al. (2004), Kang et al. (2007), and Stornetta et al. (2006). Recent findings of cerebellar and forebrain sites described in the Allen (Lein et al., 2007) and GenSat (Gong et al., 2003) atlases are not included in this Figure 1. (Figure created by MSC and CMR).

Figure 2.

T2-relaxometry procedures, which quantify free water content, indicate neural injury or failed development of neurons in CCHS children; alterations appear in the cerebellum, a column extending from the preoptic areas through the hypothalamus, the cingulate cortex, medial prefrontal cortex, and hippocampus. Specific functions related to physiological deficits in CCHS are noted for the structures; the functions represent only selected actions served by those structures. Adapted from Kumar et al. 2005, reproduced with permission.

Figure 3. Increased axial diffusivity from diffusion tensor imaging (DTI) in children with CCHS. Abnormalities appear in the midbrain (A), raphé (B), midline of the caudal basal pons (C), and the right lateral medulla (D, E, and F). Adapted from Kumar et al. (2008b), reproduced with permission).

Figure 4. fMRI responses to 5% CO₂/95% O₂ (red bar) in control adolescents. Signal increases in yellow-red scale appear in the cerebellar cortex as indicated with arrows. “FN” is cerebellar fastigial nucleus. Signal decreases in blue-green scale appear in the caudate nucleus, posterior thalamus, hippocampus and medial midbrain. Adapted from Harper et al. (2005), reproduced with permission.

Figure 5.

fMRI responses to 5% CO₂ in CCHS subjects, compared to age- and gender-matched control adolescents. Signals increase (yellow-red scale), in CCHS subjects (relative to controls), in the dorsal medulla, cerebellum, and amygdala. In the parabrachial pons/locus coeruleus, midbrain and hippocampus, signals decline (blue-green scale). Arrows indicate response differences in the posterior and medial thalamus (1 and 2), medial midbrain (3), and dorsolateral pons (4). The warm colors represent an increase in signal responses in CCHS cases compared to controls, the cool colors represent a greater decline in CCHS over values in controls. Adopted from Harper et al (2005), reproduced with permission.

Figure 6. fMRI responses to 5% CO₂ in CCHS subjects relative to control adolescents, demonstrating other significant clusters of enhanced signals. In CCHS subjects, signal increases (yellow-red scale) appear unilaterally in the insula and cerebellar cortex. Arrows indicate response differences in globus pallidus, putamen, and thalamus (1, 2, 3), caudate (4), and hippocampus (5). Color coding of responses as in Figure 4. Adopted from Harper et al. (2005), reproduced with permission.

Figure 7.

DTI and fMRI images of structural injury (yellow areas), as assessed by axial and radial diffusivity, and functional deficits (blue areas; both blue and yellow areas are highlighted with circles to show comparable regions in structural and functional panels) for hypercapnic, hypoxic, and cold pressor challenges in CCHS patients. Structural injury and functional deficits appear in cerebellum, lateral medulla, and a region of tissue extending from the posterior thalamus through the midbrain. Adopted from Harper et al. (2005), Kumar et al. (2008b), Macey et al. (2005a, b), reproduced with permission.

Table 1
CCHS publications including CO₂ challenge, in chronological order (note no *PHOX2B* testing available until 2003).

Citation	N	Type of study	Genotype	Age at onset	Age at test	Challenge method	State	Gas mixtures CO ₂ /O ₂ /N ₂	Results & comments
Melins, et al. 1970	1	CR	NA	12 do	12 mo	RB SP: >100	W	4%/U/U	Case compared to 30 previous mostly adult onset cases with reduced CO ₂ sensitivity. Established definitional diagnostic criteria and behavioral state dependence.
Shannon, et al. 1976	2	CR	NA	birth	4 mo	D	R	5%/U/U	Compared 6 unmatched controls. Decreased V _I to challenge.
Haddad, et al. 1978	2	CR	NA	birth	1-2 mo	SP:48-75 D	W/R/N	2%/U/U	No response in f or V _I to challenge.
Wells, et al. 1980	1	CR	NA	birth	19 mo	SP: 71	W/S	U/A & 100%	No ventilatory response in R or N
Fleming, et al. 1980	1	CR	NA	birth	9 mo	D	W/R/N	4%/U/U	No ventilatory response in W or R, small response in N. Parents also challenged; mother shows very low-normal response.
Guillemainault, et al. 1982	2	CR	NA	early	2 mo, 16 mo	D	W/S	4.75%/U/U 7.25%/ U/U 2%/ U/U 5%/ U/U	Minimal ventilatory response to 4.75%; some response to 7.25%. Some response to 5%; stage-dependent arousals.
Oren, et al. 1987	6	CR/CU	NA	early	5 wo + follow-up	D	W	3.5%/A	Ventilatory response muted or absent
Paton, et al. 1989	5	CU	NA	<1 yo	6-11 yo	RB-13	W	5%/95%	No consistent ventilatory response to challenge
Marcus, et al. 1991	8	CU	NA	<1 yo	0.4-12 yo	D/RB	S	10%/A	7/8 cases aroused to challenge with no ventilatory response, but required more time (reached higher P _{at} CO ₂)
Weese-Mayer, et al. 1992	32	CU	NA	early	median: 3mo	SP: >70 CB	W/S	U	No ventilatory response to challenge.
Shea, 1993	5	CU	NA	birth	8-17 yo	RB-2 CB	W	14%/A	Blunted ventilatory response. No subjective 'breathlessness'.
Gozal, et al. 1993	5	CM	NA	<1yo	9-14 yo	RB-U	W	15%/ U/U 5%/95% 5%/0% 95%	Designed to elicit mainly transient peripherally mediated response. No significant case-control differences in CO ₂ response slopes; significant changes in V _I (and f in some challenges).
Nakahara, et al. 1995	1	CR	NA	birth	birth	U	W/S	U	Normal response in W; flat in S.

Citation	N	Type of study	Genotype	Age at onset	Age at test	Challenge method	State	Gas mixtures CO ₂ /O ₂ /N ₂	Results & comments
Kerbl, et al. 1996	1	CR	NA	birth	8mo	RB	W/S	U	Blunted f and arousal response to challenge.
Croaker, et al. 1998	5	CR	NA	birth	birth	SP: varied	W/S	U	No ventilatory response to hypercapnia.
Macey, et al. 2003	14	CA	NA	U	8-15 yo	D	W	5%/95% 0%/15%/85%	Measured fMRI global BOLD response. Gases measured in subset. Muted hypercapnic response in cases.
Macey, et al. 2004	12	CM	U	U	8-15 yo	D	W	5%/95% 0%/15%/85%	Slow/muted response. Slow f response to hypercapnia.
Bajaj, et al. 2005	1	CR	NPARM	pre	pre	SP: 120	S		No ventilatory response in extreme preterm with Hirschsprung disease.
Chiaretti, et al. 2005	3	CM	U	1-4 wo	1-3 mo	SP: 75	S		No ventilatory response.
Harper, et al. 2005	14	CA	U	U	8-15 yo	D	W	5%/95% 0%/15%/85%	Measured fMRI localized BOLD response. Most areas muted or inverse response in cases. Group differences in midline dorsal medulla, etc.
Chen, et al. 2005	5	CA	U	<1 yo	mean: 21 yo	RB-13	W	5%	No ventilatory response, with normal cardiovascular response. BP response preserved.
Antic, et al. 2006	5	CR	20/25	varied	22-36yo	CB SP: 60-82	W/S		Mild phenotype in adult diagnoses (LO-CCHS) with possible antecedent symptoms.
Bachetti, et al. 2006	2	CR	U	birth	3 mo <15 mo	V	N	U	No ventilatory response.
Barratt, et al. 2007	1	CR	20/25	32 yo?	41 yo	RB	W	U	No quantification.
Diedrich, et al. 2007	1	CR	20/25	U	27 yo	CB	W	U	Blunted response to challenges. No EMG response to breath hold. Reduction in some HRV measures. BP similar to control.
Doherty, et al. 2007	5	CR	20/25	varied	4-41 yo	RB-5	W	7%/93%	LO-CCHS. Reduced response in all <i>PHOX2B</i> mutation-confirmed family members.
Huang, et al. 2008	7	CM	20/25-27	U	mean: 13 yo	V	W/N/R	U	More severe hypoventilation in N and R. Arousals in 41% of sleep trials. Summary data pooled from 7 CCHS and 2 non-CCHS with normal genotype.
Lee, et al. 2009	3	CU	20/25	22-30 yo	22-53 yo	RB-7	W	5%	Reduced slope response to CO ₂ challenge

Citation	N	Type of study	Genotype	Age at onset	Age at test	Challenge method	State	Gas mixtures CO ₂ /O ₂ /N ₂	Results & comments
Fine-Goulden, et al. 2009	1	CR	20/25	12 yo	12 yo	SP: >112	S		Respiratory failure after anesthesia.

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Abbreviations:

N: Number of subjects exposed to hypercapnic challenges

Type of Study: CR: Case report CU: Cohort, unmatched CA: Cohort, approximately matched CM: Cohort, matched

PHOX2B Genotype: NA: not available pre 2003 U: unknown; not tested NPARM: non-polyalanine repeat mutation

Challenge Method: D: Direct through ventilator, pneumotach or mask RB-X: Rebreathing, with X liter reservoir CB: Voluntarily controlled breathing rate V: Ventilator withdrawal SP: X: Spontaneous hypercapnia to X mm Hg.

Behavioral State: W: Wake S: Sleep, unspecified stage N: Non-REM R: REM

Gas Mixtures: xx/xx/xx: Specific blend of CO₂/O₂/N₂ U: Unspecified A: Ambient room air

Comments: f: Respiratory frequency V_t: End-tidal volume HRV: Heart-rate variability EMG: Electromyograph Pet: End-tidal partial pressure fMRI: Functional magnetic resonance imaging **BOLD**: Blood-oxygen-level dependent BP: Blood pressure LO-CCHS: Late onset CCHS