Early postnatal chronic intermittent hypoxia modifies hypoxic respiratory responses and long-term phrenic facilitation in adult rats

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Reeves, Stephen R., Gordon S. Mitchell, and David Gozal. Early postnatal chronic intermittent hypoxia modifies hypoxic respiratory responses and long-term phrenic facilitation in adult rats. Am J Physiol Regul Integr Comp Physiol 290: R1664 –R1671, 2006. First published February 2, 2006; doi:10.1152/ajpregu.00851.2005.—Acute isocapnic intermittent hypoxia elicits time-dependent, serotonin-dependent enhancement of phrenic motor output in anesthetized rats (phrenic long-term facilitation, pLTF). In adult rats, pLTF is enhanced by chronic intermittent hypoxia (CIH). To test the hypothesis that early postnatal CIH induces persistent modifications of ventilation and pLTF, we exposed male Sprague-Dawley rat pups on their first day of life to a CIH profile consisting of alternating room air and 10% oxygen every 90 s for 30 days during daylight hours (RAIH) or to comparable exposures consisting of room air throughout (RARA). One month after cessation of CIH, respiratory responses were recorded using whole body plethysmography, and integrated phrenic nerve activity was recorded in urethane-anesthetized, vagotomized, paralyzed, and ventilated rats at baseline and after exposures to three 5-min hypoxic episodes [inspired O2 fraction (FiO2) = 0.11] separated by 5 min of hyperoxia (FiO2 = 0.5). RAIH rats displayed greater normoxic ventilation and also increased burst frequency compared with RARA rats (P < 0.01). Ventilatory responses to hypoxia and short-term phrenic responses during acute hypoxic challenges were reduced in RAIH rats (P < 0.01). Although pLTF was present in both RAIH and RARA rats, it was diminished in RAIH rats (minute activity: 74 ± 2% in RARA vs. 55 ± 5% in RAIH at 60 min; P < 0.01). Thus we conclude that early postnatal CIH modifies normoxic and hypoxic ventilatory and phrenic responses that persist at 1 mo after cessation of CIH (i.e., metaplasticity) and markedly differ from previously reported increased neural plasticity changes induced by CIH in adult rats.

ANESTHETIZED ADULT RATS exposed to acute intermittent hypoxia (IH) exhibit a prolonged enhancement of phrenic motor output, a phenomenon termed phrenic long-term facilitation (pLTF) (3, 14, 36). pLTF exemplifies the ability of neural pathways underlying respiratory control to exhibit functional plasticity (16). Although intermittent stimulation of the carotid sinus nerve (CSN) as well as multiple IH protocols elicit pLTF (16, 34–36), pLTF is not elicited by equivalent exposures to sustained hypoxia (SH) (2). Diminished pLTF has been demonstrated in CSN-denervated rats, demonstrating that at least a portion of pLTF can be elicited in the absence of functional carotid body chemoreceptors (5). pLTF is a form of serotonin-dependent plasticity (16). According to current models, activation of postsynaptic 5-HT receptors, principally 5-HT2A subtypes, activates downstream signaling cascades involving several protein kinases, facilitating new protein synthesis (3) and increasing glutamatergic synaptic transmission between bulbospinal respiratory premotor neurons and phrenic motoneurons (3, 14, 33, 37).

Genetic, developmental, and gender-related factors modulate pLTF (8, 17). For example, both phrenic and hypoglossal LTF decrease in middle-aged male Sprague-Dawley rats, an effect possibly associated with age-associated changes in serotonin and/or its receptors (52). However, the opposite (i.e., increased pLTF) is observed in middle-aged female rats, suggesting complex interactions between respiratory control and sex hormones in modulating IH-induced respiratory plasticity (53). pLTF can be enhanced by previous experience, such as prior exposure of adult rats to chronic intermittent hypoxia (CIH) (27, 41).

The respiratory control system exhibits developmental plasticity, a unique effect due to experiences during development that persist into adulthood (9, 37, 45). However, it is unclear whether developmental exposures to CIH influence the expression of other forms of plasticity later in life. Thus a fundamental goal of the present study was to determine whether early developmental exposures to CIH influence the subsequent response of adult animals to acute IH and the expression of pLTF. This issue is of considerable concern to the human population because large numbers of children exhibit sleep-disordered breathing (20). The potential impact of such respiratory disorders on ventilatory control later in life is unknown.

In a series of studies addressing the ventilatory changes elicited by long-term CIH exposures (1 mo), Reeves and Gozal (44) demonstrated that ventilatory plasticity progressively declines with advancing age. Furthermore, long-term CIH present either prenatally (23) or postnatally (47) elicited sustained increases in normoxic ventilation that persist into adulthood. In the present study, we examined the hypothesis that early postnatal exposures to CIH lead to persistent modifications of the ventilatory plasticity exemplified by pLTF. In addition, we also studied the hypoxic ventilatory responses (HVR) in similarly exposed freely behaving adult rats to compare and contrast the responses observed in both experimental models.

METHODS

Timed-pregnant Sprague-Dawley rats were purchased from Charles River Laboratories (Portage, MI), and their male offspring...
were used for all experiments. Litters were routinely culled to eight pups at birth. The experimental protocols were approved by the Institutional Animal Care and Use Committee and are in close agreement with the National Institutes of Health Guide for the Care and Use of Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Experimental Protocol

This study encompasses two series of experiments using identically exposed rats. Male rats were exposed to 30 consecutive days of 12-h IH during daylight starting at 6 h after delivery in room air (RAH animals). Animals were exposed with their dams and weaned at the age of 21 days while the IH exposure continued. Control animals were exposed in identical chambers to continuous normoxic conditions with room air being flushed in the chambers in a similar periodicity as that used for IH exposures (RARA animals). After 30-day IH exposures were completed, animals were removed from the environmental chambers and returned to normal rearing conditions in room air until experimentation. In the first series of experiments, adult male rats (n = 8 per group from at least four different litters per exposure group) were used in acute ventilatory studies. The second series of experiments were conducted in separate rats (n = 10 per group from at least four different litters per exposure group) and used an anesthetized model to study pLTF.

IH Exposures

Animals were placed in four identical commercially designed chambers (30 × 20 × 20 in.; Oxycycler model A44XO; Biospherix, Redfield, NY), which were operated under a 12:12-h light-dark cycle (6:00 AM–6:00 PM). Gas was circulated around each of the chambers, attached tubing, and other units at 60 l/min (i.e., 1 complete cycle/hr). Gas was circulated around each of the chambers and returned to normal rearing conditions in room air until experimentation. In the first series of experiments, adult male rats were exposed (n = 8 per group from at least four different litters per exposure group) were used in acute ventilatory studies. The second series of experiments were conducted in separate rats (n = 10 per group from at least four different litters per exposure group) and used an anesthetized model to study pLTF.

Acute Ventilatory Experiments

All ventilatory measurements are reported as BTPS values. Tidal volume (VT), respiratory frequency (f), and expired volume over time (VE) were computed and stored for subsequent off-line analysis.

Acute hypoxic ventilatory challenges. Animals were weighed and allowed at least 60 min to acclimate to the barometric recording chamber. After stable baseline normoxic values were obtained for at least 30 min, rats were switched to 10% O2-balance N2 with the use of a premixed gas mixture. The hypoxic challenge lasted for 20 min, after which room air was reintroduced into the recording chamber and recovery was recorded for 10 min. Ventilatory measures were averaged in 1-min intervals and plotted.

Acute Neurophysiological Experiments

Preparation. When the rats were between 7 and 9 wk of age, anesthesia was induced with isoflurane (Isoflo; Harvard Apparatus) and then maintained [2.5% isoflurane, inspired oxygen fraction (FIO2) = 0.5, balance N2] through a nose cone, followed by a tracheal cannula placed for mechanical ventilation. After implantation of a catheter (0.80 outer diameter × 0.50 mm inner diameter) in the femoral vein, rats were slowly converted to urethane anesthesia (1.6 g/kg iv) over a period of several minutes. A second identical catheter was implanted in the femoral artery for monitoring of arterial blood pressure and also was used to withdraw blood samples for pH and arterial blood gas analysis (ABL-510; Radiometer, Copenhagen, Denmark). Arterial blood gas values were corrected to rectal temperature measured at the time of sampling, with arterial blood pressure also measured via this catheter. Body temperature was maintained at ~37°C with a homeothermic blanket system (model 50-7053; Harvard Apparatus). Adequacy of anesthesia was assessed periodically throughout the experiment by the observation of blood pressure responses to noxious stimuli (i.e., toe pinch); supplemental urethane (0.2 g/kg iv) was given as necessary. Continuous intravenous infusion (2.5 ml/h) of a 1:11 solution of sodium bicarbonate (5%) and lactated Ringer solution was used to maintain acid-base balance beginning shortly after urethane administration. Animals were bilaterally vagotomized in the midcervical region to avoid entrainment by the ventilator and were paralyzed with pancuronium bromide (2.0 mg/kg iv) to prevent spontaneous respiratory movement. End-tidal carbon dioxide partial pressures (PETCO2) were continuously monitored throughout the protocol with the use of a flow-through carbon dioxide analyzer placed in the exhaust line of the ventilator circuit (Capnogard model 1265; Novametrix). The left phrenic nerve was isolated using a dorsal approach, cut distally, desheathed, placed on a bipolar silver wire electrode, and submerged in mineral oil. Nerve activity was amplified (>10,000), band-pass filtered (100 Hz to 10 kHz; model 1800; A-M Systems, Carlsborg, WA). The signal was then digitized (PowerLab 8/SP; ADInstruments, Sydney, Australia), rectified and integrated (time constant = 50 ms), and processed with commercially available computer software (Chart 5.2; ADInstruments).

Phrenic LTF protocol. Preparations were allowed to stabilize for ~30 min after surgery (PETCO2 ~40 Torr). Apneic threshold was determined by hyperventilating the rat until phrenic nerve activity ceased and then slowly increasing PETCO2 by decreasing ventilator rate or supplementing inspired carbon dioxide until rhythmic activity returned. To stabilize baseline activity, we maintained preparations 3 Torr above the PETCO2 value at which respiratory activity resumed. After baseline phrenic activity was established (30 min), an arterial blood sample (0.2–0.3 ml in a heparinized glass syringe) was drawn; subsequent blood samples were compared with this baseline value. Rats were then exposed to either three 5-min bouts of hypoxia [arterial oxygen partial pressure (Pao2) ~40 Torr] as the pLTF induction protocol or maintained under baseline conditions as a time control (n = 3 for RAIH and RARA exposure groups). Arterial carbon dioxide partial pressure (Paco2) was strictly maintained at ±2 Torr of baseline during hypoxia. Blood samples were collected during the final 30 s of the first treatment bout, and if blood gases were not within the acceptable range, FiO2 and/or inspired carbon dioxide fraction

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(FIO₂) was adjusted during the next bout. Rats were returned to FIO₂ = 0.5 for 5 min between hypoxic episodes. After the hypoxic episode, phrenic nerve activity was monitored for 1 h while baseline conditions were strictly maintained. Blood samples were collected at 15, 30, and 60 min after PLTF induction to ensure that blood gases remained isocapnic (PaCO₂ within 1 Torr of baseline). After the 60-min blood sample was collected, a hypercapnic challenge (PETCO₂ > 70 mmHg for 10 min) was performed to elicit maximal phrenic output.

Data Analysis

Phrenic activity was averaged online in 30-s bins and stored for subsequent off-line analysis. Changes from baseline in burst amplitude and minute phrenic activity (MPA) were normalized as percentages of maximal values due to significant differences between the groups during basal conditions (RARA = 36.1 ± 1.4 vs. RAIH = 42.0 ± 1.3 burst frequency; P < 0.02). Acute hypoxic responses and time-dependent changes in phrenic activity, blood gases, and blood pressure were compared among treatment groups by using one-way or two-way repeated-measures ANOVA followed by Fisher’s least significant difference post hoc tests as appropriate. Effects were considered statistically significant at P < 0.05.

RESULTS

Normoxic Ventilation

RAIH animals weighed significantly less than controls (271.3 ± 4.1 vs. 301.4 ± 9.0 g, respectively; P < 0.01); thus all reported ventilation data were normalized for weight as appropriate. Basal normoxic VE as measured using whole body plethysmography was significantly increased in adult male rats exposed to 30 days of postnatal IH (P < 0.05; Fig. 1A). Ventilatory enhancements were related to significantly greater VT (P < 0.05; Fig. 1B) as well as increased f (P < 0.05; Fig. 1C).

Peak Hypoxic Ventilatory Response

Examination of ventilatory measures during peak HVR revealed that weight-normalized VE was not significantly different between the groups (Fig. 2A). Furthermore, no significant differences occur with respect to weight-normalized VT [RARA = 3.6 ± 0.3 μl vs. RAIH = 3.5 ± 0.2 μl; P = not significant (NS)] or f (RARA = 285.8 ± 23.1 min⁻¹ vs. RAIH = 235.6 ± 36.9 min⁻¹; P = NS) during peak HVR. Conversely, when measures were corrected for their corresponding baseline values, RAIH animals displayed substantially reduced responses to acute hypoxia (P < 0.01; Fig. 2B). Relative changes in VE were accounted for primarily through relative changes in f (RARA = 248.6 ± 27.2% vs. RAIH = 56.3 ± 15.3%; P < 0.001) without significant relative changes in VT (RARA = −1.6 ± 8.9% vs. RAIH = −4.4 ± 5.7%; P = NS).

Apneic Threshold, Baseline Conditions, Arterial Blood Gases, and Arterial Blood Pressure

The CO₂ apneic threshold was not significantly different between RARA and RAIH animals (43.0 ± 0.63 and 42.7 ± 0.42 mmHg PETCO₂, respectively; n = 7/group). Because the baseline CO₂ for all experiments was set at 3 mmHg above the individual apneic threshold and isocapnia was strictly maintained (PaCO₂ ± 1 mmHg relative to baseline) throughout the duration of the experiment (see Table 1), any changes in
the PaO2 is unlikely to significantly contribute to changes in mmHg at all times following the LTF induction protocol; thus emerging between the groups with regard to PaO2 with the displayed significantly elevated burst frequency compared with neurograms for each group are shown in Fig. 3. RAIH rats phrenic output. Representative rectified and integrated phrenic nerve output should not have been influenced by relative differences in PaCO2 regulation. No differences emerged between the groups with regards to PaO2 with the exception of the 15-min post-IH stimulus time point (P < 0.02). Furthermore, no significant differences in PaCO2 occurred between the groups. Table 1. PaO2 and PaCO2 measurements collected during the time course of a pLTF experiment in RARA and RAIH rats

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<td>RARA</td>
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Values are means ± SE (n = 7/group) for arterial partial pressure of oxygen (PaO2) and carbon dioxide (PaCO2) collected during the time course of a phrenic long-term facilitation (pLTF) experimental protocol in rats exposed to room air conditions (RARA) or early postnatal chronic intermittent hypoxia (RAIH). No differences emerged between the groups with regards to PaO2 with the exception of the 15-min post-IH stimulus time point (P < 0.02). Furthermore, no significant differences in PaCO2 occurred between the groups.

Phrenic nerve output should not have been influenced by relative differences in PaCO2 regulation. No differences emerged between the groups with regard to PaO2 with the exception of the 15-min post-IH stimulus time point (P < 0.02; Table 1). It is important to note that PaO2 is well above 100 mmHg at all times following the LTF induction protocol; thus the PaO2 is unlikely to significantly contribute to changes in phrenic output. Representative rectified and integrated phrenic neurograms for each group are shown in Fig. 3. RAIH rats displayed significantly elevated burst frequency compared with RARA rats during baseline conditions (P < 0.02; Fig. 4C); however, burst amplitude (Fig. 4B) and MPA (Fig. 4A) were not significantly different. No significant differences emerged between the groups during exposure to hypercapnia to elicit maximal phrenic activity with respect to burst frequency, burst amplitude, or MPA. Thus all measurements are expressed as percentages of the maximum value.

Examination of mean arterial blood pressure revealed no significant differences between the groups during baseline conditions (RARA = 88.1 ± 5.7 mmHg vs. RAIH = 86.5 ± 2.6 mmHg; P = NS). Decreases in arterial blood pressures during hypoxic challenges did not differ between groups (RARA = 44.7 ± 1.2 mmHg vs. RAIH = 43.2 ± 1.0 mmHg; P = NS). Measurements of mean arterial blood pressure at 60 min after the LTF induction protocol also revealed no significant differences between the groups (RARA = 85.7 ± 3.81 mmHg vs. RAIH = 86.6 ± 3.80 mmHg).

**Short-Term Acute Hypoxic Responses**

Phrenic short-term hypoxic responses (STHR), as demonstrated by MPA, were significantly attenuated in RAIH rats despite having similar values (see Table 1) for PaO2 and PaCO2 (P < 0.01; Fig. 5A). The diminished MPA was primarily attributable to decreased burst amplitude (P < 0.01; Fig. 5B) without significant differences in burst frequency (Fig. 5).

**Phrenic Long-Term Facilitation**

Both groups demonstrated significantly increased phrenic output (MPA and burst amplitude) 60 min after the pLTF induction protocol, indicating the development of pLTF (P < 0.05 for each). In addition, RAIH rats demonstrated significantly attenuated phrenic LTF compared with RARA rats (P < 0.05; Fig. 6, A and B). Furthermore, phrenic burst frequency 60 min after the pLTF induction protocol was not significantly different between the groups (Fig. 6C) despite significant differences occurring during baseline conditions.

**DISCUSSION**

The present study demonstrates that early postnatal exposure to CIH of 1 mo in duration leads to persistent alterations of ventilatory control in 2-mo-old rats. Specifically, developmental CIH resulted in enduring enhancements of both normoxic ventilation and basal phrenic burst frequency. Furthermore,
early postnatal CIH was associated with considerable attenuations of the STHR in both freely behaving and acutely instrumented adult rats. Finally, developmental CIH also reduced the magnitude of pLTF. These findings suggest that developmental CIH elicits long-lasting functional modifications of ventilatory control-related networks.

Methodological Considerations

Before we further discuss the findings of the present study, it is important to acknowledge several technical issues pertaining to portions of the experiments described. First, the acutely instrumented animal preparation used in the present study is an anesthetized, vagotomized, paralyzed, and artificially ventilated model. Although a priori, such a model could compromise the integrity of the normal physiology, the preparation has the powerful advantages that it enables rigorous control of blood gases and the elimination of multiple uncontrolled factors that could modify or mask the expression of pLTF. Furthermore, this is a widely studied model, and key elements of the underlying mechanism of pLTF have been revealed (3, 14, 36). Thus, using this same model, we are able to make comparisons between the present data and a wealth of experience using the same experimental procedures.

Experiments were conducted in adult rats treated with 30 days of CIH beginning on the first day life. After CIH exposure, animals were returned to room air for an additional 30 days and allowed to mature in normoxia until the experiments were initiated. Thus any residual effects of early postnatal CIH shown represent the persistence of a CIH-induced effect long after the IH had ended and thus reflect a long-lasting form of plasticity and metaplasticity induced during development. We do not know whether such effects will endure during longer periods of normoxia after the IH exposure has ended. Indeed, several recent studies have suggested that varying durations and severity of IH profiles are important determinants of the magnitude and duration of the physiological responses elicited by IH (22, 32, 42, 49). Thus it is critically important to take into account the type of IH profiles used in any given experimental setting when attempting to compare among different studies. The profile used in this study was chosen for two major reasons, namely, its unique resemblance to the gas exchange alterations occurring in patients with sleep apnea (21) and also by virtue of the profile’s previously demonstrated ability to elicit alterations in ventilatory control in either adult (46) or neonatal rats (23, 47).

One additional point that deserves further comment is that IH may affect arterial blood pressure and that acute hypoxia also leads to pressor and depressor responses. Thus a component of the differences in respiratory responses presented could be secondary to altered cardiovascular responses. Indeed, a recent study from our laboratory (48) revealed that early postnatal CIH leads to long-term altered baroreflex function. However, although significant differences in heart rate responses and baroreflex sensitivity were identified in adults

Fig. 4. Measurements (means ± SE; n = 7/group) of minute phrenic activity (MPA; A), burst amplitude (ΔAmp; B), and burst frequency (C) during baseline conditions. No significant differences occurred in either MPA or AMP; however, phrenic burst frequency was significantly elevated in RAIH animals (*P < 0.02). Data are expressed as %maximal responses to hypercapnic challenge.

Fig. 5. Measurements (means ± SE; n = 7/group) of MPA (A), ΔAmp (B), and burst frequency (C) during hypoxic conditions (inspired O2 fraction = 0.11). Short-term hypoxic responses (STHR) were significantly attenuated in RAIH animals as demonstrated by reduced MPA and were primarily related to significant ΔAmp. *P < 0.01 compared with RARA animals. No significant differences occurred in phrenic burst frequency during STHR.
exposed postnatally to CIH, there were no differences in mean arterial blood pressure during normoxia, a finding that is now replicated by this study. Furthermore, we now report that the depressor responses during acute hypoxia and blood pressures responses upon induction of pLTF also are similar in the two experimental groups.

**Developmental Plasticity of Ventilatory Control**

Recent evidence has accumulated to support the concept that environmental influences, in addition to genetic influences, may play an important role in determining the functional response properties of pathways underlying ventilatory control (9, 37). In addition, numerous studies have documented profound differences in adaptive responses between adults and developing animals. Such findings have led to the formulation of the concept of “critical periods” of susceptibility during development (for further reading, please refer to Refs. 9 and 24). Briefly, the term critical period as it relates to developmental plasticity refers to a discrete temporal window during which structural and/or functional development of neural networks is uniquely susceptible to environmental influences. This period of time is characterized by accelerated brain growth and synaptogenesis (10, 26), developmental regulation of receptors and associated signaling cascades (25), and neuronal pruning that is regulated through tightly controlled apoptotic mechanisms (43). Consequently, the occurrence of external, environmental modifiers such as early postnatal CIH during this critical period of susceptibility could disrupt the normal maturation of the system and thereby alter its final configuration. In this study, we did not attempt to define the temporal boundaries of the previously identified critical period but, rather, conducted exposures throughout the first month of postnatal life to be all inclusive of this previously defined temporal window of plasticity.

The effects of early postnatal exposure to environmental stimuli have been investigated in several models of ventilatory plasticity. Indeed, developmental hypoxia (7, 12, 15, 38, 39) and hyperoxia both impair ventilatory and phrenic responses in adult rats (6, 18, 29, 30). Such effects can last for the lifetime of the animal (18). Because long-term effects are not observed after hyperoxic exposures in adult rats (28, 29), these forms of plasticity are unique to development.

Recent evidence presented by Bavis et al. (7) has demonstrated that hypoxic phrenic responses are unaffected by perinatal SH in adult rats. Therefore, altered respiratory mechanics are most likely responsible for the changes in ventilatory responses vs. nervous system plasticity following development.

The effects of CIH on pLTF in adult rats have now been investigated in multiple studies (19, 27, 54). In adult male rats, pretreatment with CIH enhances the STHR, as well as pLTF. In fact, CIH restores diminished pLTF in geriatric female rats (54). Ling et al. (21) demonstrated that enhanced pLTF following CIH is serotonin dependent, albeit not necessarily involving the 5-HT\textsubscript{2A} serotonin receptors most frequently associated with pLTF (36). In the present study, developmental CIH exposures were associated with markedly different metabolic changes in phrenic nerve motor output responses. Indeed, developmental CIH attenuated both STHR and pLTF. There are some similar reports of diminished HVR following a CIH profile consisting of 30 min a day for 6 days in piglets (50, 51). However, because previous studies of pLTF have demonstrated a correlation between STHR and pLTF, we cannot rule out the possibility that the attenuation of pLTF following developmental CIH may be linked to the reductions in STHR (16). Furthermore, these results seem to contrast with previous findings by McGuire and Ling (31). In that study, a 7-day CIH profile consisting of alternating 11% \textsubscript{O}2 for 5 min with 5 min of 21% \textsubscript{O}2 for 12 h during the night (6:00 PM to 6:00 AM) was...
associated with enhanced ventilatory LTF in poikilocapnic freely behaving rats. These effects lasted for more than 3 wk but not more than 7 wk. It is critically important to note that the preparation used in the present study is considerably different from the one described by McGuire and Ling. Indeed, we used an anesthetized and vagotomized animal preparation in which isocapnia was strictly maintained (please refer to Methodological Considerations). Thus the two models are obviously and intrinsically different, which in our opinion precludes direct comparisons of the two data sets. Furthermore, notwithstanding inherent differences between the models used in these studies, it also is important to note that the dissimilar CIH profiles also may have contributed to the seemingly disparate results.

In conclusion, we have shown that early postnatal 30-day exposures to CIH lead to lasting alterations in STHR and pLTF in 2-mo-old male rats. In addition, this exposure paradigm results in sustained enhancements of basal respiratory motor output, as well as overall increased normoxic ventilation. However, the mechanisms underlying these phenomena remain to be established.

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GRANTS

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