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

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\textit{Running Title: Postnatal CIH and pLTF}

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ABSTRACT

Acute isocapnic intermittent hypoxia elicits a time-dependent, serotonin-dependent enhancement of phrenic motor output in anesthetized rats, i.e., 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, male Sprague-Dawley rat pups were exposed as of the 1st day of life to a CIH profile consisting of alternating room air and 10% oxygen every 90 sec for 30 days during daylight hours (RAIH), or to a 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-anaesthetized, vagotomized, paralyzed and ventilated rats at baseline and following exposures to three, 5-min hypoxic episodes (FIO2 = 0.11) separated by 5 min hyperoxia (FIO2 = 0.5). RAIH rats displayed greater normoxic ventilation and also increased burst frequency compared to RARA rats (p<0.01). Ventilatory responses to hypoxia and short-term phrenic responses during acute hypoxic challenges were reduced in RAIH (p<0.01). Although pLTF was present in both RAIH and RARA rats, it was diminished in RAIH (minute activity: 74 ±2% in RARA vs. 55 ±5% in RAIH rats at 60 min; p<0.01). Thus, we conclude that early postnatal CIH modifies normoxic and hypoxic ventilatory and phrenic responses that persist at one month after cessation of CIH (i.e., metaplasticity), and markedly differ from previously reported increased neural plasticity changes induced by CIH in adult rats.
Key Words: respiratory control; intermittent hypoxia; development; plasticity; metaplasticity
INTRODUCTION

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-HT$_{2A}$ subtypes, activates downstream signaling cascades involving several protein kinases, facilitating new protein synthesis (3) and increasing glutamatergic synaptic transmission between bulbo-spinal 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 (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 if developmental exposures to chronic intermittent hypoxia influence the expression of other forms of plasticity later in life. Thus, a fundamental goal of the present study was to determine if early developmental exposures to intermittent hypoxia influence the subsequent response of adult animals to acute intermittent hypoxia and the expression of pLTF. This issue is of considerable concern to the human population since 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 month), we demonstrated that ventilatory plasticity progressively declines with advancing age (44). Furthermore, long-term CIH presented either pre-natally (23) or post-natally (47) elicited sustained increases in normoxic ventilation that persist into adulthood. In the present study, we examined the hypothesis that early long-term post-natal 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 in order to compare and contrast the responses observed in both experimental models.
METHODS

Time 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 8 pups at birth. The experimental protocols were approved by the Institutional Animal Use and Care Committee and are in close agreement with the National Institutes of Health Guide in 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 hours IH during daylight (RAIH) starting at 6 hours after delivery in room air. 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. 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 series one, adult male rats (n=8 per group from at least 4 different litters/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 4 different litters/exposure group) and employed an anesthetized model to study pLTF.

Intermittent Hypoxia Exposures
Animals were placed in four identical commercially designed chambers (30 X 20 X 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., one complete change per 10 s). The O₂ concentration was continuously measured by an O₂ analyzer and was changed by a computerized system controlling the gas valve outlets. Deviations from the desired concentrations were met by computer-controlled addition of N₂ or O₂ through solenoid valves. Ambient CO₂ in the chamber was periodically monitored and maintained at <0.01% by adjusting flow rates through the chambers. Humidity was measured and maintained at 40–50% by circulating the gas through a freezer and silica gel. Ambient temperature was kept at 22–24°C. The IH profile consisted of alternating room air and 10% O₂ every 90 s for the 12 hour light period for 30 days.

**Acute Ventilatory Experiments**

*Ventilatory recordings:* Respiratory measures were continuously acquired in the freely behaving animals using the barometric method (Buxco Electronics, Willmington, NC) (4; 40). To minimize the long-term effect of signal drift due to temperature and pressure changes outside the chamber, a reference chamber of equal size in which temperature was measured using a T-type thermocouple was used. In addition, a correction factor was incorporated into the software routine to account for inspiratory and expiratory barometric asymmetries (13). Environmental temperature was maintained slightly below the thermoneutral range (24–26°C). At least 60 min before the start of each protocol, animals were allowed to acclimate to the chamber, in which humidified air (70–
90% relative humidity) was passed through at a rate of 5 l/min, by use of a precision flow pump-reservoir system. Pressure changes in the chamber due to the inspiratory and expiratory temperature changes were measured by using a high-gain-differential pressure transducer (Validyne, model MP45-1). Analog signals were continuously digitized and analyzed on-line by a microcomputer software program (BioSystem XA, Buxco Electronics). A rejection algorithm was included in the breath-by-breath analysis routine and allowed for accurate rejection of motion-induced artifacts. All ventilatory measurements are reported as BTPS values. Tidal volume ($V_T$), respiratory frequency ($f$), and $\dot{V}_E$, were computed and stored for subsequent off-line analysis.

**Acute hypoxic ventilatory challenges:** Animals were weighed and allowed at least 60 minutes to acclimate to the barometric recording chamber. After stable baseline normoxic values were obtained for at least 30 min, rats were switched to 10% O$_2$-balance N$_2$, using a premixed gas mixture. The hypoxic challenge lasted for 20 min, after which room air was reintroduced in 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 weeks of age, anesthesia was induced with isoflurane (Isoflo, Harvard Apparatus) and then maintained (2.5% isoflurane, $F_{I_{O_2}} = 0.5$, balance N$_2$) through a nose cone and followed by a tracheal cannula placed for mechanical ventilation. Following the implantation of a catheter (OD 0.80 X ID 0.50mm) in the femoral vein, rats were slowly converted to urethane anesthesia
(1.6 g/kg i.v.) over a period of several minutes. A second identical catheter was implanted in the femoral artery for the monitoring of arterial blood pressure and was also 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 being also measured via this catheter. Body temperature was maintained at \(~37^\circ\text{C}\) 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 i.v.) was given as necessary. Continuous intravenous infusion (2.5 ml/h) of a 1:11 solution of sodium bicarbonate (5%) and lactated Ringer solution were 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 i.v.) to prevent spontaneous respiratory muscle movement. End-tidal carbon dioxide partial pressures were continuously monitored throughout the protocol using 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 (\(\times10,000\)), 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, Sydney, Australia).

**Phrenic LTF protocol.** Preparations were allowed to stabilize for approximately 30 minutes after surgery ($E_{\text{CO}_2} \sim 40$ Torr). Apneic threshold was determined by hyperventilating the rat until phrenic nerve activity ceased and then slowly increasing $E_{\text{CO}_2}$ by decreasing ventilator rate or supplementing inspired carbon dioxide until rhythmic activity returned. To standardize baseline activity, preparations were maintained 3 Torr above the $E_{\text{CO}_2}$ 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-minute bouts of hypoxia ($P_{\text{O}_2} = \sim 40$ Torr) as the pLTF induction protocol, or maintained under baseline conditions as a time control ($n=3$ for RAIH and RARA exposure groups). $P_{\text{CO}_2}$ was strictly maintained $\pm 2$ Torr of baseline during hypoxia. Blood samples were collected during the final 30 s of the first treatment bout, and, if blood gases are not within the acceptable range, $F_{\text{O}_2}$ and/or inspired carbon dioxide fraction were adjusted during the next bout. Rats were returned to $F_{\text{O}_2} = 0.5$ for 5 min between hypoxic episodes. After the hypoxic episode, phrenic nerve activity was monitored for 1 h while strictly maintaining baseline conditions. Blood samples were collected at 15, 30, and 60 minutes post pLTF induction to ensure that blood gases remained isocapnic ($P_{\text{CO}_2}$ within 1 Torr of baseline). Following the 60-minute blood sample, a hypercapnic challenge ($E_{\text{CO}_2} > 70$ mmHG for 10 minutes) was performed to elicit maximal phrenic output.
Data Analysis

Phrenic activity was averaged online in 30-s bins and stored for subsequent offline analysis. Changes from baseline in burst amplitude and minute phrenic activity (MPA) were normalized as a percentage of maximal values (% Max) 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 use of one-way or two-way repeated-measures ANOVA followed by Fisher LSD 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, control; p < 0.01), thus all reported ventilation data were normalized for weight as appropriate. Basal normoxic \( V_E \) as measured by whole body plethysmography was significantly increased in adult male rats exposed to 30 days of postnatal IH (p < 0.05; Figure 1A). Ventilatory enhancements were related to significantly greater \( V_T \) (p < 0.05; Figure 1B) as well as increased \( f \) (p < 0.05; Figure 1C).

Peak Hypoxic Ventilatory Response

Examination of ventilatory measures during peak HVR revealed that weight normalized \( V_E \) was not significantly different between the groups (Figure 2A). Furthermore, no significant differences occur with respect to weight normalized \( V_T \) (RARA 3.6 \( \mu l \) ± 0.3 \( \mu l \) vs. RAIH 3.5 \( \mu l \) ± 0.2 \( \mu l \); p=ns) or \( f \) (RARA 285.8 min\(^{-1}\) ± 23.1 min\(^{-1}\) vs. RAIH 235.6 min\(^{-1}\) ± 36.9 min\(^{-1}\); p=ns) during pHVR. Conversely, when measures were corrected for their corresponding baseline values, RAIH animals displayed substantially reduced responses to acute hypoxia (p < 0.01; Figure 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 \( V_T \) (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 apnoeic threshold was not significantly different between RARA and RAIH treated animals (43.0 ± 0.63 mmHg and 42.7 ± 0.42 mmHg PₑT₇CO₂, respectively; n=7/group). Since the baseline CO₂ for all experiments was set at 3 mmHg above the individual apneic threshold and isocapnia strictly maintained (PaCO₂ ± 1 mmHg relative to baseline) throughout the duration of the experiment (see Table 1) any changes in 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 minutes 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 partial pressure of O₂ is unlikely to significantly contribute to changes in phrenic output. Representative rectified and integrated phrenic neuronograms for each group are shown in Figure 3. RAIH rats displayed significantly elevated burst frequency compared to RARA rats during baseline conditions (p < 0.02; Fig 4C); however, burst amplitude and MPA 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 a percentage of the maximum value (% Max).

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-not significant). 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-not significant). Measurements of mean arterial blood pressure at 60 minutes
following 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 (STHR)**

Phrenic STHR as demonstrated by MPA, was significantly attenuated in RAIH rats despite having similar values (see Table 1) for PaO$_2$ and PaCO$_2$ (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 (pLTF)**

Both groups demonstrated significantly increased phrenic output (MPA and burst amplitude) 60 minutes after the pLTF induction protocol indicating the development of pLTF (p < 0.05 for each). In addition, RAIH treated rats demonstrated significantly attenuated phrenic LTF compared to RARA rats (p < 0.05; Figs. 6A, 6B). Furthermore, phrenic burst frequency 60 minutes 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 post-natal exposure to CIH of 1 month duration leads to persistent alterations of ventilatory control in 2-month-old rats. Specifically, developmental CIH resulted in enduring enhancements of both normoxic ventilation and basal phrenic burst frequency. Furthermore, early postnatal CIH was associated 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 herein. First, the acutely instrumented animal preparation used in the present study is an anesthetized, vagotomized, paralyzed and artificially ventilated model. While a priori such 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 uncovered (3; 14; 36). Thus, by use of 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 in the first day life. Following 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 here represent the persistence of a CIH-induced effect long after the intermittent hypoxia 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 physiologic 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 2 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 intermittent hypoxia 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 herein could be secondary to altered cardiovascular responses. Indeed, a recent study from our laboratory revealed that early postnatal chronic intermittent hypoxia leads to long-term altered baroreflex function (48). However, although significant differences in heart rate responses and baroreflex sensitivity were identified in adults exposed post-
naturally 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 are also 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 lead to the formulation of the concept of “critical periods” of susceptibility during development (for further reading please refer to 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 post-natal chronic intermittent hypoxia 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 in order to be all inclusive of this previously defined temporal window of plasticity.

The effects of early post-natal 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). Since long-term effects are not observed following hyperoxic exposures in adult rats (28; 29), these forms of plasticity are unique to development.

Recent evidence presented by Bavis et al. has demonstrated that hypoxic phrenic responses are unaffected by perinatal sustained hypoxia in adult rats. Therefore, altered respiratory mechanics are most likely responsible for the changes in ventilatory responses versus nervous system plasticity following developmental sustained hypoxia (7). Notably, similar exposures to sustained hypoxia in adult rats augment ventilation and hypoxic ventilatory responses via distinct central and peripheral neural mechanisms that are readily reversible (1; 11; 46).

In the present study, we found increased basal phrenic nerve burst frequency as well as increased normoxic ventilation following early post-natal IH exposure. These findings are compatible with previous plethysmographic findings in freely-behaving RAIH-exposed rats (47). Developmental exposures to CIH alter ventilatory output when presented either pre-natally (i.e. gestational IH) (23) or post-natally (44). Furthermore, the effects of developmental CIH are not mechanistically similar to those induced by similar exposure to hyperoxia or sustained hypoxia (SH), when assessed in awake, freely behaving rats (47). However, much like the effects of hyperoxia and SH, early post-natal
exposure to CIH elicits an age-dependent plasticity, such that identical exposures at later ages result in relatively smaller changes in normoxic ventilation (44).

**Effect of early post-natal CIH on pLTF**

The effects of CIH on pLTF in adult rats have now been investigated in multiple studies (19; 27; 54). In adult male rats, pre-treatment with CIH enhances the STHR as well as pLTF. In fact, CIH restores diminished pLTF in geriatric female rats (54). Ling and colleagues (2001) demonstrated that enhanced pLTF following CIH is serotonin-dependent, albeit not necessarily involving the 5-HT$_{2A}$ serotonin receptors most frequently associated with pLTF (36). In the present study, developmental CIH exposures were associated with markedly different metaplastic 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 minutes a day for 6 days in piglets (50; 51). However, since previous studies of pLTF have demonstrated a correlation between STHR and pLTF, we can not 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 this study, a 7-day CIH profile consisting of alternating 11% O$_2$ for 5 minutes with 5 minutes of 21% O$_2$ for 12 hours during the night (6:00 PM to 6:00 AM) was associated with enhanced vLTF in poikilocapnic freely behaving rats. These effects lasted for more than 3 weeks, but not more than 7 weeks. It is critically important to note that the preparation used in the present study is considerably different than 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 above). Thus, the two models are obviously and intrinsically different, which in our opinion precludes direct comparisons of the 2 datasets. Furthermore, notwithstanding inherent differences between the models used in these studies it is also important to note that the dissimilar CIH profiles may have also contributed to the seemingly disparate results.

In conclusion, we have shown that early post-natal 30-day exposures to CIH lead to lasting alterations in STHR and pLTF in 2-month 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.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1: Mean measurements (± SEM; n=8/group) of baseline normoxic $V_E$ measured by whole body plethysmography were increased in adult male RAIH rats ($p < 0.05$; A). Ventilatory enhancements were related to significantly greater $V_T$ ($p < 0.05$; B) and increased $f$ ($p < 0.05$; C).

Figure 2: Mean measurements (± SEM; n=8/group) of absolute weight normalized $V_E$ (Panel A) and ventilation expressed as percent change from baseline during peak HVR. Weight normalized $V_E$ did not significantly differ between the groups ($p = ns$). However, ventilatory measures corrected for corresponding baseline values revealed that RAIH animals demonstrated significantly attenuated ventilatory responses to acute hypoxia ($p < 0.01$, Panel B).

Figure 3: Phrenic neurograms of representative pLTF experiments illustrating integrated phrenic motor output in animals exposed to either room air conditions (RARA) or early post-natal chronic intermittent hypoxia (RAIH). RAIH animals demonstrated attenuated phrenic responses to hypoxia as well as diminished pLTF.

Figure 4: Mean measurements (± SEM; n=7/group) of minute phrenic activity (MPA, A), burst amplitude (AMP, 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 is expressed as % maximal response to hypercapnic challenge.

Figure 5: Mean measurements (± SEM; n=7/group) of minute phrenic activity (MPA, A), burst amplitude (AMP, B), and burst frequency (C) during hypoxic conditions (FIO2 = 0.11). Short-term hypoxic responses (STHR) were significantly attenuated in RAIH as demonstrated by reduced MPA (p < 0.01); and were primarily related to significant changes in AMP (p < 0.01). Furthermore, no significant differences occurred in phrenic burst frequency during STHR.

Figure 6: Mean measurements (± SEM; n=7/group) of minute phrenic activity (MPA, A), burst amplitude (AMP, B), and burst frequency (C) 60 minutes following the pLTF induction protocol. Greater pLTF occurred in RARA animals as demonstrated by elevated MPA and AMP (p < 0.05). Furthermore, no significant differences occurred in phrenic burst frequency 60 minutes following the LTF induction protocol.

Table 1: Mean (± SEM; n=7/group) PaO2 and PaCO2 collected during the time course of a pLTF experimental protocol in RARA- and RAIH-exposed
rats. 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 2 groups.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5

A

Minute Phrenic Activity (%Max)

B

Amp (%Max)

C

Phrenic Burst Frequency (min⁻¹)

CHANGE IN PHRENIC ACTIVITY
Figure 6
Table 1

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