Chronic intermittent hypoxia reduces ventilatory long-term facilitation and enhances apnea frequency in newborn rats

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Julien C, Bairam A, Joseph V. Chronic intermittent hypoxia reduces ventilatory long-term facilitation and enhances apnea frequency in newborn rats. Am J Physiol Regul Integr Comp Physiol 294: R1356–R1366, 2008. First published February 20, 2008; doi:10.1152/ajpregu.00884.2007.—Ventilatory long-term facilitation (LTF; defined as gradual increase of minute ventilation following repeated hypoxic exposures) is well described in adult mammals and is hypothesized to be a protective mechanism against apnea. In newborns, LTF is absent during the first postnatal days, but its precise developmental pattern is unknown. Accordingly, this study describes this pattern of postnatal development. Additionally, we tested the hypothesis that chronic intermittent hypoxia (CIH) from birth alters this development. LTF was estimated in vivo using whole body plethysmography by exposing rat pups at postnatal days 1, 4, and 10 (P1, P4, and P10) to 10 brief hypoxic cycles (nadir 5% O2) and respiratory recordings during the following 2 h (recovery, 21% O2). Under these conditions, ventilatory LTF (gradual increase of minute ventilation during recovery) was clearly expressed in P10 rats but not in P1 and P4. In a second series of experiments, rat pups were exposed to CIH during the first 10 postnatal days (6 brief cyclic exposures at 5% O2 every 6 min followed by 1 h under normoxia, 24 h a day). Compared with P10 control rats, CIH enhanced hypoxic ventilatory response (estimated during the hypoxic cycles) specifically in male rat pups. Ventilatory LTF was drastically reduced in P10 rats exposed to CIH, which was associated with higher apnea frequency during recovery. We conclude that CIH from birth enhances hypoxic chemoreflex and disrupts LTF development, thus likely contributing to increase apnea frequency.

The RESPIRATORY CONTROL system, whose primary functions include proper oxygen delivery to the tissues, is subjected to rapid postnatal development. The fact that environmental factors may influence this development is well acknowledged, and several studies have revealed the importance of stable normoxic conditions for proper development of the respiratory control system (8, 24, 43). However, such condition remains ideal since intermittent hypoxia (IH) associated with apneas still represents the most important cause of morbidity in pre-term neonates (21).

Acute IH exposure, such as occurring during a cycle of apneas, induces a form of ventilatory plasticity known as ventilatory long-term facilitation (LTF) and defined as a gradual increase of minute ventilation (recorded in vivo in awake animals) (35) or phrenic nerve activity (recorded in vitro in anesthetized animals) (23). The function of LTF remains largely hypothetical, but the actual consensus attributes a protective role for LTF to maintain regular breathing and avoid dramatic elevation of apnea frequency (1, 33, 38, 44). In newborn mammals, respiratory LTF may be observed in anesthetized newborn rats (36) and on in vitro brain stem preparation (7), but several important questions concerning LTF in nonanesthetized newborn rats are still unresolved. In particular, we have no knowledge on the postnatal pattern of development of LTF, and on the impact of chronic IH (CIH) on LTF in the newborn.

Some long-term effects of CIH on both hypoxic ventilatory response (HVR) and LTF have been assessed by exposing rats during the first postnatal month (34, 45). However, these models did not target specifically the critical period of peripheral chemoreceptor development, which in rats is mainly concentrated during the first 10 postnatal days (12, 19, 31) and approximates late fetal development in humans (9).

Accordingly, the present study was undertaken to test the hypothesis that 1) LTF and HVR are subjected to a pattern of postnatal development and 2) exposure to CIH during the first 10 postnatal days enhances HVR and alters postnatal development of LTF. Since gender is a common contributing factor to specific response to acute, chronic, or IH exposures in newborn or in adult rats (6, 50), we used both male and female rats to test any sex-specific effects of CIH during development.

METHODS

Ethical approval. All procedures have been approved by the local committee of animal care and use of Laval University and are in accordance with the guidelines of the Canadian Council of Animal Care.

Animals. We used a total of 37 male and 38 female Sprague-Dawley rat pups between 1 and 10 postnatal days of age. All pups were born in our animal care facility from 11 virgin females (Charles Rivers Canada, St. Constant, QC, Canada). Rats were supplied with food and water ad libitum and maintained in standard laboratory conditions (21°C, 12:12-h light-dark cycle; lights on at 7:00 AM and off at 7:00 PM). On postnatal day 1 (P1), litters were culled to 12 pups, with a roughly equal number of males and females.

Whole body plethysmography. Respiratory recordings were performed in freely behaving, unrestrained male and female rat pups by whole body flow through plethysmography (Emka Technologies, Paris, France) according to our standard method (30). Air flow through the chamber was set at 0.1 L/min (monitored by a mass flowmeter, model 4140; TSI, Shoreview, MN). The temperature inside the chamber was fixed using a temperature-control system (Physitemp, Clifton, NJ) at 34°C, 32°C, and 30°C for P1, P4, and P10 rats, respectively. Rat pups were introduced in the plethysmograph and allowed 10 min of stabilization; oral (P1 and P4 rats) or rectal (P10 rats) temperature was then measured using a thermocouple for small rodents. Rat pups were then returned to the plethysmograph, and
the normoxia period was started when ventilatory parameters were stable (>10 min). Body temperature was also measured at the end of the experiment. Mean body temperature was used for correction of tidal volume using standard equations (4, 18). In- and out-flowing gases were analyzed using a dual channel oxygen analyzer (AEI Technologies) for calculation of oxygen consumption as previously described (30). Signals from the plethysmograph, gas analyzers, and flowmeter were directed toward a computer for storage and online calculation of respiratory parameters (Ve, fR, and VT) and oxygen consumption \( [\text{VO}_2 = (\text{O}_2\text{in}-\text{O}_2\text{out}) \times \text{flow}] \) using IOX software (Emka Technologies). Ve, VT, and VO2 are expressed per 100 g body wt to allow comparison between groups. Oxygen convection ratio was calculated as Ve/VO2.

**Baseline and HVR.** We used male and female pups at P1 (n = 7 males and 9 females), P4 (n = 8 males, 8 females), or P9–P10 (n = 12 males, 12 females) from five different litters. Individual animals were used only for one session of recording using whole body plethysmography. After a baseline period of 10 min in normoxia, inflowing air was switched to a nitrogen tank ensuring a rapid decrease of oxygen from 21% to 5% in 60 s, and returned to 21% in 120 s. This hypoxic cycle was repeated 10 times, each separated by 5 min of normoxia for a peak-to-peak period of 8 min (Fig. 1, A and B). Baseline ventilatory and metabolic data were averaged during the last 5 min of the baseline period. For HVR, ventilatory data were averaged every second and analyzed at 20%, 15%, 10%, and 5% O2 during acute hypoxia and return to normoxia for each cycle (see Fig. 1B for O2 profile). To test the hypothesis that HVR remained similar throughout the 10 hypoxic cycles, a nonparametric Friedman test for repeated measures was performed to compare HVR during the first, fifth, and tenth hypoxic cycles. Since there was no statistical difference \( (P > 0.05) \), the data from the 10 cycles were averaged and expressed in percent of baseline values.

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**Fig. 1.** A: experimental protocol for hypoxic cycles and long-term facilitation (LTF) studies. **Top:** baseline, 10 intermittent hypoxic (IH) cycles and 2-h recovery periods. **B:** inspired oxygen in plethysmograph during baseline, hypoxic ventilatory response (HVR; during hypoxic cycles), and LTF (during recovery). Hx, Decrease in inspired oxygen from 21% to 5% in plethysmograph; reox, reoxygenation from 5% to 21% of oxygen in plethysmograph. C: individual plethysmographic recordings during baseline, HVR, and LTF in rat pups at postnatal day 1 (P1), day 4 (P4) and day 10 (P10) after birth and at day 10 after exposure to chronic intermittent hypoxia (P10-CIH).
Recovery and ventilatory LTF. At the end of the 10 hypoxic cycles used for HVR, the animals were left undisturbed in the chamber for a recovery period of 2 h during which respiratory and metabolic parameters were continuously measured (Fig. 1). Ventilatory and metabolic parameters were averaged every 5 min and expressed in percent of baseline values.

CIH. An additional group of three pregnant female rats were housed in a Plexiglas chamber (internal volume 0.05 m³) 1 day before delivery and IH was started at P1 using an Oxycycler (Biosperix, Redfield, NY) controlling two actuator pods (Biosphere) connected to the chamber. Silicon perforated tubes were positioned in the ceiling of the chamber to ensure fast and homogenous gas diffusion. This setup allows a rapid oxygen cycling and limits environmental disturbances that may be associated with rapid air pulses through the original actuator pods. Oxygen dropped from 21% to 5% in 100 s and then returned to 21% in 140 s, followed by normoxia during 6 min. This 10-min cycle was repeated six times, followed by 1 h of normoxia and repeated 24 h a day during 10 consecutive days.

At P10, individual pups (n = 10 males, 9 females) were used as described above for recordings of HVR and LTF using whole body plethysmography. The values obtained for this group of animal (CIH) were compared with the values obtained in P10 rats raised in normoxia (defined as control).

Apneas. Apneas were analyzed by visualizing individual respiratory recordings on a computer display. This analysis was performed during the 10 min of baseline recordings, the first 10 min following the last hypoxic cycle, and the last 10 min of the recovery period, using standard criteria to identify apneas as an absence of flow for at least two normal respiratory cycles (37). Two types of apneas were distinguished, 1) spontaneous apneas with interruption of flow and 2) postsigh apneas preceded by a breath with amplitude at least twice the resting tidal volume. The apnea index was reported as number per hour and mean apnea duration in milliseconds.

Statistics. Results were expressed as means ± SE. Because the data did not have a normal distribution, we used Wilcoxon and Mann-Whitney nonparametric tests for intragroup and intergroup comparison, respectively. Correlation was analyzed using a Spearman nonparametric test. Significance was defined at P < 0.05.

RESULTS

Individual recordings and baseline values during development. Representative respiratory recordings obtained during baseline, hypoxic cycle, and at the end of the recovery period in P1, P4, P10, and CIH rats are shown in Fig. 1C.

During postnatal development, respiratory frequency increased progressively and tidal volume decreased between P1 and P10 rats. Oxygen consumption decreased, while the Ve/V̇O₂ increased significantly between P4 and P10 rats (Table 1).

HVR during development. Under our dynamic conditions of acute hypoxic exposure, in P1 and P4 rats ventilation showed an early peak as the oxygen level switched rapidly from 21 to 10%, followed by a step decrease as oxygen dropped below 10% (Fig. 2; points during hypoxic exposure are at 20, 15, 10, and 5% O₂, respectively). The peak response was higher in P4 (+58% vs. baseline) compared with P1 rats (+39%; Fig. 2). By contrast, in P10 rats, the response was maintained above baseline until 5% O₂ and during the return to normal air. This specific response pattern resulted from the contribution of both respiratory frequency and tidal volume in P1, while respiratory frequency alone was increased in P4. In P10 rats, the increase in minute ventilation was due to the increase of respiratory frequency and tidal volume. There was no sex-specific effect on this response at any age.

Table 1. Baseline values for body weight, ventilation, and metabolism in rat pups during development

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<th>Age, postnatal days</th>
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<th>4</th>
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Values are means ± SE; data from male and female rat pups were pooled. V̇E, minute ventilation; fR, respiratory frequency; V̇T, tidal volume; V̇O₂, oxygen consumption; Ve/V̇O₂, oxygen convection ratio. *P < 0.05 Mann-Whitney U-test vs. postnatal day 1.

Note that in Fig. 2 the deviation from 100% for the values at zero reflects the changes occurring between baseline (i.e., while the animal lay quiet in the plethysmograph before hypoxia), and the values under 20% O₂ averaged between each hypoxic cycle. The lower respiratory frequency observed at zero occurs after the first hypoxic cycle for each normoxic period between hypoxic cycles. This lower respiratory frequency most probably reflects the posthypoxic ventilatory decline (44). In P4 rats, this was accompanied by a higher tidal volume.

Ventilatory LTF during development. Ventilatory LTF, assessed as a progressive increase of minute ventilation throughout the 2 h following the acute 10 hypoxic cycles, is presented in Fig. 3. In P1 and P4 rats, minute ventilation was unchanged during the recovery period. In P10 rats, however, minute ventilation (and tidal volume) increased progressively. At the end of the recovery period, minute ventilation and tidal volume were 23% and 28% above baseline value, respectively.

There were only modest changes in oxygen consumption during the recovery period as shown in Fig. 4. However, as a general trend during the recovery period oxygen consumption (V̇O₂) declined in P1, remained stable in P4, and increased (although this was not significant) in P10 rats (Fig. 4). The resulting Ve/V̇O₂ during recovery period was substantially higher compared with baseline in P10 rats, while only minor fluctuations were found in P4 and P1 rats (Fig. 4). There was no sex-specific effect on this response at any age.

To confirm that the response in P10 rats was specifically due to the IH exposure, we recorded minute ventilation during 3.5 h in normoxia in an additional group of P10 pups (6 males, 6 females). Throughout this period, minute ventilation remained stable (Fig. 5).

CIH enhances HVR specifically in male pups. In P10 pups exposed to CIH from birth, body weight was reduced compared with controls (Table 2). In baseline conditions, there was a slight reduction of respiratory frequency compared with controls (Table 2 and Fig. 1C). However, minute ventilation and metabolic rate were similar between control and CIH rats. There was no intragroup or intergroup sex-specific effect for baseline values.
During acute hypoxia, HVR was altered in CIH male pups compared with controls, with a higher magnitude (+53% vs. +32% of baseline value) and a sustained elevation during lowest oxygen level (Fig. 6). By contrast, in female pups, there was no observable difference between CIH and control pups.

**CIH abolishes ventilatory LTF.** During recovery, the progressive increase in minute ventilation observed in P10 control pups was abolished in P10 rats raised under CIH from birth (Fig. 7). Indeed, minute ventilation progressively decreased during the recovery period in CIH pups (Fig. 7), this being largely due to a progressive decrease of tidal volume and respiratory frequency. There was no intragroup or intergroup sex-specific effect during recovery period.

**Correlation between HVR and apnea frequency.** It has been previously suggested that elevated hypoxic sensitivity of peripheral chemoreceptors may be critical to generate apneas, specifically in preterm neonates (2, 11, 41), consistently with the well-supported role of peripheral chemoreceptors on generation of apneas (17, 49). But the existence of a relationship between hypoxic sensitivity and apnea frequency is not commonly tested in experimental studies. Since our experimental approach to test HVR used short exposure time, we assume that it is a reliable index of peripheral chemoreceptor function. Under these conditions, in P10 rats, we found a strong correlation between HVR and apnea frequency in baseline conditions (Fig. 8, left); however, this correlation is lost in CIH rats (Fig. 8, right).

**Enhanced apnea frequency during recovery in CIH rats.** There was no difference in apnea frequency or duration under baseline conditions between controls and CIH pups (Fig. 9). In control pups there was a moderate increase in apnea index at the end of the recovery period compared with baseline. In CIH pups, however, this effect was drastically amplified, leading to an apnea frequency two times higher than controls (Fig. 9, left).
This effect was of similar magnitude for spontaneous vs. postsight apneas. The mean apnea duration was also higher in CIH vs. control at the beginning and at the end of the recovery period (Fig. 9, right). There was no gender-specific effect on this response.

**DISCUSSION**

The main findings of this study are as follows: 1) ventilatory LTF appears gradually during postnatal development in newborn rats, and 2) CIH increases HVR exclusively in male rat pups and drastically impairs LTF development. It is remarkable that the abolition of ventilatory LTF following CIH is associated with a higher apnea frequency, thus suggesting a link between ventilatory LTF and reduction of apnea frequency in newborn rats. These results are critical to better understand the complex series of events leading to the dramatically elevated frequency of apneas, which remains the major cause of morbidity in preterm neonates (21).

**Methodological considerations.** The main limitation to this study comes from the utilization of whole body plethysmography to assess ventilatory parameters in newborn rats. The basis of this approach relies on measuring the pressure changes induced by humidification and heating as the inspired air moves from the chamber to the lungs and to the opposite changes as this air leaves the lungs (5). This approach requires precise determination of several parameters during recording to ensure adequate calculation of tidal volume using the standard equation proposed by Bartlett and Tenney (5).

Even when taking care to assess these parameters, substantial errors are still present on the calculated tidal volume (27, 51). However, this does not preclude the use of this estimation of tidal volume for comparisons between experimental groups, providing that the error on tidal volume is constant. The error made on the calculation of tidal volume depends on the difference between the chamber temperature and the rectal temperature of the animal, and this error becomes higher as this difference becomes smaller (40). This clearly may have an impact on our study since this gradient of temperature differs across ages, being very small in P1 rats and more important in P4 and P10 rats. However, this is not a concern when assessing the pattern of LTF at different ages.

Fig. 3. Changes in minute ventilation, respiratory frequency, and tidal volume during recovery period after 10 hypoxic cycles in rat pups at P1 (n = 16), P4 (n = 16), and P10 (n = 24) after birth. All values are %baseline (means ± SE). Data from male and female rat pups are pooled. *P < 0.05, Wilcoxon vs. baseline; †P < 0.05, Mann-Whitney U-test vs. P1. Solid lines correspond to successive significant differences vs. baseline.
Most significant errors may nonetheless appear since we did not measure rectal temperature throughout the recordings in our study. This is not ideal, and continuous measurement of body temperature should be preferred. However, this would have caused several concerns, since the animal should either be handled at different time points for measurements or instrumented with an adequate temperature probe before the experiments. We rather prefer to limit the manipulation of the animals, and measured rectal temperature at the beginning and at the end of the experiment. Actually, rectal temperature slightly increases between these two points to a similar extent between all groups (about 0.5°C). Nonetheless, a similar increase of body temperature was observed in a control group of P10 rats maintained in the plethysmograph for 3.5 h (without hypoxic exposure). Minute ventilation in these animals remained stable throughout the recordings; they did not show any sign of LTF (Fig. 5), which is therefore a specific response to the hypoxic stimuli and not an artifact due to changing temperature conditions.

We have not taken sleep/wake states into account during our recordings. However, in newborn rats, sleep/wake cycles are very brief, lasting around 10 s in 2-day-old rats, and then

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<th>Table 2. Effect of chronic intermittent hypoxia on baseline values for body weight, ventilation, and metabolism in 10-day-old control rats and in rats raised under chronic intermittent hypoxia (CIH) from birth</th>
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<td><strong>Ventilation</strong></td>
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Values are means ± SE; data from male and female rat pups were pooled. *P < 0.05 Mann-Whitney U-test vs. P10-control.
progressively increasing during the first postnatal week to reach 30 s in 8-day-old rats. But the percentage of time spent asleep (around 70%) does not evolve significantly during this period (10). Accordingly potential state-dependent expression of LTF could not be a confounding factor in our results showing postnatal development of LTF.

HVR during development. Our experimental setup allows the dynamic analysis of ventilatory response to a brief, but profound hypoxic exposure (i.e., 5% O2 reached in 60 s). Because the time of exposure was short, the response observed likely represents the output of the peripheral chemoreceptors (16). Our findings are consistent with previous studies (12, 19, 31), showing that the magnitude of the response to acute hypoxia increased between P1 and P4, but at both ages, ventilation rapidly returned to baseline, while %oxygen was still decreasing. In addition, the oxygen level at which ventilation peaked changed from 10 to 15% O2 between P1 and P4 (Fig. 2), which may either represent an increased sensitivity and/or a faster dynamic response of peripheral chemoreceptors. By contrast in P10 rats, while the magnitude of HVR was lower than in P4, it was maintained during the deepest level of hypoxia and during the early phase of reoxygenation. This difference with earlier studies showing gradual increases of HVR during development may be explained by our pattern of rapid dynamic hypoxic exposure vs. sustained exposure to a single fraction of inspired oxygen (12, 19, 31). The pattern of the ventilatory response also evolved during development, since both respiratory frequency and tidal volume increased at P1 but only respiratory frequency increased at P4.

It is also worth mentioning that another key component of the HVR is the posthypoxic frequency decline, characterized by a decline of respiratory frequency after hypoxic exposure (7, 13–15, 44). Consistent with this notion, respiratory frequency was lower between hypoxic cycles vs. baseline in all groups.

Ventilatory LTF appears gradually during postnatal development in newborn rats. LTF is defined as a progressive increase in ventilation and respiratory nerve activity following IH exposure (38, 39, 44). IH-induced LTF has been demonstrated in several species including rats (23, 35), cats (39), and humans (25), and is not restricted to lung ventilation, but also affects upper airway motor nerves (36). The functional implication of ventilatory and upper airway LTF is still debated, but it is accepted, although not directly demonstrated, that it may help to reduce apnea frequency, specifically in the “gray zone” when a subclinical syndrome is apparent (33, 38, 44). In the central nervous system, and more specifically in ventral spinal segments containing the phrenic nucleus, IH exposure induces
Previous studies have shown the occurrence of LTF in newborn rats and mice (36). In anesthetized rat pups (2–3 days after birth), LTF was limited to the activity of the genioglossus muscle, while ventilation did not increase following IH exposure (36). In newborn mice, phrenic nerve LTF was observed by using the in vitro isolated brain stem preparation (7). Furthermore, LTF occurs also in vitro (isolated brain stem-spinal cord preparation) after intermittent administration of serotonin in newborn rats (P0-P2) (32). These data suggest that the molecular machinery and neural circuitry necessary for LTF expression is functional early in development.

In our study, using nonanesthetized animals, ventilatory (i.e., increased minute ventilation) LTF was clearly observed only in P10 pups. While the absence of LTF in P1 pups is evident, it is slightly different in P4 rats, since tidal volume was high compared with baseline value following hypoxic exposure. The detailed analysis shows that tidal volume increased between the fifth and the tenth hypoxic cycle (not shown), but since respiratory frequency remained low, minute ventilation did not increase. Accordingly, it is tempting to speculate that ventilatory LTF in rat pups does not occur in P1 rats, but appears gradually between P1–P10.

CIH increases HVR in male but not in female rat pups. Ten-day-old rats exposed to CIH from birth had a notable reduction (i.e., about 20%) of body weight vs. P10 control, as well as a slightly reduced respiratory frequency (~6%). Other recorded parameters in normoxia were similar. It is worth mentioning that a decreased respiratory frequency was also reported in human preterm neonates with apnea of prematurity compared with age-matched nonapneic preterm babies (2).

In P10 male rats exposed to CIH, the HVR was substantially increased compared with control P10 rats, consistent with previous studies in newborns (34, 43). Similar responses have been reported in adult male rats (46), piglets (29), cats (48), and humans (22). In preterm apneic babies, enhanced sensitivity of peripheral chemoreceptor was also described (2, 11, 41). However, our study shows a clear gender discrepancy of this response, as the enhanced HVR was observed specifically in male pups. Testosterone released during the end of gestation and after birth in male rat pups (53) may explain gender-specific responses to hypoxic stimuli. In adult cats, testosterone increases hypoxic sensitivity of carotid body and HVR (52). Accordingly, it is tempting to speculate that concomitant testosterone exposure and IH are both required to enhance HVR in newborn rats. An enhanced HVR may result in higher vulnerability to ventilatory instability and apneas (2, 11, 41), since responses to transient alterations of arterial oxygen would result in respiratory “overshoot” and exaggerated CO₂ washout.

CIH drastically impairs LTF development. While ventilatory LTF was clearly apparent in P10 rat pups, this was not the case in CIH rats. In other words, our results likely indicate that CIH abolishes or delays the postnatal development of ventilatory LTF. A previous study reported that rats raised during 1 mo (from birth) under IH showed a long-lasting reduction of ventilatory LTF, estimated by measuring phrenic nerve activity in 2-mo-old rats (47). Combined with our results, this suggests that exposure to postnatal IH impairs the development of ventilatory LTF. However, when newborn rats are exposed to 7 days of CIH, ventilatory LTF (assessed in vivo using whole body plethysmography) was enhanced later during develop-

**Fig. 7.** Changes in minute ventilation, respiratory frequency, tidal volume (VT), and V˙E/V˙O₂ during recovery period after 10 hypoxic cycles in rat pups at P10 (P10-control, n = 24) or CIH (P10-CIH, n = 19). All values are %baseline (means ± SE). Data from male and female rat pups are pooled. *P < 0.05, Wilcoxon vs. baseline, †P < 0.05, Mann-Whitney U-test P10-CIH vs. P10-control. Solid lines correspond to successive significant differences vs. baseline or between groups.
ment (in 1-mo-old rats) (34), indicating that varying exposure protocol or recording approach may yield contradictory results. In adult rats (35) CIH enhances ventilatory LTF. Accordingly, the impact of CIH on ventilatory LTF appears to be age dependent.

Impaired ventilatory LTF is associated with higher apnea frequency. From a functional point of view, it is remarkable that the impaired LTF in pups raised in CIH results in higher frequency of apnea at the end of the recovery period. Although, our study was limited in time (we computed apnea only for 10 min, 2 h after cessation of the intermittent exposures), this result suggests that the establishment of a robust LTF is an efficient endogenous protective mechanism against apnea in rat pups. To our knowledge, this is the first study in newborn rats showing an inverse relationship between ventilatory LTF and apnea frequency, corresponding to the general agreement that LTF may reduce apneas by ensuring upper airway patency and increased respiratory drive (1, 33, 38, 44).

Despite the fact that rats raised under CIH had higher HVR compared with controls, they did not have higher apnea frequency under baseline conditions, as would have been predicted by the strong correlation between HVR and apnea frequency reported in control animals (Fig. 8). This result may suggest that apart from enhancing HVR and reducing LTF, CIH from birth induces more subtle changes in the respiratory control system, which disrupts the relationship between HVR and apneas under resting conditions. However, these subtle changes are apparently not strong enough to overcome the deleterious effects of the impaired LTF induced by CIH.

**Perspectives and Significance**

Newborn humans are more likely to experience apneas or hypoventilation due to the contribution of specific factors. The first factor is related to the small “CO₂ reserve” (i.e., the difference between eupneic and apneic Pa₄CO₂) compared with adults (28). From our results it is tempting to speculate that a second factor is also important since a weak or absent ventilatory LTF will further contribute to the development of apneas. This could help to understand the well-known inverse relationship between apnea frequency and gestational age at birth in preterm neonates (26). An additional factor emerges from the fact that CIH enhances hypoxic chemoreflex, promoting potent “overshoot” responses to hypoxic exposure, and further contributing to enhance apnea, as previously suggested (2, 11, 41, 49), particularly when the protective mechanisms of LTF are not present or have been impaired (present study). Since ventilatory LTF development is delayed by CIH, apneas should then persist for a longer period of time during postnatal life reflecting the delayed development of protective mechanism. Interestingly, a previous study reported that apnea persisted for a longer period of time in preterm infants born between 24 and 28 gestational weeks compared with infants born after 28 wk of gestation (20). Accordingly, our study may help to better understand the dramatic occurrence and the persistence in time of apneas in preterm neonates, by providing evidence showing the establishment of reciprocal positive feedback mechanisms promoting instability of respiratory control system (i.e., im-

**Fig. 8. Relationship between HVR (%baseline) and apnea index during baseline (number/hour) in P10 control (left) and CIH (right) male and female rat pups. Correlation was tested using Spearman nonparametric test; significance, P < 0.05.**
paired LTF and enhanced HVR) as proposed by Mahamed and Mitchell in adults (33).

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