Differences in time-dependent hypoxic phrenic responses among inbred rat strains

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Golder, Francis J., Andrea G. Zabka, Ryan W. Bavis, Tracy Baker-Herman, David D. Fuller, and Gordon S. Mitchell. Differences in time-dependent hypoxic phrenic responses among inbred rat strains. J Appl Physiol 98: 838–844, 2005. First published November 5, 2004; doi:10.1152/japplphysiol.00984.2004.—Hypoxic ventilatory responses differ between rodent strains, suggesting a genetic contribution to interindividual variability. However, hypoxic ventilatory responses consist of multiple time-dependent mechanisms that can be observed in different respiratory motor outputs. We hypothesized that strain differences would exist in discrete time-dependent mechanisms of the hypoxic response and, furthermore, that there may be differences between hypoglossal and phrenic nerve responses to hypoxia. Hypoglossal and phrenic nerve responses were assessed during and after a 5-min hypoxic episode in anesthetized, vagotomized, and ventilated rats from four inbred strains: Brown Norway (BN), Fischer 344 (FS), Lewis (LW), and Piebald-viral-Glaxo (PVG). During baseline, burst frequency was higher in PVG than LW rats (P < 0.05), phrenic burst amplitude was higher in PVG vs. other strains (P < 0.05), and hypoglossal burst amplitude was higher in PVG and BN vs. FS and LW (P < 0.05). During hypoxia, burst frequency did not change in BN or LW rats, but it increased in PVG and FS rats. The phrenic amplitude response was smallest in PVG vs. other strains (P < 0.05), and the hypoglossal response was similar among strains. Short-term potentiation posthypoxia was slowest in FS and fastest in LW rats (P < 0.05). Posthypoxia frequency decline was absent in PVG, but it was observed in all other strains. Augmented breaths were observed during hypoxia in FS rats only. Thus genetic differences exist in the time domains of the hypoxic response, and these are differentially expressed in hypoglossal and phrenic nerves. Furthermore, genetic diversity observed in hypoxic ventilatory responses in unanesthetized rats may arise from multiple neural mechanisms.

hypoxia; breathing; hypoglossal; genetic

Both experience (i.e., plasticity; reviewed in Ref. 29) and genetics (reviewed in Ref. 17) influence the neural control of breathing. Genetic influences on respiratory control have been described in rats (13, 15, 20, 37), mice (40), and humans (42). Because rats are commonly used to investigate fundamental aspects of breathing, an understanding of the genetic determinants of ventilatory control in this species is essential. Furthermore, understanding of the range of genetic variation in respiratory control may have important clinical implications in a genetically diverse population, such as humans.

Differences in ventilation among inbred and outbred rat strains have been documented during eupnea (quiet breathing), hypoxia, hypercapnia, and exercise (13, 20, 37), and some of these changes have been associated with genotypic differences, localized to specific chromosomes (13). For example, in Brown Norway (BN) rats the frequency response to hypoxia is small compared with some other strains (20, 37). Hodges et al. (20) reported that, although BN rats responded to hypoxia with less change in minute ventilation than other strains, hypoxic arterial P\textsubscript{CO\textsubscript{2}} (Pa\textsubscript{CO\textsubscript{2}}; and therefore magnitude of hyperventilation) was similar to other strains. Although not directly measured, these data suggest BN rats experience greater hypoxia-induced hypermetabolism, an effect that may be associated with genetic differences on chromosomes 9 and 18 (13). Thus it may be that the unique ventilatory response of BN rats reflects differences in the genetic determinants of metabolic regulation vs. ventilatory control per se.

Hypoxic ventilatory responses are complex, and they consist of multiple discrete neural mechanisms operating in different time domains (33). For example, the onset of hypoxia in anesthetized rats elicits an acute response, followed by slower (seconds to minutes) short-term potentiation (STP) of nerve burst amplitude and short-term depression of burst frequency (19, 33). Collectively, these mechanisms during brief hypoxia comprise the short-term hypoxic response (23). After hypoxia in rats, nerve burst amplitude slowly declines, reflecting the off-response of STP (19), and nerve burst frequency decreases below prehypoxic levels for several minutes, an effect termed posthypoxia frequency decline (PHFD; Refs. 7, 33). Thus it is plausible that genetic influences on ventilation may be unique to these time-dependent mechanisms during or after hypoxia. Some investigators have begun to dissect the respective components of the hypoxic ventilatory response in unanesthetized rats (38, 39). The advantage of such analyses in unanesthetized rats is that responses are observed without the complications attendant to anesthesia; on the other hand, it is difficult to control all of the relevant variables in such experiments, and it is difficult to isolate specific time-dependent mechanisms without complications resulting from, for example, changes in arterial blood gases. Thus there can be advantages in assessing time-dependent hypoxic responses in both anesthetized preparations, where greater control of relevant variables is possible, and unanesthetized preparations, where the complications of anesthesia are avoided. Through both approaches, we are most likely to achieve a full understanding of genetic differences in components of the hypoxic ventilatory response.

Another level of complexity in the hypoxic ventilatory response is that the hypoxic respiratory behavior occurs via increased respiratory drive to diverse populations of respiratory motoneurons, each under distinct premotor control (30, 31). For example, hypoxia increases respiratory drive to motoneurons innervating the respiratory pump muscles (e.g., phrenic motoneurons), as well as to motoneurons that innervate muscles that control upper airway resistance (e.g., hypoglossal motoneurons). As such, genetic or strain differences in discrete...
RAT STRAIN DIFFERENCES IN HYPOXIC PHRENIC RESPONSES

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The time domains of the hypoxic response may be unique to specific motor outputs. To date, there is no information available concerning this possibility.

Given that ventilation in awake rats can be influenced by genetic differences in metabolic rate, pulmonary mechanics, body temperature, and gas exchange, we chose to investigate rodent strain differences in anesthetized, vagotomized, and mechanically ventilated rats. In this preparation, body temperature, arterial blood gases, and vagal afferent feedback are more effectively controlled. Furthermore, this preparation enabled an evaluation of differences in the regulation of a phrenic vs. hypoglossal motor output. We hypothesized that genetic differences would be observed among inbred rat strains with respect to the following: 1) baseline phrenic and hypoglossal nerve activity, 2) short-term hypoxic phrenic and hypoglossal responses (STHR), 3) the time course of STP, and 4) the magnitude and/or time course of PHFD. Four inbred rat strains were chosen on the basis of known differences in their hypoxic response (37) and selection for the rat genome project (BN; Ref. 34), common use in studies of aging and ventilatory control in our laboratory and in others [Fischer 344 (FS); Ref. 5], differential motor responses after spinal cord injury [Lewis (LW); 32], and a propensity for high [Plebald-viral-Glaxo (PVG)] or low (LW) spontaneous running wheel activity (22). The experimental protocol was nearly identical to previous investigations concerning time domains of the hypoxic response originating from our laboratory (15, 19, 23, 44; for reviews see Refs. 14, 28, 33), thereby facilitating comparisons with published studies.

METHODS

Animals. Forty-four male rats were used in this study from four inbred strains, all provided by the same vendor (Harlan, Indianapolis, IN) in an effort to minimize variations in rearing practices. The strains were BN (n = 9; 108 ± 5 days old; 287 ± 7 g (mean ± SE); colony 217], FS (n = 12; 108 ± 5 days old; 285 ± 7 g; colony 212B)], LW (n = 11; 110 ± 4 days old; 364 ± 40 g; colony 202B), and PVG (n = 12; 103 ± 4 days old; 279 ± 10 g; colony 205). Animal husbandry and all procedures were approved by the Institutional Animal Care and Use Committee of the School of Veterinary Medicine at the University of Wisconsin, Madison.

Measurement of respiratory motor output. Rats were initially anesthetized with isoflurane in 50% O2 (balance N2). The trachea was exposed and cannulated to permit mechanical ventilation throughout the experiment (2.0–2.5 ml, Rodent respirator model 683; Harvard Apparatus, South Natick, MA). Bilateral vagotomy was performed to prevent entrainment of respiratory nerve activity with the ventilator. Subsequently, the femoral artery and vein were cannulated to measure arterial blood pressure (model P122, Grass Telefactor, West Warwick, Subsequently, the femoral artery and vein were cannulated to measure arterial blood pressure (model P122, Grass Telefactor, West Warrick, MA). Bilateral vagotomy was performed to prevent entrainment of respiratory nerve activity with the ventilator.

Arterial blood samples (0.4 ml) were collected for pH and blood-gas measurements during baseline conditions and during hypoxia. Peak integrated hypoglossal and phrenic neurogram burst amplitude and burst frequency were measured for 30-s periods immediately before each blood sample. Peak integrated burst amplitude was chosen as the neural correlate to tidal volume because this parameter is positively correlated to muscle activity and muscle force output (12). Furthermore, we recently demonstrated that awake respiratory frequency and tidal volume (barometric plethysmography) were highly correlated in the same rat with phrenic burst frequency and amplitude (stimulus) from nerve recordings made as described in the present study (Gold and Mitchell, unpublished data). Animals were included in the final analysis only if 1) arterial PO2 (PaO2) during the hypoxic period was between 35 and 45 Torr, 2) PaCO2 during baseline conditions was above 150 Torr, and 3) PaO2 during hypoxia was within 2 Torr of the baseline value. At the end of each experiment, inspired PaCO2 was increased to 80–85 Torr to obtain near maximal hypercapnic nerve activity.

Statistical analysis. Baseline integrated burst amplitude and minute phrenic activity (amplitude × burst frequency) are reported as a percentage of the burst amplitude during hypercapnia. Changes in amplitude and minute phrenic activity during hypoxia are expressed as a percentage of baseline values. Burst frequency during hypoxia was reported as a change from the baseline value. To assess PHFD, burst frequency was measured after the hypoxic episode and averaged during 30-s intervals for 5 min posthypoxia.

STP has been described as gradual increase of postsynaptic neural responses during a continuous stimulus (hypoxia) followed by an exponential decline after the stimulus ceases (43). The exponential decay is believed to represent decay of the potentiating mechanism. STP was measured from the decay of phrenic burst amplitude after hypoxia and indexed to the difference between hypoxic and baseline burst amplitudes [(posthypoxic amplitude – baseline amplitude)/ (peak hypoxic amplitude – baseline amplitude)], thus providing a ratio from 0 to 1.0. The magnitude of STP was compared between rat strains by using the half-life and time constant for the decay of indexed amplitude.

Before parametric statistical tests were performed, the assumptions of normality of the data and equal variances were confirmed. When these assumptions were not satisfied, nonparametric analyses were performed. Nonparametric analysis consisted of Kruskal-Wallis ANOVAs followed by Mann-Whitney U-tests. All other means were compared by using an ANOVA, with individual comparisons made by using the Student-Neuman-Keuls post hoc test. PHFD was analyzed by using a two-way ANOVA with repeated measures applied to time. Differences were considered significant if P <0.05. All values are expressed as means ± SE.

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RESULTS

Baseline conditions. The CO₂ apneic threshold was not different among BN, FS, and LW rats. Thus baseline PaO₂ values were similar in these strains (Table 1). In contrast, PVG rats had a higher apneic threshold and PaCO₂ than LW rats (Table 1; *P < 0.05). Baseline PaCO₂ was lower in BN and LW rats relative to PVG and FS strains (Table 1; *P < 0.05), but it was never <150 Torr. Mean arterial pressure was lower in LW rats than all other strains (Table 1; *P < 0.05).

Baseline phrenic burst frequency ( fictive respiratory rate ) was higher in PVG rats vs. LW rats (Table 1; *P < 0.05); the other two strains were intermediate but were not significantly different from any other. Baseline phrenic burst amplitude, normalized to the maximal hypercapnic amplitude, was similar in BN, FS, and LW rats (Fig. 1), but it was higher in PVG relative to other strains (Fig. 1A; *P < 0.05). Similar strain effects were observed in phrenic minute activity when it was compared across groups (Fig. 1B; *P < 0.05). Strain differences in baseline respiratory activity were not restricted to spinal (pump) motoneurons, because differences were also noted in hypoglossal motor output. Normalized hypoglossal burst amplitude was higher in BN and PVG rats vs. FS and LW rats (Fig. 1C; *P < 0.05), and similar differences were present in hypoglossal minute activity (Fig. 1D; *P < 0.05).

Hypoxia. Arterial pH and mean arterial blood pressure decreased during hypoxia, and the magnitude of change was similar in all strains (Table 1; *P < 0.05). PaO₂ during hypoxia was similar between strains (Table 1). At the time of blood sampling, fictive respiratory rate was elevated in FS and PVG strains only (Table 1, see Fig. 3A; *P < 0.05). The frequency response during the final minute of hypoxia was higher in FS than BN rats and was elevated in PVG rats relative to all other strains (see Fig. 3A; *P < 0.05). Phrenic and hypoglossal burst amplitude increased during hypoxia in all rats; however, strain differences were observed only in phrenic, and not hypoglossal, amplitude. The change in phrenic amplitude from baseline was lower in PVG vs. all other groups (Figs. 2 and 3, A and B; *P < 0.05). In comparison, there were no differences in the hypoxic response of hypoglossal burst amplitude (Fig. 4). Phrenic and hypoglossal minute activity responses were not different among strains (Figs. 3 and 4).

The normalization method used to characterize phrenic and hypoglossal burst amplitude responses to hypoxia had minimal impact on conclusions regarding strain effects. For example, similar conclusions were reached whether burst amplitudes were expressed as a value normalized to maximal CO₂-stimulated activity or baseline amplitude, or as a nonnormalized (i.e., raw) voltage taken from the integrated neurogram. In addition, the hypercapnic burst amplitude, expressed as a nonnormalized voltage, was not different across strains (P = 0.93; data not shown), suggesting that normalization to this value would not create artifacts due to differences in the maximal hypercapnic output among strains.

PHFD. The posthypoxic period was characterized by decreased burst frequency below prehypoxic baseline values for at least 5 min in FS, LW, and BN rats but not in PVG rats (Fig. 5A; *P < 0.05). This PHFD was largest in FS and LW rats during the first 2 min, and then it was similar in magnitude to that in BN rats for the duration of the observed posthypoxic period (Fig. 5A; *P < 0.05).

STP. STP was assessed in each rat by measuring the decay of phrenic burst amplitude after hypoxia (Fig. 5B). The decay half-life was longest in FS vs. other strains (FS 44 ± 4; BN

| Table 1. Rectal temperature, arterial blood gas, phrenic burst frequency, and mean arterial blood pressure measurements during baseline conditions and hypoxia in four strains of inbred rats |
|-----------------|--------|--------|--------|--------|
|                  | Brown  | Fischer 344 | Lewis  | PVG    |
| Baseline         | nor    | nor    | nor    | nor    |
| Temperature, °C  | 37.2   | 37.3   | 37.4   | 37.7   |
| pH               | 7.37   | 7.39   | 7.37   | 7.38   |
| PaO₂, Torr       | 45    | 43     | 46     | 43     |
| PaCO₂, Torr      | 211   | 271‡   | 225    | 257†   |
| RR, breaths/min  | 44    | 44     | 40     | 47     |
| Mean BP, mmHg    | 88    | 88     | 71     | 91     |
| Hypoxia          | nor   | nor    | nor    | nor    |
| Temperature, °C  | 37.1   | 37.2   | 37.3   | 37.4   |
| pH               | 7.36   | 7.36   | 7.35   | 7.35   |
| PaO₂, Torr       | 46    | 45     | 46     | 42     |
| PaCO₂, Torr      | 41    | 40     | 40     | 38     |
| RR, breaths/min  | 43    | 50     | 43     | 60     |
| Mean BP, mmHg    | 56    | 68     | 42     | 62     |

Values are means ± SE. BP, mean arterial blood pressure; RR, respiratory rate; PaO₂, arterial PO₂; PaCO₂, arterial PCO₂; PVG, Piebald-viral-Glaxo. *Significantly different from Brown Norway, *P < 0.05. †Significantly different from Fischer, *P < 0.05. ‡Significantly different from Lewis, *P < 0.05. §Significantly different from the baseline value, *P < 0.05.

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Augmented breaths. Fictive augmented breaths were observed during hypoxia in FS rats (Fig. 2) but in no other strain. Augmented breaths were identified based on the criteria of Cherniack et al. (6). Inspiration during an augmented breath in spontaneously breathing animals is biphasic, where phase 1 volume is similar to preceding breaths and phase 2 is augmenting. Furthermore, phase 1 and 2 inspiratory time combined is similar to inspiratory time for preceding breaths, and expiratory time is often prolonged after an augmented breath in awake rats. Fictive augmented breaths occurred at a frequency of 2.3 breaths/min and were always followed by prolonged expiratory times (normal breath in hypoxia, expiratory time = 0.86 ± 0.03 s; augmented breaths, expiratory time = 1.04 ± 0.04 s; Fig. 2B; P < 0.05). Phase 1 burst amplitude was similar in magnitude to the preceding hypoxic burst (Fig. 2B), and phase 2 was augmenting (Fig. 2B). Augmented breath inspiratory time (phases 1 and 2 combined) was equivalent to the inspiratory duration for preceding breaths (inspiratory time: hypoxia, 0.40 ± 0.01 s; augmented breath, 0.43 ± 0.01 s; Fig. 2B). Fictive augmented breaths were not observed during baseline conditions or hypercapnia in FS rats, or at any time in other rat strains.

Discussion

Baseline respiratory nerve activity, the STHR, the time course of STP, the magnitude and duration of PHFD, and the frequency of augmented breaths recorded from hypoglossal and phrenic nerves vary among rat strains. Strain effects on the amplitude response to hypoxia were restricted to spinal (i.e., phrenic), and not brain stem (i.e., hypoglossal), respiratory motoneurons. These results suggest that genetics influence the pattern and magnitude of the diaphragmatic response to hypoxia, with less pronounced effects in upper airway muscles, at least in the rat strains chosen for this study.

Interindividual variation in the hypoxic ventilatory response has been recognized in normal humans for 30 yr; however, genetic studies have progressed into animal models because of difficulties controlling for environmental factors in the human population (42). By using inbred rodents from controlled environments, genetic determinants of ventilatory control can be identified more readily. In our study, rats were obtained from the same vendor to reduce variability associated with different rearing practices and environmental conditions, although there is still some possibility that developmental and/or environmental effects contributed to some of the strain differences reported here.

STHR. BN is the only strain in our study previously evaluated by others investigating strain-genetic effects on ventilatory control (13, 20, 37, 38). Strohl et al. (37) compared BN, Sprague-Dalwey, Koletsky, and Zucker rats, and they reported

![Fig. 2. Representative integrated phrenic neurograms before, during, and after 5-min hypoxic episode (A) in 4 rat strains: BN, FS, LW, and PVG. The y-axis scales (in V) are similar between individuals. B: fictive augmented phrenic burst from a FS rat during hypoxia with characteristic biphasic inspiration and fictive postsigh prolongation of expiratory time.](http://jap.physiology.org/)

![Fig. 3. Short-term hypoxic phrenic response expressed as a change in burst frequency (A), integrated amplitude (B), and minute phrenic activity (C) during final minute of a 5-min hypoxic episode in 4 rat strains: BN, FS, LW, and PVG. Changes (Δ) in burst amplitude (B) and minute activity (C) are normalized to baseline values (%Baseline). MIN-1, bursts/min. * Significantly different from BN, P < 0.05. †Significantly different from FS, P < 0.05. ‡Significantly different from, P < 0.05.](http://jap.physiology.org/)
no strain effects on resting minute ventilation. However, BN and Zucker rats utilized a more rapid, shallow breathing pattern relative to the other strains. Strain differences in hypoxic ventilatory responses were also observed; BN rats had a smaller increase in frequency than Sprague-Dawley rats during hypoxia, but the change in tidal volume was similar (37, 38). Although no differences were found in baseline breathing when comparing male BN, Dahl salt-sensitive, Fawn-hooded hypertensive, and Sprague-Dawley rats (20), BN rats again had the smallest change in breathing frequency during early hypoxia compared with the other strains.

In our study using anesthetized, vagotomized, and ventilated rats, the frequency response to hypoxia was also weak in BN rats. Thus the pattern of frequency and amplitude change in the phrenic nerve during hypoxia was strain dependent, even in the absence of lung volume feedback (i.e., after vagotomy). These strain differences in the control of respiratory frequency suggest genetic influences on the supraspinal control of breathing. However, this tentative conclusion must be tempered with the realization that other afferent pathways may underlie differences in breathing frequency.

Hodges et al. (20) reported that BN rats respond to hypoxia with less change in minute ventilation, but that the magnitude of hypoxic hyperventilation was similar to other strains (based on equivalent PaCO2). The authors interpret this as a greater reduction in metabolic rate in BN rats during hypoxia compared with other strains. In our study, where PaCO2 and temperature were adjusted continuously to reduce the impact of changes in metabolic rate, the change in phrenic minute activity from baseline activity also was not different between strains, consistent with their observations and interpretation.

In addition to supraspinal factors, strain effects on phrenic and hypoglossal burst amplitude may occur from genetic variations in motoneuron or premotoneuron properties, or modulatory influences from descending or segmental modulatory inputs. In Sprague-Dawley rats, hypoglossal and phrenic motoneurons receive respiratory drive from different brain stem premotoneurons (30, 31). Thus there is considerable potential for genetic differences to be expressed uniquely in select (pump muscle or upper airway) motoneuron populations. In PVG rats, the hypoxic amplitude response was smaller than in other strains but only in phrenic nerve activity. Thus the genetic variation responsible for this strain effect occurs within a component of the respiratory control network that is unique to phrenic motoneurons and that does not directly influence hypoglossal nerve activity. One factor that must be considered is that the diminished STHR of PVG rats was associated with an increased baseline phrenic nerve activity relative to the maximal hypercapnic phrenic activity (which was not different among strains). Thus the hypoxic response may be capped in this strain because there was less difference between baseline and maximal phrenic burst amplitude in PVG rats.

Caution is needed when comparing unanesthetized respiratory behavior with the pattern of respiratory motor output from anesthetized, vagotomized, and ventilated preparations. Breathing in unanesthetized animals is modulated by a complicated network of feedforward and feedback mechanisms, some of which were removed by denervation or anesthesia in the present study. The specific advantage of our reduced, in
vivo preparation over an unanesthetized animal is that it enables more control of relevant variables (e.g., blood gases); permits isolation of specific, time-dependent mechanisms or neural outputs; decreases the impact of changes in metabolic rate on ventilation; and minimizes the impact of often underappreciated factors in awake animals, such as stress or an uncontrolled response to a novel stimulus. On the other hand, the major limitation is that our data must be regarded carefully because the impact of anesthesia (or other procedures such as vagotomy) on any of the time-dependent hypoxic responses observed is unknown. We recently correlated data collected from a single group of rats for which ventilatory measurements were made when unanesthetized in a barometric plethysmograph, followed by an assessment of neural responses under conditions described in the present study (F. J. Golder and G. S. Mitchell, unpublished data). These data demonstrate that respiratory frequency and tidal volume in the unanesthetized rats highly correlated with phrenic burst frequency and integrated burst amplitude (expressed as a raw voltage) in the same anesthetized rat studied several days later. Thus there appears to be an inherent “personality” for a given rat that is robust in the face of anesthesia, vagotomy, and phrenic nerve recording. These unpublished observations provide reassurance that strain differences reported here for anesthetized rats may be pertinent to the same rat strains when unanesthetized and spontaneously breathing.

**PHFD.** Hypoxia induces PHFD by central neural mechanisms associated with lateral pontine neurons (9, 36). Furthermore, PHFD is modulated by the activation of NMDA (8), serotonin type 2 (23), and α2-adrenergic (2) receptors. Other factors alter the magnitude of PHFD, such as prior exposure to hypoxia; both acute (2) and chronic hypoxia (24) attenuate PHFD. On the other hand, PHFD is exaggerated in phrenicotomized rats, an effect that is eliminated by spontaneous exercise (3). Thus PHFD is an example of respiratory frequency plasticity that is subject to metabolic plasticity.

Strain differences in the incidence and magnitude of ventilatory depression posthypoxia have been reported in unanesthetized rats; specifically, BN rats exhibited greater posthypoxic ventilatory decline than Sprague-Dawley rats (38, 39). Whether the posthypoxic decrease in respiratory frequency in these unanesthetized rats represents true PHFD (as defined in Ref. 33) remains uncertain because hypocapnia attendant to the poikilocapnic hypoxic exposure used in these studies persists into the posthypoxic period and may secondarily depress respiratory frequency. In the present study, we monitored and controlled arterial blood-gas composition during (and after) hypoxia, thereby demonstrating that the observed PHFD was central neural in origin. Thus differential expression of PHFD in FS and LW rats relative to BN rats and the lack of any PHFD in PVG rats are novel observations that confirm differences in the central neural mechanisms that give rise to this time domain of the posthypoxic ventilatory response.

**STP.** Phrenic STP manifests as a progressive increase (on-phase) in phrenic burst amplitude during the onset of hypoxia, and a slow return (off-phase) to its original baseline (33). The on- and off-phases are believed to reflect the same mechanism, although the off-response has a slightly longer time constant (43). The mechanism responsible for STP is still unknown. STP reflects a type of short-term synaptic plasticity that may involve second-order relay neurons of the nucleus tractus solitarii (27) or bulbospinal synaptic inputs to phrenic motoneurons in the spinal cord (26). The magnitude of STP increases with duration of hypoxia (10), but it does not appear affected by carotid chemosensitivity or age (1, 18). Genetic influences on the time course of STP have not been previously reported. Thus ours is the first report of strain (and presumably genetic) differences in the expression of STP posthypoxia. We did not quantitate the on-response in STP because it is more difficult to be certain that the observed kinetic results from STP rather than slow progression of the hypoxic stimulus in our experimental setup.

**Augmented breaths.** Augmented breaths increase in frequency during hypoxia in spontaneously breathing rats (13, 20, 21, 25), and the magnitude of this change is strain dependent (13, 20). Augmented breaths are thought to be airway protective reflexes that require vagal afferent feedback from lower airways (4, 21). Strain differences in the incidence of augmented breaths may originate at pulmonary receptors and/or sites of central neural integration of these afferent inputs. Spontaneous augmented breaths are abolished after vagotomy but can reappear after 2–5 h during hypoxia (25). Thus, although some studies propose that augmented breaths require vagal afferent feedback (4, 21), the studies of Marshall and Metcalfe (25), and the present experiments, suggest that augmented breaths can still be generated in the absence of vagal afferent feedback, at least in some strains. Our results demonstrate a strain (presumably genetic) dependence of this vagally independent expression of augmented breaths because they could only be observed in FS rats.

In addition to biphasic inspiration, augmented breaths are characterized by prolongation of the expiratory duration, [post sigh apneas; Cherniack et al. (6)]. Increased vagal afferent feedback during augmented tidal volume has been suggested to contribute to postsigh apneas (6, 41). However, in spontaneously breathing animals, postsigh apneas may also reflect a transient reduction in PaCO2 after overshoots in ventilation (11). Augmented breaths in our study were always followed by prolongation of the expiratory duration, despite bilateral vagotomy and precise regulation of arterial blood gases. Thus postsigh apneas in FS rats do not require vagal feedback or changes in arterial blood gases, and they are most likely to be of central neural origin. Rodent strain differences have been described in the duration of postsigh apneas, being longer in BN rats than some other strains (13, 20). We were not able to make similar comparisons in this study because only one strain expressed augmented breaths.

**Conclusions.** In summary, rat strain differences exist in multiple aspects of the central neural control of breathing during and after hypoxia, including differences in the baseline ventilatory activity, the STHR, the duration of STP potentiation, the magnitude of PHFD, and an airway protective reflex expressed as augmented breaths. Strain differences were not generalized to both respiratory neural outputs but predominated in phrenic motoneurons innervating the diaphragm (a pump muscle) vs. hypoglossal motoneurons innervating the tongue, an upper airway resistance-regulating muscle. These data provide a more clear understanding of how the choice of rat strain can influence conclusions concerning the control of breathing in rodents. Furthermore, our results strongly indicate that it is critical to carefully choose and then specify rat strain in all studies concerning the neural control of breathing.

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Each time-dependent mechanism of the hypoxic ventilatory response is associated with a unique combination of neurochemicals and their associated receptors (29, 33). Thus the identification of strain and/or genetic differences in specific time-dependent mechanisms may help to guide further studies aimed at the identification of candidate genes contributing to genetic differences in ventilatory control. By identifying genetic influences on the neural control of breathing, it may be possible to gain insights concerning observed interindividual differences in the control of breathing in genetically diverse populations, such as humans.

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