Preinspiratory and inspiratory hypoglossal motor output during hypoxia-induced plasticity in the rat

Kun-Ze Lee and David D. Fuller

Department of Physical Therapy, McKnight Brain Institute, University of Florida, College of Public Health and Health Professions, Gainesville, Florida

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Lee K-Z, Fuller DD. Preinspiratory and inspiratory hypoglossal motor output during hypoxia-induced plasticity in the rat. J Appl Physiol 108: 1187–1198, 2010. First published February 11, 2010; doi:10.1152/japplphysiol.01285.2009.—Respiratory-related discharge in the hypoglossal (XII) nerve is composed of preinspiratory (pre-I) and inspiratory (I) activity. Our first purpose was to test the hypothesis that hypoxia-induced plasticity in XII motor output is differentially expressed in pre-I vs. I XII bursting. Short-term potentiation (STP) of XII motor output was induced in urethane-anesthetized, vagotomized, and ventilated rats by exposure to isocapnian hypoxia (PaO2 of ~35 Torr). Both pre-I and I XII discharge abruptly increased at beginning of hypoxia (i.e., acute hypoxic response), and the relative increase in amplitude was much greater for pre-I (507% baseline) vs. I bursting (257% baseline; P < 0.01). In addition, STP was expressed in I but not pre-I bursting following hypoxia. Specifically, I activity remained elevated following termination of hypoxia but pre-I bursting abruptly returned to prehypoxia levels. Our second purpose was to test the hypothesis that STP of I XII activity results from recruitment of inactive or “silent” XII motoneurons (MNs) vs. rate coding of active MNs. Single fiber recordings were used to classify XII MNs as I, expiratory-inspiratory, or silent based on baseline discharge patterns. STP of I XII activity following hypoxia was associated with increased discharge frequency in active I and silent MNs but not inspiratory-inspiratory MNs. We conclude that the expression of respiratory plasticity is differentially regulated between pre-I and I XII activity. In addition, both recruitment of silent MNs and rate coding of active I MNs contribute to increases in XII motor output following hypoxia.

Address for reprint requests and other correspondence: K.-Z. Lee, Univ. of Florida, College of Public Health and Health Professions, McKnight Brain Institute, Dept. of Physical Therapy PO Box 100154, 100 Newell Dr, Gainesville, FL 32610 (e-mail: kzlee@ufl.edu).

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1187
Table 1. Arterial blood gas parameters during baseline and hypoxia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{aO}_2}$, Torr</td>
<td>233 ± 2</td>
<td>34 ± 1*</td>
</tr>
<tr>
<td>$P_{\text{aCO}_2}$, Torr</td>
<td>30 ± 1</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.01</td>
<td>7.36 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE. $P_{\text{aO}_2}$, arterial partial pressure of O$_2$; $P_{\text{aCO}_2}$, arterial partial pressure CO$_2$. *$P < 0.01$, compared with the baseline value.

expiratory phase. Three such expiratory bursting patterns have been described including exclusively phrenic discharge (E MNs), bursting that initiates during late expiration (E-I MNs), and bursting that begins during inspiration but terminate by mid-expiration (I-E MNs; Ref. 23). Thus pre-I activity observed in the XII nerve results from the activation of E-I MNs that initiate bursting during late expiration (29, 30). Hwang et al. (23) also suggest that as many of 50% of I XII MNs are inactive during eupnea but can be recruited during respiratory challenge such as hypercapnia and/or hypoxia. Thus the XII motor pool represents a diverse population of MNs, many of which are silent during normal breathing.

It is presently unknown if the expression of respiratory plasticity is different between phenotypically classified populations of XII MNs (e.g., I, E-I, etc.; Ref. 29). Moreover, it is unknown if recruitment of previously silent XII MNs provides a mechanism for sustained increases in XII output following respiratory stimulation. Accordingly, the second purpose of our study was to investigate XII MN behavior, including discharge frequency, onset, duration, and total spikes per breath during hypoxia-induced STP of XII output. We hypothesized that the onset of STP would occur in parallel with the recruitment of previously silent XII MNs and that these cells would continue to burst following return to normoxia.

MATERIALS AND METHODS

Animals. These experiments used a total of 27 Sprague-Dawley rats weighing 399 ± 5 g and obtained from Harlan (Indianapolis, IN). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

General animal preparation. Isoflurane anesthesia (3–4%) was introduced in a closed chamber and then maintained at 2–3% via the nose cone. The body temperature was monitored by an electrical thermometer and maintained at 37.5 ± 1°C via a servo-controlled heating pad (model TC-1000, CWE; Ardmore, PA). The trachea was cannulated with PE-240 tubing below the larynx, and the rat was then pump ventilated during the experiment (model 683; Harvard Apparatus; South Natick, MA). A PE-50 catheter was placed into the femoral artery for blood pressure monitoring (Statham P-10EZ pressure transducer, CP122 AC/DC strain gage amplifier; Grass Instruments, West Warwick, RI) and blood sampling for subsequent analysis (i-Stat; Heska, Fort Collins, CO). The arterial partial pressure of O$_2$ ($P_{\text{aO}_2}$), CO$_2$ ($P_{\text{aCO}_2}$), and pH were analyzed from 0.2-ml blood samples (see Experimental Protocol). Arterial blood gases were analyzed in 26 of the 27 rats used in these experiments. The femoral vein was cannulated with PE-50 tubing, and the animal was then gradually converted from isoflurane to urethane anesthesia (1.6 g/kg iv; Sigma, St. Louis, MO) over a period of 30 min. A paralytic drug (pancuronium bromide, 2.5 mg/kg iv; Hospira, Lake Forest, IL) was administrated to prevent spontaneous muscle movement. Bilateral cervical vagotomy was performed to prevent entrainment of respiratory activity with ventilator-induced lung inflation. End-tidal CO$_2$ partial pressure ($P_{\text{ETCO}_2}$) was monitored using a Capnogard neonatal CO$_2$ monitor placed on the expired line of the ventilator circuit (Novametrix Medical Systems, Wallingford, CT).

Nerve recordings. The cut, central end, of one phrenic and XII nerve were recorded as previously described (29, 30, 31, 32, 33). Recordings were made from the main trunk of the XII nerve and accordingly reflect neural drive to the tongue protrudor and retractor muscles (18) and possibly the intrinsic tongue muscles as well (4, 5, 6). In addition, action potentials from individual XII MN axons were recorded from the contralateral XII nerve (29, 30). Briefly, the phrenic nerve was isolated in the cervical region and cut distally. The XII nerve was then exposed bilaterally by removing the digastric muscle and then cut distally. The left XII nerve was then desheathed and separated into thin filaments to enable recording of individual XII MN action potentials (e.g., see Figs. 4, 5, and 9). Single fiber recordings were always done with a monopolar configuration using silver electrodes. Whole nerve recordings were done using either a monopolar or bipolar recording configuration with silver electrodes. Neural signals were amplified (×1,000; Model 1700; A-M Systems, Carlsborg, WA) and band-pass filtered (0.3–10 KHz). The electrical signal from the whole phrenic and XII nerves was integrated to enable quantification of burst amplitude (time constant 100 ms; model MA-1000; CWE.

Fig. 1. Sample phrenic (Phr) and XII neurograms demonstrating the method for calculating the respiratory cycle. Inspiratory duration (T$_i$) was defined as the period between the onset of the phrenic inspiratory burst (vertical dashed line) and the time point when integrated phrenic activity amplitude reduced by 50% of the peak value (vertical solid line; see ref. 31). Onset of the XII burst was identified from the integrated XII trace (vertical dotted line) and then used to calculate the difference between phrenic and XII burst onset time. Amplitudes of the preinspiratory (pre-I) and inspiratory (I) XII bursts were measured as the peak height of the integrated XII signal during expiration and inspiration, respectively. Tonic XII bursting during the hypoxic challenge is indicated by the upward displacement of the integrated XII neurogram just before the pre-I burst onset. Is moving time averaged or “integrated” neurogram.
Ardmore, PA). All data were digitized using a CED Power 1401 data acquisition interface and recorded on a PC using Spike2 software (Cambridge Electronic Design, Cambridge, England).

**Experimental protocol.** Once stable recordings of the whole phrenic and XII nerves had been established during hyperoxic (FIO2 = 0.50–0.60) normocapnic condition, the CO2 apneic threshold for I bursting was determined. The ventilator rate was gradually increased to reduce PETCO2 to a level at which phrenic I bursting ceased for 2 min. The ventilator rate was then gradually reduced until rhythmic I phrenic bursting resumed. The PETCO2 was then maintained at 2–3 Torr above this recruitment threshold during the subsequent baseline recording condition. During baseline conditions, recordings of individual XII MN action potentials were established in the left XII nerve using a “single fiber” approach (23, 24, 29, 30, 31, 33). Once stable recordings were obtained in both whole nerve and single fiber recordings, an arterial blood sample was drawn to establish baseline blood gases values. The animal was then exposed to a 3-min bout of isocapnic hypoxia with an arterial blood sample obtained during the final minute (FiO2 = 0.13–0.15). While PETCO2 is a reliable index of PacO2 during normoxic baseline conditions in this preparation, it is not accurate during hypoxic conditions. Accordingly, we raised the PETCO2 values to 3 Torr above baseline during hypoxia by decreasing ventilator rate, and this resulted in arterial isocapnia (see Table 1). Following hypoxia, rats were returned to baseline FiO2 and PETCO2 was again set at 2–3 Torr above the CO2 recruitment threshold.

**Data analyses.** All data were acquired and analyzed using Spike 2 software as previously described (33). Figure 1 depicts how pre-I and I XII activity and TI and TE were measured from the integrated phrenic and XII neurograms. Briefly, TI was defined as the period between I phrenic onset and the time point when phrenic amplitude reduced by 50% of the peak value, and TE was defined as periods between end of inspiration and phrenic onset of the subsequent breath (Fig. 1). As illustrated by Fig. 1, using the point where the phrenic signal had declined by 50% of the peak value prevented the inclusion of post-I activity in the calculation (33). Pre-I and I XII activity were defined as the peak height of XII signals during TE and TI, respectively (Fig. 1). The signals were expressed as a percentage of baseline both during and following hypoxia. Hypoxia-induced changes in nerve activity were expressed relative to the percentage of the maximum value during hypoxia (%max). The onset of phrenic and XII bursting was considered to be the time point when the signals rose above the baseline as assessed during TE (see Fig. 1).

The XII MNs were classified as E-I, I, and post-I according to their discharge pattern relative to the respiratory cycle (23, 29, 30). Silent

| Table 2. Cardiovascular responses during baseline, hypoxia, and 3 min posthypoxia |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Baseline | Onset | Mid | End | 3 min |
| MAP, mmHg      | 115 ± 3   | 113 ± 3 | 113 ± 5  | 104 ± 4† | 119 ± 3         |
| HR, beats/min  | 448 ± 5   | 459 ± 7* | 480 ± 10† | 473 ± 7† | 453 ± 4         |
| Values are means ± SE. MAP, mean arterial blood pressure; HR, heart rate; Onset, Mid, and End, initial, middle, and last 30 s of the hypoxic episode; 3 min, 3 min following cessation of hypoxia. *P < 0.05, †P < 0.01, compared with the baseline value. |

Fig. 2. Phrenic and XII neurogram activity during and following hypoxia. Peak height of the integrated (∫) phrenic and XII burst and the pre-I XII burst are expressed relative to baseline activity (%baseline) in A. In B, the change (Δ) in amplitude is expressed relative to the maximum activity (Δ %max). Hypoxia-induced changes in nerve activity were expressed relative to the percentage of the maximum value during hypoxia (%max). The onset of phrenic and XII bursting was considered to be the time point when the signals rose above the baseline as assessed during TE (see Fig. 1).

The XII MNs were classified as E-I, I, and post-I according to their discharge pattern relative to the respiratory cycle (23, 29, 30). Silent
XII MNs were defined as neurons that were inactive during baseline conditions but could be recruited during hypoxia. The mean discharge frequency of the XII MNs was calculated as the total number of spikes divided by total burst duration (i.e., the period between the first and last spike within each neural breath). I and E discharge burst frequencies were calculated by dividing the spike number by the burst duration during $T_I$ and $T_E$, respectively. The difference between discharge onset of the XII MN and phrenic burst was analyzed to determine whether E discharge of XII MN was influenced by hypoxia. For example, when the value of XII MN onset is changed from “positive” to “negative” during hypoxia, this represents the transformation of an I to E-I discharge pattern during hypoxia (see Table 4).

All data were averaged over a 30 s period immediately before the hypoxic treatment (baseline); during first, middle, and last 30 s of hypoxia (0–30, 75–105, and 150–180 s following onset of hypoxia, respectively); and at 3 min posthypoxia. One-way repeated measures ANOVA was used to compare respiratory pattern (i.e., $T_I$, $T_E$, and respiratory frequency), cardiovascular responses (i.e., blood pressure and heart rate), and XII discharge onset across time points (i.e., baseline, onset, mid, and end of hypoxia, 3 min posthypoxia). Comparisons of nerve activity and XII MN firing behavior were done by using two-way repeated measurement ANOVA [factor one = nerve activity (phrenic, pre-I XII, and I XI) or XII MN type (E-I, I, or silent), factor two = time point] followed by the Student-Newman-Keuls post hoc test. A paired $t$-test was used to compare blood gas parameters during baseline and hypoxia. Data are expressed as the means ± SE. Statistical significant difference was considered when $P < 0.05$.

RESULTS

Arterial blood gases and cardiovascular responses. $P_{O_2}$ was reduced during hypoxia as expected (Table 1). $P_{CO_2}$ was similar between baseline and hypoxic conditions indicating an isocapnic hypoxic challenge (Table 1). The arterial pH was slightly reduced during hypoxia ($P < 0.01$; Table 1) consistent with prior reports (25, 33). Mean arterial blood pressure decreased during hypoxia ($P < 0.01$; Table 2) but returned to baseline values by 3 min posthypoxia ($P > 0.05$; Table 2). Heart rate increased during hypoxia ($P < 0.05$; Table 2) but returned to baseline values at 3 min posthypoxia ($P = 0.43$; Table 2).

XII and phrenic nerve activity. Pre-I activity was observed in recordings from the main trunk of the XII nerve during baseline conditions as expected (Fig. 1). Accordingly, the XII burst onset preceded the phrenic burst by 278 ± 34 ms at baseline. During the initial phase of hypoxia, the onset difference between the XII and phrenic burst was reduced to 173 ± 10 ms ($P < 0.01$ vs. baseline). However, the XII-phrenic onset difference returned to baseline values by 3 min posthypoxia ($P = 0.43$; Table 2).

Hypoxia caused differential changes in pre-I compared with I XII burst amplitude. Specifically, hypoxia-induced increases in pre-I activity (507 ± 46% baseline) assessed at the end of hypoxia were considerably greater than for I XII activity (257 ± 16% BL; $P < 0.01$; Fig. 2A). However, both the pre-I and I XII responses were greater than the I phrenic hypoxic response (186 ± 7% baseline; $P < 0.01$; Fig. 2A). Qualitatively similar differences between amplitude of XII and phrenic neurograms were observed when burst amplitude was expressed as %max (Fig. 2B).

Posthypoxia STP (i.e., the “offset” phase) was assessed 3 min after return to baseline conditions. The amplitude of the pre-I I XII burst rapidly diminished after hypoxia and by 3 min was not significantly different than the baseline (116 ± 8% baseline; $P = 0.49$; Fig. 2A). In contrast, STP was evident in both I phrenic (138 ± 5% baseline) and II XII activity (159 ± 9% baseline) at 3 min posthypoxia (both $P < 0.05$ vs. baseline). These differences were even more robust when the change in burst amplitude was normalized as %max (Fig. 2B).

Indeed, differences between posthypoxia and baseline activity were 3 ± 1%max for pre-I I XII activity compared with 21 ± 2 and 19 ± 2%max for I I XII and I phrenic activity, respectively (both $P < 0.01$ vs. pre-I). Accordingly, posthypoxia STP was robust in I phrenic and I I XII activity but was minimal or absent in pre-I I XII bursting.

Hypoxia also evoked an upward shift in the I XII amplitude recorded just before pre-I onset (see Fig. 1). This upward shift indicates that tonic and/or E XII output was evoked by hypoxia. However, our single fiber recordings (see below) did not indicate the presence of tonic activity during hypoxia; accordingly, the upward shift of the XII baseline likely reflects excitation and/or prolongation of E XII activity. After termi-

Table 3. Respiratory pattern during baseline, hypoxia, and 3 min posthypoxia

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Onset</th>
<th>Mid</th>
<th>End</th>
<th>3 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_I$, s</td>
<td>0.39 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>$T_E$, s</td>
<td>1.09 ± 0.05</td>
<td>0.52 ± 0.02</td>
<td>0.91 ± 0.04</td>
<td>0.96 ± 0.04</td>
<td>1.44 ± 0.07</td>
</tr>
<tr>
<td>Frequency, burst/min</td>
<td>42 ± 1</td>
<td>72 ± 1</td>
<td>54 ± 2</td>
<td>51 ± 2</td>
<td>35 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. $T_I$, inspiratory duration; $T_E$, expiratory duration. *$P < 0.05$, †$P < 0.01$, compared with the baseline value.

Fig. 3. Distribution of XII motoneurons (MN) discharge onset relative to the respiratory cycle. XII MN discharge onset time was expressed as percentage of inspiratory ($T_I$) or expiratory ($T_E$) duration as measured from the phrenic neurogram (see Fig. 1). Onset time of inspiratory-inspiratory (E-I, white) and inspiratory (I, gray) XII MNs was assessed during the baseline condition. Recruited, previously silent XII MNs (S, dark grey) were inactive during baseline and thus their onset time was assessed during the first 30 s of hypoxia.
nation of hypoxia, the E/fXII amplitude recovered to the original baseline level.

Changes in the respiratory pattern during hypoxia are summarized in Table 3. Briefly, T₁ was gradually reduced during hypoxia, and remained below baseline following hypoxia (P < 0.01). Tₑ was initially reduced at onset of hypoxia (P < 0.01) and then returned toward the baseline value. However, Tₑ was significantly elongated at 3 min posthypoxia (P < 0.01), resulting in posthypoxia frequency decline as previously described (33, 48).

XII MN discharge patterns during baseline. The distribution of XII MN discharge onset relative to the onset of the phrenic burst is depicted in Fig. 3, and representative examples of bursting are provided in Figs. 4 and 5. Those XII MNs that showed rhythmic bursting at baseline and were classified as either I (n = 17; Fig. 4) or E-I (n = 8; Fig. 5). The I MNs burst exclusively during T₁ (Fig. 4B), whereas E-I MNs began bursting before phrenic burst onset (i.e., during Tₑ) and ceased firing at end of inspiration (Fig. 5B). E-I XII MNs had different baseline burst frequencies during Tₑ (34 ± 5 Hz) vs. T₁ (59 ± 4 Hz; P = 0.004; Fig. 6, A and B). In addition, the discharge frequency of E-I cells during T₁ was greater than was observed for purely I XII MNs (37 ± 5 Hz; P < 0.01, Fig. 6A). The total number of spikes per respiratory cycle and the overall discharge duration (i.e., spanning T₁ and Tₑ) were greater in E-I vs. I XII MNs (P < 0.01; Figs. 7C and 8C).

XII MN discharge frequency during and following hypoxia. Hypoxia caused an abrupt increase in I MN discharge frequency during the T₁ phase (Fig. 6A). The burst frequency of I MNs continued to increase throughout the hypoxic exposure suggesting the onset of STP (Fig. 6). In addition, hypoxia also caused I MNs to burst during the Tₑ phase (Figs. 4B and 6B).
In contrast to the E-I response (see above), a robust offset STP of I MN burst frequency was present at 3 min posthypoxia (Fig. 4B). This response was only present during the TI phase as I MN bursting during TE did not persist following hypoxia (Figs. 4B and 6B).

During the onset of hypoxia (i.e., the acute response), E-I burst frequency was not significantly changed during TI or TE ($P > 0.05$; Figs. 6, A and B). However, the overall discharge frequency (i.e., including both $T_I + T_E$) of E-I MNs was significantly increased after 1.5 min of hypoxia suggesting the onset of STP (Fig. 6C). E-I MN burst frequency returned to baseline values by 3 min posthypoxia as assessed during both TI and TE (i.e., no offset STP; Fig. 6, A and B).

A population of previously inactive or “silent” MNs were observed as separate and clearly distinguishable spikes during hypoxia. These recordings were obtained in preparations that were already set up to record a XII neuron that was active under baseline conditions. The majority of silent MNs (14/16) were initially recruited during the $T_I$ phase at the onset of hypoxia. However, as hypoxia progressed, these silent MNs began to show an earlier onset time (Table 4) and thus began to initiate bursting during the $T_E$ phase (Fig. 9B). Silent XII MNs also showed a robust STP as reflected by persistent bursting during the posthypoxia period (Figs. 6 and 9). However, posthypoxia STP of silent MNs burst frequency was only observed during TI. Indeed, TE bursting in these cells promptly ceased upon return to normoxia (Figs. 6 and 9).

**XII MN spikes per breath and burst duration during and following hypoxia.** Burst frequency analyses (e.g., Fig. 6) does not fully characterize XII MN activity because the overall duration of bursting within each neural breath changed during and following hypoxia. Accordingly, we also quantified the total number of action potential “spikes” occurring during each breath (Fig. 7) as well as MN burst duration (i.e., the time from the 1st to last spike; Fig. 8).

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Fig. 5. Representative example of phrenic and XII neurograms (whole nerve recordings) and E-I XII MN bursting (single fiber recording) before, during, and after hypoxia. Figure labels and orientation are the same as in Fig. 4. Note that the E-I MN begins bursting during the late E or pre-I period during baseline (Bo), and this pattern is maintained both during (Bb–c) and following hypoxia (Bd).
The total number of I XII MN spikes per breath showed a robust increase during hypoxia (Fig. 7C). Further analyses of E-I spikes relative to inspiration and expiration revealed that hypoxia caused a reduction in spike number during TI ($P < 0.01$; Fig. 7A) but increased spike number during TE ($P < 0.01$; Fig. 7B). The overall E-I spike duration was not changed by hypoxia (Fig. 8C). However, closer analyses revealed that hypoxia reduced E-I duration during TI ($P < 0.01$; Fig. 8A) and tended to increase duration during TE (Fig. 8C).

The total number of spikes from recruited silent MNs increased during hypoxia and remained elevated during the posthypoxia s of hypoxia but was elevated by 1.5 min (i.e., “mid-hypoxia”; Fig. 7C). Further analyses of E-I spikes relative to inspiration and expiration revealed that hypoxia caused a reduction in spike number during TI ($P < 0.01$; Fig. 7A) but increased spike number during TE ($P < 0.01$; Fig. 7B). The overall E-I spike duration was not changed by hypoxia (Fig. 8C). However, closer analyses revealed that hypoxia reduced E-I duration during TI ($P < 0.01$; Fig. 8A) and tended to increase duration during TE (Fig. 8C).

The total number of I XII MN spikes per breath showed a robust increase during hypoxia (Fig. 7C). Further analyses of spike numbers relative to the respiratory cycle revealed progressive increases in spikes during both TI (Fig. 7A) and TE (Fig. 7B). However, as expected from the MN frequency data (Fig. 6), following hypoxia spike numbers were elevated only during TI (Fig. 7A). The total discharge duration of I MNs increased during hypoxia (Fig. 8C) as a result of an increase in TE but not TI discharge duration (Fig. 8, A and B).

Contrary to I XII MN spike number, the total number of E-I spikes per breath was not changed during the initial 30
The hypothesis that hypoxia differentially influences XII MN bursting during TI vs. TE. To better illustrate this concept, we expressed XII MN burst frequency relative to the maximum observed frequency (i.e., %max) and then plotted the normalized frequency over the course of experimental protocol (Fig. 10). This analysis indicates that hypoxia induces a greater enhancement in E vs. I discharge frequency of E-I (\( P < 0.05 \); Fig. 10A) and I MNs (\( P < 0.01 \); Fig. 10B).

**DISCUSSION**

There are two primary findings from the present study. First, pre-I and I XII motor output show differential responses both during and following respiratory stimulation with hypoxia. More specifically, the relative increase in pre-I bursting during hypoxia is far greater than is observed for I XII output, and conversely, the posthypoxia “offset” phase of STP is robust for I activity but absent for pre-I XII activity. These findings are consistent with the hypothesis that pre-I and I motor outputs are regulated separately by the central nervous system (1, 26, 45, 52). Second, XII MN recruitment and discharge patterns differ between the onset (i.e., during hypoxia) and offset phases of hypoxia-induced STP. During hypoxia, the I, E-I, and previously silent XII MNs show an expected and progressive increase in discharge frequency. In addition, I MNs show an earlier onset during hypoxia thereby contributing to the onset of pre-I STP. Following hypoxia, E-I MNs quickly resume baseline bursting patterns, whereas both I and silent MNs show persistent increases in burst frequency.

**I XII and tonic XII activity.** We observed that XII motor output during baseline conditions was associated with activation of XII MNs with both E-I and I bursting patterns as previously described (23, 29, 30). However, tonic XII MN discharge was not apparent during the baseline condition in our preparation. In contrast, both tonic and phasic XII motor output (assessed via genioglossus muscle electromyogram activity) can be observed in awake rats during spontaneous breathing (57). Similarly, both tonic and phasic genioglossus electromyogram signals can be recorded in awake humans (7, 61). Recent data (7) show that the relative degree of tonic genioglossus motor unit activity is highly state dependent. In fact, Bailey et al. (7) suggest that GG motor units should not be designated as tonic vs. phasic, since most, if not all, of the motor units they studied exhibited both behaviors depending on the state (e.g., waking vs. non-rapid eye movement sleep). Accordingly, the lack of tonic XII discharge at baseline in the present study may reflect a general effect of urethane anesthesia on respiratory motor output. Tonic XII activity also appears to be much more prevalent under vagal-intact conditions (7, 57). Accordingly, vagal afferent feedback associated with activation of slow adapting receptors in the lung inhibits phasic I XII discharge (23, 30) but may facilitate tonic XII activity.

**Table 4. Discharge onset of XII motoneurons during baseline, hypoxia, and 3 min posthypoxia**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Onset</th>
<th>Mid</th>
<th>End</th>
<th>3 min</th>
</tr>
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<tbody>
<tr>
<td>E-I</td>
<td>-221 ± 64</td>
<td>-213 ± 58</td>
<td>-286 ± 22</td>
<td>-294 ± 25</td>
<td>-267 ± 67</td>
</tr>
<tr>
<td>I</td>
<td>41 ± 6</td>
<td>-14 ± 13</td>
<td>-202 ± 34*</td>
<td>-213 ± 38*</td>
<td>1 ± 28</td>
</tr>
<tr>
<td>S</td>
<td>—</td>
<td>48 ± 7</td>
<td>-114 ± 33</td>
<td>-118 ± 36</td>
<td>93 ± 20</td>
</tr>
</tbody>
</table>

Values are means ± SE. E-I, expiratory-inspiratory; I, inspiratory; S, silent. *\( P < 0.01 \), compared with the baseline value.
During respiratory stimulation with hypoxia, I XII burst amplitude showed a robust increase that was associated with the recruitment of silent XII MNs and increased burst frequency of previously activity I cells. In contrast, E-I MNs showed little change in burst frequency during the I phase, suggesting that these cells do not contribute to the I portion of the XII response to hypoxia. Consistent with this suggestion, the total number of E-I action potential spikes per breath actually decreased during hypoxia.

The overall XII I response to hypoxia was considerably greater than the phrenic response as previously reported in anesthetized rats (3, 19). The relatively greater XII response could be due to several factors. First, XII MNs or premotor neurons (46) may be more sensitive to inputs from carotid chemoreceptors and/or baroreceptors (9). For example, Salamone et al. (53) observed that decreases in arterial blood pressure have a greater impact on XII compared with phrenic respiratory motor output. Another possibility is that the XII motor pool contains a relatively greater number of silent MNs, which can be recruited during respiratory stress. Similar to the earlier observations of Hwang et al. (23), we found that ~40% of XII MNs were inactive during baseline conditions. After they were recruited during hypoxia, these previously silent XII MNs showed greater and more persistent enhancement of discharge frequency compared with responses observed in silent, recruited phrenic MNs (33).

Pre-I XII activity. During baseline conditions, the pre-I discharge observed in XII nerve recordings (Fig. 1) reflects activation of E-I MNs. Similarly, during the initial (acute) phase of the hypoxic response, E-I MNs continue to be the dominant source of pre-I XII bursting although I MN bursting begins to contribute as their bursting starts to occur slightly before the phrenic burst (Fig. 4). As hypoxia progressed, I MNs began to burst even earlier (i.e., the cells adopted an E-I burst pattern) and a population of recruited, silent XII MNs also began bursting during the pre-I period. Accordingly, during sustained hypoxia, the progressive increase in the pre-I XII bursting reflects activation of all three XII MN types that we were able to identify in this study (i.e., I, E-I, and silent).

**Fig. 9.** Representative example depicting recruitment of a previously silent XII MN during hypoxia, and the persistent bursting of this neuron following hypoxia. Labels and orientation are the same as in Figs. 4 and 5. Note that the MN (XII MN) initially begins to burst with an I pattern (Ba) but then adopts an E-I pattern (Bc). The previously silent neuron continues to burst upon return to normoxia (Bd).
recruitment patterns associated with XII vs. phrenic STP following hypoxia and thus did not contribute to posthypoxia STP (33). Phrenic MNs promptly ceased bursting upon termination of response. However, the majority (90%) of recruited silent MNs is recruited recently observed that a population of previously silent phrenic MNs represents an important component of the onset of phrenic STP response. However, the majority (90%) of recruited silent phrenic MNs promptly ceased bursting upon termination of hypoxia and thus did not contribute to posthypoxia STP (33). Thus there appear to be fundamental differences in the MN recruitment patterns associated with XII vs. phrenic STP following hypoxia. This observation is consistent with the hypothesis that there are distinct I inputs to phrenic vs. XII MNs (32, see below).

Regulation of pre-I vs. XII activity. A considerable body of evidence indicates that pre-I and I XII discharge can be differentially modulated by respiratory-related stimuli including lung volume feedback (11, 30, 52) and pulmonary C-fiber activation (29, 32). For example, Lee et al. (30) showed E discharge of E-I XII MN could be uncoupled from the phrenic burst under higher PEEP condition (9 and 12 cmH2O). In contrast, the firing of I XII MNs remained synchronized with the phrenic nerve discharge under similar conditions. In addition, activation of central μ-opiate receptors and orexin B receptors can also cause different responses in pre-I compared with I XII activity (10, 26). Our present results confirm and extend these previous studies by reporting that hypoxia-induced short-term plasticity is differentially expressed in pre-I vs. I XII activity.

The mechanisms underlying the differential control of pre-I vs. I bursting are not precisely known (11, 52). This may partly reflect a heterogeneous distribution of XII MN excitability. For example, the earlier onset and greater discharge frequency of E-I vs. I XII MNs may indicate that that the former cells have a higher excitability (i.e., lower rheobase current). The designation of XII MNs as either I or E-I in our study was based on their bursting patterns as previously reported (23, 29, 30). However, recordings of GG motor units in humans suggest that XII MN firing is flexible and that discharge in a particular phase (or phases) of the respiratory cycle can vary as a function of the level of respiratory drive (7). Similarly, we also observed that XII MN discharge patterns were not fixed. For instance, I XII MNs changed to an E-I MN discharge pattern during hypoxia, and silent XII MNs could be recruited with either I or E-I discharge patterns. In this regard, the XII MN classification scheme of I vs. E-I could be viewed as being arbitrary. However, this method enabled us to examine our fundamental hypothesis that STP of XII motor output is differentially expressed between pre-I vs. I XII discharge.

Another possibility that could explain the differential control of pre-I vs. I bursting is that E-I XII MNs may receive a pre-I-specific synaptic input during the late E phase from neurons in the pons. Indeed, pre-I activity can be eliminated by surgical removal of pontine inputs to respiratory motoneurons (1, 55). In addition, Ezure and Tanaka (12) demonstrated that E-I neurons within the pontine Kölliker-Fuse (KF) nucleus have direct projections to the hypoglossal motor nucleus and that the discharge patterns of E-I KF neurons are similar to E-I XII MNs. Finally, activation of receptors for the neuropeptide orexin within the KF nucleus can specifically modulate the duration of pre-I XII activity. Following orexin microinjection, I duration (T1) was unchanged, but the pre-I duration was significantly increased (10).

The retrotrapezoid nucleus/parafacial respiratory group is another possible locus of cells driving pre-I XII activity (44). These cells have been suggested to represent an "expiratory rhythm generator" (44) with I rhythm originating from the well-documented medullary pre-Bötzinger complex (26). Mellen et al. (39) found that an opioid receptor agonist specifically inhibited pre-Bötzinger I neurons but not pre-I neurons in or near the retrotrapezoid nucleus/parafacial respiratory group. In addition, Janczewski and Feldman (26) further demonstrated I but not pre-I/E
activity of the genioglossus is inhibited by opioid agonists in the juvenile rat. These observations indicate that pre-I and I XII activity might be driven by the E and I rhythm generator, respectively. The suggestion that E-I XII MNs receive distinct inputs during expiration and inspiration is also supported by our observations of the differential E and I firing behaviors of XII MNs (Figs. 6–8, 10).

**Possible mechanisms associated with STP.** STP probably reflects a central neural mechanism because it can be evoked by electrical stimulation of the carotid sinus nerve (59). There is evidence that brainstem “integration” of afferent signals may be enhanced during STP (40). Specifically, carotid sinus nerve stimulation can enhance the response of neurons within the NTS to subsequent activation (40). Electrical stimulation of the spinal cord can also evoke STP of phrenic motor output in spinalized rats (22, 38) and cervical respiratory output in turtles (27). Thus STP may reflect a combination of enhanced central integration of peripheral afferent inputs and changes in the inputs to and/or intrinsic properties of spinal (or medullary) motoneurons and/or interneurons.

It is unclear if the induction (onset) vs. recovery (decline) phases of respiratory STP reflect distinct mechanisms (see Refs. 19, 33). The posthypoxia decrease of respiratory output could reflect a time-dependent “decay” of the mechanisms that induced STP (48). Alternatively, unique mechanisms may contribute to each phase since STP onset and decline typically follow a different time course (59). A recent study from our laboratory (33) is consistent with the idea that the onset and offset of phrenic STP reflect distinct mechanisms. We observed that previously silent phrenic MNs (i.e., recruited cells not active before hypoxia) are active during the onset of phrenic STP but do not appear to make a significant contribution to the posthypoxia phase of phrenic STP. Accordingly, the onset and offset phases of respiratory STP are associated with distinct MN recruitment strategies (33). The present data also support this concept by the showing onset of XII STP can be observed in both I and pre-I bursting, but the offset phase was only observed in I activity.

There have been relatively few studies of the cellular/molecular mechanisms associated with STP. Poon et al. (47) showed that systemic blockade of NMDA receptors with MK-801 dramatically alters the time constant associated with induction and recovery of STP in rats. They suggested that the relevant neural circuitry included both the respiratory central pattern generator and motoneurons (47). Nitric oxide also appears to play a role in STP as demonstrated by experiments in knockout mice deficient in nitric oxide synthase (28). Similarly, a nitric oxide synthase-I inhibitor can abolish STP in wild-type mice (28).

**Physiological significance.** Our results indicate that STP is differentially expressed in pre-I vs. I XII activity, suggesting that the determinants of neural plasticity in respiratory control circuits may be distinct across respiratory phases (i.e., I vs. E). Since the generation of the typical three-phase eupneic respiratory rhythm is dependent on pontine and medullary respiratory networks (50), it will be valuable to examine neuromodulation of pontine respiratory activity in future studies. Our data also indicate that posthypoxia STP is evoked primarily in I and silent XII MNs but not E-I XII MNs. This finding implies that expression of plasticity is not a general phenomenon in all respiratory MNs and probably depends on both intrinsic membrane properties and extrinsic synaptic inputs.

While the functional significance of hypoxia-induced XII STP is not precisely known, it may serve to enhance the stability of breathing during and following respiratory stimulation (19, 48). For example, increased I activity in the extrinsic and possibly intrinsic tongue muscles (4, 6) may minimize upper airway narrowing when greater transmural pressures tend to collapse the airways during or following hypoxia. Pre-I activity of XII nerves may also play a role in dilating and/or stiffening the upper airway before I airflow enters the airways (16, 43). In addition, pre-I XII activity increases in parallel with abdominal late-E discharge and therefore may reduce the upper airway resistance during active expiration as occurs during and following hypoxia (1). However, we observed that pre-I XII STP was not present after hypoxia. Accordingly, reduced E airflow after hypoxia may prolong the period for gas exchange in the lung upon reexposure to normoxic gas. In other words, the absence of pre-I XII STP following hypoxia may prevent excessive E airflow and thus maintain gas exchange efficiency and suitable end-expired lung volumes.

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**DISCLOSURES**

No conflicts of interest are declared by the author(s).

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