Uncoupling of upper airway motor activity from phrenic bursting by positive end-expired pressure in the rat

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Lee K-Z, Fuller DD, Tung LC, Lu I-J, Ku L-C, Hwang J-C. Uncoupling of upper airway motor activity from phrenic bursting by positive end-expired pressure in the rat. J Appl Physiol 102: 878–889, 2007. First published November 2, 2006; doi:10.1152/japplphysiol.00934.2006.—Phasic bursting in the hypoglossal nerve can be uncoupled from phrenic bursting by application of positive end-expired pressure (PEEP). We wished to determine whether similar uncoupling can also be induced in other respiratory-modulated upper airway (UAW) motor outputs. Discharge of the facial, hypoglossal, superior laryngeal, recurrent laryngeal, and phrenic nerves was recorded in anesthetized, ventilated rats during stepwise changes in PEEP with a normocapnic, hyperoxic background. Application of 3- to 6-cmH2O PEEP caused the onset inspiratory (I) UAW nerve bursting to precede the phrenic burst but did not uncouple bursting. In contrast, application of 9- to 12-cmH2O PEEP uncoupled UAW neurograms such that rhythmic bursting occurred during periods of phrenic quiescence. Single-fiber recording experiments were conducted to determine whether a specific population of UAW motoneurons is recruited during uncoupled bursting. The data indicate that expiratory-inspiratory (EI) motoneurons remained active, while I motoneurons did not fire during uncoupled UAW bursting. Finally, we examined the relationship between motoneuron discharge rate and PEEP during coupled UAW and phrenic bursting. EI discharge rate was linearly related to PEEP during prespiration, but showed no relationship to PEEP during inspiration. Our results demonstrate that multiple UAW motor outputs can be uncoupled from phrenic bursting, and this response is associated with bursting of EI nerve fibers. The relationship between PEEP and EI motoneuron discharge rate differs during prespiratory and I periods; this may indicate that bursting during these phases of the respiratory cycle is controlled by distinct neuronal outputs.

Uncoupled activity; motoneurons; expiratory-inspiratory; prespiratory

The mammalian upper airway (UAW) consists of the airflow passages extending from the trachea to the external nares and is thus composed of the nasal cavity, pharynx, and larynx (34). The compliance and geometry of this region are influenced by a number of skeletal muscles. Among them are the alae nasi, tongue, and laryngeal muscles, which are innervated by the facial (FN), hypoglossal (HN) and superior (SLN), and recurrent laryngeal nerves (RLN), respectively (1, 26, 41).

Many studies have shown that the inspiratory (I) discharge of the HN precedes the phrenic I burst (5, 6, 13, 17, 23, 33). This prespiratory (Pre-I) activity has been suggested to ben-
phrenic bursting is a common property of UAW motor pools that normally show respiratory-related activity. Hence, our first aim was to determine whether phasic bursting in the FN, SLN, and RLN would continue or uncouple during periods of phrenic quiescence induced with PEEP.

UAW motoneurons have been classified as Pre-I, I, inspiratory-expiratory (IE), expiratory-inspiratory (EI), or tonic based on the time of their discharge onset and termination relative to the phrenic burst (14, 15). However, the behavior of these subsets of UAW motoneurons during PEEP-induced uncoupling is not known. Accordingly, our second aim was to thoroughly characterize the discharge patterns of individual UAW motoneurons (relative to phrenic bursting) during application of PEEP. We hypothesized that the uncoupled activity recorded in UAW motor nerves would reflect activation of a particular subset of respiratory-related neurons during the periods of PEEP-induced phrenic quiescence.

**MATERIALS AND METHODS**

**Animal preparation.** Male Wistar rats obtained from the Animal Center of the National Taiwan University were used in the present study. Fifty-two animals (386.2 ± 6.3 g) were used: 27 for studies involving only neurogram recording, and an additional 25 for motoneuron recordings. All procedures were approved by the Animal Care and Use Committee of the National Taiwan Normal University.

Rats were initially treated with atropine (0.5 mg/kg im, Sigma, St. Louis, MO) and 30 min later were anesthetized with urethane (1.2 g/kg ip, Sigma). An adequate level of anesthesia was confirmed by a lack of withdrawal reflex during toe pinch. After paralysis (see below), the adequacy of anesthesia was assessed by monitoring blood pressure, heart rate, and phrenic nerve bursting during the same stimulus. Rectal temperature was monitored by electrical thermometer and maintained at 36–37°C with a heating pad. The trachea was intubated, and the femoral artery and vein were catheterized for measurement of blood pressure and drug administration, respectively. The rat was artificially ventilated (volume = 10 ml/kg; frequency = 60–70 strokes/min) and paralyzed with gallamine triethiodide (5 mg/kg iv, Sigma). PEEP was applied by inserting a short length of tubing from the outlet port on the ventilator into a beaker of water. The amount of PEEP varied from 0 to 15 cmH2O, depending on the particular phase of the experimental protocol (see below). The tracheal pressure was sampled in the tracheostomy tube. End-tidal fraction concentration of CO2 was analyzed with a CO2 analyzer (Electrochemistry CD3A, Ametek, Pittsburgh, PA) and kept at 4–5% by adjusting the ventilator volume and frequency as necessary.

**Nerve recording.** Nerves were isolated for neurogram recording as follows. The phrenic nerve was isolated with a ventral approach at the C4-C5 spinal level, as described previously (23). The HN was exposed by a ventral approach and cut proximal to the bifurcation of this nerve into distinct medial and lateral branches (13, 23). The SLN was isolated near the larynx and cut distally (25). The FN was dissected with a lateral approach similar to that in our previous report (16) and

![Fig. 1. Method for calculating the percentage of uncoupled bursts occurring in uncoupled upper airway (UAW) motor nerves. In this example, during the period of positive end-expired pressure (PEEP) application, the hypoglossal nerve (HN) shows 8 rhythmic bursts, whereas the phrenic nerve (PN) shows 5. Thus the percentage of uncoupled bursts was quantified as 3/8 or 37.5%. HNA, HN activity; PNA, PN activity; Int., “integrated” nerve activity; TP, tracheal pressure.](http://jap.physiology.org/)

**Fig. 2.** Representative examples of facial nerve (FN), HN, superior laryngeal nerve (SLN), and PN discharge during varying levels of PEEP. PEEP was maintained at 0 (A), 3 (B), 6 (C), 9 (D), 12 (E), and 15 cmH2O (F). The onset of the UAW motor nerve discharge precedes the phrenic burst by a progressively longer duration as PEEP is increased from 0- to 6-cmH2O PEEP (small arrows in A–C). The rhythm of UAW motor nerve discharge was always coupled with phrenic bursting at PEEP values between 0 and 6 cmH2O. However, UAW and phrenic rhythms were uncoupled when PEEP was maintained between 9 and 12 cmH2O (triangles in D and E). An extra uncoupled burst was observed in FN activity (FNA) (E, marked with downward triangle). Bursting of both UAW and PN was totally inhibited when PEEP was set at 15 cmH2O (F). SLNA, SLN activity.
The RLN was isolated at the cervical level, as previously described (25). To enable recording of individual motoneuron action potentials, efferent thin filaments of the FN, HN, SLN, and RLN were dissected with a no. 5 forceps and surgical scissors under the aid of surgical microscope (Wild) in the same manner as in our previous studies (14–16).

Neurograms were recorded via a bipolar electrode connected to an AC preamplifier (Grass, P511, Quincy, MA) and filtered at 0.3–3 kHz. The amplified signal was then integrated (time constant $/H_{11005}/H_{11005}$ 50 ms), digitized (PowerLab, ADI Instruments Pty NES, Australia), and stored on the hard disk. Individual motoneuron action potentials were recorded with a platinum bipolar electrode connected to an AC preamplifier (Grass P511) and digitized as above. Single motoneurons were confirmed as representing individual action potentials by examining the amplitude and waveform of the action potential on an oscilloscope (Tektronix, 5113), as described previously (Refs. 14, 16, also see Fig. 7, $\text{Ah}–\text{Dh}$).

**Experimental protocols.** Three separate experimental protocols were performed. In protocol 1, phrenic neurograms were simultaneously recorded with neurograms in the FN and HN, and either the SLN or RLN. This protocol was designed to examine whether changes in PEEP could uncouple UAW and phrenic bursting and was based on prior work (4, 36). Baseline neural recordings were obtained during application of 3-cmH$_2$O PEEP. After stable bursting was observed in all neurograms, rats were exposed to 0-, 6-, 9-, 12-, and 15-cmH$_2$O PEEP in random order. Exposures were limited to 10 s and were separated by duration sufficient to return activity to control levels.

Protocol 2 was similar, except we also recorded action potentials from individual motoneurons in the FN, HN, SLN, and RLN, as described above. In these studies, single motoneuron recordings were performed on one side, and the activity of the corresponding nerve was recorded on the contralateral side. The purpose of protocol 2 was to determine whether uncoupled neurogram activity resulted from activation of a particular type of UAW motoneuron (i.e., Pre-I, I, EI, etc.; Ref. 14). In addition, we wished to determine whether the discharge rate of UAW motoneurons varied across the respiratory cycle in response to PEEP manipulation.

The third protocol was designed to determine whether PEEP-induced uncoupling was dependent on afferent inputs associated with the ventilator pump. Rats ($n=3$) were treated as described above (protocol 1), but the ventilator was briefly turned off during periods of uncoupled UAW and phrenic bursting (e.g., see Fig. 6).

**Data analysis and statistics.** Data stored in the hard disk were retrieved and analyzed with software written using the Visual C++ language. Inspiratory time ($T_I$) was defined as the duration of the phrenic burst, and expiratory time ($T_E$) was calculated as the duration between phrenic bursting. The software automatically distinguished a respiratory burst from the baseline noise, if the signal remained above the baseline for at least 20% of the average duration of the 10 previous respiratory cycles. If the baseline changed after treatment, the program could be manually adjusted such that the calculation would accommodate the new baseline. Ten neural respiratory cycles were measured for each condition described above.

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### Fig. 3. Influence of PEEP on the respiratory cycle. Stepwise increases in PEEP progressively decreased the I period (A) with a concomitant elongation of the expiratory period (B). **$P < 0.01$ compared with PEEP of 3 cmH$_2$O. $N$, number of animals observed.**

### Fig. 4. Influence of PEEP on respiratory frequency. A: the burst frequency of the UAW and PN were similar (i.e., “coupled”) when PEEP was maintained at 0, 3, and 6 cmH$_2$O. However, this coupling was dissociated at PEEP values between 9 and 12 cmH$_2$O, such that average rhythmic frequency was greater in UAW vs. phrenic neurograms. Rhythmic bursting was often absent in all nerves at 15-cmH$_2$O PEEP. However, bursts were occasionally observed at 15 cmH$_2$O, and thus respiratory frequency does not reach zero in any of the neurograms. B: changes in amplitude of the UAW and phrenic bursts decreased in parallel as PEEP increased, and these reductions were statistically significant at 12 and 15 cmH$_2$O. **$P < 0.01$ compared with PEEP of 3 cmH$_2$O; # $P < 0.05$; ## $P < 0.01$ compared with the phrenic burst at the same level of PEEP. The numbers in the parentheses indicate the number of animals observed.**
The influence of PEEP on neurogram and motoneuron activity was determined using a one- or two-way ANOVA, as appropriate, and the Student-Newman-Keuls post hoc test (SigmaStat version 2.0) (44). A linear regression was used to examine the relationship between motoneuron discharge and PEEP during periods of coupled UAW and phrenic bursting (i.e., 0–6 cmH2O; see RESULTS). All data are expressed as means ± SE. A value of P < 0.05 was considered to represent a statistically significant difference.

RESULTS

Cardiovascular effects of PEEP. The mean arterial pressure was 88 ± 4 mmHg during baseline. While PEEP tended to cause a decrease in mean arterial pressure, the drop was statistically significant only when PEEP reached 12 cmH2O (71 ± 5 mmHg) and 15 cmH2O (64 ± 5 mmHg) (both P < 0.01 vs. control). Heart rate was 465 ± 7 beats/min during baseline and was not significantly changed in response to the manipulation of PEEP.

Influence of PEEP on the respiratory cycle. The I duration was 0.31 ± 0.01 s during baseline (PEEP = 3 cmH2O; see Fig. 3A). This value was reduced to 0.26 ± 0.01, 0.23 ± 0.01, 0.15 ± 0.02, and 0.05 ± 0.02 s during 6-, 9-, 12-, and 15-cmH2O PEEP, respectively (all P < 0.01, Fig. 3A). The Tc was 0.64 ± 0.01 s at baseline and was not significantly changed during application of 6- and 9-cmH2O PEEP (Figs. 2 and 3). However, the Tt was increased to 5.95 ± 0.79 and 10.19 ± 0.53 s when PEEP was increased 12 and 15 cmH2O, respectively (both P < 0.01, Fig. 3B). Removal of PEEP did not significantly affect the Ti and Tt (Figs. 2 and 3).

Influence of PEEP on the respiratory frequency. The frequency of UAW and phrenic bursting was reduced in a dose-dependent manner as PEEP was increased (P < 0.01, Fig. 4A). Respiratory frequency was not altered when PEEP was reduced to zero from the control value of 3 cmH2O. Respiratory responses to PEEP were vagally mediated as they were abolished by midcervical bilateral vagotomy (n = 3, data not shown).

Phrenic neurogram activity. Increasing PEEP caused a reduction in phrenic activity. This inhibitory effect was mild (P > 0.05) at PEEP of 6 and 9 cmH2O (Figs. 2, C and D, and 4B). However, when PEEP reached 12 and 15 cmH2O, the phrenic neurogram amplitude was decreased to 61 ± 8 and 16 ± 7% of the control (both P < 0.01, Fig. 4B), respectively. PEEP-induced inhibition of phrenic activity was not observed after midcervical bilateral vagotomy (n = 3, data not shown).

UAW neurogram activity. The amplitude of the integrated burst in each UAW nerve remained relatively constant in response to changes in PEEP over the range of 0–9 cmH2O (Fig. 4B). However, when PEEP was increased to 12 and 15 cmH2O, UAW neurogram burst amplitudes were significantly reduced. Specifically, during 12- and 15-cmH2O PEEP, FN amplitude was reduced to 54 ± 11 and 14 ± 8% of control, whereas HN amplitude was reduced to 58 ± 10 and 13 ± 10% of control, SLN amplitude was diminished to 63 ± 0 and 21 ± 11% of control, and RLN amplitude was reduced to 48 ± 12 and 17 ± 11% of control (all P < 0.01 vs. control).

Uncoupling of UAW and phrenic neurograms. The rhythmic burst or respiratory frequency in all UAW neurograms was similar to phrenic burst frequency at 0- and 3-cmH2O PEEP (Fig. 4A) and was time locked to the ventilator frequency as expected in vagally intact animals (see Figs. 2 and 6B).
Respiratory frequency of UAW neurograms remained constant when PEEP was raised from 6 and 9 cmH₂O; however, this increase in PEEP was accompanied by a decrease in phrenic burst frequency (P < 0.01, Fig. 4A). Increasing PEEP to 12 cmH₂O caused a significant decrease in the burst frequency of all neurograms except the FN (Fig. 4A). Moreover, UAW neurogram bursting clearly became uncoupled from phrenic bursting (Figs. 2 and 4A). The relative degree of “uncoupled activity” (e.g., Fig. 1) increased as PEEP was raised from 9 to 12 cmH₂O (Fig. 5A, P < 0.01). Consistent uncoupled bursting was not observed when PEEP was raised to 15 cmH₂O.

The uncoupling of UAW and phrenic bursting during PEEP manipulation was dependent on the mechanical ventilator. Thus the pattern of uncoupled bursting was immediately replaced with a pattern of coupled UAW and phrenic bursting when the ventilator was briefly shut off (Fig. 6A). The uncoupled pattern resumed when the ventilator was turned back on (Fig. 6A). These results were highly reproducible in three animals.

At 9-cmH₂O PEEP, a notch between Pre-I and I activity was always observed (Fig. 6Ab, low amplitude marked with upward arrow). This was discernible on all of the animals observed (please see the DISCUSSION for an interpretation of this phenomenon). Overlay plots of respiratory activity during PEEP (Fig. 6B) showed that the onset of the phrenic burst synchronized with the ventilator as PEEP was increased (dotted lines in Fig. 6B) and also that the uncoupled bursting occurred at the nadir of the PEEP (dashed line in Fig. 6B at 12-cmH₂O level).

Onset of the UAW nerve I discharge. The onset of UAW motor nerve I bursting always preceded the corresponding phrenic burst during baseline (Fig. 2B). Relative to phrenic onset, the mean onset times for the FN, HN, SLN, and RLN were -188.0 ± 27.2, -125.0 ± 10.2, -148.0 ± 14.7, and -116.0 ± 29.0 ms (Fig. 5B), respectively. When PEEP was removed, FN and RLN burst onsets were delayed such that they occurred 9.6 ± 21.8 and 18.0 ± 18.7 ms after the phrenic burst, respectively (both P < 0.01 vs. control). However, in the absence of PEEP, the onset of HN (-12.0 ± 12.4 ms) and SLN discharge (-30.8 ± 8.9 ms) still occurred before the phrenic burst, although the relative onset was delayed (both P < 0.05 vs. control, Fig. 5B). When PEEP was raised to 6 and 9 cmH₂O, UAW nerve burst onset commenced earlier relative to the phrenic burst as follows: FN, -298.0 ± 36.5 and -316.0 ± 46.0 ms; HN, -212.0 ± 18.7 and -251.0 ± 30.8 ms; SLN, -261 ± 26.3 and -262.0 ± 27.1 ms; and RLN, -223.0 ± 35.8 and -246.0 ± 56.2 ms (all P < 0.05 vs. control; Fig. 5B). The onset of UAW nerve bursting was delayed (i.e., occurred closer to phrenic burst onset) by increasing PEEP to 12 cmH₂O (Fig. 5B) and occurred approximately simultaneous with phrenic burst onset when PEEP reached 15 cmH₂O (P < 0.05, Fig. 5B).

Effects of PEEP on UAW motoneuron discharge. A total of 72 UAW motoneurons were recorded (Table 1). Motoneurons classified as EI and I were most commonly observed in UAW recordings.

EI motoneurons. During baseline, UAW EI motoneurons initially burst at a relatively slow discharge rate and then gradually increased discharge to a peak at the transition from TE to TI. Discharge rate then reached a plateau before gradually decreasing (Fig. 7). Both the discharge rate and onset time of EI motoneurons were modulated by PEEP. During late TE, both parameters were significantly reduced upon removal of PEEP (i.e., 0 cmH₂O; Table 2, see Fig. 7, Aa–Da). Due to these reductions, some EI motoneurons (n = 29/37) commenced discharge at the same time as phrenic burst, indicating that their discharge pattern became similar to I neurons (see Fig. 7, Aa–D). In contrast, EI discharge rate was increased, and onset was advanced relative to phrenic onset when PEEP was increased to 9 cmH₂O (Table 2, see Fig. 7, A; c and d, through D, c and d). EI motoneuron activity was clearly coupled with phrenic bursting when PEEP was set at 0–6 cmH₂O (see Fig. 7, A, a–c, through D, a–c).

![Figure 6](http://jap.physiology.org/10.1152/jappl.00565.2006)

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The majority of EI motoneurons became uncoupled from phrenic bursting when PEEP was held between 9 and 12 cmH₂O. Specifically, these neurons maintained a rhythmic discharge during the period of phrenic quiescence induced by PEEP (shown by triangles in Fig. 7, A, e and f, through D, e and f). Sometimes, motoneurons did not fire during uncoupled neurogram bursting. Importantly, the shape and amplitude of the EI action potentials recorded before and after PEEP-induced uncoupling were identical (Fig. 7, Ah–Dh). This confirms that the recordings obtained during coupled and uncoupled activity originated from the same neuron. EI motoneurons were almost always inhibited when PEEP was raised to 15 cmH₂O (Fig. 7, Ag–Dg), although we occasionally observed sporadic uncoupled bursting (Fig. 7Dg). A histogram plot depicting discharge rate of EI neurons across the respiratory cycle is provided in Fig. 7, Ad–Dd. These plots show that, as PEEP was increased from 0 to 3 cmH₂O, the mean onset of EI neuron discharge was advanced, peak discharge (i.e., the smallest interspike interval) was shifted to the left (indicated by the horizontal leftward dashed arrow in Fig. 7, Ad–Dd), and the overall discharge rate was increased. EI motoneuron discharge recorded in the FN and RLN became tonic with PEEP at 6- and 9-cmH₂O PEEP (Fig. 7, Ad, Ae, Dd, and De). EI motoneurons in the RLN continued tonic discharge at 12-cmH₂O PEEP (Fig. 7Df).

Table 1. Numbers of motoneurons recorded from the upper airway motor nerves

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<tr>
<th>Nerves</th>
<th>Patterns of Motoneurons</th>
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<tr>
<td></td>
<td>EI</td>
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<tr>
<td>Facial</td>
<td>11</td>
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<tr>
<td>Hypoglossal</td>
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<td>Superior laryngeal</td>
<td>8</td>
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<tr>
<td>Recurrent laryngeal</td>
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El, expiratory-inspiratory; I, inspiratory; Pre-I, preinspiratory; IE, inspiratory-expiratory; Early E, early expiratory; T, tonic.

Fig. 7. Representative examples of expiratory-inspiratory (EI) motoneurons recorded from the FN (A), HN (B), SLN (C), and recurrent laryngeal nerves (RLN; D) in responses to alterations of PEEP. The onset of EI motoneuron bursting preceded phrenic bursting during the control condition (PEEP = 3 cmH₂O, e.g., see panels labeled b). When PEEP was increased to 6 cmH₂O, the onset of EI bursting was advanced even more relative to the phrenic burst (e.g., see panels labeled c). Removal of PEEP to reduce lung inflation caused EI motoneuron bursting to synchronize with the phrenic burst (e.g., see panels labeled a). The average onset time (±SE) of all observed EI discharge caused by varying PEEP from 0 to 6 cmH₂O is depicted in the plots presented in panels labeled d. The rhythm of EI motoneuron bursting was uncoupled from phrenic bursting when PEEP was raised to 9 and 12 cmH₂O (indicated by the triangles in panels e and f). EI bursting was absent during the highest level of PEEP (15 cmH₂O, see panels labeled g). Individual action potentials recorded throughout the respiratory cycle (before and during PEEP application) are presented in panels labeled h. These tracings are provided to document that the recordings do in fact represent the activity of the same neuron. UA, unit activity.
I motoneurons. Discharge of UAW I motoneurons occurred only during phrenic bursting, and uncoupling of I bursting from the phrenic neurogram was never observed (Fig. 8). The onset of I motoneuron bursting was significantly delayed (i.e., occurred after phrenic burst onset) during the baseline and was further delayed by removal of PEEP (Table 2, Fig. 8, Aa–Da). The discharge rate of I motoneurons tended to decrease with removal of PEEP, but this was significant ($P < 0.05$) only for the SLN (Table 2). Discharge rate was not significantly altered by increasing PEEP to 6 cmH$_2$O ($P > 0.05$, Table 2). Discharge of I motoneurons was inhibited as PEEP was increased, and discharge stopped completely during the highest PEEP levels (Fig. 8, Ag–Dg).

Pre-I motoneurons. Two Pre-I motoneurons were observed in the FN. These neurons discharged during the late expiratory or Pre-I period and gradually increased their discharge rate to a peak at the end of neural expiration (see Fig. 9A, b and d). They maintained rhythmic Pre-I activity during PEEP values from 0 to 6 cmH$_2$O (Fig. 9A, a–c). The discharge of both Pre-I neurons was reduced by removing PEEP and was augmented by increasing PEEP to 6 cmH$_2$O. Both Pre-I motoneurons showed uncoupled bursting when PEEP was set at 12 cmH$_2$O (indicated by triangles in Fig. 9Af). One of them showed an uncoupled discharge pattern during 9-cmH$_2$O PEEP (not shown), whereas the other did not (Fig. 9Ae). However, their rhythmic discharge was totally inhibited when PEEP was set at 15 cmH$_2$O (Fig. 9Ag).

IE motoneurons. A single IE motoneuron was observed in the FN. This neuron discharged from inspiration to early expiration during baseline (Fig. 9Bb). However, this IE motoneuron discharged with a tonic pattern when PEEP was removed (Fig. 9Ba). Its discharge rate was decreased and onset was delayed when PEEP was set at 6 cmH$_2$O (Fig. 9Bc). Interestingly, this IE motoneuron also fired during PEEP-induced phrenic quiescence (triangle in Fig. 9Bf), although it was not activated each time the FN showed uncoupled bursting (e.g., the first triangle in Fig. 9Bf). Like all respiratory-related motoneurons, this IE motoneuron was inhibited by the highest level of PEEP (Fig. 9Bg).

Tonic motoneurons. Tonic motoneurons were only observed in the FN ($n = 3$, Table 1) and SLN ($n = 2$). These motoneurons burst throughout the respiratory cycle and were apparently not affected by changes in PEEP (data not shown).

Early expiratory motoneurons. Three early expiratory motoneurons were recorded from the RLN (Table 1). These neurons discharged immediately after phrenic burst cessation. Their discharge rate increased to peak quickly and decreased gradually to cease around the midpoint of expiration. Removal of PEEP resulted in an extension of their overall burst duration, but did not advance the onset of the bursting. However, increasing PEEP from 3 to 12 cmH$_2$O evoked a gradual inhibition of their discharge, but bursting remained coupled with the phrenic burst. When PEEP reached 15 cmH$_2$O, these neurons stopped bursting.

Relationship of motoneuron discharge rate to level of PEEP. To provide insight regarding the source of synaptic inputs onto UAW motoneurons, we examined the relationship between motoneuron discharge and PEEP values associated with “normal” (i.e., coupled) UAW and phrenic bursting. We transformed the discharge rate of EI motoneurons during the Pre-I period into percentage of the peak discharge observed during 0- to 6-cmH$_2$O PEEP; this value was plotted against the level of PEEP. This analysis revealed a linear relationship between EI neuron discharge and PEEP during the Pre-I period (Fig. 10A). A similar relationship was observed in the limited sample of Pre-I neurons ($n = 2$) recorded only from the FN. In contrast, no relationship between PEEP and EI discharge frequency was observed during the I period in the HN, SLN, and RLN (Fig. 10B). Discharge rate of FN EI motoneurons,
however, was linearly augmented with PEEP during the I period (Fig. 10B).

The duration of EI neuron discharge during the Pre-I phase was variable. However, when duration was standardized as a percentage of the longest observed burst (i.e., %maximum), the duration was constant at a given level of PEEP (Fig. 10C). The relationship between discharge duration and PEEP was linear and had a similar slope for all UAW EI motoneurons (Fig. 10C).

Fig. 8. Representative examples of I motoneurons recorded from the FN (A), HN (B), SLN (C), and RLN (D) during alterations of PEEP. I motoneuron discharges remained coupled to phrenic bursting over PEEP values of 0–12 cmH2O and were totally inhibited at PEEP of 15 cmH2O. a, b, and c: I motoneuron bursting at 0, 3, and 6 cmH2O, respectively. d: Average response of I motoneuron bursting throughout the respiratory cycle at PEEP values of 0–6 cmH2O. e, f, and g: I motoneuron bursting during 9-, 12-, and 15-cmH2O PEEP, respectively.
DISCUSSION
There are three primary findings of our study. First, rhythmic bursting recorded in several UAW motor nerves (FN, HN, SLN, and RLN) can be uncoupled from phrenic bursting by manipulation of PEEP. This uncoupling phenomenon did not persist when mechanical ventilation was transiently stopped and thus appears to be dependent on afferent inputs associated with the ventilator. Second, EI motoneurons recorded from UAW motor nerves remained active during periods of uncoupled bursting and thus may contribute to the uncoupled discharge observed in the UAW neurograms. We also observed that Pre-I and IE neurons recorded from the FN showed bursting during periods of PEEP-induced phrenic silence. Third, during coupled UAW and phrenic bursting, the relationship between EI motoneuron discharge rate and PEEP was linear during the Pre-I stage but unrelated during the I period.

Fig. 9. Examples of Pre-I (A) and IE (B) motoneuron discharge recorded in the FN during stepwise changes in PEEP. Pre-I motoneuron discharge increased as PEEP increased from 0 (a) to 3 (b) to 6 cmH2O (c). d: The response of discharge rate to varying PEEP from 0 to 6 cmH2O is depicted. f: Pre-I neurons continued to discharge during PEEP-induced phrenic cessation, resulting in uncoupling in the FN bursting (marked by triangles). g: Pre-I neurons were inhibited by 15-cmH2O PEEP. B: IE motoneuron bursting appeared to be inversely related to PEEP of levels of 0–6 cmH2O (a–d). This IE motoneuron continued to discharge during PEEP-induced uncoupling of the FN at levels of 12 cmH2O (f) and was inhibited by the highest level of PEEP at 15 cmH2O (g). e: 9-cmH2O PEEP.
These latter results are consistent with the hypothesis that UAW motoneurons may receive distinct inputs during the Pre-I and I periods.

Critique of methods. We would like to comment on a few aspects of our experimental methods. First, PEEP was used as a tool to uncouple phasic HN bursting from phrenic bursting, as previously described (4, 36). PEEP will increase lung volume and, because rats were vagally intact, will initiate the Hering-Breuer inflation reflex to reduce or inhibit inspiration. Furthermore, because rats were vagally intact, the respiratory burst frequency entrained with the rate of the mechanical ventilator (29, 37). Previous studies showed that the uncoupling response does not require sustained lung inflation, but rather phasic inflations during PEEP application (4). Thus the frequency of the ventilator in combination with lung volume appears to play a critical role in producing the uncoupling of UAW and phrenic outputs. As discussed subsequently, the uncoupling response could therefore reflect variability in the threshold for ventilator entrainment of UAW vs. phrenic motor activity.

Another point is that the method used to discriminate a respiratory burst may have actually underestimated the degree of uncoupling. We frequently observed small UAW bursts during PEEP application that did not meet our criteria (see MATERIALS AND METHODS) for respiratory bursting. For example, this response was observed in 9 of 24 HN recordings (e.g., first triangle in Fig. 2D, HNA). A similar phenomenon is seen in Fig. 2E in which HN and SLN bursting does not quite reach the threshold to be considered a respiratory burst.

Uncoupling of UAW and phrenic bursting. Our study extended the observations of Saito et al. (36), who found that Pre-I hypoglossal activity can be uncoupled from phrenic bursting by PEEP-induced changes in lung volume. Our data show that uncoupled activity is not a unique response to the HN but rather appears to be common for all respiratory modulated UAW motor outputs. Although our data do not allow specific conclusions regarding the source of afferent input required to uncouple UAW bursting, PEEP-induced changes in lung volume are known to activate pulmonary stretch receptors, vagal Aδ and C fiber receptors (45). Interestingly, activation of vagal Aδ and C fiber receptors via capsaicin does not produce uncoupled bursting in the HN (23, 25). Moreover, the PEEP values used on our study were not high enough to activate vagal C fiber receptors (12). Hence, we speculate that the uncoupled bursting observed in our study may be reflexively caused by PEEP-induced modulation of pulmonary stretch receptors (9) and/or Aδ fiber receptors.

Previous studies have shown that the relative changes of UAW and phrenic motor output are different in response to changes in lung volume, chemoreceptor stimulation, and anesthetics (7, 13, 36, 42). Hence, uncoupled bursting may reflect a different PEEP sensitivity between the neurons and/or networks controlling UAW and phrenic motor output. One possibility is that the relative threshold for entrainment of respiratory bursting to the ventilator is different between UAW and phrenic outputs. Previous studies have demonstrated that entrainment to the ventilator during apnea occurs for only some central respiratory neurons, and that respiratory neurons that are reciprocally related during spontaneous breathing can be entrained during the same phase of the ventilator cycle (32). However, as shown by Fig. 6B, the PEEP-threshold for entrainment of respiratory bursting with the ventilator appeared to be similar for UAW I and phrenic outputs.

We speculate that PEEP may inhibit the respiratory-related discharge of I UAW motoneurons, while Pre-I UAW neurons continue to discharge, thus creating the uncoupled burst. This speculation is based on several aspects of our data. First, the
advanced onset of EI motoneuron discharge during PEEP suggests that the bursting of these neurons could begin to dissociate from their I discharge. This notion is compatible with studies showing a gap between HN Pre-I and I activity during hypothermia (39). Furthermore, we observed a distinct “notch” between Pre-I and I activity when phrenic and UAW bursting was still coupled (just before the onset of uncoupled bursting) (Fig. 6A, downward arrow). This notch could represent the separation of EI and I motoneuron discharge. The small I burst following the notch was still coupled with the phrenic burst, suggesting an incomplete inhibition of I motoneurons. Although speculative, the subsequent UAW burst could involve primarily EI motoneurons. In addition, FN Pre-I neurons displaying uncoupled bursting provides evidence that uncoupling may reflect continued discharge of the Pre-I neurons (39). Thus the uncoupling phenomenon may depend on advancement of Pre-I activity and simultaneous inhibition of I discharge during application of PEEP.

Data from power spectral analyses indicate that hypoglossal activity during inspiration and expiration exhibits different peak frequencies (24). Moreover, Pre-I hypoglossal activity disappears and apparently becomes part of the I burst during gasping. This finding suggests a differential modulation for the hypoglossal activity during TI and TE (24). This notion is consistent with our present observations.

Recently, it has been suggested that the overall respiratory neural rhythm may derive from distinct rhythm generators driving inspiration and expiration (for review, see Ref. 46). One is located in the pre-Bötzinger complex (the locus of the putative I rhythm generator) (21, 35, 38), and the other is situated in the region of the retrotrapezoid nucleus and/or parafacial respiratory group, the proposed expiratory rhythm generator (18, 19, 31). These two respiratory rhythm generators observed in juvenile rats are normally coupled but can be differentially influenced by μ-opic agonist fentanyl and by continuous positive airway pressure. Therefore, rhythmic bursting in the genioglossus and abdominal muscles may be driven by the expiratory rhythm generator during periods of I inhibition (18, 27, 28).

**Contribution of UAW motoneurons to uncoupled bursting.** Our results indicate that the EI and I type motoneurons are commonly observed in respiratory-related UAW motor output. Pre-I and IE motoneurons were only observed in the FN, consistent with our previous studies (14, 16) and other reports (20, 36). Specifically, EI motoneurons continued to discharge during periods of phrenic apnea induced by PEEP, and their discharge corresponded to the response recorded in UAW motor nerves.

The rhythmic frequency of EI motoneuron discharge remained at a constant ratio to the phrenic burst over PEEP values from 0–6 cmH₂O; however, the ratio was not maintained at higher levels of PEEP. As PEEP was increased, EI motoneurons burst earlier such that their discharge during the Pre-I phase was ultimately dissociated from their activity during inspiration. This manner of dissociation was specific for EI neurons (and a small sample of Pre-I neurons recorded in the FN) (please see above). The discharge rate of EI neurons during the Pre-I stage was linearly related to PEEP over the range of 0–6 cmH₂O (P < 0.01, Fig. 10C). These results strongly suggest that UAW motoneurons receive a common source of modulation during the expiratory or Pre-I phase during 0- to 6-cmH₂O PEEP.

A recent study demonstrates that there are three components underlying FN discharge: Pre-I, I, and postinspiratory (31). The Pre-I bursting remains active during I (phrenic) inhibition caused by the infusion of DAMGO (an agonist for μ-opioid receptor) (31). The authors suggested that Pre-I FN activity could be used as an indicator of Pre-I neurons in the para facial respiratory group, which has been proposed to be the primary respiratory rhythm generator (31) or the putative expiratory rhythm generator (18). Our data indicate that Pre-I facial motoneurons remain rhythmically active during PEEP-induced phrenic apnea. This observation provides support for the notion that UAW motoneurons might receive input from an expiratory pattern generator (18).

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