Ventrolateral medullary respiratory network participation in the expiration reflex in the cat

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Submitted 25 July 2003; accepted in final form 3 February 2004

The expiration reflex is an airway defensive response characterized by a brief, intense expiratory effort and coordinated adduction and abduction of the laryngeal folds. It can be elicited by mechanical or chemical stimulation of the vocal folds of humans and laboratory animals. The expiration reflex prevents entry of foreign materials into the lower airways and removes mucus from the subglottal region; it is considered distinct from cough because there is no preceding large inspiratory effort. The reflex has been studied extensively over the past 30 years by investigators at Jessenius Medical School, Martin, Slovakia [see reviews by Korpas and Tomori (28) and Korpas and Jakus (27)].

The expiration reflex is divided into three phases (28, 36, 44). First, the compressive phase is characterized by a simultaneous increase in abdominal and expiratory intercostal muscle activity and activation of laryngeal adductor muscles (thyroarytenoid) leading to narrowing of the glottis. This coordinated muscle activity results in a large increase in intrathoracic pressure. The next expulsive phase is marked by decreased glottal resistance due to relaxation of the adductor and contraction of the abductor muscles (posterior cricoarytenoid), along with continued activity in the expiratory pump muscles. During the subsequent constrictive phase, there is declining expiratory pump muscle activity, increased laryngeal adductor activity, and increased laryngeal resistance.

The most effective sites for eliciting independent expiratory efforts are the medial margins of the vocal folds. The reflex response is likely due to stimulation of subepithelial rapidly adapting receptors (22, 45). In cats and other laboratory animals, laryngeal afferent fibers travel in the superior laryngeal nerve and project to various subnuclei of the caudal nucleus tractus solitarius (24, 31). In humans, the inferior laryngeal nerve is the critical pathway (27). The connections through which laryngeal tractus solitarius second-order neurons distributeafferent information to the medullary respiratory network are unknown.

Although much work has been done to describe the afferent pathway and motor effort, the brain stem network that coordinates the reflex is not well understood. Extracellular recordings in the caudal ventral lateral medulla (ventral respiratory group, VRG) of cats have found that expiratory neurons have altered peak firing rates during the FER. Increased firing rates of bulbospinal neurons and expiratory laryngeal premotor and motoneurons during the expiratory burst of FER were accompanied by changes in the firing patterns of putative propriobulbar neurons proposed to participate in the eunopic respiratory network. The results support the hypothesis that elements of the rostral and caudal ventral respiratory groups participate in generating and shaping the motor output of the FER. A model is proposed for the participation of the respiratory network in the expiration reflex.

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major components of the ventrolateral medullary respiratory network in the expiration reflex. Spike trains of simultaneously recorded neurons were evaluated for responses during fictive expiration reflex (FER). The neurons were further characterized with tests for bulbospinal projections with antidromic stimulation methods and for functional relationships with laryngeal motoneurons using spike-triggered averaging of recurrent laryngeal motor nerve activities. A model is proposed for the participation of the respiratory network in the expiration reflex.

METHODS

General Methods

Most of the methods have been described in detail elsewhere (1, 39, 40). Experiments were performed under protocols approved by the University of South Florida’s Animal Care and Use Committee. Data were obtained from 17 adult cats (2.8–5.6 kg) of either sex. Animals were initially anesthetized and later decerebrated using the technique of Kirsten and St. John (25). Eight animals were anesthetized with intravenous sodium thiopental (22.0 mg/kg) and the remaining nine with isoflurane (2–5%).

Before surgery, atropine (0.5 mg/kg im) was administered to reduce mucus secretion in the airways, and dexamethasone (2.0 mg/kg iv) was given to help prevent hypotension and minimize brain stem swelling. Femoral arteries and veins were catheterized for monitoring arterial blood pressure, acquisition of arterial blood samples, and administration of intravenous fluids and drugs. Throughout the experiment, arterial blood samples were periodically analyzed for 

\[\text{Po}_2, \text{Pco}_2, \text{and pH}; \text{these parameters were maintained within normal limits.} \]

Solutions of 5% dextrose in 0.45% NaCl, 5% dextran, or lactated Ringer’s solution were administered intravenously as needed to maintain a mean blood pressure of at least 100 mmHg. Until decerebration was completed (to render the animal insentient), the level of anesthesia was adjusted with end-expiratory pressure to prevent lung collapse; i.e., at positive-pressure ventilation. The end-expiratory lung volume was allowed to fill the thoracic volume. When necessary, the lungs were hyperinflated to obtain a return to control inspiratory and expiratory motor patterns. A phrenic driven ventilator was used to monitor the effectiveness of the stimuli to elicit the expiration reflex.

Neuron Recordings and Characterization

An occipital craniotomy was performed, and portions of the caudal cerebellum were removed by suction to expose the medulla. Medullary respiratory neurons were monitored with two independently controlled planar arrays of tungsten microelectrodes (10–12 MΩ) positioned on the right side of the medulla (Fig. 1A). Each array consisted of six to eight microelectrodes. In the initial experiments, individual electrodes in an array were fixed to each other. In later experiments, the depth of each electrode was adjusted with micrometer controllers. Signals were amplified and filtered (band pass 0.1–5 kHz). The medullary surface was covered with a pool of warm mineral oil.

Regions of the ventral lateral medulla searched for respiratory-modulated neurons included 1) the rVRG, which contains the Botzinger and pre-Botzinger complexes (Bö/FrVRG), 2.3–5.7 mm rostral to obex, 2.5–4.5 mm lateral to midline, 2.6–5.7 mm below dorsal surface; and 2) the caudal VRG (cVRG), obex to 2.6 mm caudal to obex, 2.9–4.3 mm lateral to midline, 2.3–4.4 mm below dorsal surface (5, 13, 30, 38, 40).

Neurons antidromically activated (positive collision test) from the spinal cord were designated bulbospinal. Bipolar stainless steel electrodes were placed in the ventral spinal cord at the T1 level contralateral to medullary recording sites. Single pulses ranging in intensity from 1–10 V with 0.1-ms duration were used. Spike-triggered averaging of contralateral lumbar and ipsilateral recurrent laryngeal nerve efferent activities was used to identify putative premotor neurons and laryngeal motoneurons (see Spike-Triggered Averaging of Efferent Nerve Signals).

Evoking the FER

A tracheal cannula was inserted caudal to the larynx. The FER was elicited during the expiratory phase by inserting a thin piece of polylethylene tubing into the cannula and gently probing the medial margins of the laryngeal folds (Fig. 1B). The reflex was characterized by a brief, large increase in lumbar motor nerve discharge (Fig. 1, C and D). The response was similar to that reported previously in unanesthetized or anesthetized spontaneously breathing animals and anesthetized or decerebrate neuromuscular-blocked animals [see reviews by Korpas and Tomori (28) and Korpas and Jakus (27)]. After each stimulus period, the polylethylene tubing was retracted into the cannula. At least five separate expiration reflexes were produced in each recording. Each stimulus trial was separated by at least 1 min. This interval was sufficient for a return to control inspiratory and expiratory motor patterns. A phrenic driven ventilator was used to allow matching of pulmonary stretch receptor activity with central inspiratory and expiratory activity.

Data Acquisition, Entry, and Preprocessing

During the experiments, signals were monitored on oscilloscopes, a polygraph, and audio monitors, and recorded on magnetic tape. These data included signals from the microelectrode arrays, multiferent efferent nerve activities, arterial blood pressure, tracheal pressure, and stimulus timing signals. From the tape, phrenic, lumbar, and recurrent laryngeal nerve signals were integrated with a leaky resistor-capacitor circuit (0.2-s time constant) and recorded on a polygraph to monitor the effectiveness of the stimuli to elicit the expiration reflex.

Nerve Recordings

The right lumbar iliohypogastric (L1), right recurrent laryngeal nerve, and left phrenic (C3) nerves were desheathed and cut, and their efferent activities were recorded with bipolar silver electrodes covered with mineral oil. Nerve signals were amplified and filtered (band pass 0.1–5 kHz). Phrenic, lumbar, and recurrent laryngeal nerve discharges were integrated with a leaky resistor-capacitor circuit (0.2-s time constant) and recorded on a polygraph to monitor the effectiveness of the stimuli to elicit the expiration reflex.

Evoking the FER

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Xscope (29) provided a graphical representation of the times of action potentials and other digital and analog signals (Fig. 1C) and enabled integration of the spike trains (Fig. 1E). This program also allowed additional event codes to be added and graphically confirmed the selection of data segments to be written as separate files for later analysis.

The taped signals of efferent multiunit nerve activities were also high-pass filtered (40 Hz, 3-dB cutoff) and, along with the common synchronization timing pulses, were digitized (5 kHz) with a 16-bit ADC488/16 analog-to-digital converter hosted by a Hewlett-Packard 9000/380 computer. These files were subsequently analyzed with the corresponding spike files for spike-triggered averaging as described below.

**Spike Train Analysis Methods for Each Single Neuron**

The following measures were computed from a 5-min control period that preceded the beginning of the expiration reflex stimuli. Spike trains were subjected to two statistical evaluations of respiratory modulation, and a measure of respiratory modulation, \( \eta^2 \), was calculated (33, 34). Cycle-triggered histograms were used to classify cells with statistically significant respiratory modulation according to the phase (inspiratory (I) or expiratory (E)) in which they were more active (Fig. 3). Neurons with peak firing rates in the first half of the phase were classified as decrementing (Dec), whereas those cells with peak firing rates in the second half of the phase were denoted as augmenting (Aug). Cells with a relatively constant discharge rate...
throughout a respiratory phase were classified as plateau (Plat). Neurons that could not be placed in one of these major classifications, generally those with low firing rates, were denoted as I-Other or E-Other. Respiratory-modulated cells with activity traversing the transition between the respiratory phases that could not be placed in one of the preceding categories were denoted phase spanners. Cells that were silent during control cycles and elicited during the expiration reflex were denoted as recruited (ER-Recruit; e.g., peak activity during the expiration reflex). Neurons with no preferred phase of maximum activity were classified as nonrespiratory modulated.

Autocorrelograms were computed for each spike train to ensure that it represented the activity of one neuron (Fig. 1F). A spike train with action potentials from two or more neurons would include short intervals not constrained by refractoriness. Spike trains were also evaluated statistically for changes in peak discharge rate. These values during the FER, averaged over five trials, were compared with the mean for five expiratory periods preceding the reflex ($P < 0.05$, Student’s $t$-test).

**Spike-Triggered Averaging of Efferent Nerve Signals**

Spike-triggered averaging of neurograms is widely recognized as an effective means for identifying motoneurons and detecting putative relationships between motoneurons and functionally antecedent neurons. The evidence for neuronal interactions is based on measured time-locked fluctuations in the recruitment and firing rates of motoneurons due to direct, indirect, or parallel paths. Advantages and limitations of these methods have been considered elsewhere (8, 10, 11, 32, 35). Statistical measures of significance for spike-triggered averages have not been developed. As described in the literature, the detection and evaluation of features departing from background activity were based on visual inspection. As a result, only very large departures were reported in this study.

Each average was computed over the entire recording period. Occurrence times of action potentials in each neuron defined intervals of the digitized recurrent laryngeal nerve activity that were used to generate corresponding “triggered” averages of both unrectified and full-wave rectified signals. A short-latency (1.5–3.5 ms) (9), short-duration biphasic feature in the unrectified recurrent laryngeal nerve average provided evidence that the recorded neuron was a laryngeal motoneuron (8, 32, 35) (see Fig. 8B). The biphasic feature represents “motoneuron” action potentials. The presence of an offset positive feature in the rectified but not the unrectified signal (see Fig. 3, B and C) suggested that the trigger neuron was 1) a premotor neuron that projected to the monitored motoneuron pool or 2) a neuron that was tightly synchronized with premotor neurons by functionally antecedent shared inputs (8, 11, 32, 35). Presence of an offset sharp positive feature in a rectified lumbar nerve average was used as evidence that the neuron was either 1) or 2).

**RESULTS**

**Respiratory Motor Pattern Changes During the Fictive Expiration Reflex**

Mechanical stimulation of the vocal folds during the expiratory phase elicited a brief increase in lumbar and recurrent laryngeal motor nerve discharge. In the absence of muscle activity, this discharge pattern is termed the FER. A variety of respiratory pattern changes accompanied the expiratory burst (Fig. 2). In 14 recordings where the FER was the only reflex response, the expiratory phase was prolonged in six, was shortened in seven, and showed no change in one recording. In six recordings, augmented phrenic activity closely followed the expiratory burst. A “coughlike” pattern occurred in the cycle after the FER in 12 recordings, i.e., increased phrenic followed by increased lumbar motor discharge.

**Neuron Recordings from the Ventral Lateral Medulla**

In 32 recordings (17 cats), the spike trains of 232 neurons were monitored in the rVRG (including Bötzingen and pre-Bötzingen complexes) and cVRG and evaluated for responses during the FER. Each recording included 3–20 spike trains from single cells and lasted 30 min to 1 h. Among the 232 neurons, 208 were respiratory modulated and 24 were nonrespiratory modulated.
spontaneous; 104 of the respiratory and 6 of the nonrespiratory-modulated neurons had altered peak firing rates during the FER. Table 1 presents a summary of neuronal discharge patterns during control periods, results from spinal antidromic stimulation, spike-triggered averaging of the recurrent laryngeal nerve, and alterations in peak firing rate during the FER. Some neurons were not tested for spinal projections or laryngeal function with these methods. Figure 1C shows multiple simultaneously recorded neurons changing activity during the FER. Figures presented subsequently do not include the complete data sets of neurons recorded simultaneously. Rather, they include examples of the responses of representative classes of neurons.

**Bulbospinal/Premotor Expiratory Neurons of the Caudal VRG**

Seven augmenting expiratory neurons (E-Aug) and three decrementing expiratory neurons (E-Dec) neurons were identified as having spinal axonal projections (bulbospinal) with collision tests. Two additional E-Aug neurons were designated as presumptive premotor neurons with spike-triggered averaging of the lumbar nerve (not in Table 1). These 12 neurons increased firing rate coincident with the burst of activity in the lumbar nerve during the FER. Figure 3A shows integrated activities of four simultaneously recorded E-Aug neurons together with phrenic, lumbar, and recurrent laryngeal nerves and representative cycle-triggered histograms and spike-triggered averages. The rectified averages of lumbar nerve activity triggered by spikes in neurons 351 and 355 showed short-latency offset positive peaks (Fig. 3, B and C), suggesting that the neurons were functionally premotor to lumbar motoneurons. The responses during the FER of four neurons in another animal are shown in Fig. 4. This sample included two bulbospinal neurons classified as E-Aug and E-Dec on the basis of their cycle-triggered histograms (neurons 351 and 353). The summary circuits in Figs. 3 and 4 show inferred functional connections. These and subsequent summary diagrams were placed adjacent to corresponding results to facilitate further considerations in the DISCUSSION.

**Other Caudal VRG Expiratory Neurons**

The 41 cVRG expiratory neurons without detected spinal projections or features in their spike-triggered averages or not tested (Table 1) had various discharge patterns: augmenting, decrementing, plateau, other, and active only during the FER (ER-Reruit). Seventy-six percent of these neurons increased activity during the FER (11 E-Aug, 9 E-Dec, 5 E-Plat, 2 E-Other, and 4 ER-Reruit). Examples include recordings of E-Aug neurons 353 and 354 (Fig. 3A), E-Dec neurons 356 and 362 (Fig. 4) and E-Plat neuron 308 (Fig. 5A).

The remaining 10 cVRG expiratory neurons showed either a decrease or no change in peak firing rate during the FER. Decrementing expiratory neurons were the only population with more than one cell showing these responses. Examples of data are shown in Fig. 5B; neuron 67 had no change in peak firing rate, and neuron 69 became inactive during the FER.

**Expiratory Neurons of the Böt/vrVG**

Putative propriobulbar E-Aug neurons. Twelve E-Aug cells were considered to be putative propriobulbar (axons limited to the brainstem); none had detected spinal projections or features in recurrent laryngeal or lumbar nerve spike-triggered averages. Eight of these neurons increased activity during the FER (see records from neurons 41 and 47 in Fig. 6). The two E-Aug cells in Fig. 6 also increased activity during the subsequent coughlike pattern. Four E-Aug neurons became inactive during the FER (two examples are shown in Fig. 7).

**Expiratory laryngeal motoneurons.** Five expiratory neurons were identified as laryngeal motoneurons. Three of these cells had E-Dec patterns during control cycles; two others were silent and recruited during the FER. The averages of recurrent

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**Table 1. Neuronal discharge patterns during control periods, results from spinal antidromic stimulation, spike-triggered averaging of the recurrent laryngeal nerve, and alterations in peak firing rate during the FER**

<table>
<thead>
<tr>
<th></th>
<th>cVRG</th>
<th>Böt/vrVG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recurrent laryngeal nerve</td>
<td>Recurrent laryngeal nerve</td>
</tr>
<tr>
<td>n</td>
<td>Bulbospinal</td>
<td>Premotor</td>
</tr>
<tr>
<td>E-Aug</td>
<td>21 0/14</td>
<td>0/13</td>
</tr>
<tr>
<td>E-Dec</td>
<td>18 3/13</td>
<td>0/18</td>
</tr>
<tr>
<td>E-Plat</td>
<td>7 0/7</td>
<td>0/6</td>
</tr>
<tr>
<td>E-I/E</td>
<td>2 0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>E-Other</td>
<td>3 0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>ER Recruit</td>
<td>4 0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>I-Aug</td>
<td>15 3/8</td>
<td>3/13</td>
</tr>
<tr>
<td>I-Dec</td>
<td>5 2/5</td>
<td>0/5</td>
</tr>
<tr>
<td>I-Plat</td>
<td>7 0/5</td>
<td>0/4</td>
</tr>
<tr>
<td>I-I/E</td>
<td>3 0/1</td>
<td>0/3</td>
</tr>
<tr>
<td>I-Other</td>
<td>2 0/2</td>
<td>0/1</td>
</tr>
<tr>
<td>Phase spanners</td>
<td>13 0/6</td>
<td>0/3</td>
</tr>
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</table>

n. No. of neurons; Bulbospinal, spinal antidromic activation testing (numerator is the no. of neurons with positive spinal collision tests; denominator is the no. of neurons tested); FER, fictive expiratory reflex; cVRG, caudal ventral respiratory group; Böt/vrVG, rostral ventral respiratory group, which contains the Bötzinger and pre-Bötzinger complexes; E, expiratory; Aug, augmenting; Dec, decrementing; Plat, plateau; E-I/E, inspiratory-expiratory phase spanning; Other, neurons that could not be placed in 1 of the major classifications; ER Recruit, cells with activity only during the FER; I, inspiratory; I-E/I, expiratory-inspiratory phase spanning; NRM, nonrespiratory modulated. Arrows indicate change in peak firing rate during FER: ↑, increase; ↓, decrease; →, no change.
laryngeal nerve activity triggered by neuron 42 (Fig. 8, B and C) show representative offset features with positive time lags. A short-onset latency, short-duration peak was found in the rectified average (Fig. 8B). The unrectified average had a biphasic feature (Fig. 8C) indicative of spikes arising from a motoneuron with an axon in the recorded nerve. The integrated activities of two E-Dec neurons (42 and 43) and one ER-Recruit neuron (35) are shown in Fig. 8A. During the FER,
there was an increase in the firing rates of the three motoneurons during the interval of increased integrated recurrent laryngeal nerve activity.

Putative expiratory laryngeal premotoneurons. Rectified averages of recurrent laryngeal nerve activity triggered by five expiratory neurons had offset peaks suggestive of functional associations between the trigger neuron and expiratory laryngeal motoneurons; there were no features in the unrectified averages triggered by this subset of neurons, which included two decrementing, one plateau, one recruited, and one “other” expiratory cell. During the FER, the firing rates of these neurons increased concurrently with the augmented recurrent laryngeal nerve activity (e.g., see E-Dec neuron 61 in Fig. 9).

Other rVRG expiratory neurons. Thirty-seven rVRG expiratory neurons with no detected spinal projections or features in their spike-triggered averages had various discharge patterns (Table 1), including decrementing, plateau, inspiratory-expiratory (E-I/E) phase spanning, recruited, and other.

Three distinct pattern changes during the FER were observed in 11 rVRG E-Dec neurons. Two cells increased activity during the FER, whereas seven were not excited. If these latter neurons were discharging when the stimulus occurred, their activity ceased (see cVRG neuron 69 in Fig. 5B). If they were inactive when the stimulus occurred, they remained inactive. Two other E-Dec cells that were active
throughout the control expiratory phases remained active without any change in peak firing rate during the FER.

Of the 12 E-Plat neurons recorded in the rVRG, two increased, six decreased, and four showed no change in peak firing rate coincident with the FER. Figure 10 shows data from two simultaneously recorded E-Plat neurons; the firing rates of neurons 121 and 124 decreased and increased, respectively, during the FER.

Eight neurons were silent during control periods, not identified as laryngeal motor or premotor cells, and discharged only during the FER (ER-Recruit). Four other neurons were designated E-Others; three increased and one showed no change in peak activity during the FER. Two inspiratory-expiratory phase-spanning neurons (E-I/E) were not activated during the FER.

**Rostral and Caudal VRG Inspiratory Neurons**

The recorded inspiratory-modulated neurons had augmenting, decrementing, plateau, expiratory-inspiratory phase-spanning (I-E/I), and other discharge patterns (Table 1). Eighty-three of 91 inspiratory neurons were not active during the FER (e.g., see neurons 41 and 72 in Fig. 8) and included some I-Aug and I-Dec cells that tested positive for spinal projections and others that had offset peaks in spike-triggered averages suggesting they were inspiratory laryngeal premotor and motor neurons. Eight neurons increased activity during the FER; these had negative bulbospinal and spike-triggered averaging tests.

**Respiratory Phase-Spanning Neurons**

Five cells showed peak firing rates at the transitions between respiratory phases. Three cells spanned the inspiratory and expiratory phases; two increased and one was not activated during the FER. Two cells spanned the expiratory to inspiratory phases; one increased and one decreased activity during the FER. None of these cells had features in their spike-triggered averages or antidromically activated spinal projections.

**Nonrespiratory Modulated Cells**

Six of 24 nonrespiratory-modulated neurons increased activity during the FER. None of the cells tested had significant spike-triggered average features or antidromically activated spinal projections.

**DISCUSSION**

The concurrent responses and inferred connectivity reported here support the hypothesis that elements of both the rVRG, including the Bötzinger and pre-Bötzinger complexes (Böt/rVRG), and the cVRG are involved in configuring motor patterns during the expiration reflex. When considered together...
with our previous reports on cough (1, 39, 40), the results are consistent with the concept of “functional plasticity” (multi-functional nature) of the respiratory network (14, 15, 43).

Respiratory Motor Pattern Changes During the FER

Although the primary expiratory component of the FER was apparent in all reported experiments, other changes in the respiratory pattern were also noted. Cough or an augmented inspiration occurred after the FER in approximately half of the recordings. These varied responses have been well documented previously and shown to be elicited by mechanical stimulation of the same site, the medial margins of the vocal folds [see reviews by Korpas and Tomori (28) and Korpas and Jakus (27)]. Available evidence suggests that rapidly adapting receptors in the subepithelial neuronal structure of the medial margins of the vocal folds produce these reflex responses (28). The mechanisms for these varied pattern changes are unknown. One possibility is that the motor changes depend on the duration and intensity of the applied stimulus.

The state (excitability) of the brain stem may also affect the characteristics of the response. Although the expiratory reflex is robust and resistant to anesthesia and antitussives (26), the “paired responses” that also include cough or augmented inspiration may be more vulnerable to the excitability of brain

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Fig. 6. Böth/VRG and cVRG E-Aug neurons, control cycle-triggered histograms (148 cycles averaged), and summary circuit of potential connectivity of neurons 41 and 47.
stem neural networks. In regard to laryngeal stimulation, Wid-dicombe stated “the type of response discovered was strongly influenced by wakefulness or sleep pattern” (46).

**Caudal VRG Expiratory Premotor/Bulbospinal Neurons**

A group of cVRG E-Aug neurons with positive spinal collision tests and offset peaks in lumbar spike-triggered averages showed increased firing rates coincident with lumbar motor activity during the FER. These results are consistent with a contribution to the enhanced activity of expiratory pump muscles during the expiration reflex and support the hypothesis that this subset of neurons serves a premotor function with respect to spinal expiratory motoneurons (Fig. 3D, summary circuit; Fig. 11, connection 1). Collectively, the data confirm and extend earlier reports (12, 16, 20, 21).

Bulbospinal decrementing expiratory neurons that also increased activity during the FER were not included in the model (Fig. 11). To our knowledge, there is no evidence in the literature that these neurons are premotor to spinal expiratory motoneurons; they may have inhibitory actions on spinal inspiratory motoneurons (Fig. 4, summary circuit). Furthermore, the propriobulbar neurons shaping their discharge pattern are unknown.

Additional expiratory neurons with unidentified axonal projections also increased activity simultaneously with lumbar nerve activity during the FER. These cells had augmenting, decrementing, plateau, and other discharge patterns and included some that were active only during the FER (ER-Recruit). This group of cells could include untested or nonactivated bulbospinal neurons, untested laryngeal premotor or motoneurons, or propriobulbar interneurons with unknown function.

**Putative Neurons Exciting/Inhibiting Bulbospinal/Premotor Expiratory Neurons**

Previous studies support the excitation of cVRG E-Aug neurons by Böt/rVRG E-Aug neurons during eupnea and cough (6, 7, 23, 30, 39, 40). In the present study, the pattern changes of a subset of rVRG E-Aug neurons during the FER was similar and coincident with increased activities in cVRG E-Aug neurons and the lumbar nerve. These neurons also increased activity during the expiratory phase of coughlike patterns that occurred subsequent to the FER. These cells had no features in their recurrent laryngeal or lumbar nerve spike-triggered averages and tested negative for spinal projections; they were considered to be putative propriobulbar (axons...
limited to the brain stem). These results are consistent with the observed rVRG E-Aug neurons being excitatory to cVRG premotor neurons (Fig. 6, summary circuit; Fig. 11, connection 2). Neurons proposed to have connections onto E-Aug premotor, as well as I-Aug premotor, ILM and ELM, neurons arise from the Böt/rVRG (“core”) network.

Another subset of putative propriobulbar Böt/rVRG E-Aug neurons that discharged primarily during the latter part of the expiratory phase became inactive during the FER; their activity returned after the FER in those trials with a delayed onset of inspiratory activity. A similar response has been observed in Böt/rVRG E-Aug neurons during cough (40). Böt/rVRG E-Aug neurons are also known to inhibit cVRG bulbospinal E-Aug neurons during eupnea (23, 30). In our cough model, we hypothesized that their attenuated activity during cough would lead to disinhibition of bulbospinal neurons (40). The decline in activity of Böt/rVRG E-Aug neurons during the FER is also suggestive of a similar disinhibitory action, leading to increased burst activity in cVRG bulbospinal E-Aug neurons (Fig. 7, summary circuit; Fig. 11, connection 3) and the lumbar nerve.

**Expiratory Laryngeal Motoneurons**

Laryngeal motoneurons identified with spike-triggered averaging of recurrent laryngeal nerve activity included rVRG E-Dec neurons and neurons recruited during the reflex (Fig. 8, summary circuit; Fig. 11, connection 4). The evoked changes

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**Fig. 8.** A: laryngeal motoneurons in Böt/rVRG and an inspiratory neuron in the cVRG with selected cycle triggered histograms (49 cycles averaged). ELM, expiratory laryngeal motoneuron; ILM, inspiratory laryngeal motoneuron. B and C: rectified and unrectified spike-triggered averages with short latency (4.0 ms; 21,489 trigger events) offset features supporting the role of motoneuron for cell 42. D: summary circuit of inferred connectivity for neurons 35, 42, and 43.
in firing rates of these cells during the FER matched the increase in integrated recurrent laryngeal nerve activity. These motoneurons activate laryngeal adductor muscles (thyroarytenoid) leading to narrowing of the glottis. Our observations are in general agreement with previous reports on the response of expiratory laryngeal motoneurons and thyroarytenoid muscles during the expiration reflex (28, 36, 44).

**Putative Expiratory Laryngeal Premotoneurons**

Previous studies showed that VRG E-Dec neurons are the only respiratory-modulated neurons exciting expiratory laryngeal motoneurons during eupnea (4, 37) and cough (1). In the present study, rVRG E-Dec neurons identified as laryngeal premotoneurons were excited during the FER coincident with recurrent laryngeal nerve discharge (Fig. 9, summary circuit; Fig. 11, connection 5). The similarity of the activity profiles of these neurons and expiratory laryngeal motoneurons during both eupnea and the FER support their function as premotor neurons.

Offset peaks were also observed in laryngeal nerve averages triggered by plateau and “recruited” expiratory neurons. Although these results, included for the sake of completeness, are suggestive of excitation of expiratory laryngeal motoneurons, there is to our knowledge no other evidence in the literature to support this inference. These neurons also increased activity coincident with the recurrent laryngeal nerve during the FER.

**Other Rostral VRG Expiratory Neurons**

Most of the rVRG E-Dec neurons were not identified as bulbospinal or laryngeal premotor and responded differently than E-Dec premotor neurons during the FER. This group may be considered putative propriobulbar neurons. The largest subset of these neurons ceased activity if they were discharging when the stimulus occurred; if they were inactive when the stimulus occurred, they remained inactive. There are E-Dec neurons in the core respiratory network of the rVRG proposed to influence respiratory phase timing and neuron discharge patterns through inhibitory actions on expiratory neurons (3, 13, 30). The observed group of cells discharged in the early expiratory (postinspiratory) phase during control recordings and could be involved in inhibition of expiratory laryngeal motoneurons and E-Aug neurons (Fig. 11, connections 6 and 7). Their inactivity during the FER would result in disinhibition of the target expiratory cells. In the suggested model, the cessation in activity of these E-Dec neurons during the FER would reflect inhibition by propriobulbar E-Aug neurons (30, 40) that were excited during the FER (Fig. 11, connection 8).

Another subset of unidentified E-Dec neurons, which discharged throughout the expiratory phase during control periods, remained active without any change in firing rate during the FER. A possible function of these cells could be suppression of inspiratory neurons and control of the expiratory phase (Fig. 11, connection 9).

Unidentified expiratory neurons with control plateau and phase-spanning discharge patterns responded during the FER with an increase, decrease or no change in peak firing rate. A few neurons that were silent during control periods were recruited during the FER. The functions of these types of cells are unknown.

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**Fig. 9.** Böt/RVRG I-Aug and E-Dec neurons with control cycle triggered histogram (89 cycles averaged), spike-triggered average (1.5 ms lag; 3.0 ms half width; 10,651 trigger events), and summary circuit representing potential connectivity for E-Dec neuron 61.
Inspiratory neurons of both the rVRG and cVRG tended to be silent during the FER. This observation has been reported previously (12, 16, 20, 21). All studies reported no activity in the diaphragm or phrenic nerve during the expiration reflex, thus no activity in medullary bulbospinal inspiratory neurons would be expected. The suppression of inspiratory neuron activity may, at least in part, reflect inhibition by E-Dec and E-Aug neurons (Fig. 11, connections 9, 10, and 11).

A few inspiratory cells were activated during the FER in the present study. They were not bulbospinal or associated with laryngeal motoneurons; their function is unknown. The present study and the work of Strański and Tomori (44) did not detect any activity in inspiratory laryngeal motoneurons during the FER in paralyzed, decerebrated, or anesthetized cats, respectively. A recent report by Poliacek et al. (36) showed that a burst of activity was regularly recorded in laryngeal abductor muscles (posterior cricoarytenoid) during the expulsive phase of ER in anesthetized, spontaneously breathing cats. The function would be to decrease airway resistance during the expulsive phase. The reason for the differences between these studies is unknown but could be related to the presence of neuromuscular blocking drugs in the first two studies.

Nonrespiratory Modulated Cells

A population of continuously active, nonrespiratory-modulated neurons increased activity during the FER. Their function is unknown; they may be interneurons transmitting afferent information to the respiratory network. These putative interneurons are not included in the ER model in Fig. 11.

Network Model for the Control of Expiratory Laryngeal Motoneurons and Spinal Premotoneurons During Expiration Reflex

Figure 11 shows a schematic model of the ventrolateral medullary respiratory neuronal network proposed by us to play a role in the generation of eupneic and cough motor patterns in pump and laryngeal muscles (1, 3, 39–42). Data from the present experiments support the participation of elements of this network in the expression of pump and laryngeal motor activity during the expiration reflex. The numbered connections are proposed interactions that occur during the expiration reflex. Connections onto spinal premotor (bulbospinal) neurons of the cVRG and laryngeal motoneurons of the rVRG and cVRG arise from the core network, which is composed of neurons of the Böt/rVRG. The following paragraphs give a summary of hypotheses, suggested and supported by the re-
results, that lead to excitation of expiratory pump and laryngeal motoneurons and inhibition of inspiratory activity. This model does not include proposed mechanisms for the brief excitation of inspiratory laryngeal motoneuron activity reported by Poliacek et al. (36).

Expiration reflex afferents in the larynx excite second-order neurons in the nucleus tractus solitarius (NTS) ER receptor 2nd-order neurons. E-Aug Early and E-Aug Late, neurons that begin discharging before and during the latter part of the expiratory phase, respectively. I-Drive, inspiratory neuron also active before the expiratory-inspiratory phase transition and with a relatively constant discharge rate throughout the inspiratory phase (I-Plat); definition specifically limited to Böt/rVRG neurons with previously identified excitatory functional links to other inspiratory neurons (3). Neuron connections onto ILM, ELM, I-Aug premotor, and E-Aug premotor neurons arise from the core network (enclosed by dotted line box). Arrows indicate change in peak firing rate during FER (↑, increase; ↓, decrease; →, no change). Locations of each population are labeled, indicating their position within the ventral respiratory group. Other abbreviations have been described in detail in the text. For detailed description of the core of the model, see Shannon and coworkers (39, 40).
expression of the reflex and suppression of inspiratory activity by E-Dec and E-Aug neurons (Fig. 11, connections 9, 10, and 11).

Other Putative Modulatory Influences on the Expiration Reflex

Nonrespiratory modulated bulbo spinal neurons located in the gigantocellular tegmental field of the medulla have been shown to be excited during the expiration reflex (12). The role of these neurons is unknown; they may also excite spinal respiratory neurons. A potential role for this region was suggested by the elimination of the reflex after kainic acid inactivation of cell bodies in the lateral tegmental field and adjoining areas (19). The reflex is also absent after kainic acid injection in the midline raphe nuclei (18) or ablation of the dorsal lateral pons (21). A decline in facilitatory input from raphe neurons to the Böt/VRG network was proposed to be responsible for the attenuation of the reflex. The effect of the pontine lesions could be due to the alteration in the breathing pattern and resultant decreased excitability of the medullary network to afferent input.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical support of Jan Gilliland, Rebecca McGowan, Kimberly Ruff, Kathryn Hodgson, Andrew Ross, Zhongzeng Li, and Peter Barnhill.

A preliminary account of the results has been reported (2).

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grant HL-49813.

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