Respiratory neuronal activity during apnea and poststimulatory effects of laryngeal origin in the cat

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Bongianni, Fulvia, Donataella Mutolo, Marco Carfi, Giovanni A. Fontana, and Tito Pantaleo. Respiratory neuronal activity during apnea and poststimulatory effects of laryngeal origin in the cat. J Appl Physiol 89: 917–925, 2000.—We investigated the behavior of medullary respiratory neurons in cats under pentobarbitone anesthesia, vagotomized, paralyzed, and artificially ventilated to elucidate neural mechanisms underlying apnea and poststimulatory respiratory depression induced by superior laryngeal nerve (SLN) stimulation. Inspiratory neurons were completely inhibited during SLN stimulation and poststimulatory apnea. During recovery of inspiratory activity, augmenting inspiratory neurons were depressed, decrementing inspiratory neurons were excited, and late inspiratory neurons displayed unchanged bursts closely locked to the end of the inspiratory phase. Augmenting expiratory neurons were either silenced or displayed different levels of tonic activity during SLN stimulation; some of them were clearly activated. These expiratory neurons displayed activity during poststimulatory apnea, before the onset of the first recovery phrenic burst. Postinspiratory or decrementing expiratory neurons were activated during SLN stimulation; their discharge continued with a decreasing trend during poststimulatory apnea. The results support the three-phase theory of rhythm generation and the view that SLN stimulation provokes a postinspiratory apnea that could represent the inhibitory component of respiratory reflexes of laryngeal origin, such as swallowing. In addition, because a subpopulation of augmenting expiratory neurons displays activity during SLN stimulation, the hypothesis can be advanced that not only postinspiratory, or decrementing expiratory neurons, but also augmenting expiratory neurons may be involved in the genesis of apnea and poststimulatory phenomena. Finally, the increase in the activity of decrementing inspiratory neurons after the end of SLN stimulation may contribute to the generation of poststimulatory respiratory depression by providing an inhibitory input to bulbospinal augmenting inspiratory neurons.

STUDIES PERFORMED IN NEWBORN ANIMALS have shown that apnea-producing superior laryngeal nerve (SLN) electrical stimulation can activate an inhibitory memory-like process that produces poststimulatory apnea followed by prolonged respiratory depression in phrenic nerve activity (20, see Ref. 13 for review). Recently, we have provided characterization of poststimulatory respiratory phenomena induced by SLN stimulation using a quantitative analysis of their development as well as of the recovery process of inspiratory activity in the adult cat (25).

Richter and colleagues (21, 32, 33, 36) have shown that, in various animal species, the central respiratory rhythm generation involves three phases: one phase of inspiration and two phases of expiration, termed stage I or postinspiration (passive expiration) and stage II (active expiration). Both chemical and electrical stimulation of laryngeal afferents prolong the duration of the stage I expiration in the cat (31). Furthermore, medullary postinspiratory neurons are activated by SLN stimulation (31, 34). The hypothesis has been advanced that stimulation of laryngeal afferents holds the whole respiratory network in stage I expiration; i.e., it causes a postinspiratory apneic state (31). A three-phase pattern of breathing, as well as the effects of electrical and chemical stimulation of laryngeal afferents, have been confirmed in neonatal animals (namely, piglets) by recording the activity of ventral respiratory neurons (11, 22). Laryngeal stimulation induces apnea, activation of postinspiratory neurons, and stable membrane potentials in medullary inspiratory and expiratory cells consistent with postinspiratory inhibition; usually the membrane potential of each neuronal type shows levels characteristic of stage II expiration before the onset of the first recovery phrenic ramp after such apneic periods (22). These results support the three-phase theory of rhythm generation, as well as the hypothesis that laryngeal afferent stimulation induces a postinspiratory apnea (31, 33).

In the present study, an attempt was made to investigate the discharge patterns of different types of medullary respiratory neurons (see Refs. 6, 33, and 36a for review) during apnea and poststimulatory respiratory depression induced by SLN stimulation in the adult cat. The aim was to gain further insights into the neuronal processes underlying these phenomena. To
this purpose, the activity of medullary respiratory neurons was recorded from the dorsal respiratory group (DRG), as well as from different components of the ventral respiratory group (VRG), i.e., the rostral expiratory neurons of the Bötzing complex, the expiratory population of the caudal VRG (cVRG), and the intermediate inspiratory portion of the VRG (iVRG).

**METHODS**

**Animal preparation.** Experiments were performed on 16 cats of both sexes (2.6–3.8 kg) anesthetized with pentobarbital sodium (35 mg/kg intraperitoneally, supplemented by 2–4 mg·kg⁻¹·h⁻¹ intravenously; Nembutal, Abbott, Saint-Remy-sur-Avre, France). All animal care and experimental procedures were conducted in accordance with the Italian legislation and the official regulations of the European Communities Council on the use of laboratory animals (Directive 86/609/EEC).

The trachea was cannulated, and polyethylene catheters were inserted into a femoral artery and vein for the measurement of arterial blood pressure and the administration of drugs, respectively. Both C5 phrenic roots were dissected free and cut distally. Both vagosympathetic trunks were cut in the neck. Both SLNs (internal branches) were exposed, dissected free, and cut distally. The animals were then placed in a prone position, held by vertebral clamps and a stereotaxic head holder. Their head was ventroflexed at an angle of 45°, relative to the Horsley-Clarke horizontal plane, to facilitate recordings from the medulla. The dorsal surface of the medulla was widely exposed by occipital craniotomy. The posterior part of the cerebellum was removed by gentle suction to provide access to the rostral part of the medulla. All exposed tissues were covered with warm paraffin oil (37–38°C). The animals were vagotomized, paralyzed with gallamine triethiodide (5 mg/kg intravenously, supplemented with 2 mg · kg⁻¹·h⁻¹, Sigma Chemical), and artificially ventilated. The adequacy of anesthesia was assessed by a stable and regular pattern of phrenic discharge and by the absence of fluctuations in arterial blood pressure or phrenic nerve activity in response to nociceptive stimulation. Body temperature was maintained at 37 ± 0.5°C by a heating pad controlled by a rectal thermistor probe.

**Recording procedures.** Efferent phrenic activity was recorded from desheathed C5 phrenic roots placed on bipolar platinum electrodes, amplified, full-wave rectified, and “integrated” using a resistance-capacitance circuit leaking, with a time constant of 100 ms to obtain a “moving average” of the activity usually referred to in the literature as “integrated activity” (see Refs. 7–9, 18, 20, 22, 31, 34). Extracellular recordings from medullary neurons were made with tungsten microelectrodes (5–12 MΩ impedance, as tested at 1 kHz). As far as possible, single-unit activity was recorded from relatively pure inspiratory or expiratory neuronal populations (see Refs. 6, 13). Thus recordings were performed from the Bötzing complex (3.8–5.2 mm rostral to the obex, 2.8–3.6 mm lateral to the midline, and 3.7–5.2 mm below the dorsal medullary surface), from the iVRG (0.5–2.5 mm rostral to the obex, 2.4–3.0 mm lateral to the midline, and 2.4–3.5 mm below the dorsal medullary surface), and from the DRG (0.5–2.0 mm rostral to the obex, 2.0–2.5 mm lateral to the midline, and 1.5–2.0 mm below the dorsal medullary surface). On some occasions, to discriminate single-unit action potentials before further processing, a window discriminator that provided an output of standard pulses was used. Discharges from single neurons were amplified and processed in the same way as phrenic activity; integrated neuronal activity provided a unidirectional signal, the amplitude of which gave an approximate quantitative evaluation of neuronal firing rate and could not be characterized in terms of calibration, because its amplitude depended not only upon the frequency of discharge but also upon the amplitude and duration of action potentials. The amplitude and duration of single-unit action potentials vary during an individual trial, especially when changes in discharge frequency occur. This could be overcome by using a window discriminator with standard pulse output. However, because we were mainly focused on the qualitative aspects of neuronal behavior, we did not systematically use the window discriminator. Extracellular action potentials were, in all probability, recorded from cell bodies because they displayed biphasic or triphasic shapes, relatively high amplitudes (200–450 μV), and long durations (≥1 ms); furthermore, recordings were stable and could be held over a long (50–100 μm) microelectrode travel (see Ref. 19 for further references). In some experiments, recordings from cell bodies were made with small cathodal electrolytic lesions (20 μA for 20 s) to facilitate later histological identification. Phrenic and neuronal activities were monitored as “raw” signals on an oscilloscope (model 5112, Tektronix, Beaverton, OR) and fed to an audiometer. Strain-gauge manometers were used for monitoring arterial blood pressure and intratracheal pressure. End-tidal fractional concentration of CO₂ was measured by an infrared CO₂ analyzer (Datex CD-102, Normocap, Helsinki, Finland). End-tidal CO₂ was kept at desired levels (4–5%) by adjusting the frequency and stroke volume of the respiratory pump. Integrated phrenic and neuronal activities, as well as the signals of the other variables studied, were recorded on an eight-channel rectilinearly writing chart recorder (model 8K20, NEC San-ei, Tokyo, Japan). On some occasions, the raw signals of phrenic and neuronal activities were displayed on an oscilloscope (model 5112, Tektronix) and photographed by means of a kymograph camera.

**Stimulation procedures.** Bipolar silver electrodes were used for electrical stimulation of the left or right SLN. Rectangular pulses (0.5-ms duration) were delivered by a stimulator (S8, Grass Instruments, Quincy, MA) driven by a stimulator (S8, Grass Instruments). Current intensity was monitored as voltage drop through a small-series resistor using a differential amplifier and an oscilloscope. The threshold intensity was defined as the lowest stimulus strength necessary to induce persistent apnea (≥5 s). Tetanic SLN stimulation, at an intensity of 2 × threshold intensity (15–30 μA) and frequency of 20 Hz, was applied for durations ranging from 5 to 60 s to induce apnea. Stimulations of different duration were applied in random order; their onsets were always during the expiratory time. After each trial, at least 6 min of recovery were allowed before the application of another train of stimuli (25).

Antidromic stimulation of the vagus nerve or SLN was used to identify vagal or laryngeal motoneurons and to exclude them from this study. Only six neurons with respiration-related discharge patterns were found to be antidromically activated by vagus nerve stimulation; they were encountered in different sites of the VRG. Bipolar silver electrodes were used for electrical stimulation of both vagus and SLN, both ipsi- and contralateral to the sites of neuronal recordings. Rectangular pulses (0.1-ms duration, intensity up to 200 μA) were used. Stimulation was performed with single shocks or trains of stimuli (200 Hz, 40–100 ms). Standard criteria for antidromic activation of medullary respiratory neurons were used (23) and, in particular, the
ability to follow high-frequency stimulation (200 Hz) and collision tests (23, 30). No attempt was made to investigate other axonal projections of sampled neurons, such as those to the glossopharyngeal nerve or to the pharyngeal branches of the vagus nerve (see Ref. 6 for review), because of the obvious difficulties in maintaining single-unit recordings for long periods.

Histological control of recording sites. The histological analysis was performed on 11 preparations. At the end of the experiment, the brain was perfused with 0.9% NaCl solution and then with 10% formalin solution via a carotid artery. After at least 48 h of immersion in 10% formalin solution, the brain was placed in a hypertonic sucrose solution. Frozen serial sections 50 μm thick were made in the frontal plane to confirm, according to the atlas of Berman (5), the stereotaxically located recording sites.

RESULTS

SLN stimulation produced apnea that outlasted the stimulation period (poststimulatory apnea); when respiration resumed, it was depressed, as revealed mainly by decreases in phrenic minute output, rate of rise of inspiratory activity, and respiratory frequency due to increases in both inspiratory and expiratory times (Fig. 1). We reported, in a previous study (25), a quantitative analysis of both the magnitude and time course of poststimulatory depression induced by SLN stimulation. As already shown (25), the intensity of such respiratory depression proved to be a function of the duration of SLN stimulation, whereas phrenic output recovered gradually to control levels after the end of SLN stimulation.

In the present study, our attention was mainly focused on the qualitative aspects of the behavior of medullary respiratory neurons during apnea and poststimulatory effects induced by SLN stimulation. To provide a more complete and effective description, neuronal discharge patterns have been represented in the figures, as far as possible, not only as integrated traces but also as raw signals.

A total of 80 respiratory units were studied. Stereotaxic coordinates and extracellular recordings of neuronal discharge patterns allowed us to localize the recording sites within the different medullary respiratory regions. The location of recorded units was confirmed in 11 preparations by histological analysis, which revealed that the recording sites were actually within the desired medullary respiratory regions corresponding to those described in previous literature (6, 36a). In more detail, Bötzinger complex units were found in the vicinity of the retrofacial nucleus, iVRG units in the para-ambigualis region, cVRG neurons in the region of the nucleus retroambigualis, and DRG units in the ventral and ventrolateral aspects of the nucleus tractus solitarii. Respiratory units of each type were grouped according to their behavior during SLN stimulation to make description easier. Data are summarized in Table 1; the amplitude of the first neuronal burst after apnea and poststimulatory apnea has been taken as an index of the intensity of neuronal discharge during poststimulatory depression of respiratory activity.

Inspiratory neurons of different types were encountered, i.e., augmenting, late and early, or decrementing. All tested inspiratory neurons (n = 30) were completely inhibited during the apneic period (i.e., during SLN stimulation and poststimulatory apnea). During the recovery period, augmenting inspiratory neurons (DRG, n = 4; iVRG, n = 17) were depressed and displayed a delayed onset compared with that of phrenic nerve activity (Fig. 1A); depressant phenomena consisted of slight decreases in peak amplitude and marked decreases in rate of rise of their activity, which displayed obvious increases in duration. They recovered progressively in parallel with phrenic motor output. Late inspiratory neurons, i.e., with late onset of firing, were encountered in the DRG (n = 2) or in the iVRG (n = 3). During the recovery period, despite considerable increases in inspiratory duration, their firing patterns remained closely locked to the end of the inspiratory phase, without appreciable changes in the duration and intensity of neuronal discharge (Figs. 1B and 2B). This phenomenon was particularly evident (Fig. 2B) after long-lasting stimulation periods lead to marked depression of the first recovery breath (25). Early or decrementing inspiratory neurons encountered in the iVRG (n = 4) displayed, after the apneic period, excitatory responses consisting of increases in the duration and intensity of their discharge. Their activity recovered gradually, with a time course similar to that of phrenic motor output (Figs. 1C and 2A). No inspiratory neurons were encountered in the Bötzinger complex region, probably due to the deep anesthesia of our preparations (6, 16).

![Fig. 1. Behavior of medullary inspiratory neurons during apnea and poststimulatory respiratory depression induced by 10-s superior laryngeal nerve (SLN) stimulation. Inspiratory motor output was monitored as integrated phrenic nerve activity (IPA). Integrated discharge patterns allowed us to localize the recording sites within the different medullary respiratory regions. The location of recorded units was confirmed in 11 preparations by histological analysis, which revealed that the recording sites were actually within the desired medullary respiratory regions corresponding to those described in previous literature (6, 36a). In more detail, Bötzinger complex units were found in the vicinity of the retrofacial nucleus, iVRG units in the para-ambigualis region, cVRG neurons in the region of the nucleus retroambigualis, and DRG units in the ventral and ventrolateral aspects of the nucleus tractus solitarii. Respiratory units of each type were grouped according to their behavior during SLN stimulation to make description easier. Data are summarized in Table 1; the amplitude of the first neuronal burst after apnea and poststimulatory apnea has been taken as an index of the intensity of neuronal discharge during poststimulatory depression of respiratory activity.

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A total of 50 units with expiration-related discharge patterns were sampled. During SLN stimulation, augmenting expiratory neurons (n = 45) were either silenced or displayed different levels of tonic activity (Figs. 3 and 4, B and C). Among these neurons, 16 were completely inhibited (Bötzinger complex, n = 10; cVRG, n = 6), 20 showed a tonic level of activity similar to (Bötzinger complex, n = 4; cVRG n = 2) or lower (Bötzinger complex, n = 7; cVRG, n = 7) than the peak level of control breaths, and 9 were clearly activated (Bötzinger complex, n = 7; cVRG, n = 2), displaying a tonic level of activity higher than during control expiration. Despite these different behaviors during SLN stimulation, all augmenting expiratory neurons displayed activity during poststimulatory apnea, before the onset of the first recovery phrenic burst. Neurons presenting tonic activity during SLN stimulation could maintain or increase their level of activity, showing a ramp discharge pattern during poststimulatory apnea (Figs. 3, A and B, and 4B). In the recovery period, neurons presenting tonic activity lower than or similar to that of control expiratory activity showed a decrease in their peak activity and a subsequent progressive recovery. On the contrary, neurons activated by SLN stimulation displayed bursts of larger duration and higher discharge frequency (Figs. 3A and 4B) that progressively resumed control values in parallel with the recovery of the expiratory time. Neurons com-

### Table 1. Behavior of medullary inspiratory and expiratory neurons during apnea and poststimulatory respiratory phenomena induced by SLN stimulation

<table>
<thead>
<tr>
<th>Respiratory Neurons</th>
<th>n</th>
<th>Activity During SLN Stimulation</th>
<th>Activity During Poststimulatory Apnea</th>
<th>Amplitude of the First Recovery Burst³</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-AUG</td>
<td>4 (DRG)</td>
<td>Silenced</td>
<td>Silenced</td>
<td>Lower</td>
</tr>
<tr>
<td></td>
<td>17 (iVRG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-LATE</td>
<td>2 (DRG)</td>
<td>Silenced</td>
<td>Silenced</td>
<td>Unchanged</td>
</tr>
<tr>
<td></td>
<td>3 (iVRG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-DEC</td>
<td>4 (iVRG)</td>
<td>Silenced</td>
<td>Silenced</td>
<td>Higher</td>
</tr>
<tr>
<td></td>
<td>10 (Böt c)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6 (cVRG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-AUG</td>
<td>7 (Böt c)</td>
<td>Tonic, low level²</td>
<td>Ramp</td>
<td>Lower</td>
</tr>
<tr>
<td></td>
<td>7 (cVRG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (Böt c)</td>
<td>Tonic, control level³</td>
<td>Tonic</td>
<td>Lower</td>
</tr>
<tr>
<td></td>
<td>2 (cVRG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-DEC</td>
<td>7 (Böt c)</td>
<td>Tonic, high level⁴</td>
<td>Tonic</td>
<td>Higher</td>
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<td></td>
<td>2 (cVRG)</td>
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</tr>
<tr>
<td></td>
<td>3 (Böt c)</td>
<td>Tonic, high level⁴</td>
<td>Tonic</td>
<td>Higher</td>
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<tr>
<td></td>
<td>2 (cVRG)</td>
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</table>

I-AUG, augmenting inspiratory neurons; I-LATE, late inspiratory neurons; I-DEC, decrementing inspiratory neurons; E-AUG, augmenting expiratory neurons; E-DEC, decrementing expiratory neurons. DRG, dorsal respiratory group; iVRG, intermediate portion of the ventral respiratory group; Böt c, Bötzinger complex; cVRG, caudal ventral respiratory group; n, number of neurons. ¹As compared with control activity. ²Lower than the peak level of control expiratory activity. ³Similar to the peak level of control expiratory activity. ⁴Higher than the peak level of control expiratory activity.
pletely inhibited during SLN stimulation showed a poststimulatory pause and, thereafter, a resumption of their ramp activity before the first recovery phrenic burst (Figs. 3C and 4C). At the beginning of the recovery period, their peak activity was lower than control levels and then displayed a progressive recovery. According to our previous report (25), poststimulatory apnea increased by increasing the stimulation period, reaching its maximum levels for stimulations lasting 30–40 s and decreasing thereafter. Correspondingly, an increase was also observed in the duration of the activity of augmenting expiratory neurons (see Fig. 4, B and C).

During SLN stimulation, decrementing expiratory neurons or postinspiratory neurons encountered either in the Bötzinger complex \((n = 3)\) or in the iVRG \((n = 2)\) displayed a tonic level of activity higher than the peak level displayed during control breaths. After the end of SLN stimulation, the discharge continued during poststimulatory apnea, with a decreasing trend (Fig. 4A). At the beginning of the recovery period, the activity of these neurons displayed increases in frequency and duration (Figs. 3 and 4), progressively resuming control values thereafter.

**DISCUSSION**

This report describes the behavior of different types of medullary respiratory neurons during apnea and poststimulatory respiratory depression induced by SLN electrical stimulation in the adult cat. Extensive literature reports results obtained with electrical stimulation of the SLN (see Refs. 8, 22, 25, 37); however, the functional type of receptors corresponding to the afferent fibers affected by electrical stimuli is quite uncertain. Doubts even remain as to which laryngeal receptors are responsible for respiratory reflexes evoked by more natural stimuli applied to the laryngeal mucosa (37). Chemosensitive and irritant-sensitive endings appear to be the most likely candidates for the apneic response, although other types of receptors, such as mechanosensitive endings, may contribute to this response (25, 37). Electrical stimulation of the SLN cannot be considered the equivalent of physiological stimulation of peripheral receptors; however, the reflex responses closely resemble those obtained with physiological stimuli (see Refs. 8, 22, 25, 37). Electrical stimulation has the advantage over mechanical and chemical stimulation in that the strength and the du-
ration of the stimulus can be more precisely and easily controlled. In addition, it avoids the confounding problem of the possible persistence of receptor activation, for instance, after withdrawal of chemical stimuli, and its role in causing prolonged apneic effects (11, 13, 22, 25). The relatively low intensities of stimulation used suggest that the largest diameter laryngeal afferents capable of inhibiting central inspiratory activity were involved; this group of afferents includes water-sensitive, as well as a variety of mechanically activated, afferents (24). Repetitive electrical stimulation of SLN at low frequency may evoke swallowing, coughing, and even prolonged apnea (6, 8, 11, 15, 20, 25, 28, 31, 37). Nevertheless, the most common response in deeply anesthetized animals seems to be prolonged apnea (37); increasing depth of anesthesia progressively depresses the swallowing reflex (26). Moreover, chemical and mechanical stimulation of laryngeal afferents provokes apnea in anesthetized animals with thresholds lower than those needed for cough or expiratory reflexes (37). In addition, in pentobarbitone-anesthetized, spontaneously breathing animals, our laboratory did not succeed in evoking obvious swallowing either in previous studies (8) or in the preliminary trials of the present study using the same stimulation parameters. Also, in our deeply anesthetized animals, we never observed the occurrence of short bursts of phrenic nerve activity during the prolonged SLN-induced apnea, i.e., so-called phrenic “breakthroughs” that are probably due to the activation of the swallowing reflex during SLN stimulation (18, 26).

In an attempt to provide a coherent summary of our results and related hypotheses, we especially considered the models of the medullary network underlying respiratory rhythm generation proposed by Rybak et al. (35), whose performances extensively predict the behaviors of the different types of medullary respiratory neurons during SLN stimulation. We also took into account the models by Balis et al. (2) and by Ogilvie et al. (27) in the interpretation of some features of neuronal responses. In all three-phase models by Rybak et al. (35), SLN afferent stimulation excites postinspiratory neurons and inhibits all other types of inspiratory and expiratory medullary neurons. Simulations performed with these models have shown that decrementing expiratory neurons and postinspiratory neurons may have different roles in respiratory pattern generation. Nevertheless, because the difference between the firing patterns of these neurons is not significant, we considered these two neuronal types equivalent in accordance with previous suggestions (6, 27, 36a). Keeping this equivalence in mind, our results fit the outcomes of the simulations performed with the models by Rybak et al. (35) quite well for all types of inspiratory neurons, for postinspiratory neurons, and, at least in part, for augmenting expiratory neurons.

The present data on inspiratory neurons confirm and extend previous findings (4, 10, 12, 22). In more detail, they show that not only augmenting inspiratory neurons, but also late and early inspiratory neurons, are completely inhibited during SLN stimulation and post-stimulatory apnea (see Figs. 1 and 2). The augmenting inspiratory neurons that we sampled could be either propriobulbar neurons or bulbospinal premotoneurons. Bulbospinal inspiratory neurons are expected to start their discharge at or near the onset of phrenic nerve activity. However, a number of them may have a different threshold of recruitment, and therefore a delayed onset (10), to progressively activate phrenic motoneurons and generate the phrenic ramp. Thus the finding that all sampled augmenting inspiratory neurons displayed a delayed onset compared with that of phrenic nerve activity during the recovery period suggests that most of recorded neurons are not bulbospinal neurons; nevertheless, at least some of them may be bulbospinal premotoneurons presenting a rise in their threshold of activation due to SLN stimulation. Late inspiratory neurons encountered in the present study recall type I late inspiratory neurons, the firing patterns of which remain closely locked to the end of inspiration despite considerable changes in the inspiratory duration produced by lung afferent excitation (10). These neurons have been suggested to be involved in promoting the inspiratory off-switch (10) and are also considered to play this role in the models by Rybak et al. (35). However, because we did not test late inspiratory neurons for vagal influences, we do not know whether these neurons correspond to those described by Cohen et al. (10). Interestingly, early inspiratory neurons displayed excitation and gradual recovery after poststimulatory apnea of laryngeal origin; the increase in their activity may be due to a postinhibitory rebound, which is in agreement with the suggestions reported by Lawson et al. (22) in their study on piglets. In addition, the pattern of discharge of early inspiratory neurons after the end of SLN stimulation indicates that these neurons may contribute to the generation of poststimulatory respiratory depression and increases in inspiratory time by providing an inhibitory input to bulbospinal augmenting inspiratory neurons and, hence, to phrenic motoneurons. The gradual recovery of the activity of these neurons may have a role in the modulation of the recovery process of inspiratory activity (6). The inhibitory influences exerted by early inspiratory neurons are not considered in the models of Rybak et al. (35). However, it is worth mentioning that our interpretation is consistent with the simulations performed with the model reported by Balis et al. (2). Moreover, according to the three-phase theory proposed by Richter et al. (33) and the models by Rybak et al. (35), the observed increases in the activity of early inspiratory neurons during the recovery period contribute to the inhibition of late inspiratory neurons, the delay in onset of their discharge, and, hence, the increase in inspiratory time. Finally, the inhibitory influences of early inspiratory neurons on expiratory neurons (2, 27, 33, 35) may conceivably contribute to shape the pattern of expiratory neuronal activity during the recovery period.

As to the expiratory neurons, we believe that augmenting expiratory neurons encountered in the cVRG most likely belong to the population of bulbospinal
expiratory neurons that are densely packed in this region (6, 36a). However, the possibility exists that some of them are propriobulbar expiratory neurons (1). The location and discharge patterns of rostral augmenting expiratory neurons sampled in this study suggest that they belong to the same Bötzinger complex population of propriobulbar and bulbospinal neurons previously studied (6, 7, 36a). Some of the sampled augmenting expiratory neurons could also be pharyngeal motoneurons (6, 7, 36a); however, we believe that it is uncommon to encounter such neurons in decerebrate cats (7) and even more in anesthetized cats, as most cranial motoneurons with respiratory pattern of activity are depressed and seldom fire spontaneously, especially in barbiturate-anesthetized preparations (6, 36a).

As already mentioned, the present findings on neurons active in the expiratory phase are, to a great extent, consistent with the simulations performed with the models by Rybak et al. (35), which, on the other hand, are largely based on Richter's three-phase theory of rhythm generation (31, 33, 34). Indeed, our results are in keeping, at least in part, with those of previous studies performed on piglets or adult cats (11, 22, 31, 34). These studies show that electrical SLN stimulation, as well as laryngeal receptor activation by chemical stimuli (such as water, diluted hydrochloric acid solution, and smoke), activates postsynaptic neurons that are inhibitory for both inspiratory and expiratory medullary neurons (3, 11, 22, 33, 34). Accordingly, our results show that SLN stimulation activates postsynaptic neurons and inhibits not only all inspiratory neurons but also part of both rostral and caudal augmenting expiratory neurons. However, at variance with previous results (11, 22, 31) as well as with the performances of the models by Rybak et al. (35), tonic activity that could reach relatively high levels was observed in some rostral and caudal expiratory neurons during SLN-induced apnea. Thus, whereas on one hand our data support the notion that SLN stimulation produces apnea (31), on the other hand, they are in contrast with the finding that postsynaptic effects of sln stimulation may suggest that they share a common role with postinspiratory or decrementing expiratory neurons. Noticeably, a subset of augmenting expiratory neurons whose excitation is characteristic of stage I expiration induced by cervical vagus nerve stimulation, cVRG expiratory neurons were released from inspiratory inhibition and remained at an intermediate level of membrane potential (tonic activation) that is characteristic of stage I expiration. At this time, the reasons for the discrepancies in the results of these studies are not clear (17, 18). Neural responses to single stimuli applied to the SLN may be relevant to the present discussion and help in the interpretation of our findings. In fact, previous studies have reported both inhibitory (8, 29) and excitatory (7) effects on Bötzinger complex augmenting expiratory neurons. Inhibitory effects on cVRG augmenting expiratory neurons have been described (17). On the other hand, postsynaptic potentials evoked in medullary expiratory neurons of the piglet (11) displayed either early hyperpolarization or early hyperpolarization followed by late depolarization, thus revealing that respiratory neurons receive both a fast initial input derived reflexly from laryngeal afferents and late inputs that probably represent the involvement of the whole respiratory network in the response. Thus these findings (11) show that the membrane potential of expiratory neurons during SLN-induced apnea may represent a net effect of excitatory and inhibitory inputs activated by SLN afferents. Similarly, the level of tonic expiratory activity observed in our experiments may result from a balance of excitatory and inhibitory inputs derived by complex multisynaptic interactions brought into action by SLN stimulation. The finding of tonic activation of a relatively large number of caudal and rostral augmenting expiratory neurons during SLN stimulation may suggest that they share a common role with postsynaptic or decrementing expiratory neurons in generating apnea and poststimulatory effects of laryngeal origin. This subpopulation of augmenting expiratory neurons activated by SLN stimulation is not included in the models by Rybak et al. (35). We believe that it would be of interest to analyze the performance of a three-phase model that includes such a population of expiratory neurons. Noticeably, a subset of augmenting expiratory neurons whose excitation produces apnea is considered in the two-phase model by Balis et al. (2).

Interestingly, all expiratory neurons display activity during poststimulatory apnea before the onset of the first recovery phrenic burst; this phenomenon is particularly evident in expiratory neurons either partially or completely inhibited by SLN stimulation (Figs. 3 and 4) and clearly indicates that these neurons may have to complete their three-phase pattern (i.e., to always present an activity characteristic of stage II expiration) before the onset of the first recovery phrenic burst. These results extend previous observations in the piglet (22) to the adult cat and are in agreement with the finding that postsynaptic neurons display chloride-mediated postsynaptic potentials characteristic of stage II expiration before the first recovery phrenic burst after apnea induced by SLN stimulation (31). It has been proposed that respiratory
rhythm generation involves reciprocal inhibition between postinspiratory and early inspiratory neurons (33). However, the present results, in agreement with previous findings obtained in piglets (22), also suggest that stage II expiratory mechanisms are of importance in the control of respiratory rhythm. Inhibitory connections of augmenting and decrementing expiratory neurons with augmenting inspiratory cells may well account for the occurrence and duration of poststimulatory apnea (see Refs. 33 and 35). Similarly, the enhanced discharge of decrementing expiratory neurons and of a subset of augmenting expiratory neurons (Figs. 3 and 4) may help in the interpretation of some features of poststimulatory inspiratory depression, such as, for instance, the increase in the expiratory time. Furthermore, an increase in the intensity and duration of the discharge of decrementing expiratory neurons and their mutual inhibitory connections with early inspiratory neurons may contribute to explain the increase in the expiratory time (35).

The interpretation of present results has been mainly addressed to support the three-phase theory of rhythm generation. In addition, apnea induced by SLN stimulation has been interpreted as a postinspiratory apneic state (31). As already mentioned, SLN stimulation can induce swallowing and coughing (6, 8, 11, 15, 20, 25, 28, 31), although these latter reflexes are apparently relatively rare or do not occur at all in deeply pentobarbitone-anesthetized preparations. Even in the absence of manifest swallowing, the inhibitory components of this reflex could be still present and cause inhibitory effects on respiration or even apnea.

In conclusion, the present findings are consistent, to a great extent, with the three-phase theory of rhythm generation and with the view that SLN stimulation provokes a postinspiratory apnea. The latter could be an expression of the inhibitory component of the neural mechanisms subserving swallowing in unanesthetized or slightly anesthetized animals. Moreover, because not only postinspiratory or decrementing expiratory neurons but also part of the augmenting expiratory neurons are activated during SLN stimulation, our results suggest that a subpopulation of augmenting expiratory neurons may have a role in the genesis of apnea and poststimulatory phenomena. The increase in the activity of decrementing inspiratory neurons after the end of SLN stimulation may contribute to generate poststimulatory respiratory depression and to modulate, with its progressive decay, the recovery process of augmenting inspiratory neurons and phrenic motoneurons. Finally, the present finding that augmenting expiratory neurons present after the end of SLN stimulation, an activity characteristic of stage II expiration, extends to the adult cat, for the first time, previous observations obtained in the piglet (22).

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