Respiratory-related neurons of the fastigial nucleus in response to chemical and mechanical challenges

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Xu, Fadi, and Donald T. Frazier. Respiratory-related neurons of the fastigial nucleus in response to chemical and mechanical challenges. J. Appl. Physiol. 82(4): 1177–1184, 1997.—Responses of cerebellar respiratory-related neurons (CRRNs) within the rostral fastigial nucleus and the phrenic neurogram to activation of respiratory mechano- and chemoreceptors were recorded in anesthetized, paralyzed, and ventilated cats. Respiratory challenges included the following: 1) cessation of the ventilator for a single breath at the end of inspiration (lung inflation) or at functional residual capacity, 2) cessation of the ventilator for multiple breaths, and 3) exposure to hypercapnia. Nineteen CRRNs having spontaneous activity during control conditions were characterized as either independent (basic, n = 14) or dependent (pump, n = 5) on the ventilator movement. Thirteen recruited CRRNs showed no respiratory-related activity until breathing was stressed. Burst durations of expiratory CRRNs were prolonged by sustained lung inflation but were inhibited when the volume was sustained at functional residual capacity; it was vice versa for inspiratory CRRNs. Multiple-breath cessation of the ventilator and hypercapnia significantly increased the firing rate and/or burst duration concomitant with changes noted in the phrenic neurogram. We conclude that CRRNs respond to respiratory inputs from CO2 chemosensitive and pulmonary mechanoreceptors in the absence of skeletal muscle contraction.

respiratory control; cerebellum; hypercapnia; lung inflation; movement of skeletal muscles

The relative importance of the cerebellum in the regulation of responses to respiratory challenges has received previous attention. Partial or whole ablation of the cerebellum significantly attenuated the ventilatory responses to hypercapnia or hypoxia in cats and dogs, primarily by reduction of respiratory frequency (14, 22, 23). In vagotomized cats, cerebellectomy inhibited the diaphragmatic response to inspiratory tracheal occlusion, and this inhibition was significantly diminished by spinal cord dorsal rhizotomies at C3–7 (6, 24). Cerebellar respiratory-related neurons (CRRNs) have been reported in the cerebellum of the carp (1) and, more specifically, in the rostral fastigial nucleus (FNR) of spontaneously breathing cats (7, 11). With use of extracellular recording, investigators (11) observed that units in the FNR with spontaneous phasic activity correlated with the respiratory rhythm. About one-half of the CRRNs were responsive to intracarotid infusion of sodium cyanide and tracheal occlusion applied for several breaths. These results suggest that the cerebellum (FNR) is capable of modulating respiratory chemosensitive and mechanoreflexes. CRRNs can be driven by activation of carotid body chemoreceptors and stimulation of mechanoreceptors (respiratory muscle). However, questions still remained as to whether CRRNs are responsive to activation of CO2-sensitive chemoreceptors and/or vagal pulmonary mechanoreceptors and whether spontaneously active CRRNs depend on inputs emanating from contracting respiratory and other skeletal muscles.

To examine these questions, anesthetized, paralyzed, and ventilated cats were used to test CRRN and phrenic neurogram (PN) responses to selective stimulation of pulmonary mechanosensitive and CO2 chemoreceptors. On the basis of their phase relationship with the PN neurogram, 32 CRRNs were classified as follows: basic (independent of ventilator movement), pump (dependent on ventilator movement), and recruited (showed no respiratory-related activity until breathing was stressed). With the exception of pump CRRNs, the firing pattern of CRRNs was significantly modulated during single-breath cessation of the ventilator at end-inspiratory lung volumes (lung inflation [LI]) or at functional residual capacity (FRC), multibreath (MB) cessation of the ventilator, and inhalation of a hypercapnic gas mixture. The characteristics of the responses of the CRRN were mirrored by concomitant changes in the PN. Recruited CRRNs that were either silent or displayed a tonic discharge pattern during control developed phasic respiratory activity when respiratory challenges were applied. The finding that the firing behavior of CRRNs could be altered by hypercapnia and manipulation of vagal input in paralyzed cats suggests that CRRNs in the FNR are capable of responding to and integrating information from CO2 chemosensitive and pulmonary mechanoreceptors in the absence of skeletal (respiratory) muscle contraction.

METHODS

Eight adult cats of either gender were initially anesthetized with an intraperitoneal injection of thiopental sodium (50 mg/kg), and the anesthetic level was maintained with chloralose (40 mg/kg iv). To prevent brain edema during surgery, 4 mg dexamethasone were injected the day before and 2 mg on the day of the experiment. The left femoral vein and artery were cannulated. The former was utilized for anesthetic supplement and the latter for monitoring arterial blood pressure (ABP; model P23AA, Statham) and periodic analysis of arterial blood gases (1306 pH/blood-gas analyzer, Instrumentation Laboratory). Rectal temperature was monitored continuously (model 73ATA, Yellow Springs Instruments) and maintained at 38°C via a heating pad and a radiant-heat lamp. A tracheotomy was performed below the larynx by blunt dissection. Tracheal pressure (Ptr) was monitored via a differential pressure transducer (model PM5T, Statham) attached to the cannulas. Animals were subsequently paralyzed with gallamine triethiodide (4 mg/kg for induction followed by continuous supplement of 4 mg·kg−1·h−1) and artificially ventilated (model 55-0798, Harvard Apparatus). In the spontaneously breathing animals, supplemental anesthesia was administered as needed to suppress corneal and withdrawal
reflexes. After paralysis of the animal, supplemental anesthetic was administered when irregularities were observed in ABP, heart rate, and respiratory rate and pattern. To minimize movement of brain by mechanical ventilation, a bilateral pneumothorax was created. The inlet of the ventilator was controlled by a three-way valve so that the animal was allowed to inhale room air (appropriate supplemental gas mixture) or hypercapnic gas mixtures.

Animals were placed in a Kopf stereotaxic apparatus, and openings were made in the occipital skull (detailed in Ref. 24). Bleeding was controlled by bone wax and absorbable hemostat (Surgicel and/or Gelfoam). The dura was removed, and the underlying tissue was covered with mineral oil.

The right C5 cervical phrenic nerve rootlet was isolated via a dorsal approach and cut. The central end of the nerve was mounted on a bipolar recording electrode and then covered with petroleum jelly to prevent drying. Raw signals of the PN were filtered (300–3,000 Hz) and amplified by using a preamplifier (model P15, Grass Instruments) before being displayed on a storage oscilloscope (model 5103n, Tektronix). The amplified signals were in turn processed by an integrator (moving average; model MA-821RSP, Charles Reel Enterprises) to obtain an integrated PN (\( \int \)). Stereotaxic coordinates (15) were used to position a tungsten microelectrode (–5 MΩ) in the vicinity of the FNr. CRRN activity was electrically isolated and amplified through a preamplifier (model P511K, Grass Instruments) and displayed on the storage oscilloscope described above. Single units were identified by using the criterion of spike-amplitude uniformity.

Protocol. A recording microelectrode held in a micromanipulator (Wells Hydraulic Micro-Drive) was positioned into the FNr by utilizing stereotaxic coordinates, and the prescribed area was searched for units having respiratory phasic discharge. When such units were encountered, they were tested as described below. However, if there was no discernable electrical activity (silent) or if tonic, non-respiratory-modulated activity was present, MB cessation of the ventilator was applied to determine whether respiratory stress could recruit CRRNs. Recruited CRRNs were also exposed to the stimulating protocols. The electrode was repositioned when testing was completed or a CRRN could not be isolated at a given site.

Arterial blood was sampled periodically throughout the course of the experiment, and arterial pH and arterial PO2 were kept within control conditions (7.3–7.4 and \( \geq 100 \) Torr, respectively). End-tidal PCO2 (PETCO2) was continuously monitored (model 78356A, Hewlett-Packard) and kept at \( 35 \) Torr by adjustment of the ventilator and the inhaled gas mixtures. Tidal volume of the ventilator was usually adjusted at \( 25 \) ml with a frequency \( 35 \) breaths/min to minimize inputs from afferents of the chest wall and diaphragm. These ventilator parameters were kept constant before and during a given respiratory challenge.

When the neuronal baseline activity and/or PN became stable under control conditions, the animal was randomly exposed to 1) cessation of the ventilator at either LI or FRC for a single breath to manipulate the stimulation of pulmonary mechanoreceptors; 2) cessation of the ventilator for MBs to initially manipulate the activity of pulmonary mechanoreceptors and, subsequently, the activity of chemoreceptors because of developing hypercapnia and hypoxia; and 3) hypercapnic gases (7% CO2–93% O2) for \( 1 \) min to activate CO2 sensory receptors. The intervals allowed for recovery after the stimulation were 10 breaths for LI and FRC and 5 min for MBs and hypercapnia, respectively. By utilizing the initial site as a reference, the recording microelectrode was then moved to another tract and the protocols repeated. ABP, PTR, PN, \( \int \) PN, and unit activity of CRRNs (inspiratory or expiratory) were continuously recorded on videotape via a pulse code modulation recording adaptor (model 3000A, Vetter Digital) and monitored by Grass polygraph (model 7D recorder) for later data analysis. After completion of the experiment, the accuracy of the electrode placements was confirmed by direct-current lesion (20 µA, 2 min).

The animals were killed by administration of additional anesthetic, and the brain stem and the cerebellum were removed and placed in 10% Formalin. After at least 3 days of immersion fixation, the brain stem and the cerebellum were frozen, and 50-µm sections were cut and mounted. The location of the marking lesions was drawn with camera lucida.

Data analysis. A window slope-height discriminator (model 74-60-1, Frederick Haer) was used to electrically isolate the discharge of a single unit, and a histogram of the integrated neural spike frequency was constructed from an rate-interval monitor (model 74-40-5, Frederick Haer). In general, time base settings in the rate mode were 5 Hz/0.2 s or 10 Hz/0.1 s. The level of the band-pass filter was adjusted to minimize the background noise. To delineate the burst characteristics of neurons and the PN with and without added respiratory challenges, the signals were replayed onto a high-speed thermographic recorder (model TA2000, Gould) in real time.

Inspiratory- or expiratory CRRNs were classified by identifying whether the neuronal burst duration occurred primarily during the corresponding phase of the PN. Neurons that displayed respiratory phasic activity under control conditions (without respiratory challenges) were subcategorized as basic CRRNs, whereas neurons having respiratory phasic activity that depended on either ventilator movement or respiratory stress were listed as pump and recruited CRRNs, respectively. The population of each group was expressed as percentage of all recorded CRRNs. ABP, PETCO2, end-tidal PO2 (PETO2), firing rate and duration of CRRNs, inspiratory time (TI) and expiratory time (TE) (denoted on PN), and peak of \( \int \) PN were recorded. The control values were obtained by the average of the relevant variables of five breaths just before application of respiratory stimulii. The responses were determined by measuring respiratory variables of 1) the first inspiratory phase after cessation of the ventilator at FRC; 2) the first expiratory phase after cessation of the ventilator at LI, and 3) the maximum response (both respiratory phase) during application of MB cessation of the ventilator and hypercapnia. One-way analysis of variance with the Student-Newman-Keuls post hoc test was used to identify significance of the differences among the three types of neuronal firing patterns during control. The differences of the control activity of the CRRNs and their responses to respiratory challenges (LI, FRC, MB cessation of the ventilator, and hypercapnia) were compared and examined by using the same statistics. All data are presented as means ± SE. Significance was considered at \( P < 0.05 \).

RESULTS

Thirty-two CRRNs were recorded, which included 15 inspiratory (basic = 5, recruited = 5, and pump = 5) and 17 expiratory (basic = 9 and recruited = 8). Fourteen (44%) of these neurons displayed phasic respiratory-related activity during control conditions and were arbitrarily classified as basic neurons. Thirteen (41%) were initially either silent or fired tonically during control but showed phasic-related activity with respiratory challenges (recruited neurons), and five (15%)
were clearly driven by the ventilator (pump neurons). The average firing rate of the CRRNs was 40.5 ± 5.1 Hz with a range from 0 (recruited) to 110 Hz. Figure 1 illustrates the location of CRRNs and the distribution of basic, recruited, and pump CRRNs (inspiratory and expiratory neurons). For all data reported, the placement of the recording electrode within the vicinity of the FNr was verified histologically.

An example of a basic CRRN firing pattern is shown in Fig. 2. This unit displayed bursting behavior under control conditions that was correlated with the expiratory phase of the preceding PN activity. Five CRRNs displayed respiratory-related phasic activity dependent on the pumping action of the ventilator. As shown in Fig. 3, the discharges of a pump CRRN was closely related to the volume changes precipitated by the ventilator (see Ptr trace). When the ventilator was stopped the unit was silent although the PN activity persisted. After MB cessation of the ventilator, an immediate reappearance of the neuronal discharge was coordinated with the ventilator but not associated with phrenic nerve activities. The peak value of \( f_{PN} \) was enhanced during MB cessation because sustaining the lung volume at FRC reduced the vagal inhibitory effect on inspiration.

Recruited CRRNs exhibited either tonic firing behavior (\( n = 10 \)) or no activity at all (\( n = 3 \)) under control conditions. Their respiratory-related phasic activity did not emerge until respiratory challenges were applied. Representative discharge patterns of CRRNs exposed to respiratory challenges are presented in Figs. 4–8. This expiratory CRRN showed phasic discharge that was clearly coordinated with silent period of phrenic nerve activity. imp/sec: Impulses/s.

![Fig. 1. Cross section of brain stem and cerebellum illustrating regions where recording electrode was positioned and distribution of basic, recruited, and pump cerebellar respiratory-related neurons (CRRNs). Open symbols, inspiratory CRRNs; solid symbols, expiratory CRRNs; circles, basic CRRNs; triangles, recruited CRRNs; squares, pump CRRNs; triangles with '1', silent recruited CRRN. FN, rostral fastigial nucleus; IN, interposed nucleus; CBL, lateral cerebellar nucleus; V4, fourth ventricle; VII, facial nucleus; P, pyramidal tract; IFC, infracerebellar nucleus.](image)

![Fig. 2. Example of basic CRRN. Traces from top to bottom are CRRN discharge, CRRN unit, neuronal firing rate, integrated phrenic neurogram (\( f_{PN} \)), and tracheal pressure (Ptr) (similar to those in Figs. 3–8). This expiratory CRRN showed phasic discharge that was clearly coordinated with silent period of phrenic nerve activity. imp/sec, Impulses/s.](image)
Recruited CRRNs are defined as those neurons that were silent or displayed tonic activity during control conditions but were phasically modulated when the breathing was stressed. An example of a recruited-tonic CRRN is shown in Fig. 7. This expiratory CRRN displayed tonic discharge that was not correlated with the respiratory cycle during control conditions. However, respiratory-related phasic discharges emerged within the background of tonic discharges during MB cessation of the ventilator. An inspiratory recruited-silent CRRN is illustrated in Fig. 8. This neuron was silent during control, but respiratory-related phasic discharges appeared during MB cessation of the ventilator.

The group data of responses of CRRNs to cessation of the ventilator for MBs and hypercapnia are listed in Table 1. MB cessation of the ventilator increased firing rates (inspiratory and expiratory) and peak values of \( \int PN \) of basic and recruited CRRNs, and it prolonged neuronal and PN burst duration (\( P < 0.05 \)). Cessation of the ventilator for MBs eliminated pump neuronal firing. Among 27 basic and recruited CRRNs, 10 neurons failed to respond to single-breath cessation, but 17 were responsive to both single-breath and MB cessation of the ventilator. Successful recording of single units throughout hypercapnic stimulation was made for 13 CRRNs. Hypercapnia enhanced neuronal firing rate of 11 inspiratory and expiratory basic and recruited CRRNs with no effect on the activity of two pump neurons. Neurons that responded to hypercapnia also responded to MB cessation of the ventilator (7 of which responded with single-breath cessation). In addition, responses of CRRNs to respiratory challenges are qualitatively similar to changes noted on the PN (inspiratory duration and peak values of \( \int PN \)).

It should be noted that previous studies have reported large populations of neurons having a tonic firing pattern that was not associated with respiratory phase even with respiratory challenges (7, 11). In our paralyzed preparation, we encountered many neurons that displayed no detectable respiratory modulation with and without stressed breathing. Seven of them were recorded and showed no significant difference between control and MB cessation of the ventilator (29.1 ± 4.8 vs. 34.5 ± 6.3 Hz).
In the present study, LI and FRC, compared with control, did not significantly alter values of ABP (130 ± 6.11.0 vs. 135.5 ± 13.3 mmHg), PETCO2 (34.7 ± 1.6 vs. 33.8 ± 2.6 Torr), and PETO2 (97.8 ± 3.0 vs. 98.5 ± 2.6 Torr). However, ABP and PETCO2 values obtained in response to MB cessation of the ventilator (152.7 ± 5.8 mmHg and 44.2 ± 5.2 Torr, respectively) and to hypercapnia (155.6 ± 6.2 mmHg and 54.5 ± 5.8 Torr, respectively) were markedly increased (P < 0.05). With MB cessation of the ventilator, PETO2 values were reduced to 82.2 ± 3.2 Torr (P < 0.05), whereas during hypercapnia PETO2 elevated to the values >100 Torr.

**DISCUSSION**

Three types of respiratory-related neurons (basic, pump, and recruited) were found within the cerebellar fastigial nucleus with no apparent clustering. The firing behavior of these neurons was modulated by selective stimulation of vagal afferents. Manipulation of lung volume was used to vary vagal activity emanating from pulmonary mechanoreceptors. Potentially complicating effects of respiratory and skeletal muscles afferents on the CRRNs during sustained LI were minimized by muscle paralysis. For expiratory CRRNs, we observed that a sustained LI resulted in a prolongation of the burst duration with a concomitant increase in the interburst interval of the PN. Conversely, when the lung volume was sustained at FRC, the burst duration of the expiratory CRRNs was inhibited while the inspiratory duration and amplitude of the PN were increased. Responses of inspiratory CRRNs to these stimuli were just the opposite of those of expiratory CRRNs. Neurons possessing some of the firing characteristics of pump-type cells (4) were also encountered. These neurons fired when the lungs were inflated by the ventilator but were silent when inflation was withheld, suggesting dependency on vagal input (see Fig. 3). Our postulate that CRRNs neurons are involved in regulation of vagal-mediated respiratory responses is supported by other anatomic and functional data. First, projections from vagal afferents to the cerebellum and the FN have been documented. Hen-

Table 1. Responses of CRRNs to respiratory challenges

<table>
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<tr>
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<th>Control</th>
<th>LI</th>
<th>FRC</th>
<th>MB</th>
<th>CO2</th>
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<tr>
<td><strong>Inspiratory CRRNs</strong></td>
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<td>n</td>
<td>22 †</td>
<td>5</td>
<td>11</td>
<td>6</td>
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<tr>
<td>Spikes/s</td>
<td>42.9 ± 5.4</td>
<td>85.3 ± 7.4*</td>
<td>102.4 ± 10.1*</td>
<td>83.4 ± 10.9*</td>
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<tr>
<td>Burst duration, s</td>
<td>1.2 ± 0.2</td>
<td>2.6 ± 0.3*</td>
<td>3.1 ± 0.4*</td>
<td>2.1 ± 0.4</td>
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<tr>
<td>Ti, s</td>
<td>3.8 ± 0.2</td>
<td>2.9 ± 0.5*</td>
<td>3.8 ± 1.0*</td>
<td>1.9 ± 0.3</td>
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<tr>
<td>Peak /PN</td>
<td>6.6 ± 0.4</td>
<td>12.3 ± 0.9*</td>
<td>26.9 ± 2.5*</td>
<td>13.6 ± 2.1*</td>
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<td><strong>Expiratory CRRNs</strong></td>
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<td>n</td>
<td>28 ‡</td>
<td>7</td>
<td>16</td>
<td>5</td>
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<tr>
<td>Spikes/s</td>
<td>39.8 ± 3.9</td>
<td>64.7 ± 3.5*</td>
<td>95.7 ± 11.7*</td>
<td>95.7 ± 9.3*</td>
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<tr>
<td>Burst duration, s</td>
<td>0.7 ± 0.2</td>
<td>2.0 ± 0.1*</td>
<td>1.8 ± 0.1*</td>
<td>1.5 ± 0.4</td>
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<tr>
<td>Te, s</td>
<td>1.4 ± 0.1</td>
<td>2.1 ± 0.1*</td>
<td>2.2 ± 0.2*</td>
<td>1.9 ± 0.4</td>
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<tr>
<td>Peak /PN</td>
<td>6.2 ± 1.0</td>
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<td><strong>Pump CRRNs</strong></td>
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<td>n</td>
<td>5 §</td>
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<tr>
<td>Spikes/s</td>
<td>54.2 ± 7.6</td>
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<td>0.0 ± 0.0*</td>
<td>18.4 ± 2.6*</td>
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<tr>
<td>Burst duration, s</td>
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<td>Ti, s</td>
<td>1.2 ± 0.2</td>
<td>1.9 ± 0.4*</td>
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<tr>
<td>Peak /PN</td>
<td>6.2 ± 1.0</td>
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Values are means ± SE; n, no. of neurons. CRRNs, cerebellar respiratory-related neurons; LI, lung inflation; FRC, functional residual capacity; MB, multiple breath; Te, expiratory time; Ti, inspiratory time; /PN, integrated phrenic neurogram. Durations of MBs were 11.5 ± 2.1 s for pump CRRNs and 16.9 ± 4.9 s for others. *Significant difference between data obtained before and during respiratory challenges, P < 0.05. Because nos. of tested neurons indicated by † (n = 2), ‡ (n = 3), and § (n = 2) are too small, no statistics were made in these cases (also see text).
nemann and Rubia (8) recorded evoked potentials in lobes V and VI of the cerebellum when the cervical vagus was electrically stimulated. By injecting horseradish peroxidase into various parts of the cerebellar cortex and nuclei, Zheng et al. (25) demonstrated that there are direct projections from vagal nuclei to the FN in the cat. Second, functional interaction between the cerebellum and vagal afferents have also been reported to influence respiration. For example, cerebellectomy had little effect on inspiratory muscle response to inspiratory tracheal occlusion; however, cerebellectomy in concert with bilateral vagotomy significantly inhibited this response (24). In addition, removal of the cerebellum decreased inspiratory and expiratory duration, but these changes were no longer obtained if cerebellectomy was subsequently followed by bilateral vagotomy (18).

Another major finding was that firing rates of basic and recruited CRRNs were significantly increased when the animal was exposed to a hypercapnic gas mixture, suggesting that CRRNs receive and respond to information from CO₂ chemoreceptors. Several lines of studies support cerebellar (FNr) contribution to respiratory responses to hypercapnia. Functional projections from central CO₂ chemoreceptors to the cerebellum and its nuclei has been documented recently (9). By using c-fos as a marker to identify the chemoreceptors on medullary ventral surface of rats, James et al. (9) found that when these chemoreceptors were activated by acidic stimulation, c-fos-positive cells were detected in the

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**Fig. 6.** Activity of basic-phasic CRRN in response to hypercapnic challenge. A: control. B: hypercapnia. Compared with control, firing rate and duration of this inspiratory CRRN were significantly altered during hypercapnia.

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**Fig. 7.** Response of recruited-tonic CRRN to cessation of ventilator for multiple breaths. Expiratory CRRN showed tonic discharge unrelated to respiratory cycle during control. However, respiratory-related phasic discharges are present on background tonic discharges with multibreath cessation of ventilator at FRC (↑, ventilator on; ↓, ventilator off).
brain stem as well as cerebellar nuclei. By utilizing whole or partial ablation, investigators have shown an important role of cerebellum and FNr in respiratory response to CO2 (12, 14, 21, 22). Mansfeld and Tyukody (12) described that cerebellectomy depressed the ventilatory response to inhalation of 10 and 20% CO2 in anesthetized dogs. With the same preparation, Sanapati et al. (14) observed that eupneic breathing and the ventilatory response to 4 or 6% CO2 were depressed after ablation of the anterior lobe of the cerebellum. Our laboratory (21, 22) reported that cerebellectomy or FNr lesions reduced the ventilatory response to progressive hypercapnia primarily by decreasing respiratory frequency. In addition, electrical stimulation of the FNr showed that phrenic nerve activity was dramatically altered in decerebrated or anesthetized cats (3, 10, 17).

Combining these results with our data, we infer that CRRNs are able to integrate the information from CO2 chemoreceptors and pulmonary mechanoreceptors and in turn modulate the respiratory motor output.

All CRRNs recorded in our experiments responded to MB cessation of the ventilator, whereas 37% of them failed to respond to single-breath cessation of the ventilator (FRC or L1). These results show that CRRNs have differential responses to mechanical and chemical perturbations and thereby suggest that a more profound cerebellar involvement in chemo- rather than pulmonary mechanoreflexes. Indeed, investigators, using ablation of the cerebellum or the FNr, have demonstrated cerebellar (FNr) contributions to respiratory response to hypercapnia (12, 14, 21, 22) and hypoxia (11, 23). In contrast, with respect to respiratory mechanoreflexes, cerebellectomy failed to affect diaphragmatic activities evoked by application of inspiratory tracheal occlusion (24), although it altered electromyographic activity of the transversus abdominis muscle elicited by a continuous expiratory threshold load (19).

Interestingly, we found a population of neurons that show no respiratory-related activity (silent or tonic) during control conditions but display phasic respiratory-related activity with increasing respiratory challenges. This finding is in agreement with the acknowledged cerebellar role in the control of breathing; i.e., the cerebellum is involved in respiratory-stressed responses but is not critical for eupneic breathing. It is generally accepted that during eupneic breathing, cerebellectomy or FNr lesions have little effect on minute ventilation, diaphragm electromyographic activity, and inspiratory activity of phrenic efferents in decerebrate (16) and anesthetized cats (22, 24).

We addressed the question as to whether the presence of CRRNs is dependent on the input from skeletal muscle contraction. The rationale was derived from the fact that recruited FN neurons (not related to respiration) have been recorded in the monkey that were silent until skeletal muscle voluntary movements occurred (2). In addition, firing patterns of cerebellar respiratory-movement-sensitive neurons in the carp were shown to be sensitive to mechanical loading of the buccal or the opercular system (1). Some CRRNs of spontaneously breathing cats have also been shown to respond to passive limb movement (11). Moreover, as described in the introduction, respiratory responses elicited by respiratory muscle voluntary contraction are modulated by the cerebellum (5, 6, 19, 24). Our results that paralysis of respiratory muscles or cessation of the ventilator (to eliminate passive movement of respiratory muscles) failed to abolish activity of CRRNs (except pump neurons) demonstrate that the phasic nature of these CRRNs does not depend on sensory feedback elicited by voluntary and/or passive movements of respiratory (skeletal) muscles.

In our experiment, the average value and the range of firing frequencies of CRRNs recorded during control
conditions are very close to those previously reported in spontaneously breathing cats (28.8 ± 2.9 Hz with a range of 0–90 Hz, Ref. 11). The percentages of inspiratory and expiratory neurons recorded in the present study were 47 and 53%, respectively. These data show a little more balance between inspiratory and expiratory neurons than do those reported by Gruart and Maria (7), who found a predominance of expiratory CRRNs (76%). This difference of cell populations (inspiratory vs. expiratory CRRNs) may be due to the fact that our data were obtained in anesthetized and paralyzed cats, whereas theirs were collected in alert cats. Moreover, our recording region was localized in the FNr, but theirs included other cerebellar nuclei (interposed nuclei).

The amplitudes of the discharges of CRRNs recorded in this study could be varied continuously by moving the electrode tip over ~50 µm, suggesting that most of the recordings were related to somata rather than axons. Previous experiments involving electrical stimulation of the FNr also support neuronal involvement (20). After regional injection of kainic acid into the FN to destroy cell bodies (13), the respiratory responses to electrical stimulation previously observed in anesthetized cats were abolished.

In summary, three types of CRRNs were observed in this study, i.e., basic, recruited, and pump neurons. The presence of activity of basic and recruited CRRNs was not dependent on the inputs elicited by contraction of respiratory and other skeletal muscles. The responses of CRRNs to cessation of the ventilator for a single respiratory challenge were applied. We conclude that there are CRRNs that have a presence that is independent of inputs from respiratory and skeletal muscles. These CRRNs are capable of responding to and/or integrating information from CO2 chemoreceptors and vagal afferents (pulmonary mechanoreceptor). The appearance of recruited CRRNs supports the cerebellar involvement in respiratory control during stressed breathing.

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