Responses of Simultaneously Recorded Respiratory-Related Medullary Neurons to Stimulation of Multiple Sensory Modalities

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Li, Zhonzeng, Kendall F. Morris, David M. Baekey, Roger Shannon, and Bruce G. Lindsey. Responses of simultaneously recorded respiratory-related medullary neurons to stimulation of multiple sensory modalities. J. Neurophysiol. 82: 176–187, 1999. This study addresses the hypothesis that multiple afferent systems share elements of a distributed brain stem network that modulates the respiratory motor pattern. Data were collected from 18 decerebrate, bilaterally vagotomized, paralyzed, artificially ventilated cats. Up to 28 neurons distributed in the rostral and caudal ventral respiratory group, nucleus tractus solitarius, and raphe obscurus were recorded simultaneously with microelec-trode arrays. Phases of the respiratory cycle and inspiratory drive were assessed from integrated efferent phrenic nerve activity. Carotid chemoreceptors were stimulated by injection of CO2-saturated saline solution via the external carotid artery. Baroreceptors were stimulated by increased blood pressure secondary to inflation of an embolectomy catheter in the descending aorta. Cutaneous nociceptors were stimulated by pinching a footpad. Four hundred seventy-four neurons were tested for respiratory modulated firing rates and responses; 403 neurons were tested with stimulation of all 3 modalities. Chemoreceptor stimulation and pinch, perturbations that tend to increase respiratory drive, caused similar responses in 52 neurons; 28 responded oppositely. Chemoreceptor and baroreceptor stimulation resulted in similar primary responses in 45 neurons; 48 responded oppositely. Similar responses to baroreceptor stimulation and pinch were recorded for 38 neurons; opposite effects were measured in 26 neurons. Among simultaneously recorded neurons, distinct combinations of firing rate changes were evoked in response to stimulation of the different modalities. The results show a functional convergence of information from carotid chemoreceptors, baroreceptors, and cutaneous nociceptors on respiratory-modulated neurons distributed in the medulla. The data are consistent with the hypothesis that brain stem neurons have overlapping memberships in multifunctional groups that influence the respiratory motor pattern.

INTRODUCTION

Stimulation of different types of sensory receptors can evoke similar changes in breathing. For example, a decrease in blood oxygen tension or rise in CO2 stimulates peripheral chemoreceptors, leading to an increase in peak integrated efferent phrenic nerve activity and respiratory frequency (Morris et al. 1996a,b; Shannon 1976). Nociceptive reflexes triggered by cutaneous stimulation may include similar changes in the respiratory motor pattern (Duranti et al. 1991; Waldrop et al. 1984). Conversely, a rise in arterial blood pressure and baroreceptor stimulation produce a decline in respiratory drive (Bishop 1974; Brunner et al. 1982; Heymans and Bouckaert 1930; Nishino and Honda 1982).

Current models of the respiratory brain stem include medullary raphe neurons in a distributed system that modulates breathing. Functional links have been found among raphe neurons and the ventral respiratory group (VRG) of the lateral medulla (Lindsey et al. 1994), a system of neurons widely considered to have an essential role in the generation of the respiratory motor pattern (Bianchi et al. 1995). Medullary raphe neurons respond to carotid chemoreceptor (Morris et al. 1996a), baroreceptor (Lindsey et al. 1998; Morrison and Gebber 1984; Yen and Blum 1984), and nociceptive stimulation (Anderson et al. 1977; Fields et al. 1983; Gao et al. 1998; Yen and Blum 1984). Collectively, these observations suggest the hypothesis that multiple afferent systems influence breathing through actions on a distributed system that includes shared raphe neurons and the BOT-VRG.

Medullary neurons are organized into transiently configured correlational assemblies with overlapping membership (Lindsey et al. 1992b, 1994, 1997). Functional convergence of different afferent systems on raphe neurons (Yen and Blum 1984) and other cells of the respiratory network (Arita et al. 1988) may have roles in defining such dynamic groupings and the modulation of breathing. Measurement of fluctuations in neuronal activity and effective connectivity can be used to assess parallel processing, and concurrent changes in the respiratory motor pattern can serve as a “reporter system” for the detection of physiological stimuli by the respiratory network. The neural representation of different combinations of stimulus conditions is relevant to category formation by the nervous system (de Sar and Ballard 1998).

In addressing these issues, we measured concurrent changes in the firing rates of neurons in n. raphe obscurus, nucleus tractus solitarius (NTS) and the VRG to sequential stimulation of baroreceptors, carotid chemoreceptors, and cutaneous nociceptors. A companion paper describes related measurements of short-time scale correlations among neurons tested for responses to more than one modality and considers cooperative actions in the control of breathing during perturbations of different sensory modalities (Li et al. 1999). Preliminary accounts of this work have been reported (Li et al. 1996, 1997).

METHODS

Surgical preparation and general methods

Experiments were performed on 18 adult cats of either gender. Each cat was anesthetized with sodium thiopental (28 mg/kg) until a mid-
collicular decerebration was done. Femoral veins were catheterized for administration of intravenous fluids and drugs and for monitoring central venous pressure. Femoral arteries were catheterized to monitor arterial blood pressure, obtain blood samples for analysis of pH, pCO₂, and pO₂, and insert an embolectomy catheter into the descending aorta to just rostral to the origin of the renal arteries. A region of the right common carotid artery caudal to the sinus area and the right external carotid and lingual artery rostral to the sinus area were cleared. The common carotid and lingual arteries were clamped to allow placement of a concentric catheter into the external carotid artery. The tip of the catheter was placed at the level just below the sinus. The outer barrel of the concentric catheter was connected to an infusion pump that slowly infused heparinized normal saline solution (buffered to pH 7.4) to prevent clot formation; the inner barrel was used for injection of CO₂ saturated saline solution to stimulate carotid chemoreceptors.

Each animal received a tracheotomy and bilateral vagotomy. Animals were artificially ventilated with room air. Supplemental 100% oxygen was added if there was a ventilation-perfusion mismatch causing hypoxemia. End-tidal CO₂ was monitored and maintained at 4–5%. Core temperature was maintained at 38 ± 0.5°C by a servo-controlled heating pad. Animals were maintained with an intravenous drip of 2.5% dextrose, 0.005% potassium, and 0.042% sodium bicarbonate in 0.45% sterile saline solution. Atropine (0.5 mg/kg) was administered intramuscularly at the beginning and middle of each experiment to reduce mucus secretion in the airways. Dexamethasone (2 mg/kg i.v bolus followed 2 h later by constant infusion at 0.5 mg kg⁻¹·h⁻¹) was administered to help prevent hypotension and to minimize brain stem swelling. When necessary, a mean arterial blood pressure of at least 100 mmHg was maintained by infusion of lactated Ringer solution, 2.5% dextran in normal saline solution, or a 0.002% dopamine solution. Bicarbonate solution (8.4%) was administered intravenously as needed to correct metabolic acidosis. Arterial blood samples were analyzed hourly for pH, pCO₂, and pO₂. These parameters were maintained within normal limits.

Animals were placed in a prone position in a stereotaxic head holder and decerebrated at the midcollicular level. The left C₃ phrenic nerve rootlet was approached through a dorsal route, isolated, and desheathed. Animals were paralyzed with a bolus of gallamine triethiodide (2.2 mg/kg) followed by constant infusion (0.4 mg · kg⁻¹ · h⁻¹) via an infusion pump throughout each experiment. An occipital craniotomy was performed, and the caudal portion of the cerebellum was removed to expose the brain stem. In six animals, a laminectomy was performed at the C₃ level to insert stimulating electrodes for antidromic testing of axonal projection of monitored brain stem neurons; these results are considered in a companion paper (Li et al. 1999).

A thoracic spinal process (T₃) was cleared for placement of a spinal clamp to help minimize brain stem movements due to ventilation. A bilateral thoracotomy was performed to further minimize brain stem movements. To prevent lung collapse in an open chest preparation, the functional residual capacity was maintained within normal range by adjustment of the expiratory resistance. The upper airways were suctioned periodically to remove mucus that accumulated in the trachea; the lungs were hyperinflated periodically to counteract atelectasis.

Neuronal recording from brain stem

Four planar arrays of 8–11 individual tungsten microelectrodes (10 MΩ) were inserted into the brain stem. Each array was controlled by hydraulic microdrives mounted on the calibrated bars of the stereotaxic head holder. Movements of each electrode in three of the arrays (NTS, caudal VRG, and rostral VRG) were controlled by individual motors and associated electronics. Neuronal activity was amplified and filtered (100–5,000 Hz band pass) and recorded on three 16-channel FM instrumentation recorders, along with end-tidal PCO₂, blood pressure, tracheal pressure, phrenic nerve activity, and stimulus markers. A common 5-Hz synchronization pulse was recorded on each tape for subsequent merger of the data files.

Experimental protocol and data acquisition

Systemic arterial blood pressure, end-tidal CO₂, tracheal pressure and integrated phrenic nerve activity (rectified, filtered 200–3,000 Hz band-pass, time constant of 200 ms) were recorded continuously on a polygraph throughout the recording. Control neuronal activity was recorded for 5 min before the onset of stimulation. Baroreceptors were stimulated for 30 or 60 s by inflation of the embolectomy catheter and occlusion of the descending aorta; four to five stimulus periods were separated by an interval of 60 s. This method generated an initial increase in arterial blood pressure of at least 25 mmHg above the mean arterial blood pressure. Carotid chemoreceptors were stimulated by injection of 0.2–0.5 ml of a CO₂-saturated normal saline solution into the common carotid artery over a period of 30 s; a series of five stimuli were each separated by 5-min intervals. Footpads were pinched with a hemostat for 30 or 60 s. A series of five to eight pinches was applied with intervals of 60 s between stimuli. This protocol was considered sufficient to stimulate nociceptors as judged by associated increases in arterial blood pressure. A particular footpad was pinched only one time during a recording to minimize the effects of a previous stimulation history of the cutaneous nociceptors (e.g., sensitization).

Data entry and spike sorting

Action potentials of single neurons and timing pulses from each experiment were converted to arrays of occurrence times with Datawave software, transferred to Hewlett-Packard 9000 series computers, merged into one master file, and analyzed (Morris et al. 1996b). The phrenic nerve activity, along with the synchronization pulse channel was digitized at 5 kHz with 16-bit accuracy. In parallel, the phrenic signal was also fed into a resistor-capacitor “leaky” integrator with a time constant of 200 ms. The resultant signal, along with signals corresponding to arterial blood pressure, end-tidal pCO₂, tracheal pressure, and stimulus marker was amplified and digitized with 12-bit accuracy at 40 Hz.

Identification of respiratory modulation

All spike trains of neurons were tested for respiratory modulation using two statistical tests. The first was the subject-by-treatment experimental design (Orem and Dick 1983). Fifty consecutive breaths (subjects) were each divided into 20 equal time partitions (treatments). The variance of means of partition spike counts within breaths was tested for a significant difference (P < 0.05; F test) from variance of means among breaths. The second test was a nonparametric sign test (Morris et al. 1996b). Each respiratory cycle was divided into 20 equal time partitions during the entire period of recording. For each spike train, the first 50 consecutive cycles were used to develop a hypothesis as to the half of the respiratory cycle in which the cell was more active. The hypothesis was formed by counting spikes and dividing the cycles to give the greatest total difference between halves. All remaining respiratory cycles were then halved accordingly and inspected to determine in which half of the respiratory cycle the neuron fired more often. The sign test was performed at the 5% significance level to determine whether the neuron’s greater activity in one half of the respiratory cycle, over the entire course of the recording, was greater than chance. Neurons confirmed by either test with respiratory modulation were considered respiratory modulated neurons (RM). Otherwise, they were considered to be not respiratory modulated (NRM). The times of onset of the inspiratory and expiratory phases were derived from the digitized, integrated phrenic nerve signal: the I
The average firing rate of each neuron as a function of time in the respiratory cycle was measured with cycle-triggered histograms (CTHs) (Cohen 1968) and compared with overlaid integrated efferent phrenic nerve activity. CTHs were used to classify neurons with significant respiratory modulation as inspiratory (I) or expiratory (E) neurons according to the phase during which they were more active. Neurons with peak firing rates in the first half of the phase and longer periods of decrementing activity were categorized as decrementing (DEC) neurons. Neurons with peak firing rates in the second half of the phase and longer periods of augmenting activity were classified as augmenting (AUG) neurons. Phase-spanning neurons were classified first by the phase with peak average activity and then by the phase transition of greater activity, e.g., I-EI. RM neurons with patterns not easily classified were denoted as "Others" (OTH) (Segers et al. 1987).

Individual neurons were labeled with a number and abbreviations that denoted the site at which their spike trains were recorded: CM, caudal midline in the region of nucleus raphe obscurus; CV, caudal VRG; NTS, nucleus tractus solitarius.

### TABLE 1. Boundaries of stereotaxic coordinates of recorded cells in four brain stem regions

<table>
<thead>
<tr>
<th>Brain Stem Region</th>
<th>From obex</th>
<th>From midline</th>
<th>From dorsal surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rostral VRG</td>
<td>5.40</td>
<td>3.04</td>
<td>2.51</td>
</tr>
<tr>
<td>Caudal VRG</td>
<td>2.00</td>
<td>3.20</td>
<td>1.95</td>
</tr>
<tr>
<td>Raphe</td>
<td>6.19</td>
<td>-0.10</td>
<td>3.30</td>
</tr>
<tr>
<td>NTS</td>
<td>-0.11</td>
<td>0.60</td>
<td>1.41</td>
</tr>
</tbody>
</table>

Rostral-caudal: positive numbers indicate locations rostral to obex, whereas negative numbers caudal to obex; left-right: positive numbers indicate locations on right side to midline. VRG, ventral respiratory group; NTS, nucleus tractus solitarius.

The average firing rate of each neuron as a function of time in the respiratory cycle was measured with cycle-triggered histograms (CTHs) (Cohen 1968) and compared with overlaid integrated efferent phrenic nerve activity. CTHs were used to classify neurons with significant respiratory modulation as inspiratory (I) or expiratory (E) neurons according to the phase during which they were more active. Neurons with peak firing rates in the first half of the phase and longer periods of decrementing activity were categorized as decrementing (DEC) neurons. Neurons with peak firing rates in the second half of the phase and longer periods of augmenting activity were classified as augmenting (AUG) neurons. Phase-spanning neurons were classified first by the phase with peak average activity and then by the phase transition of greater activity, e.g., I-EI. RM neurons with patterns not easily classified were denoted as “Others” (OTH) (Segers et al. 1987).
RV, rostral VRG; N, the NTS. The rostral VRG includes neurons referred to as Bötzinger and pre-Bötzinger by some and is widely considered to contain the basic respiratory rhythm-generating network (Bianchi et al. 1995). To ensure an inclusive nomenclature, we refer to the local network in this region as the BÖT-VRG.

**Identification of neuronal responses**

All spike trains were evaluated for primary, short-term (30 – 60 s) responses to chemoreceptor, baroreceptor stimulation and footpad pinch. Neuronal firing rates during the stimulation and during an equivalent period before onset of each stimulus were averaged for each spike train. A peristimulus time histogram (PSTH) was constructed for each neuron; mean firing rates greater or less than 2 SD of the mean of prestimulus levels were considered significant. The PSTHs sometimes may not detect small changes in activity. Therefore cumulative sum histograms (CUSUM) derived from PSTHs were also calculated. Confidence bands at ±3 SD were constructed for the CUSUMs (Davey et al. 1986). Only changes in activity exceeding these confidence limits were considered significant.

**RESULTS**

Examples of changes in the respiratory motor pattern and arterial blood pressure in response to each stimulus protocol are shown in Fig. 1. The peak amplitude of integrated phrenic activity and arterial blood pressure increased during carotid chemoreceptor stimulation (Fig. 1A) and pinch (Fig. 1C). These variables remained elevated transiently after stimulation. Baroreceptor stimulation resulted in a decrease in peak phrenic amplitude (Fig. 1B). Stimulation of each modality also altered respiratory phase durations; carotid chemoreceptor stimulation and pinch shortened expiratory phase duration. In contrast, baroreceptor stimulation resulted in a prolongation of expiration. Phase graphs (Fig. 1, right) document significant alterations in the peak integrated phrenic activity and inspiratory and expiratory phase durations measured before, during, and after each corresponding perturbation in the left panel. In these and subsequent similar plots, brackets to the right and dotted lines indicate means (±2 SD) of the plotted cycle parameters before stimulation.

**Responses of medullary neurons to multiple stimulus modalities**

The results are based on analyses of 474 neuronal spike trains recorded in 18 cats. The boundaries of stereotaxic coordinates of recorded neurons are given in Table 1. Responses to chemoreceptor, baroreceptor, and footpad stimulation are summarized in Table 2. Tallies are arranged by respiratory modulation and brain stem recording site. Some neurons were not tested with all three stimulus protocols because of inactivity or signal loss.

Concurrent and sequential responses during carotid chemoreceptor stimulation, baroreceptor stimulation, and pinch from one animal are shown in Fig. 2. Data include firing rate histograms from nine simultaneously monitored RM neurons recorded in the NTS, rostral VRG, and raphe obscurus, together with phrenic activity and arterial blood pressure. The activities of six of the neurons changed during carotid chemoreceptor stimulation (Fig. 2A). The firing rate of NTS neuron N1A decreased while the activities of cells N2 and N4 increased. Raphe neurons CM2, 5, and 7 also had increased firing rates.

Baroreceptor stimulation (Fig. 2B) produced a different set of responses in six of the nine recorded cells and served as a control for the effects of the changes in blood pressure during chemoreceptor stimulation. The activities of all three rostral VRG neurons increased. In contrast with their responses to chemoreceptor stimulation, the firing rates of neurons N1A and N4 were not altered, indicating that the previous changes were not a consequence of baroreceptor activation. The firing rate of N2 increased during each interval of elevated blood pressure; these transient changes were superimposed on an overall decline in mean firing rate. The activity of CM2 increased in

**Table 2**

<table>
<thead>
<tr>
<th>Modulation</th>
<th>Area</th>
<th>Chemoreceptor</th>
<th>Baroreceptor</th>
<th>Pinch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>↑ ↓ → ?</td>
<td>↑ ↓ → ?</td>
<td>↑ ↓ → ?</td>
<td></td>
</tr>
<tr>
<td>Inspiratory</td>
<td>Caudal VRG</td>
<td>46 5 4 3</td>
<td>2 17 39 0</td>
<td>13 3 41 1</td>
</tr>
<tr>
<td></td>
<td>Rostral VRG</td>
<td>44 12 17 1</td>
<td>7 19 48 0</td>
<td>9 8 55 2</td>
</tr>
<tr>
<td></td>
<td>Raphe</td>
<td>10 3 12 1</td>
<td>4 6 16 0</td>
<td>7 3 15 1</td>
</tr>
<tr>
<td></td>
<td>NTS</td>
<td>11 4 3 1</td>
<td>4 5 10 0</td>
<td>2 4 13 0</td>
</tr>
<tr>
<td>Totals</td>
<td>111 24 36 6</td>
<td>17 47 113 0</td>
<td>31 18 124 4</td>
<td></td>
</tr>
<tr>
<td>Expiratory</td>
<td>Caudal VRG</td>
<td>17 11 8 1</td>
<td>5 6 26 0</td>
<td>8 4 24 1</td>
</tr>
<tr>
<td></td>
<td>Rostral VRG</td>
<td>8 9 15 5</td>
<td>11 7 19 0</td>
<td>3 12 18 4</td>
</tr>
<tr>
<td></td>
<td>Raphe</td>
<td>11 3 21 3</td>
<td>8 8 21 1</td>
<td>8 9 20 1</td>
</tr>
<tr>
<td></td>
<td>NTS</td>
<td>6 6 16 0</td>
<td>8 5 15 0</td>
<td>3 4 20 1</td>
</tr>
<tr>
<td>Totals</td>
<td>42 29 60 9</td>
<td>32 26 81 1</td>
<td>22 29 82 7</td>
<td></td>
</tr>
<tr>
<td>NRM</td>
<td>Caudal VRG</td>
<td>2 2 8 4</td>
<td>3 5 8 0</td>
<td>2 1 11 2</td>
</tr>
<tr>
<td></td>
<td>Rostral VRG</td>
<td>4 3 5 5</td>
<td>7 2 7 1</td>
<td>4 3 6 4</td>
</tr>
<tr>
<td></td>
<td>Raphe</td>
<td>13 3 46 13</td>
<td>17 11 43 4</td>
<td>7 12 49 7</td>
</tr>
<tr>
<td></td>
<td>NTS</td>
<td>9 12 23 5</td>
<td>8 10 29 2</td>
<td>6 7 31 5</td>
</tr>
<tr>
<td>Totals</td>
<td>28 20 82 27</td>
<td>35 28 87 7</td>
<td>19 23 97 18</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>181 73 178 42</td>
<td>84 101 281 8</td>
<td>72 70 303 29</td>
<td>474 474</td>
</tr>
</tbody>
</table>

Neurons not tested (?) because of inactivity or lost signal due to movement before the onset of the stimuli (↑, increase; ↓, decrease; →, no change). See Table 1 for abbreviations.
response to baroreceptor stimulation, just as during chemoreceptor stimulation, but the other two raphe neurons responded differently. Cell CM5 showed no change, and there was a decline in CM7 activity.

A different combination of responses in a third subset of six of the nine neurons was measured during the series of pinch stimuli. One of the three rostral VRG neurons, RV1A, responded with increased activity as did two of the three NTS neurons. The firing rates of raphe neurons CM2 and CM7 also increased. However, the firing rate of CM5 declined during each pinch.

All nine neurons responded to at least one stimulus modality, and all modalities were effective in altering the firing rates of some of the sampled neurons. Comparison of the absence or presence of changes in firing rates and their directions indicate that six of the nine neurons were in similar states during both chemoreceptor stimulation and pinch. In contrast, the response rate conditions of seven of nine were different during chemoreceptor and baroreceptor stimulation, and five of nine were different when baroreceptor stimulation and pinch were compared. These results are summarized graphically in Fig. 3A.

These different combinations of neuronal responses occurred during changes in the respiratory motor pattern. Peak phrenic amplitude increased during chemoreceptor stimulation and pinch. Changes in the peak phrenic activity and expiratory phase duration during the third baroreceptor stimulation are documented in Fig. 3B. Letters in parentheses next to the identifier of each neuron indicate the respiratory phase in which the neuron had the higher average firing rate (I, inspiratory; E, expiratory). Arrows indicate direction of change in firing rate in response to stimuli: ↑, increase; ↓, decrease; →, no change. Peak firing rates are indicated at right.

**FIG. 2.** Firing rate histograms of 9 simultaneously monitored neurons, phrenic activity, and arterial blood pressure during repeated sequential stimulation of the 3 tested modalities. Data include concurrent measures of neurons in the NTS (N), rostral VRG (RV), and raphe obscurus (CM) during carotid chemoreceptor stimulation (A), baroreceptor stimulation (B), and footpad pinch (C). Peak integrated phrenic amplitude increased during carotid chemoreceptor stimulation and pinch; it decreased during baroreceptor stimulation. A reduction in phrenic activity and an increase in expiratory phase duration during the 3rd baroreceptor stimulation are documented in Fig. 3B. Letters in parentheses next to the identifier of each neuron indicate the respiratory phase in which the neuron had the higher average firing rate (I, inspiratory; E, expiratory). Arrows indicate direction of change in firing rate in response to stimuli: ↑, increase; ↓, decrease; →, no change. Peak firing rates are indicated at right.
apparent because of scaling in Fig. 2B, are documented in a phase graph (Fig. 3B). The expiratory phase duration was shortened during pinch and prolonged during baroreceptor stimulation. Table 3 shows the primary responses of 403 neurons tested with all 3 stimulus protocols. The results are arranged by the different combinations of responses to the three stimuli and by recording location.

Because of signal loss, some neurons were tested with only two stimulus protocols. Table 4 includes these additional data arranged by firing rate changes in response to three different combinations of two sequentially presented stimulus protocols. The top rows give the primary changes in firing rates of neurons in the four sampled regions to peripheral chemoreceptor stimulation and pinch. The changes in firing rates of 52 neurons to both stimuli were in the same direction: activity was enhanced in 33 neurons and decreased in 19 cells. Respiratory and stimulus-dependent firing rate modulation of inspiratory NTS neuron N4 from group 1 are detailed in Fig. 3C. The average firing rate of this I-AUG neuron increased during chemoreceptor stimulation and pinch. There was no change in firing rate during baroreceptor stimulation although peak phrenic activity declined during this perturbation (Fig. 3B). The firing rate of the I-AUG raphe obscurus neuron CM7 (Fig. 3D) did decrease during baroreceptor stimulation; it increased during chemoreceptor stimulation and pinch.

Twenty-eight neurons showed opposite responses to chemoreceptor stimulation and pinch. Neurons with either similar or opposite changes in firing probability were distributed throughout all regions sampled and included cells with and cells without RM activity.
In each multiarray recording, measured neurons had a common history and were tested under the same stimulus and state-dependent conditions. Distinct combinations of responses to the 3 stimulus protocols in 2 groups of 22 and 19 neurons, respectively, are summarized graphically in Fig. 5A. The involvement or contribution of any particular neuron to the modulation of the respiratory motor pattern that occurred in these experiments cannot be determined from these measures. However, the responses of the BÖT-VRG neurons are relevant to tests of current models that postulate particular roles for neurons with different RM discharge patterns. Group 3 included 11 BÖT-VRG neurons. CTHs (Fig. 5B) and firing rate histograms (Fig. 6) derived from seven of these neurons indicate the diversity of concurrently observed discharge patterns.

Expiratory neurons with three different firing patterns were observed (Fig. 5B, left column), including E-DEC neuron RV5A, which responded to chemoreceptor stimulation with an increase in activity. During baroreceptor stimulation, the prolonged active phase of neuron RV5A was matched by an increase in duration of the expiratory phase of the respiratory cycle. Pinch resulted in shortening of the active interval of the respiratory cycle. Table 3 indicates that the peak average firing rate of neuron CM9A was during the first half of the inspiratory phase. Pinch resulted in shortening of the active interval of the respiratory cycle.

The middle section of Table 4 shows the primary changes in firing rates of neurons tested with carotid chemoreceptors and baroreceptors. Twenty-seven neurons increased firing rates during chemoreceptor and baroreceptor stimulation, whereas 18 decreased. Activation of the two types of sensory receptors resulted in an opposite response in 48 neurons. Data from an I-DEC raphe neuron with this combination of response properties are shown in Fig. 4A. The CTH (left) indicates that the peak average firing rate of neuron CM10A was during the first half of the inspiratory phase, although the transition to a higher firing probability began late in the expiratory phase. CUSUMs (right) document increased average activity during chemoreceptor stimulation and a decreased firing rate during baroreceptor stimulation.

The primary changes in firing rates of neurons tested with baroreceptor stimulation and footpad pinch are summarized in the bottom section of Table 4. The firing rates of 18 neurons increased in response to both modalities; the activities of 20 cells decreased. Stimulation of baroreceptors and pinch produced opposite effects in 26 neurons. Data from an E-DEC raphe neuron with this combination of response properties are shown in Fig. 4B. The CTH (left) indicates that the peak average firing rate of neuron CM9A was during the first half of the expiratory phase. The CUSUM histograms (right) show the decline in average activity during baroreceptor stimulation and an increased firing rate during pinch.

For abbreviations, see Tables 1 and 3. ↑, increase in cell firing rate; ↓, decrease in cell firing rate; →, no change in cell firing rate.

### Table 3. Numbers of neurons with different combinations of responses to chemoreceptor stimulation, baroreceptor stimulation, and pinch

<table>
<thead>
<tr>
<th>Neuron Response</th>
<th>Chemo</th>
<th>Baro</th>
<th>Pinch</th>
<th>NTS</th>
<th>rVRG</th>
<th>cVRG</th>
<th>Raphe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ to ↑ 2 2 2 5 11</td>
<td>0 3 1 0 4</td>
<td>4 2 2 3 11</td>
<td>0 0 0 0 3</td>
<td>4 3 0 2 9</td>
<td>2 1 0 0 2 5</td>
<td>3 5 1 8 17</td>
<td>2 5 7 1 15</td>
<td>3 3 3 3 5 14</td>
</tr>
<tr>
<td>↑ to ↓ 8 25 32 12 77</td>
<td>0 1 0 1 2</td>
<td>4 0 2 1 8</td>
<td>0 0 1 2 3</td>
<td>3 2 0 2 7</td>
<td>5 2 1 5 13</td>
<td>0 3 1 1 5</td>
<td>2 4 3 2 4 12</td>
<td>0 4 2 1 7</td>
</tr>
<tr>
<td>↑ to → 3 8 8 12 3 26</td>
<td>0 0 4 0 4</td>
<td>0 0 4 0 4</td>
<td>8 6 2 25</td>
<td>3 1 4 6 14</td>
<td>1 3 1 8 13</td>
<td>23 20 11 40 94</td>
<td>Total 80 112 96 115 403</td>
<td></td>
</tr>
</tbody>
</table>

Chemo, chemoreceptor stimulation; Baro, baroreceptor stimulation; other abbreviations, see Table 1. ↑, increase in cell firing rate; ↓, decrease in cell firing rate.

### Table 4. Numbers of neurons with different combinations of responses to sequential stimulation of the indicated modalities

<table>
<thead>
<tr>
<th>Response to Stimulation</th>
<th>Recording Location</th>
<th>NTS</th>
<th>rVRG</th>
<th>cVRG</th>
<th>Raphe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemo</td>
<td>Pinch</td>
<td>NTS</td>
<td>rVRG</td>
<td>cVRG</td>
<td>Raphe</td>
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For abbreviations, see Tables 1 and 3. ↑, increase in cell firing rate; ↓, decrease in cell firing rate; →, no change in cell firing rate.
neuron and the expiratory phase. E-AUG neuron RV6A, and E-OTH cell RV4 had significant changes in average firing rate only during chemoreceptor stimulation and pinch, respectively.

The unusual discharge pattern of neuron RV4 suggested the possibility that both an I-AUG neuron and an E-AUG neuron were recorded on the same electrode. The absence of any events in the first 25 ms of the autocorrelogram calculated from the same data argues against that possibility. Two different spike trains with activity overlapping in time would be expected to include interspike intervals <25 ms.

All BOT-VRG inspiratory neurons in this group (Fig. 5B, right column) responded to chemoreceptor stimulation with an increase in activity (Fig. 6). The phase-spanning I-EI neuron RV7 had a low average firing rate during control intervals and did not change activity during baroreceptor stimulation or pinch. Neurons RV5 and CV2 with peak firing rates during the inspiratory ramp also responded to baroreceptor stimulation with decreased firing rates. Only I-AUG neuron CV3, with an activity profile very similar to integrated phrenic activity had an increased firing rate during pinch.

**DISCUSSION**

The results demonstrate that stimulation of three sensory modalities, each capable of altering the respiratory motor pattern, evokes many different combinations of responses in medullary neurons. Different firing rate changes were exhibited by some RM neurons in response to sequential footpad pinch and chemoreceptor stimulation, perturbations that elicit similar changes in breathing. The data support the view that brain stem respiratory-related neurons have overlapping memberships in multifunctional groups that influence their targets differently as circumstances change (Bianchi et al. 1992; Ezure 1990; Shannon et al. 1996; Lindsey et al. 1992a, b, 1994, 1997).

The multimodal response properties are also consistent with the hypothesis that some neurons limit changes in the firing rate of their target neurons in response to one stimulus modality (Morris et al. 1996a), but promote similar changes in target cell firing rates when a different modality is stimulated. For example, increased activity in a raphe neuron may “relay” inhibition to VRG neurons during stimulation of one type of modality, whereas a decline in the firing rate of that raphe cell in response to stimulation of another modality would be rate facilitating to the same VRG respiratory neurons through disinhibition. Sequential observations of single neurons cannot distinguish between a responsive cell that relays excitation and a neuron with similar responses that may act to suppress or limit the activity of the former. Simultaneous observation of multiple spike trains allows evaluation of functional connectivity and thus assessment of evidence relevant to the hypothesis. This issue is considered further in a companion paper (Li et al. 1999), which describes multimodal neurons with this putative rate limiting function.

**Multimodal medullary neurons: some functional implications**

Previous work has supported the hypothesis that medullary raphe neurons are elements of a distributed system that modulates breathing (Lindsey et al. 1994, 1998) and participates in the induction and expression of long-term facilitation of phrenic activity after chemoreceptor stimulation (Millhorn 1986; Morris et al. 1996a). Raphe neurons are dynamically organized into correlational assemblies with overlapping membership (Lindsey et al. 1992b, 1994, 1997). The present data are consistent with the hypothesis that functional convergence of different sensory modalities on medullary raphe neurons may participate in the definition of these transient configurations. The data also confirm and extend previously reported responses to carotid chemoreceptor and baroreceptor stimulation in anesthetized cats (Lindsey et al. 1998; Morris et al. 1996a,b).

Carotid chemoreceptors are well established as a primary source of respiratory drive, and their stimulation increases integrated phrenic activity and respiratory cycle frequency (Morris et al. 1996a,b; Shannon 1976). Activation of cutaneous nociceptors also enhances respiration (Duranti et al. 1991; Waldrop et al. 1984). Thermal stimulation of skin to a temperature of 46°C, sufficient to activate cutaneous nociceptors, also enhances respiration (Duranti et al. 1991; Waldrop et al. 1984). Thermal stimulation of skin to a temperature of 46°C, sufficient to activate cutaneous nociceptors, increased the amplitude of integrated phrenic activity (Waldrop et al. 1984). Duranti et al. (1991) reported that electrical stimulation of somatic nociceptive afferents in humans increases respiratory frequency, mean inspiratory flow, and rate of rise of integrated electromyographic activity of the diaphragm. At a lower level of stimulus intensity, a decrease in inspiratory phase duration contributes to increased respiratory frequency. At higher levels of stimulus intensity, the expiratory phase was also shortened.

Consistent with these previous results, we observed, in decerebrate animals, that both chemoreceptor stimulation...
and pinch increased peak integrated phrenic amplitude, respiratory frequency, and arterial blood pressure. The respiratory and cardiovascular responses to stimulation of these sensory modalities presumably constitute elements of an adaptive behavioral response of the organism (Hilton 1982; Lovick 1993). Hilton (1982) considered the inputs from peripheral chemoreceptors and somatic nociceptors to be “alerting” stimuli. The increase in ventilation and arterial blood pressure in response to chemoreceptor and footpad stimulation could be considered a “defense reaction” (Hilton 1982).

Carotid baroreceptors play an important role in the regulation of the cardiovascular system via the baroreflex. These receptors also participate in the modulation of breathing. Activation of carotid baroreceptors can reduce respiratory drive (Bishop 1974; Grunstein et al. 1975; Heymans and Bouckaert 1930; Lindsey et al. 1998; Nishino and Honda 1982), whereas deactivation of baroreceptors facilitates breathing (Brunner et al. 1982). In this study, baroreceptor stimulation decreased both integrated phrenic amplitude and cycling frequency. The prolongation of the expiratory phase during baroreceptor stimulation is consistent with previous results from anesthetized cats (Lindsey et al. 1998; Nishino and Honda 1982).

Raphe neurons have been implicated in the modulation of breathing (Holtman et al. 1986; Lalley 1986a,b; Lalley et al. 1997; Millhorn 1986; Morris et al. 1996a), the cardiovascular system (Barman and Gebber 1992, 1997; Coleman and Dampney 1995; Henderson et al. 1998; Zhong et al. 1993), and the processing and modulation of nociceptive information (Fields et al. 1983; Gao et al. 1998; Lovick 1993; Mason and Leung 1996). Raphe neurons project to the phrenic motoneurons (Holtman et al. 1984), intermediolateral nucleus (Loewy 1981; Morrison and Gebber 1984), and dorsal horn (Basbaum et al. 1978; Basbaum and Fields 1979) of the spinal cord. Baroreceptor and nociceptor stimulation had opposite effects on phrenic activity. Baroreceptor stimulation resulted in a decrease in integrated phrenic amplitude and prolongation of expiratory phase duration, whereas pinch stimulation caused an increase in integrated phrenic amplitude and a shortened expiratory phase duration. The firing rates of some raphe neurons decreased during baroreceptor stimulation but increased in response to pinch. Other raphe neurons responded oppositely.

Available evidence supports the view that there is an interaction between cardiovascular control and pain regulatory mechanisms (Randich and Maixner 1984, 1986; Zamir and Segal 1979; Zamir and Shuber 1980). Medullary raphe neurons have been implicated in anti-nociception during vagus afferent stimulation (Ren et al. 1990). In so far as baroreceptor stimulation and pinch had different effects on the same medullary raphe neurons, the present results are consistent with the hy-
hypothesis that baroreceptor stimulation may have anti-nociceptive actions in addition to its effects on breathing (Thurston and Randich 1990). Other data support the hypothesis that caudal medullary raphe neurons may contribute to decompensation (hypotension) following acute hypovolemia, and that these raphe neurons are distinct from neurons that regulate resting arterial pressure (Coleman and Dampney 1995; Henderson et al. 1998). Such neurons may also trigger hypotension in response to pain (Johansson 1962). Whether multimodal raphe neurons such as those described here participate in coordinated cardiorespiratory responses to hypovolemia, pain, and other challenges remains to be determined.

Response of VRG respiratory neurons and role of VRG E-DEC neurons in phase timing

Perturbations that alter the respiratory motor pattern probably do so by influencing the respiratory neurons in the lower brain stem (Bianchi et al. 1995). We have found that stimulation of carotid chemoreceptors, baroreceptors, and cutaneous nociceptors changes the firing rate of both inspiratory and expiratory neurons. Carotid chemoreceptor stimulation resulted in increased firing rates of VRG inspiratory neurons, which in part contribute to the increased inspiratory drive of phrenic activity during chemoreceptor stimulation (Morris et al. 1996b). Stimulation of cutaneous nociceptors also increased the activity of some inspiratory neurons including bulbospinal inspiratory neurons, which would contribute to the increased phrenic activity observed during activation of cutaneous nociceptors (Waldrop et al. 1984). Inspiratory neurons of the BÖT-VRG, including bulbospinal neurons, had decreased firing rates during baroreceptor stimulation; such responses may contribute to the decreased amplitude of phrenic activity during baroreceptor stimulation (see also Lindsey et al. 1998).

Increased firing rates of medullary expiratory neurons during baroreceptor stimulation may also contribute to the observed decreased phrenic activity. Rostral VRG E-AUG Bötzinger neurons make functional inhibitory connections both within the brain stem (Jiang and Lipski 1990; Lindsey et al. 1987) and with phrenic motoneurons (Merrill and Fedorko 1984). Perturbations of sensory modalities that alter expiratory phase duration are associated with prolongation of the active phase and firing rate of VRG E-DEC neurons (Hayashi et al. 1996; Lindsey et al. 1998; Remmers et al. 1986; Shannon et al. 1996). Functional connectivity data (Ezure 1990; Lindsey et al. 1987) and network simulations based on them (Balis et al. 1994) have suggested that rostral VRG E-DEC neurons play a role in initiating the expiratory phase and in the control of expiratory phase duration. The present data support this hypothesis.

Advantages and limitations of methods

The use of microelectrode arrays to record several single neurons and phrenic efferent activity simultaneously allowed us to detect the overlapping membership of groups of distributed neurons with multiple sensory modalities. An advantage of this approach is that changes in neuronal activity and respiratory motor pattern in response to a particular stimulus were measured under the same conditions, and were, therefore, not confounded by possible changes in the state of the animal. The
results demonstrate the feasibility of recording relatively many brain stem neurons during multiple perturbations that directly or indirectly alter blood pressure with the attendant possibility of brain stem movements that could result in signal loss. In the present sample, 403 of 474 (85%) neurons were tested for responses to all three modalities; larger percentages of the sample were tested with different combinations of two stimulus modalities. Experimental procedures including the bilateral pneumothorax presumably contributed to this success rate; the multielectrode arrays may have contributed to local stability. Simultaneously recorded neurons can also be analyzed with the complementary method of spike train cross-correlation; a companion paper (Li et al. 1999) describes results from this approach.

Chemoreceptor stimulation and pinch sometimes resulted in a delayed increase in arterial blood pressure, which would lead to secondary stimulation of carotid baroreceptors. Although the protocol of baroreceptor stimulation could differentiate this secondary baroreceptor effect on neuronal firing probabilities if a neuron responded oppositely to two types of stimulation, it could not differentiate the baroreceptor effect if the neuron responded to two types of stimulation in the same direction. However, available evidence from intracellular recordings indicates that NTS neurons receive convergent excitatory inputs from both chemoreceptors and baroreceptors (Mifflin 1993).

With the changes in pressure used in these studies, baroreceptor stimulation did not always decrease phrenic amplitude. This result in decerebrate cats is consistent with previous work from this laboratory in which baroreceptors were stimulated with three different methods in anesthetized cats (Lindsey et al. 1998). Selective stimulation of baroreceptors may influence either the durations of the respiratory phases or the amplitude of integrated phrenic nerve activity or both.

Pinch stimulation activates both noxious high-threshold mechanoreceptors (nociceptors) and nonnoxious low-threshold mechanoreceptors. Stimulation of both types of mechanoreceptors may alter medullary neuron activity (Anderson et al. 1977; Yen and Blum 1984). Therefore the experimental protocol used in this study did not allow us to differentiate the effects of stimulation of these two different types of mechanoreceptors on changes in neuronal activity and the respiratory motor pattern. However, stimulation of cutaneous receptors other than nociceptors alone would be unlikely to alter the respiratory motor pattern (Waldrop et al. 1984).

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