Multimodal Medullary Neurons and Correlational Linkages of the Respiratory Network

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Li, Zhongzeng, Kendall F. Morris, David M. Baekye, Roger Shannon, and Bruce G. Lindsey. Multimodal medullary neurons and correlational linkages of the respiratory network. J. Neurophysiol. 82: 188–201, 1999. This study addresses the hypothesis that multiple sensory systems, each capable of reflexly altering breathing, jointly influence neurons of the brain stem respiratory network. Carotid chemoreceptors, baroreceptors, and foot pad nociceptors were stimulated sequentially in 33 Dial-urethan–anesthetized or decerebrate vagotomized adult cats. Neuronal impulses were monitored with microelectrode arrays in the rostral and caudal ventral respiratory group (VRG), nucleus tractus solitarius (NTS), and n. raphe obscurus. Different phrenic nerve activity was recorded. Spike trains of 889 neurons were analyzed with cycle-triggered histograms and tested for respiratory-modulated firing rates. Responses to stimulus protocols were assessed with peristimulus time and cumulative sum histograms. Cross-correlation analysis was used to test for nonrandom temporal relationships between spike trains. Spike-triggered averages of efferent phrenic activity and antidromic stimulation methods provided evidence for functional associations of bulbbar neurons with phrenic motoneurons. Spike train cross-correlograms were calculated for 6,471 pairs of neurons. Significant correlogram features were detected for 425 pairs, including 189 primary central peaks or troughs, 156 offset peaks or troughs, and 80 pairs with multiple peaks and troughs. The results provide evidence that correlational medullary assemblies include neurons with overlapping memberships in groups responsive to different sets of sensory modalities. The data suggest and support several hypotheses concerning cooperative relationships that modulate the respiratory motor pattern. 1) Neurons responsive to a single tested modality promote or limit changes in firing rate of multimodal target neurons. 2) Multimodal neurons contribute to changes in firing rate of neurons responsive to a single tested modality. 3) Multimodal neurons may promote responses during stimulation of one modality and “limit” changes in firing rates during stimulation of another sensory modality. 4) Caudal VRG inspiratory neurons have inhibitory connections that provide negative feedback regulation of inspiratory drive and phase duration.

INTRODUCTION

A previous study found that stimulation of three sensory modalities capable of altering the respiratory motor pattern evoked different sets of responses in simultaneously recorded medullary neurons (Li et al. 1999). The data supported the hypothesis that brain stem respiratory-related neurons have overlapping memberships in multifunctional groups (Bianchi et al. 1995; Ezure 1990; Lindsey et al. 1992c, 1994; Miller 1995; Shannon et al. 1996).

The present study addresses three questions suggested by that work and earlier related studies (Arita et al. 1988; Miller 1995; Yen and Bloom 1984). 1) Are multimodal neurons members of functional assemblies defined by short-time scale correlations as well as their common response properties? 2) Do neurons responsive to only one of the tested modalities contribute to the response properties of multimodal neurons? 3) Do neurons that respond similarly to stimulation of different modalities have similar actions appropriate for the generation of correspondingly similar changes in the breathing pattern? Earlier work also suggested that some neurons “limit” the magnitude or duration of changes in firing rate evoked in other neurons by either peripheral chemoreceptors (Morris et al. 1996a) or baroreceptors (Lindsey et al. 1998). Sequential observations of single neurons cannot distinguish between responsive cells with putative “relay” functions and those that may act to limit or suppress reflexly induced changes in activity. Therefore we used spike train cross-correlation to assess the functional relationships of simultaneously recorded neurons with different combinations of responses to stimulation of baroreceptors, carotid chemoreceptors, and cutaneous nociceptors. Neurons were monitored in the nucleus of the solitary tract (NTS), raphe obscurus, and multiple subregions of the ventral respiratory group (VRG), including the rostral Böttinger (BÖT) and pre-Böttinger regions (Bianchi et al. 1995). Preliminary accounts of some of the results have been reported (Li et al. 1996, 1997; Morris et al. 1994).

METHODS

Materials and methods have been described in detail elsewhere (Li et al. 1999; Morris et al. 1996c). Data were from Dial-urethan–anesthetized (n = 15) or decerebrate (n = 18) vagotomized adult cats. Carotid chemoreceptors were selectively stimulated with the same method in both series. Baroreceptors were stimulated by inflation of an embolectomy catheter in the descending aorta of decerebrate cats (Li et al. 1999). In anesthetized animals, carotid baroreceptors were stimulated by local pressure changes in the carotid sinus (Morris et al. 1996c). Foot pads were pinched in the course of evaluation of the anesthetized state and to partially characterize recorded neurons. Because anesthesia may have blunted or suppressed some responses, the second series of pinch protocols was conducted in decerebrate cats. Changes in neuron firing rates measured in this second series were reported in the preceding paper; pinch stimuli were as described (Li et al. 1999).

Cross-correlation histograms (CCHs) were calculated for all pairs...
of simultaneously recorded neurons (Perkel et al. 1967). This method gives an estimate of the probability that an action potential in one neuron will occur at times relative to a spike in a second neuron. Short-time scale correlations permit inferences about simple classes of effective connectivity among the monitored neurons (Moore et al. 1970). Significance of primary correlogram features (peaks and troughs) was evaluated by calculation of the “detectability index” (DI) (Aertsen and Gerstein 1985). This index is the ratio of the maximum amplitude of departure from the background, to the background, divided by the standard deviation of the correlogram noise; values of DI greater than two were considered significant.

A cumulative sum histogram (CUSUM) was computed for each CCH with a significant DI value (Davey et al. 1986). The CUSUM can reveal small changes in the probability of spike occurrence obscured by random fluctuations. Successive bins in this display represent the sum of the differences of each proceeding bin and the mean count of a range of “control” bins. Statistical confidence limits were set at ±3 SD for each histogram. Ordinates were scaled to indicate the average change in the number of spikes per trigger event.

CUSUM histograms were used mainly to confirm the detection of significant changes in the firing probability of target neurons.

Two additional methods were used to characterize some neurons. Unrectified and full-wave rectified signals from the phrenic nerve were averaged using the spikes of a recorded neuron as trigger events. Nerve signals were filtered (band-pass 0.1–5 kHz) and sampled at 5 kHz with 16-bit accuracy. Signals were rectified, averaged, and interpreted as described previously (Cohen et al. 1974; Kirkwood and Sears 1991; Morris et al. 1996; Shannon et al. 1998). In six decerebrate animals, an array of four pairs of tungsten electrodes was placed in the ventral spinal cord at the C level to test for spinal projections of some recorded neurons with antidromic stimulation methods. Six of 16 BôT-VRG inspiratory neurons tested were identified as bulbospinal with positive collision tests.

RESULTS

The spike trains of 889 neurons were recorded with microelectrode arrays positioned in four brain stem regions (Fig. 1A);
stereotaxic coordinate boundaries for the sampled domains have been detailed elsewhere (Li et al. 1999; Morris et al. 1996a,c). Respiratory cycle-triggered histograms from one group of 17 simultaneously recorded neurons are shown in Fig. 1B. The abbreviations and numbers on the left denote the sites where the spike trains of the represented neurons were recorded: CM, caudal midline in the region of nucleus raphe obscurus; N, the NTS; CV, caudal VRG; RV, rostral VRG. Firing rate histograms (Fig. 1C) include repeated intervals of baroreceptor stimulation that resulted in decreased inspiratory drive; peak firing rates are indicated on the right. The response profile (Fig. 1D) gives a summary of the concurrent changes in average firing rates of the neurons, collectively designated group 1, during sequential stimulation of each tested modality.

Spike trains from 10 pairs of neurons in this set had short time scale correlations. These relationships are documented in the set of cross-correlograms illustrated in Fig. 2. An increase in the firing probability of a caudal VRG neuron following spikes in E-DEC NTS neuron N5 was indicated by the offset peak with a positive lag in Fig. 2A. Both neurons responded to two stimulus modalities. The firing rate of N5 increased during chemoreceptor stimulation and declined during baroreceptor stimulation; E-AUG neuron CV12A had a decreased firing rate both during chemoreceptor and baroreceptor stimulation.

Neurons in the region of the NTS were elements of three other correlated pairs in this group. Raphe neuron CM10A, which responded oppositely to chemoreceptor and baroreceptor stimulation, had an increased firing probability following spikes in an inspiratory NTS cell that responded only to chemoreceptor stimulation (Fig. 2B). The same NTS neuron tended to discharge synchronously with rostral VRG I-AUG neuron RV10, as indicated by a central correlogram peak (Fig. 2C). That VRG neuron responded oppositely to chemoreceptor and baroreceptor stimulation; it was associated with the tran-

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**FIG. 2.** Cross-correlograms with primary features indicative of paucisynaptic interactions among neurons represented in Fig. 1. Responses of the reference and target neurons to the tested stimulus modalities were as indicated. Some histograms were scaled-up to show significant primary features by subtraction of 80% of the counts in minimum bin from each bin. These correlograms include bin counts. A: offset peak with a positive lag of 7.5 ms; DI = 2.9; S = 0.43; HW = 3.0 ms; 4,826 reference and 31,689 target spikes. B: offset peak with a positive lag of 13.5 ms; DI = 3.0; S = 0.2; HW = 1.5 ms; 35,381 reference and 25,251 target spikes. C: central peak; DI = 2.6; S = 0.08; HW = 5.0 ms; 74,326 reference and 35,526 target spikes. D: offset peak with a positive lag of 1.5 ms; DI = 4.3; S = 0.14; HW = 1.5 ms; 103,301 reference and 22,828 target spikes. E: offset peak with a positive lag of 1.5 ms; DI = 3.4; S = 0.06; HW = 1.5 ms; 103,302 reference and 74,323 target spikes. F: central peak; DI = 3.3; S = 0.07; HW = 5.5 ms; 90,582 reference and 74,323 target spikes. G: offset trough with a positive lag of 1.5 ms and a central peak; trough DI = 3.3; S = 0.06; HW = 1.5 ms; peak DI = 4.0; S = 0.13; HW = 1.5 ms; 90,582 reference and 22,828 target spikes. H: offset trough with a positive lag of 4.5 ms; DI = 3.6; S = 0.09; HW = 5.0 ms; 90,582 reference and 74,323 target spikes. I: central trough; DI = 3.3; S = 0.11; HW = 5.5 ms; 74,315 reference and 11,553 target spikes.

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siently reduced firing probability of NTS neuron N2 (Fig. 2J), and, together with cell RV9, was one of two rostral VRG I-AUG neurons with increased firing probabilities following spikes in caudal VRG I-DEC neuron CV8 (Fig. 2, D and E).

The two rostral VRG neurons tended to discharge synchronously (Fig. 2F), consistent with convergent excitation from CV8. Cell RV9 also exhibited a transient decline in firing probability following spikes in I-DEC neuron RV8; the cross-correlogram trough with a positive lag relative to the correlogram origin was matched with a central peak (Fig. 2G). The firing probability of caudal VRG neuron I-DEC CV9 was also reduced following RV8 spikes (Fig. 2H). The rostral I-DEC neuron RV8 was also correlated with caudal I-DEC cell CV10; an asymmetric central peak was the primary correlogram feature (not shown).

Spike-triggered averages of efferent phrenic nerve activity also revealed evidence of functional interactions (Fig. 3). A transient decrease in phrenic activity followed spikes in E-AUG neuron CV12B. Increased activity followed spikes of cells RV8 and CV9 and was coincident with spikes in CV8. Summaries of functional connectivities inferred from these and subsequently described correlational linkages are deferred to and considered in the DISCUSSION.

Overall, cross-correlograms were calculated from spike trains of 6,471 pairs of neurons. Significant primary features indicative of paucisynaptic interactions, including 189 primary central peaks or troughs and 156 offset peaks or troughs, were found for 345 pairs. Table 1 gives the number of correlogram features indicative of paucisynaptic interactions for correlated pairs of reference and target neurons recorded in the indicated brain stem regions. Sixty-six pairs of neurons with primary

<table>
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<th>Reference-Target Neuron Category</th>
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<tr>
<td></td>
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<tr>
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<td>BÖT-VRG–BÖT-VRG</td>
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</tr>
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NTS, nucleus tractus solitarius; BÖT, Bötzinger; VRG, ventral respiratory group.

FIG. 3. Spike-triggered averages of full-wave rectified efferent phrenic activity sampled at 10 kHz. Other properties of the trigger neurons are shown in Figs. 1 and 2. Numbers of trigger events: CV12B, 30,352; RV8, 90,477; CV9, 50,241; CV8, 103,227.

FIG. 4. Ratios of cross-correlations with primary features or multiple peaks and troughs to totals within and between the 4 brain stem domains. Raphe, raphe obscurus; eVRG, rostral VRG; cVRG, caudal VRG; NTS, nucleus tractus solitarius.
correlogram features included a reference neuron monitored in the region of the NTS and a target cell recorded in either n. raphe obscurus or BÖT-VRG. Figure 4 shows the ratios of cross-correlograms with features to total pairs within and between the four brain stem domains sampled; it includes 80 additional pairs that exhibited multiple peaks and troughs without a primary correlogram feature.

Table 2 gives a summary of primary correlogram features for the 286 pairs composed of 2 neurons recorded during sequential stimulation of chemoreceptors, baroreceptors, and nociceptors. Correlogram features are arranged by the stimulus modalities that altered the firing rates of the reference (rows) and target (columns) neurons. Excluded from Table 2 were pairs of correlated neurons that included cells not tested with all three-stimulus protocols because of inactivity or signal loss.

Correlations of raphe neurons

Seventy-seven short time scale correlations were detected between pairs of raphe neurons (Table 1). Data from one set of simultaneously recorded raphe and caudal ventrolateral medullary neurons, designated group 2, included several correlated pairs. All of the neurons had respiratory-modulated firing rates (Fig. 5A) as judged by statistical tests (Li et al. 1999). The activities of all neurons increased in response to peripheral chemoreceptor stimulation. Fewer members of the group changed activity during baroreceptor stimulation or pinch (Fig. 5B). Spike-triggered averages indicated that CM4 spikes were associated with moments of reduced phrenic activity. In contrast, both CM6A and CV1 tended to discharge synchronously with phrenic motoneurons (Fig. 5C).

The correlation feature map (Fig. 5D) is a summary of primary cross-correlogram features detected for neurons in group 2, some of which are documented in Fig. 6. The cross-correlogram for two raphe E-DEC neurons (Fig. 6A) indicates an increased firing probability in target cell CM1 following spikes in reference neuron CM3. The firing rate of CM3 increased during chemoreceptor and nociceptor stimulation; the average activity of CM1 increased during chemoreceptor stimulation, but not during perturbations of the other tested modalities.

A central peak with bilateral troughs characterized the correlogram features included a reference neuron monitored in the region of the NTS and a target cell recorded in either n. raphe obscurus or BÖT-VRG. Figure 4 shows the ratios of cross-correlograms with features to total pairs within and between the four brain stem domains sampled; it includes 80 additional pairs that exhibited multiple peaks and troughs without a primary correlogram feature.

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relogram for two other raphe E-DEC neurons (Fig. 6B); CM2 had an increased firing rate in response to chemoreceptor stimulation; CM6A exhibited an increase in activity during chemoreceptor stimulation and pinch. The correlogram of another pair of raphe neurons had a central trough; the firing probability of one neuron was high when the other was low (Fig. 6C). Both E-OTH neuron CM6 and I-AUG neuron CM4 increased their firing rates during chemoreceptor stimulation only; they were monitored concurrently with the other neurons represented in Fig. 5.

Primary correlogram features for pairs composed of raphe and BÔT-VRG neurons included 24 central peaks, 6 central troughs, 38 offset peaks, and 15 offset troughs (Table 1). Examples of these correlations drawn from an analysis of neurons in group 2 include corresponding CUSUM histograms (Fig. 6, D–F). The I-AUG neuron CV1 had an increased firing probability following spikes in the raphe E-DEC neuron CM6A (Fig. 6D). The target cell also had an increased firing rate during chemoreceptor stimulation and exhibited a decline in activity during baroreceptor stimulation and pinch. Neuron CV1 was also correlated with raphe E-DEC neuron CM2; the firing probability of CM2 declined following spikes in CV1, although there was also a tendency for the two neurons to discharge in near synchrony (Fig. 6E). In another pair from the same group, the ventrolateral medullary I-AUG neuron CV3 had a transient decrease in firing probability following impulses in raphe I-AUG neuron CM4 (Fig. 6F). Neuron CV3 exhibited an increase in activity during chemoreceptor stimulation and a decrease during baroreceptor stimulation. The responses of CM4 were described above.

Correlations between BÔT-VRG neurons

Primary features in cross-correlograms calculated for pairs of BÔT-VRG neurons included 67 central peaks, 3 central troughs, 33 offset peaks, and 16 offset troughs. The illustrative set of eight simultaneously recorded rostral and caudal VRG neurons designated group 3 included neurons with four major features in cross-correlograms.
respiratory-modulated discharge patterns (Fig. 7A). Two rostral I-AUG neurons RV2 and RV5 responded with increased firing rates during chemoreceptor stimulation and pinch and exhibited decreased activity during baroreceptor stimulation. Both neurons were identified as bulbospinal, with projections to at least the third cervical segment identified by antidromic stimulation methods. In each of two other recordings, a bulbospinal BÔT-VRG inspiratory neuron that responded to stimulation of at least two tested modalities was identified by a positive collision test.

Other responses of neurons in group 3 are summarized in Fig. 7B. During chemoreceptor stimulation, neuronal firing rate changes were also associated with increased blood pressure (Fig. 7C). However, all neurons that responded to the perturbation of the peripheral chemoreceptors either had rate changes in the opposite direction or did not change rate when baroreceptors were stimulated separately.

The example of an offset peak in Fig. 8A documents an increased firing probability in the caudal VRG I-AUG neuron CV1 following spikes in the rostral VRG neuron RV5. Neuron RV5 was also correlated with another rostral I-AUG neuron RV1 (Fig. 8A, bottom). The responses of the latter cell to chemoreceptor stimulation and pinch were similar to those of RV5; however, the firing rate of RV1 did not change during baroreceptor stimulation. Other primary features in correlograms for group 3 are summarized in Fig. 8B.

Evidence for functional links among this group of BÔT-VRG neurons and phrenic motoneurons included several spike-triggered averages (Fig. 8C). Among the noteworthy features were the offset peaks for three rostral neurons, including the bulbospinal neurons RV2 and RV5 and the central peaks in averages triggered by spikes in CV1 and RV1.

Another set of six simultaneously recorded BÔT-VRG neurons, group 4, included caudal I-AUG neuron CV7 and rostral I/EI-DEC neuron RV1, which began to discharge late in expiration and reached peak activity during the inspiratory phase (Fig. 9A). The firing rate of CV7 increased during chemoreceptor stimulation and decreased during baroreceptor stimulation. The activity of RV1 increased only during chemoreceptor stimulation. Among the short time scale correlations of this group (Fig. 9C) was a transient decrease in the firing probability of the rostral neuron following spikes in the caudal cell.
Spikes in both neurons were followed by transient increases in phrenic motoneuron activity (Fig. 9D). Averages triggered by other caudal inspiratory neurons had central peaks (Fig. 9C).

The first two columns in Table 3 give a summary of detected correlations relevant to the hypothesis that neurons responsive to a single tested modality are elements of correlational assemblies that include multimodal neurons. The data are arranged by recording site, response properties, and correlation feature. The first column gives tallies of correlations between single modality neurons that responded to one tested (“single,” S) modality and multimodal neurons that responded to the same stimulus. The second column lists correlations between single modality neurons and multimodal neurons that did not respond to the same stimulus. Correlations between multimodal neurons are in the third column. The last two columns summarize short-time scale correlations for which simple interpretations suggest that the reference neuron acted to “promote” the response of the target neuron or “limit” the reflexly induced changes in firing rate of the target neuron, respectively. Examples of these interpretations drawn from the neuronal groups represented in the preceding figures are considered next in the Discussion.

Discussion

The results from multi-array recordings support the hypothesis that elements of a distributed brain stem respiratory network modulate breathing by both promoting and limiting changes in respiratory drive and phase durations during perturbations of multiple sensory modalities. The measured response properties and short-time scale correlations suggest cooperative functional relationships that could contribute to the observed respiratory patterns. Several simple classes of connectivity are commonly inferred from correlogram features (e.g., Aertsen and Gerstein 1985; Moore et al. 1970; Perkel et al. 1967). An offset trough suggests an inhibitory process, defined operationally as any mono- or paucisynaptic relationship that reduces target cell firing probability. An offset peak suggests excitation of the neuron with the transient increase in firing probability by the other neuron or an unobserved shared input that influences both cells with different delays. Shared inputs or cross-connections with similar action result in synchronous discharge reflected as a central peak. A central trough can be attributed to functional inputs with opposite actions on each of the monitored neurons.

Connectivity and parallel actions of group 1 neurons inferred from the results are illustrated and tabulated in Fig. 10A. The NTS neuron N5 responded to chemoreceptor stimulation with increased activity, whereas the firing rate of its putative target neuron CV12A decreased. The activities of both neurons decreased during baroreceptor stimulation. These response properties and the correlational data support the view that N5 promoted the decline in firing rate of CV12A during baroreceptor stimulation. However, the response properties during the carotid chemoreceptor stimulation were not consistent with the sign of correlation, suggesting that increased firing in N5 limited the extent of the decline in CV12A activity.

Neuron CV12A was not correlated with phrenic activity; however, spikes in CV12B, a neuron with similar discharge and

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**FIG. 7.** A: cycle-triggered histograms for group 3; 211 cycles averaged. Rate scale does not apply to phrenic activity. B: response profile for the tested modalities; abbreviations as in Fig. 1. C: firing rate histograms of neurons in group 3 during peripheral chemoreceptor stimulation, integrated phrenic activity, and arterial blood pressure.
response properties, were followed by a transient reduction in phrenic activity. The inferred inhibitory action is consistent with established connectivity for E-AUG neurons, particularly in the rostral Bo¨tzinger region (Merrill and Fedorko 1984). Other inferred actions within group 1 enumerated in Fig. 10 include the following: 1) the contribution of NTS neuron N6, which responded to one tested modality, to the multimodal response properties of raphe neuron CM10A; 2) divergent distributed inhibitory actions of rostral multimodal VRG neuron RV8; 3) the distributed excitatory actions of caudal neuron CV8; and 4) parallel excitation and inhibition of phrenic motoneurons. The summaries indicate whether each of the correlated neurons responded to stimulation of a single or multiple modalities and the putative consequences of the inferred synaptic actions. In the tabulated summaries, the term “promote” refers to any action that would contribute to the observed change in target cell firing probability during stimulation of the indicated modality. The term “limit” refers to an action that would tend to “oppose” the direction of change in the target neuron’s firing rate. “None” indicates that a putative follower neuron did not respond to stimulation of a particular modality that did influence an inferred driver neuron. The tabulated summaries exclude inferred shared inputs from unobserved sources that are shown in the graphic summary.

Evidence for local and distributed actions of raphe neurons is exemplified by results from group 2 (Fig. 10B). Neurons responsive to one or several of the tested modalities had functional linkages with caudal VRG neurons and phrenic motoneurons. Note the inferred functional connectivity between raphe neurons CM6A and CM6A. The simplest interpretation of the central correlogram peak is that two neurons had shared inputs. Both the reference and the target neurons had E-DEC discharge patterns, and both responded to chemoreceptor stimulation with increased firing rates. The extended correlational linkage of CM6A suggests that it excited tonic I-AUG neuron CVI. The inferred functional relationship between multimodal raphe neuron CM6A and caudal ventrolateral cell CVI implies a context dependent modulation of sensory information. The inferred sign of the connection is consistent with increased activity of CM6A having contributed to an increase in the firing rate of CVI during carotid chemoreceptor stimulation. However, the increase in activity of

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**FIG. 8.** A: examples of the short-time scale correlations between VRG neurons in group 3. A, top: offset peak with a positive lag of 2.5 ms; DI = 8.3; S = 0.34; HW = 1.5 ms; 71,933 reference and 34,729 target spikes. A, bottom: central peak with bilaterally mapped secondary peaks and troughs; DI = 5.8; S = 0.10; HW = 3.0 ms; same reference events as top correlogram; 68,999 target events. B: correlation feature map for group 3 neurons described in RESULTS. C: spike-triggered averages of full-wave rectified efferent phrenic activity. Numbers of trigger events: CV1, 34,729; RV1, 69,002; RV2, 51,509; RV5, 71,942; RV5A, 130,931.
CM6A during pinch would have tended to facilitate CV1 in the suggested organization, thus opposing the reduced firing rate of CV1 during pinch.

The CM2-CV1 correlation features included both an offset trough and an asymmetric central peak. The illustrated inhibitory action of CV1 on CM2 and the input shared by both neurons are simple and parsimonious inferences. Under this interpretation, the shared influences include the chemoreceptor input to which both cells were responsive. The recurrent inhibition would “limit” the response of CM2. The absence of responses to baroreceptor stimulation and pinch in CM2 would suggest that potential disinhibitory effects associated with the reduced activity in CV1 were ineffective. It is possible that the recurrent inhibition served to promote synchronous discharge of CM2 with other similar but unobserved neurons responsive to peripheral chemoreceptor inputs (cf. Lindsey et al. 1992b; Morris et al. 1996b).

TABLE 3. Numbers of pairs composed of at least one neuron responsive to stimulation of multiple modalities

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<th>Reference-Target Neuron Category</th>
<th>S-M</th>
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<th>M-M</th>
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<th>Offset feature suggestive of “limit”</th>
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<td>2</td>
<td>3</td>
<td>6 (2*)</td>
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<td>4</td>
<td>9 (4*)</td>
<td>5 (2*)</td>
</tr>
<tr>
<td>Raphe–BÖT-VRG</td>
<td>24</td>
<td>2</td>
<td>6</td>
<td>15 (9*)</td>
<td>10 (3*)</td>
</tr>
<tr>
<td>BÖT-VRG–BÖT-VRG</td>
<td>38</td>
<td>0</td>
<td>17</td>
<td>23 (5*)</td>
<td>19 (1*)</td>
</tr>
</tbody>
</table>

S-M, cross-correlation calculated from a single modality reference and a multimodal target neuron that is responsive to the same single stimulus as the reference neuron; S'-M', cross-correlation calculated from a single modality reference and a multimodal target neuron that is not responsive to the same single stimulus as the reference neuron; M-M, cross-correlation calculated from multimodal reference and target neurons; other abbreviations, Table 1. * Offset correlogram features included in a previous report on interactions of neurons responsive to peripheral chemoreceptor stimulation (Morris et al. 1996a,c).
Putative single modality raphe neurons may also have this rate-limiting function. An example is the postulated inhibitory action of CM4 on multimodal VRG neuron CV3. An increase in firing rate in CM4 would tend to have "limited" the increased activity of CV3 during chemoreceptor stimulation.

Results from the analysis of group 3 provided evidence for parallel and serial actions within the BÖT-VRG, including contributions of the observed neurons to changes in the respiratory motor pattern resulting from the perturbations. Putative actions include the convergence of rostral I-AUG neurons on caudal I-AUG neurons and both promoting and limiting actions of a multimodal caudal neuron on a rostral cell. Two subsets of rostral neurons had correlations indicative of shared inputs; neuron RV5 was an element of both. Both rostral and caudal I-AUG neurons had linkages with phrenic motoneurons (Fig. 10C). The central peak in the average triggered by the caudal neuron suggested that presynaptic synchrony was responsible for the feature: other neurons correlated with CV1, such as rostral cells RV2, RV5, and RV5A would have more direct actions on the recorded phrenic motoneurons under this interpretation of the data.

The inferred functional relationships in group 4 included serial inhibitory and disinhibitory relationships with predominantly limiting functions. The results suggest that neuron CV3 acted on RV5 by one route to limit responses to stimulation of two modalities and, via a second loop through CV5A and CV7, promote responses to the same modalities.

Relationship to previous work

The nucleus tractus solitarius is the site where most carotid sinus afferent terminals are found (Davies and Edwards 1975; Donoghue et al. 1984). Neurons responsive to peripheral chemoreceptor and baroreceptor stimulation are located within the NTS; it is the first brain stem site for mutual interactions between the two sensory systems (Felder and Mifflin 1994). The offset peaks and troughs in correlograms with NTS reference neurons and BÖT-VRG or n. raphe obscurus target cells provide additional evidence for the hypothesis that NTS neurons transmit sensory information to neurons in those two domains (see also Morris et al. 1996c).

Earlier work has also supported the hypothesis (Pitts et al. 1939) that medullary raphe neurons are elements of a distributed system that modulates breathing (Holtman et al. 1986; Lalley 1986a,b; Lindsey et al. 1992a,b, 1994). Raphe neurons have been implicated in the induction and expression of long-term facilitation of inspiratory drive after chemoreceptor stimulation (Millhorn 1986; Morris et al. 1996a), and in baroreceptor modulation of the respiratory motor pattern (Lindsey et al. 1998). Single raphe neurons respond to stimulation of different modalities and, via a second loop through CV5A and CV7, promote responses to the same modalities.
sensory modalities (Yen and Blum 1984), suggesting that they may be elements of multifunctional groups. A companion paper described the sequential responses of sets of medullary neurons to both chemoreceptor and baroreceptor stimulation, in addition to a somatic nociceptive stimulus capable of altering breathing (Li et al. 1999). The present results extend that work. The detected correlational relationships provide evidence for overlapping memberships in functional groups suggested by response properties revealed by stimulation of multiple sensory modalities.

Functional connections among BÖT-VRG neurons may contribute to changes in the respiratory motor pattern during perturbations of different afferent systems. Rostral VRG I-DRIVER neurons have been proposed to have roles in the initiation and timing of the inspiratory phase through excitation of VRG I-AUG and I-DEC neurons (Balis et al. 1994; Morris et al. 1996c; Segers et al. 1987). Other correlational evidence suggested that inspiratory neurons inhibit other inspiratory neurons (Segers et al. 1987). The present results confirm and extend the previous work and are consistent with both rostral to caudal and caudal to rostral BÖT-VRG interactions participating in the reflex modulation of breathing. Rostral BÖT-VRG inspiratory neurons may convey sensory information through modulation of the duration or magnitude of their excitatory actions on caudal inspiratory neurons. This inferred relationship was illustrated in Fig. 10C, in which the rostral BÖT-VRG I/EI-DEC neuron RV5 has an excitatory connection with caudal VRG I-AUG neuron CV1. Complementary inferred caudal to rostral excitatory actions among BÖT-VRG inspiratory neurons are also illustrated (CV8 to RV9 and RV10 in Fig. 10A).

The inferred functional connection between CV7 and RV1 (Fig. 10D) suggests that the caudal I-AUG neuron inhibited the rostral I/EI-DEC target cell. Both neurons exhibited increased firing rates during chemoreceptor stimulation; CV7 also decreased activity in response to baroreceptor stimulation. If the rostral neurons include those with an I-DRIVER function, then such connectivity could provide negative feedback to limit changes in rate and phase duration during chemoreceptor stimulation.

The inferred excitatory and inhibitory functional connectivity has led to the prediction that inspiratory neurons distributed in the rostral and caudal VRG may exhibit impulse synchrony as reflected in central correlogram peaks. The central peak on a CCH of RV8 and RV9 may reflect a shared input (Fig. 10A), whereas the offset right-side trough suggests that RV8 inhibited RV9.

Advantages and limitations of methods

Multi-array recordings have the advantage that changes in activity and the respiratory motor pattern in response to a particular stimulus are measured under the same conditions, and, therefore, not confounded by possible changes in the state of the animal. Coupled with sequential stimulation of different sensory modalities, this approach allowed us to detect the overlapping membership of groups of distributed neurons with multiple sensory modalities and make inferences about functional connectivity and information processing through cooperative phenomena.

The shapes of cross-correlogram features may depend in part on the time derivative of the postsynaptic potentials generated by the interaction. Therefore cross-correlation analysis is most likely to detect those interactions that are characterized by generation of fast excitatory or inhibitory postsynaptic potentials. Synaptic interactions that result in small postsynaptic potentials or long-lasting changes in membrane conductance may not produce significant short-time scale correlations. A polysynaptic interaction is less likely to be detected by cross-correlation analysis due to the reduction in the strength of correlation caused by temporal dispersion (Kirkwood 1979).

The absence of a change in firing rate does not necessarily indicate that a particular neuron cannot be influenced by a tested modality. Fluctuations in thresholds, network gating mechanisms, and the state of the animal all may influence responsiveness. Furthermore, “responsiveness” may not be reflected in detectable changes in the firing rates of individual neurons, but may transiently alter an emergent property such as neuronal synchrony of assemblies in which the observed neuron is a member (Arata et al. 1991; Lindsey et al. 1989, 1992c, 1997). Of course, a neuron responsive to only one of the tested modalities may be responsive to additional modalities that were not tested.

Several hypotheses on medullary network interactions inferred from the results are summarized by the corresponding numbered connections in Fig. 11. 1) Neurons responsive to a single tested modality promote changes in firing rate of multimodal target neurons. 2) Neurons responsive to a single tested modality limit changes in firing rate of multimodal target neurons. 3) Multimodal neurons contribute to changes in firing rate of neurons responsive to a single tested modality. 4) Multimodal neurons may promote responses during stimulation of one modality and limit changes in firing rates during stimulation of another sensory modality. 5) Caudal VRG inspiratory neurons have inhibitory connections that provide negative feedback regulation of inspiratory drive and phase duration.

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