Responses of ventral respiratory group neurons of the cat to natural vestibular stimulation

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Woodring, S. F., and B. J. Yates. Responses of ventral respiratory group neurons of the cat to natural vestibular stimulation. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1946–R1956, 1997.—Stimulation of vestibular otolith afferents by fore-aft tilt (pitch) elicits changes in activity of nerves innervating respiratory musculature, including the diaphragm, abdominal muscles, and tongue musculature. To determine the role of ventral respiratory group (VRG) neurons in producing these vestibulo-respiratory responses, the activity of VRG neurons was recorded during natural vestibular stimulation in multiple transverse planes. Only a small fraction of VRG neurons with inspiratory inputs (I, 20 of 80 cells), expiratory inputs (E, 11 of 59 cells), or phase spanning (4 of 16 cells) activity responded to tilts up to 15° in amplitude delivered at frequencies from 0.02 to 2 Hz. In particular, responses were infrequent in VRG neurons with projections to the spinal cord (0 of 23 E cells and 2 of 15° I cells), despite the fact that the tilts employed produced robust modulation of the activity of abdominal (expiratory) nerves. Furthermore, the characteristics of responses to tilt of the small fraction of VRG neurons with vestibular inputs did not match those of respiratory muscles. These data suggest that neurons in addition to those in the VRG must participate in generating vestibulo-respiratory responses.

The activity of respiratory muscles, including the diaphragm (major inspiratory muscle), abdominal muscles (major expiratory muscles), and upper airway muscles (which act as valves to regulate airway resistance), is modulated during movement and changes in posture (3, 5, 8, 14, 18, 22). Recent studies in the cat (16, 17, 21, 28) have shown that part of the change in activity of respiratory muscles during alterations in body position is due to influences of the vestibular system. Electrical stimulation of the vestibular nerve produces responses in nerves innervating abdominal muscles, the diaphragm, intercostal muscles, tongue musculature, laryngeal muscles, and pharyngeal muscles (20, 21, 28). Selective natural stimulation of vestibular receptors, by rotation of the head (on a fixed body) in animals with denervations to remove neck, respiratory, cardiovascular, and facial somatosensory inputs that may be produced by the movement, produces modulation in the activity of nerves innervating abdominal muscles, the diaphragm, and muscles that move the tongue (16, 17). The effects of natural vestibular stimulation on other respiratory muscles have not been studied. Vestibular stimulation at small amplitudes (10–15° rotations) routinely produces responses in the abdominal muscles; in contrast, head rotations at amplitudes over 20° are sometimes required to affect neural outflow to tongue musculature and the diaphragm (16, 17). For all respiratory muscles studied thus far, the best direction of head rotation for producing an increase in activity is typically near nose-up pitch, although ipsilateral ear-down roll is also effective in activating some abdominal muscles in ~25% of animals (16, 17). The response characteristics of vestibulo-respiratory responses suggest that they are due to stimulation of otolith organs and not semicircular canals (16, 17).

Several studies have considered the neural pathways that mediate vestibular influences on respiratory muscles. Vestibulo-respiratory responses are abolished by chemical or mechanical lesions of regions of the medial and inferior vestibular nuclei caudal to Deiter’s nucleus (16, 21, 28), suggesting that the medial and inferior vestibular nuclei are essential for producing these responses. Anatomic studies have shown that the medial and inferior vestibular nuclei project to regions of the lateral medullary reticular formation near the nucleus ambiguus and retrofacial nucleus containing neurons of the ventral respiratory group (VRG) (24). In contrast, the ventrolateral portion of nucleus solitarius containing dorsal respiratory group (DRG) neurons receives a paucity of projections from the medial and inferior vestibular nuclei (27). Electrophysiological studies confirmed that DRG inspiratory neurons receive little vestibular input (27) but that almost 50% of inspiratory bulbospinal VRG neurons (12) and over 80% of expiratory bulbospinal VRG neurons (20) respond to electrical stimulation of the vestibular nerve. However, chemical lesions that inactivated large portions of the VRG had little effect on vestibulo-respiratory responses (30). In addition, transections of axons of VRG bulbospinal neurons, which are known to be the predominant source of respiratory signals to the spinal cord (2, 6, 7), reduce but do not abolish vestibulo-abdominal responses (16, 20). These findings suggest that although VRG neurons receive vestibular inputs, other populations of neurons are also involved in relaying vestibular signals to spinal respiratory motoneurons.

As yet, the responses of VRG neurons to natural vestibular stimulation have not been characterized. It remains to be determined whether the responses of VRG neurons to natural vestibular stimulation will have the same spatial and temporal properties as vestibulo-respiratory responses recorded from the diaphragm and abdominal muscles. One possibility is that VRG neurons have similar responses to natural vestibular stimulation as do spinal respiratory motoneurons but that additional populations of premotor respiratory neurons also have similar response properties, so that lesions of the VRG do not abolish responses of respiratory muscles to vestibular stimulation. Another possibil-
ility is that VRG neurons have substantially different responses to natural vestibular stimulation than do respiratory muscles and that more powerful vestibular signals from another group of neurons mask the vestibular inputs transmitted to spinal respiratory motoneurons by VRG neurons.

To determine the role of VRG neurons in producing vestibular-respiratory responses, we recorded activity from these neurons during whole body tilts in multiple vertical planes. In many animals, nonvestibular signals that might be produced by body movements were eliminated by transection of the IXth and Xth cranial nerves and the spinal cord at C4. Similar nerve and spinal transections were previously shown to eliminate visceral inputs to the brainstem that were produced by whole body tilt (25, 26). In some animals without spinal cord transections, we also recorded activity from abdominal nerves during vertical tilts, so that vestibular-elicited changes in the activity of VRG neurons and spinal respiratory nerves could be directly compared. We additionally determined whether VRG neurons examined for vestibular inputs were bulboseptal by stimulating the C2 spinal white matter to antidromically activate descending projections from the brainstem.

Some of these data have been reported in preliminary (abstract) form (23).

METHODS

All procedures used in this study conformed with the American Physiological Society’s “Guiding Principles for the Care and Use of Animals” and were approved by the University of Pittsburgh’s Animal Care and Use Committee.

General surgical procedures. Experiments were performed on 20 adult cats of either sex. Anesthesia was induced and maintained with 1–2% halothane (Fludane, Ayerst Laboratories) vaporized in N2O and O2. Blood pressure was monitored in 20 adult cats of either sex. Anesthesia was induced and maintained by hourly injections of 5 mg/kg gallamine triethiodide (Sigma), which was supplemented by hourly injections of 5 mg/kg. While paralyzed, animals were artificially respired by use of a positive-pressure ventilator; end-tidal CO2 was typically maintained between 4 and 5% but occasionally was transiently allowed to rise as high as 6% to stimulate generation of the respiratory rhythm. In some animals, injections of doxapram hydrochloride (Dopram-V, Fort Dodge Laboratories, 3–7 mg/kg iv) were made to augment spontaneous respiratory activity in the central nervous system. At the end of the recording session, animals were killed with an overdose of pentobarbital sodium (120 mg/kg iv).

Procedures to eliminate nonvestibular inputs that could be elicited by whole body tilt. In addition to activating vestibular receptors, whole body tilt may stimulate abdominal, cardiovascular, pulmonary, and other receptors. To exclude nonvestibular inputs that could be produced by body movement, the IXth and Xth cranial nerves were cut, and the spinal cord was transected at C4 in 11 animals. We previously showed that these procedures are effective in removing visceral inputs to brainstem cardiovascular-regulatory neurons (25, 26), and so they presumably also eliminated tilt-related afferent signals other than those from the vestibular system to respiratory neurons. After the spinal cord transection, Amaran was always required to maintain mean blood pressure >100 mmHg.

Antidromic activation of neurons projecting to spinal cord. Three stainless steel floating electrodes, insulated to ~200 µm from the tip, were inserted into the lateral and ventrolateral white matter of C2 on each side. We attempted to place each electrode at a slightly different laterality, so that the entire ventrolateral white matter would be stimulated. Monopolar square-wave current pulses 0.2 ms in duration and up to 1 mA in intensity were used for stimulation; the anode was attached to muscle adjacent to the stimulated cord. In every case, the antidromic nature of responses to spinal stimulation was confirmed using collision. In some animals, the locations of the electrode tips were marked by electrolytic lesions so that they could be reconstructed. The placement of electrodes in one animal is indicated in Fig. 1.

Fig. 1. Camera lucida drawing of a transverse C2 spinal cord section showing locations of tips of stimulating electrodes (indicated by x). Three electrodes were inserted on each side to maximize area of ventrolateral white matter that was stimulated.
Vertical vestibular stimulation. Vertical vestibular stimulation was produced by tilting the entire animal about the pitch (transverse) and roll (longitudinal) axes using a servo-controlled hydraulic tilt table (Neuro Kinetics, Pittsburgh, PA). The hydraulic axes of the tilt table were driven by sinusoidal stimuli delivered by a Cambridge Electronic Design (CED) 1401-plus data collection system interfaced with a Macintosh Quadra 800 computer. To characterize the vertical vestibular inputs to a neuron, we first determined the plane of tilt that produced maximal modulation of its firing rate (response vector orientation). Response vector orientation was determined using the "wobble" stimulus, a constant-amplitude tilt whose direction moves around the animal at constant speed (19). Clockwise wobble stimuli were generated by driving the pitch axis of the tilt table with a sine wave while simultaneously driving the roll axis with a cosine wave; during this stimulus, the animal's body, viewed from above, appeared to wobble, having in succession nose down, right ear down, nose up, and left ear down. When the signal to the pitch axis of the tilt table was inverted, the stimulus vector rotated in the counterclockwise direction. The direction of the response vector orientation lies midway between the maximal response directions to clockwise and counterclockwise wobble stimulation, because the phase differences between stimulus and response are reversed during the two directions of stimulation (19). Thus, by consideration of both responses, these phase differences can be accounted for. Wobble stimuli were delivered at frequencies ranging from 0.05 to 0.5 Hz (typically including 0.2 Hz) and at amplitudes up to 15°.

Once response vector orientation was obtained, stimuli in a fixed vertical plane at or near this orientation were used to study the dynamics of the vestibular response (i.e., response gain and phase across stimulus frequencies). Planar stimuli were generated by applying sine waves to the roll axis, the pitch axis, or simultaneously to both axes of the tilt table, so that during the first half-cycle one side of the head was tilted down and during the second half-cycle the opposite side was tilted down. Driving both the pitch and roll axes simultaneously with sine waves produced tilts in a plane oriented between the pitch and roll planes; the orientation was determined by the ratio of the signal sent to the roll and pitch axes. The firing rate of most neurons that responded to tilt was modulated by stimulus amplitudes <7.5°. Thus, for most units that received vestibular inputs, we used 7.5° planar stimuli to elicit responses at frequencies <1 Hz so that the effects of the same stimulus amplitude at multiple stimulus frequencies could be compared. Because of technical limitations in our tilt table, smaller stimuli (5° at 1 Hz and 2.5° at 2 Hz) were delivered at higher frequencies.

Recording of nerve activity. Activity was recorded from the hypoglossal nerve in all animals to allow monitoring of the respiratory cycle. Bursts of activity occur in the hypoglossal nerve in synchrony with inspiration, because the tongue must be protruded to maintain airway patency during the inspiratory phase of the respiratory cycle (2, 6, 7). In addition, we recorded from an L1 abdominal nerve in four animals (with intact spinal cords), so that vestibular-elicted changes in the activity of brain stem respiratory neurons and a spinal respiratory outflow could be compared directly. Nerve activity was amplified by a factor of 10,000, filtered with a band pass of 10–10,000 Hz, full-wave rectified, and integrated (time constant of 100 ms for hypoglossal nerve and 1 ms for abdominal nerve). The signals were sampled at 500 Hz, stored, and displayed using the CED 1401-plus data collection system and Macintosh computer described above. Because of the long integrator time constant, inspiratory discharges recorded from the hypoglossal nerves appeared as an envelope of activity (see Figs. 2, 3, and 5 for examples) that could be easily compared with firing recorded from neurons.

Recording and analysis of unit activity. Electrode penetrations were made from 3 mm rostral to the obex to 4 mm caudal to the obex and from 2.5 to 4.5 mm lateral to the midline using epoxy-insulated tungsten microelectrodes with an impedance of 12 MΩ (A-M Systems). Neural activity was amplified by a factor of 10,000, filtered with a band pass of 300–10,000 Hz, and led into a window discriminator for the delineation of spikes from single units. The output of the window discriminator was led into the CED 1401-plus data collection system and Macintosh computer; the sampling rate was 10,000 Hz. Electrolytic lesions were made in the vicinity of recording sites in each experiment so that recording locations could be reconstructed.

When a unit was encountered, we initially compared its spontaneous discharges with activity recorded from the hypoglossal nerve to determine whether the cell's firing was related to the respiratory cycle. Units with respiratory-related activity were examined for responses to vestibular stimulation. Neural activity was binned (500 bins/cycle) and averaged over the sinusoidal stimulus period by the Macintosh computer. Sufficient sweeps were collected so that spontaneous fluctuations in neural activity related to the respiratory cycle were averaged out. The approximate number of sweeps typically averaged at each frequency was as follows: 25 sweeps at 0.1 Hz (and lower frequencies), 50 sweeps at 0.2 Hz, 100 sweeps at 0.5 Hz, and 200 sweeps at 1–2 Hz. However, fewer sweeps were averaged when the vestibular inputs to a neuron were very strong and masked the respiratory activity. We only considered data to be significant if responses were reproducible in several runs. For most neurons, we also examined activity during multiple frequencies of tilt to ensure that apparent responses were not due to entrainment of spontaneous discharges at a particular frequency.

Sine waves were fitted to responses with the use of a least-squares minimization technique (19). The response sinusoid was characterized by two parameters: phase shift from the stimulus sinusoid (subsequently referred to as phase) and amplitude relative to the stimulus sinusoid (subsequently referred to as gain). Gain and phase measurements were then corrected for the dynamics of the tilt table. Responses were first referred to a constant-amplitude tilt corresponding to ipsilateral ear-down roll, 90° to nose-down pitch, and 135° to nose-up pitch. In this coordinate system, the plane of the ipsilateral posterior semicircular canal is near 45°, the plane of the contralateral anterior canal is near 135°, and the planes of the ipsilateral and contralateral posterior semicircular canals are near 45° and 135°, respectively.

Responses to sinusoidal vestibular stimulation in a particular plane were classified as being the result of stimulation of otolith organs, semicircular canals, or both types of vestibular receptors. This determination was made according to phase and gain criteria based on the well-characterized responses of vestibular afferents and vestibular nucleus neurons to sinusoidal rotations (e.g., 9, 16, 25, 26, 29). Responses with gains that remained relatively flat or showed a modest increase (up to 3-fold/decade) as the frequency was increased were classified as predominantly originating from otolith organs. Otolith responses typically lead position slightly at frequencies <0.1 Hz; above this frequency, the phase leads with respect to position are usually small to moderate. In some cases,
however, central otolith responses lag stimulus position by as much as 180° at high frequencies. In contrast, responses originating from semicircular canals are characterized by steep gain increases, 5- to 10-fold per decade, and large phase leads with respect to stimulus position. The phase lead is typically around 90° in the midfrequency range and is often larger at lower frequencies. Responses resulting from convergence of otolith and semicircular canal inputs have intermediate properties. At frequencies <0.1 Hz, they have a flat gain and a small phase lead similar to the otolith response; they also show the canallike characteristics of both a phase lead >55° at 0.5 Hz and a gain increase of more than threefold from 0.1 to 1 Hz.

Histology. After the animals were killed, the brain stem and, in some cases, the upper cervical spinal cord were removed and fixed in formaldehyde solution. Sections (100 µm thick) were made in the transverse plane and stained with thionine. Locations of recorded neurons were reconstructed on standard sections with reference to placement of electrolytic lesions, relative locations of electrode tracks, and microelectrode depth.

Fig. 2. Examples of respiratory-related activity of neurons. In A–C, top traces show activity of a ventral respiratory group (VRG) neuron, and bottom traces show activity recorded from hypoglossal nerve. All traces are single, unaveraged sweeps. A: augmenting (Aug) activity recorded from an inspiratory (I; trace A1) and expiratory (E; trace A2) neuron. B: constant activity recorded from an I neuron (trace B1) and decrementing (Dec) activity recorded from an E neuron (trace B2). C: phase-spanning (PS) activity that begins during inspiration and continues into expiration (I–E, trace C1) and begins during expiration and continues into inspiration (E–I, trace C2).
RESULTS

In total, 155 neurons with respiratory-related activity were tested for responses to vertical vestibular stimulation. Most of the units (102 or 66%) were recorded in animals with a transection of the spinal cord and IXth and Xth cranial nerves to eliminate nonvestibular inputs that might be produced by whole body tilt. Most (101) of the cells were tested for a projection to the spinal cord; 40 could be antidromically activated from the C2 spinal electrodes (median threshold was 215 µA), whereas 61 could not be driven by a stimulus intensity of 1 mA.

Classification of respiratory-related activity of neurons. Neurons were classified as being respiratory-related if their spontaneous activity was synchronized with a phase of the respiratory cycle; the respiratory cycle was monitored by recording inspiratory activity from the hypoglossal nerve. Cells that fired only during bursts of activity in the hypoglossal nerve were classified as inspiratory (I); cells that fired between discharges in the hypoglossal nerve were classified as expiratory (E); and neurons that fired during a portion of both the inspiratory and expiratory phases were classified as phase spanning (PS). I and E neurons were subclassified as having augmenting, constant, or decrementing activity, as described in previous reviews (2, 6, 7). Examples of several different types of respiratory-related activity are illustrated in Fig. 2. Table 1 indicates the number of cells with different types of respiratory activity that were tested for a response to tilt. The sample was comprised predominantly of I (52%) and E (38%) neurons; PS neurons accounted for only 10% of the population. Among the E and I neurons, the augmenting discharge pattern was by far the most common. It was not possible to accurately subclassify the respiratory discharge pattern for some I and E neurons, particularly those with a slow firing rate.

Responses to sinusoidal tilt of neurons with respiratory-related activity. Typically, neurons with respiratory-related activity were first tested for responses to both clockwise and counterclockwise wobble stimuli delivered at 0.2 Hz and at an amplitude of 15°. Responses to 2 directions of wobble stimulation were similar; in CW direction, signal-to-noise ratio was 0.6 and response gain was 0.4 spikes/s per degree, whereas in CCW direction signal-to-noise ratio was 1.0 and response gain was 0.6 spikes/s per degree. Response vector orientation calculated from responses to CW and CCW wobble stimulation was near nose-down pitch (97°). CED, contralateral ear-down tilt; IED, ipsilateral ear-down tilt; ND, nose-down tilt; NU, nose-up tilt.

Table 1. Number of neurons with different types of respiratory-related activity tested for a response to tilt

<table>
<thead>
<tr>
<th>Type</th>
<th>No. (%) modulated by tilt</th>
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<tbody>
<tr>
<td>Aug</td>
<td>13 (24%)</td>
</tr>
<tr>
<td>Dec</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Con</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>?</td>
<td>7 (30%)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (25%)</td>
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<table>
<thead>
<tr>
<th>Type</th>
<th>No. (%) modulated by tilt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Dec</td>
<td>8 (53%)</td>
</tr>
<tr>
<td>Con</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>?</td>
<td>1 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>11 (19%)</td>
</tr>
</tbody>
</table>

Aug, augmenting discharge pattern; Dec, decrementing discharge pattern; Con, constant discharge pattern; I–E, response spanning between expiratory and inspiratory phases; I–E, discharge spanning between inspiratory and expiratory phases; ?, respiratory discharge pattern could not be classified.
neurons whose activity was modulated by tilt was similar in animals with transection of the spinal cord and IXth and Xth cranial nerves (27 of 102 cells) to that in animals without these transections (8 of 53 cells). The characteristics of responses to tilt were also similar in the two populations of neurons. It thus seems unlikely that visceral signals contributed appreciably to the responses of VRG neurons to tilt in those animals without transections of the spinal cord and IXth and Xth cranial nerves. The responses recorded from all VRG neurons studied were therefore pooled for subsequent analyses.

In general, most respiratory neurons that projected to the spinal cord were unresponsive to tilt. None of the 23 E cells that were activated antidromically from C2 responded to vestibular stimulation; 13 of these cells were tested for responses to 15° stimuli; 4 were tested using maximal tilt amplitudes of 10°, and 7 were studied using 7.5° stimuli. Similarly, only 2 of 15 I cells that were identified as having a spinal projection responded to tilt. However, both of the PS cells that were driven antidromically from the spinal cord responded to vestibular stimulation.

Response vector orientation, or the direction of vestibular stimulation that produced maximal excitation, was determined for all 35 neurons that responded to tilt by averaging the responses to clockwise and counterclockwise wobble stimulation. Typically, the responses to clockwise and counterclockwise wobble rotations had similar gains and signal-to-noise ratios. Vector orientations were usually determined at 0.2 Hz, and for 10 neurons, the orientation was also determined at an additional frequency (typically 0.1 Hz). When more than one frequency was employed, the responses at each frequency were typically consistent (response vector orientations measured at 0.1 and 0.2 Hz were always within 25° of each other). Figure 3 shows the responses of an E neuron to wobble stimulation, and Fig. 4 indicates the response vector orientations for all neurons whose activity was modulated by vestibular stimulation. The orientations were widely distributed and were similar for E and I neurons. Most neurons classified as receiving predominant semicircular canal inputs had response vector orientations that deviated from the canal planes, suggesting that the responses of these neurons were the result of convergence of inputs from two or more canals.

After response vector orientation was obtained, we attempted to examine the dynamics of responses of neurons to tilts in a plane near this preferred direction. However, because of the large amount of time required to analyze the responses of each neuron (due to need to perform extensive averaging to eliminate spontaneous respiratory discharges from recordings and to repeat runs to ensure that respiratory rhythm was not being entrained), 13 of the 35 neurons whose activity was modulated by tilt were lost before response dynamics could be examined. Response dynamics were typically analyzed at 0.1 and 0.5 Hz, and at additional frequencies in 16 neurons. Table 2 indicates the classification (based on response dynamics) of predominant vestibular inputs to respiratory neurons, and Fig. 5 shows the responses of an I neuron to roll tilt at four frequencies. Mean Bode plots generated for neurons classified as receiving predominant inputs from either otolith organs or semicircular canals are illustrated in Fig. 6. Response dynamics were comparable for E and I neurons. For example, the response gain at 0.1 Hz was similar for both E (mean ± SE, 1.0 ± 0.6 spikes/s per degree; n = 4) and I (1.4 ± 0.2 spikes/s per degree; n = 6) neurons classified as receiving predominant otolith inputs. Neurons classified as receiving predominant semicircular canal inputs had response phases that led stimulus position by ~90° at all frequencies; in contrast, neurons identified as receiving predominant otolith inputs had responses that were in phase with stimulus position at low frequencies but developed progressive phase lags as stimulus frequency was increased (mean lag of 71 ± 14° at 0.5 Hz; n = 12).

Locations of respiratory neurons with vestibular inputs. The locations of most respiratory neurons tested for vestibular inputs were reconstructed by reference to the placement of lesions near the recording sites. In some cases, however, the locations of lesions could not be determined, and thus not all of the recording sites were identified. However, the depth and laterality of all respiratory neurons studied suggest that they were in

<table>
<thead>
<tr>
<th>Type of Vestibular Input</th>
<th>Inspiratory neurons</th>
<th>Expiratory neurons</th>
<th>Phase-spanning neurons</th>
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<tbody>
<tr>
<td>Otolith</td>
<td>6/20</td>
<td>4/11</td>
<td>2/4</td>
</tr>
<tr>
<td>Semicircular canal</td>
<td>6/20</td>
<td>1/11</td>
<td>0/4</td>
</tr>
<tr>
<td>Otolith + canal</td>
<td>2/20</td>
<td>0/11</td>
<td>1/4</td>
</tr>
<tr>
<td>Unknown</td>
<td>6/20</td>
<td>6/11</td>
<td>1/4</td>
</tr>
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</table>

Table 2. Classification of predominant vestibular inputs to ventral respiratory group neurons.
the VRG. The locations of E and I neurons that responded to tilt as well as those without vestibular inputs are indicated in Fig. 7. There was no obvious segregation of neurons with particular types of vestibular inputs in particular regions of the VRG.

Comparison of responses to tilt of spinal respiratory nerves and VRG neurons. Our previous studies showed that vestibular stimuli 10–15° in amplitude produce robust changes in the activity of nerves innervating abdominal muscles (16). To directly compare vestibular-elicited responses in VRG neurons and a spinal respiratory nerve, activity was recorded from an L1 abdominal nerve during tilt in four animals. In all cases, 10° tilt produced modulation of abdominal nerve activity. In three animals, the direction of tilt producing maximal abdominal nerve responses was near nose-up pitch (−96°, −104°, and −116°). In the fourth animal, the response vector orientation was near ipsilateral ear-down roll (−2°). Responses recorded from this latter animal during roll tilt at a variety of frequencies are illustrated in Fig. 8. The response gain was relatively consistent across stimulus frequencies, and the response phase was near stimulus position at all frequencies. These characteristics of abdominal nerve responses to tilt are similar to those reported previously (16). For example, in this prior study, the L1 abdominal nerve responses to vestibular stimulation had a vector orientation near nose-up pitch in about three-fourths of the animals and near ipsilateral ear-down roll in the other one-fourth. Furthermore, abdominal nerve responses to vestibular stimulation were previously reported to occur in phase with stimulus position at frequencies up to 1 Hz and to have gains that were flat across stimulus frequencies (16).

In the four experiments in which abdominal nerve activity was monitored, a total of 27 respiratory neurons were studied, including 10 E neurons and 17 I cells. The activity of only four respiratory neurons (3 I cells and 1 E cell) was affected by vestibular stimulation. Seven of the respiratory neurons (5 E cells and 2 I cells) were driven antidromically from the spinal cord in these experiments, but none of these seven cells that were shown to project to the spinal cord responded to vestibular stimulation despite the fact that five of the spinally projecting E cells were tested using larger (15°) stimuli than were necessary to produce responses in the abdominal nerve.

**DISCUSSION**

Previous experiments demonstrated that stimulation of vestibular otolith receptors produces changes in

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**Fig. 5.** Responses of an I-Aug neuron to tilts at multiple frequencies in plane of ipsilateral anterior and contralateral posterior semicircular canal. A: comparison of spontaneous activity of neuron (top trace) to activity recorded from hypoglossal nerve (bottom trace). B: responses of neuron to planar tilt at multiple frequencies; stimulus amplitudes employed were 7.5° at 0.2 and 0.5 Hz, 5° at 1 Hz, and 2.5° at 2 Hz. Histograms represent average of 29 sweeps at 0.2 Hz, 56 sweeps at 0.5 Hz, 100 sweeps at 1 Hz, and 251 sweeps at 2 Hz. However, relative amplitudes of responses were adjusted to account for the fact that a different number of sweeps was averaged in each trace. Arrows indicate when stimulus position was maximal in direction of anterior or posterior semicircular canal (i.e., when tilt amplitude was maximal). C: Bode plot generated from responses of neuron to planar tilt. Gain (relative to stimulus position; top) increased sharply as stimulus frequency was increased, and response phase (bottom) led stimulus position by −90° at all frequencies. These response characteristics are similar to those of semicircular canal afferents.
activity of respiratory nerves (16, 17). However, the present study showed that most respiratory neurons in the VRG, including E neurons that project to the spinal cord, do not respond to tilts up to 15° in amplitude. This finding suggests that neurons in addition to those in the VRG must be involved in relaying vestibular signals to spinal respiratory motoneurons, particularly to abdominal motoneurons, which have been shown to be powerfully affected by small-amplitude vestibular stimulation (16). Thus the present data support the conclusions of previous ablation studies, which demonstrated that chemical lesions of the VRG or transections of the axons of VRG E neurons do not abolish respiratory nerve responses to stimulation of the vestibular nerve (16, 20, 30).

Most of the I and E neurons in the VRG whose activity was modulated by tilt had different patterns of responses to vestibular stimulation than did respiratory nerves. The direction of vertical vestibular stimulation producing a maximal change in activity of VRG neurons was highly variable from cell to cell. In contrast, in most animals, nose-up pitch is the most effective direction of rotation for producing a change in activity in the phrenic, abdominal, and hypoglossal nerves (16, 17). Furthermore, although a few neurons may have been misclassified as receiving otolith organ, semicircular canal, or convergent otolith and canal inputs, it is also clear that the dynamics of responses of most VRG neurons to tilt differed from those of respiratory nerves. The responses of the hypoglossal, phrenic, and abdominal nerves to natural vestibular stimulation suggest that the predominant vestibular influences on these respiratory outflows come from otolith organs (16, 17). In contrast, over 40% of VRG neurons had response dynamics like semicircular canal afferents. Even those VRG neurons that apparently received otolith inputs had different responses to vestibular stimulation than did respiratory nerves. The response phases of VRG neurons classified as receiving predominant otolith inputs lagged stimulus position by an average of over 70° at frequencies ≥0.5 Hz. In contrast, vestibular responses recorded from respiratory nerves have response phases near stimulus position at all frequencies tested (up to 1 Hz) (16). It thus seems unlikely that even those VRG neurons that respond to tilts <15° in amplitude contribute appreciably (either directly or indirectly) to the production of vestibular-elicited responses in spinal respiratory nerves.

The present data thus raise three questions. 1) Why does electrical stimulation of the vestibular nerve affect the activity of a large number of VRG neurons, including those projecting to the spinal cord (12, 20), but moderate-amplitude natural vestibular stimuli do not alter the firing of these cells? 2) Which cells relay vestibular signals to spinal respiratory motoneurons? 3) What is the function of vestibular inputs to VRG neurons?

The answer to the first question may lie in the fact that electrical vestibular stimulation synchronously activates all vestibular afferents and thus provides a much stronger input to the central nervous system than was produced by the 7.5–15° tilts employed in the present study. It is thus possible that larger-amplitude vestibular stimuli would have activated more VRG neurons and that these neurons may contribute to vestibular-respiratory responses elicited by large changes in body position. It is also possible that the delivery of vestibular stimuli during only one phase of the respiratory cycle would have been more effective in producing responses in VRG neurons. Nonetheless, the present data clearly demonstrate that most spinally projecting VRG neurons are not affected by the same stimuli that elicited responses in spinal respiratory nerves, indicating that premotor neurons outside of the VRG must play an important role in producing the vestibularr-respiratory reflex.
The answer to the second question is not available, since the locations of neurons outside the VRG that may contribute to vestibular-respiratory responses have not been fully identified. In general, although some data are available in the rat (4), little is known about neurons outside of the VRG and DRG that may provide inputs to respiratory motoneurons, particularly in emetic species. It is also feasible that neurons in the main respiratory groups which lack a spontaneous respiratory rhythm provide inputs to respiratory motoneurons. Further research will be required to determine which populations of brain stem neurons may

Fig. 7. Locations of I (top) and E (bottom) cells that were tested for responses to tilt and could be reconstructed from positions of lesions. Neuronal locations are plotted on transverse sections of medulla. Values to right of each section indicate relative distance (in mm) posterior to stereotaxic zero; level of obex was at −P13.5. ○, Neurons whose activity was modulated by vestibular stimulation; ●, neurons whose activity was not affected by tilt. A, nucleus ambiguous; AP, area postrema; C, cuneate nucleus; DMV, dorsal motor nucleus of vagus; EC, external cuneate nucleus; G, gracile nucleus; IO, inferior olivary nucleus; IVN, inferior vestibular nucleus; LRN, lateral reticular nucleus; SNV, spinal trigeminal nucleus; ST, solitary tract; STV, spinal trigeminal tract; XII, hypoglossal nucleus.

Fig. 8. Responses recorded from an L1 abdominal nerve during vestibular stimulation delivered at an amplitude of 10°. A: responses to roll tilt at frequencies ranging from 0.05 to 0.5 Hz. Number of sweeps averaged in each trace: 15 at 0.05 Hz, 30 at 0.1 Hz, 51 at 0.2 Hz, and 113 at 0.5 Hz. B: Bode plot indicating gain (top) and phase (bottom) of responses relative to stimulus position. Gain was normalized (by dividing gain at each frequency by maximal response gain recorded). Response gain was flat across stimulus frequency, and response phase was consistently near stimulus position. These characteristics of vestibular-abdominal responses are similar to those reported previously (16) and are similar to those of otolith afferents.
relayed vestibular signals to spinal respiratory motoneurons.

The role of vestibular inputs to the fraction of VRG neurons whose activity is modulated by moderate-amplitude tilts (third question raised above) also awaits determination. Because both the spatial and temporal characteristics of the responses of VRG neurons to vestibular stimulation differ substantially from those of the hypoglossal, abdominal, and phrenic nerves, it seems unlikely that cells in the VRG are involved in producing vestibular-respiratory responses in these nerves. However, the responses of many respiratory nerves to natural vestibular stimulation, including those innervating laryngeal, pharyngeal, and intercostal muscles, are yet to be determined. Previous studies employing electrical stimulation of vestibular afferents (21, 28) have shown that the laryngeal, pharyngeal, and intercostal muscles are influenced by the vestibular system. It is possible that VRG neurons have response properties which match those of these additional muscles and that cells in the VRG relay vestibular signals to pharyngeal, laryngeal, or intercostal motoneurons; this prospect remains to be explored.

Perspectives

The present data suggest that brain stem neurons other than those that generate the respiratory rhythm are important in relaying vestibular signals to at least some respiratory motoneurons. Previous studies have also shown that, during vomiting, neurons in addition to those in the DRG and VRG participate in controlling respiratory muscles, particularly the diaphragm (1, 10, 11, 15); because respiratory muscles contract strongly during emesis, these additional premotor neurons must have powerful influences on respiratory motoneurons. Although neurons in the medullary midline and near the parabrachial nucleus have been reported to innervate respiratory motoneurons (10, 13, 15), little is known about the influences of neurons outside of the main respiratory groups on breathing. Studies employing virus tracers for multisynaptic retrograde mapping of respiratory pathways in emetic species will be useful to determine the location of all premotor respiratory neurons. These experiments should be followed by neurophysiological studies that characterize the response properties of premotor neurons outside of the DRG and VRG which control the excitability of respiratory motoneurons.

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