Respiratory and Mayer wave-related discharge patterns of raphé and pontine neurons change with vagotomy

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Morris KF, Nuding SC, Segers LS, Baekey DM, Shannon R, Lindsey BG, Dick TE. Respiratory and Mayer wave-related discharge patterns of raphé and pontine neurons change with vagotomy. J Appl Physiol 109: 189–202, 2010. First published April 1, 2010; doi:10.1152/japplphysiol.01324.2009.—Previous models have attributed changes in respiratory modulation of pontine neurons after vagotomy to a loss of pulmonary stretch receptor “gating” of an efference copy of inspiratory drive. Recently, our group confirmed that pontine neurons change firing patterns and become more respiratory modulated after vagotomy, although average peak and mean firing rates of the sample did not increase (Dick et al., J Physiol 586: 4265–4282, 2008). Because raphé neurons are also elements of the brain stem respiratory network, we tested the hypotheses that after vagotomy raphé neurons have increased respiratory modulation and that alterations in their firing patterns are similar to those seen for pontine neurons during withheld lung inflation. Raphé and pontine neurons were recorded simultaneously before and after vagotomy in decerebrated cats. Before vagotomy, 14% of 95 raphé neurons had increased activity during single respiratory cycles prolonged by withholding lung inflation; 13% exhibited decreased activity. After vagotomy, the average index of respiratory modulation (η2) increased (0.05 ± 0.10 to 0.12 ± 0.18 SD; Student’s paired t-test, P < 0.01). Time series and frequency domain analyses identified pontine and raphé neuron firing rate modulations with a 0.1-Hz rhythm coherent with blood pressure Mayer waves. These “Mayer wave-related oscillations” (MWROs) were coupled with central respiratory drive and became synchronized with the central respiratory rhythm after vagotomy (7 of 10 animals). Cross-correlation analysis identified functional connectivity in 52 of 360 pairs of neurons with MWROs. Collectively, the results suggest that a distributed network participates in the generation of MWROs and in the coordination of respiratory and vasomotor rhythms.

pons; breathing; respiratory motor pattern

BRAIN STEM NETWORK MECHANISMS have essential roles in the control and coordination of cardiovascular and respiratory functions (cardiorespiratory coupling) (86). It is well established that sympathetic nerve activity can burst rhythmically with respiration and that this activity is modulated by sensory feedback and physiological state (1, 31, 45, 56, 75, 93, 95). Recently, we demonstrated the reciprocal relationship: respiratory activity is modulated with arterial pulse (25, 26). Although it has been known for some time that baroreceptor feedback influences the drive to breathe (4, 5, 14, 19, 20, 33, 35, 96), this new result suggests that the detailed activity patterns of respiratory premotoneurons and motoneurons are also pulse pressure modulated on a beat-by-beat basis.

The dorsolateral (dl) pons, which includes the pontine respiratory group, is an important brain region for respiratory pattern generation and the modulation of breathing and cardiovascular activity (15, 37, 46, 72, 83, 84). Withholding lung inflation during a respiratory cycle in neuromuscularly blocked, thoracotomized, decerebrated cats alters the strength and consistency of respiratory-modulated activity in some pontine neurons (15, 83). Similar results have been observed after vagotomy (10). It has been proposed that these altered discharge patterns reflect a loss of phasic lung volume feedback and stretch receptor-driven presynaptic inhibition or “gating” of an efference copy of inspiratory drive and provide a feedback inhibition that enhances intrinsic inspiratory-to-expiratory phase switching mechanisms (15).

We recently tested the hypothesis that dl pontine neuron activity patterns during a “delayed-inflation” test (similar to no-inflation tests during neural inspiration) would be comparable to those shown after vagotomy (27). Using multisite recording methods, we compared concurrent changes in the respiratory-modulated firing rates of pontine respiratory group neurons. Overall, changes in activity patterns during the delayed-inflation test cycles were similar to those after vagotomy, with notable exceptions at the phase transition from inspiration to expiration. We also recorded neurons that were functionally excited by lung inflation and neurons with firing rate changes that persisted longer than the delayed-inflation cycle.

Raphé neurons modulate sympathetic nerve activity (6, 7, 16, 32, 48, 55, 69, 87, 90). Medullary raphé neurons are integral elements of the brain stem respiratory network (71); caudal raphé networks have been postulated to provide dynamic gain control for the brain stem respiratory network (50, 52, 63–65). However, the afferent inputs and efferent projections of raphé neurons and their roles in cardiorespiratory control are not well understood. Because of the apparent overlap in action, we hypothesized that the caudal raphé neurons may have properties similar to dl pontine neurons.

In the course of the aforementioned pontine neuron recordings, we also monitored medullary raphé neuron activity as part of a larger study on the network mechanisms for cardiorespiratory coupling. We report here that the respiratory-modulated firing rates and discharge patterns of many raphé neurons were altered much like the pontine neurons both during delayed-inflation tests and after vagotomy, suggesting coordinated pontine-raphé network mechanisms for brain stem cardiorespiratory coupling.

Cherniack et al. (12), recording only the blood pressures and phrenic nerve activities of anesthetized, neuromuscularly...
blocked dogs, observed that “Maneuvers (vagotomy or arrested ventilation) which slowed the bursts of phrenic nerve activity and increased the electrical activity of each burst converted Mayer to Traube-Hering waves.” Traube-Hering waves in arterial blood pressure are associated with central respiratory drive, whereas Mayer waves are oscillations of arterial pressure of around 0.1 Hz, typically slower than respiration. Mayer waves historically have been attributed to a central oscillator or characterized as a resonant frequency of the baroreceptor reflex (for review, see Ref. 40). More recently, a “pervasive” 0.1-Hz oscillation in reflected light observed during neural imaging studies has been associated with regional cerebral blood flow (54), and neurons in the caudal raphé nuclei and ventrolateral medulla of sinoaortic denervated cats were found to have oscillations that were coherent with Mayer waves (58, 59, 77).

Using multi-electrode arrays to record raphé and pontine neuron spike trains simultaneously, we identified firing rate oscillations modulated with the respiratory rhythm, the heartbeat, and Mayer waves. Coupling of the respiratory rhythm and Mayer wave-related oscillations (MWROs) was altered by delayed lung inflation and vagotomy. Short time scale correlations indicative of paucisynaptic interactions between raphé and pontine neurons with MWROs were also detected. Collectively, the results suggest that a distributed network participates in the generation of MWROs and coordination of respiratory and vasomotor rhythms.

A preliminary account of some of the results has been reported (66).

MATERIALS AND METHODS

Surgical preparation. Experiments were performed under protocols approved by the University of South Florida’s Institutional Animal Care and Use Committee and were performed with strict adherence to all American Association for Accreditation of Laboratory Animal Care International, National Institutes of Health, and National Research Council guidelines.

Methods were as previously published (80). Data were obtained from 10 decerebrate adult cats (2.6–5.25 kg) of either sex. Animals were initially anesthetized with isoflurane alone (n = 5) or intramuscular ketamine injection (n = 5; 5.5 mg/kg) followed by isoflurane (2–5%) and later decerebrated using the technique of Kirsten and St. John (43). External carotid arteries were ligated rostral to the lingual arteries bilaterally. A craniotomy was performed in the parietal bones. John (43). External carotid arteries were ligated rostral to the lingual arteries. The brain stem was transected at the midcollicular level, and nervous tissue rostral to the transection was aspirated. Before decerebration, the level of anesthesia was assessed periodically by a noxious stimulus (toe pinch). If the stimulus evoked a withdrawal reflex or an increase in blood pressure or respiration, additional anesthesia was given until the response was absent. Femoral arteries and veins were catheterized for monitoring arterial blood pressure, acquisition of arterial blood samples, and administration of intravenous fluids and drugs. Arterial blood samples were analyzed periodically for PO2, PCO2, pH, and HCO3− concentration. Solutions of 6% dextran 70 in 0.9% sodium chloride, 0.04–0.1% dopamine, and 0.075–0.3 mg/ml phencyclidine in lactated Ringer solution administered intravenously as needed to maintain a mean blood pressure of at least 75 mmHg, and sodium bicarbonate solution (8%) was used to correct metabolic acidosis. Before surgery was initiated, atropine (0.5 mg/kg im) and, in six experiments, diphenhydramine hydrochloride (1.8 mg/kg iv) were administered to reduce mucus secretion in the airways. Dexamethasone (initial bolus of 2.0 mg/kg followed by 4.5 mg·kg−1·h−1 iv) was given to minimize brain stem swelling and to help prevent hypotension. During and subsequent to the decerebration, animals were paralyzed by continuous intravenous infusion of pancuronium bromide (initial bolus of 0.1 mg/kg followed by 0.2 mg·kg−1·h−1 iv) and artificially ventilated with 100% O2 through a tracheal cannula with a phrenic-triggered respirator. End-tidal CO2 was maintained at 4.0–5.0%. A unilateral or, if necessary, a bilateral thoracotomy was performed to minimize brain stem movement. The functional residual capacity of the lungs in thoracotomized animals was maintained within a normal range by adjustment of end-expiratory pressure. Periodically, the trachea was suctioned, and the lungs were hyperinflated. Rectal temperature was maintained at 38.0 ± 0.5°C. Animals were placed prone in a stereotaxic frame. At the end of the experiments, cats were euthanized with an injection of pentobarbital sodium (28 mg/kg) followed by 5 ml of a saturated solution of KCl in water.

Neural recordings and characterization. The left phrenic (C5) and, in 5 experiments, the right cranial iliohypogastric nerves were desheathed and cut, and their effarent activities were recorded with bipolar silver electrodes in pools of mineral oil. An occipital craniotomy was performed, and portions of the cerebellum were removed by suction to expose the medulla. We simultaneously recorded the spike trains of single neurons in the regions of the raphé and lateral pons using arrays of 8, 16, 24, or 32 tungsten electrodes (10–12 MΩ) with individual depth adjustment (e.g., Fig. 1A). In two cats, neurons in the region of the ventrolateral medullary cardiorespiratory column were also recorded as part of a larger study to be considered elsewhere. At least 10 min of baseline neural activities were recorded with the ventilator triggered by the integrated phrenic nerve signal. The baseline period was followed by 15–40 delayed-inflation tests. This protocol was similar to no-inflation tests during “neural inspiration” (15), except that triggering of the ventilator occurred after a 1-s delay (e.g., Fig. 1B). Withholding lung inflation for more than 1 s risked deflation of the lungs below functional residual capacity and stimulation of other lung receptors that might obfuscate interpretation of neural responses. Despite this precaution, in one experiment the delayed-inflation tests produced fictive coughs, indicating that rapidly adapting “cough” receptors were stimulated (see Fig. 8 in Ref. 27). In 9 of the 10 animals studied, a truncated delayed inflation was produced at the end of the inspiratory phase. After the series of delayed-inflation tests, the ventilator was switched to free run, i.e., no longer synchronized with phrenic nerve activity. The vagi were then sectioned while concurrent and subsequent neuron activities were recorded.

Data acquisition, entry, and preprocessing. Signals from the microelectrode arrays, eff erent nerve activities, arterial blood pressure, tracheal pressure, expired CO2, and stimulus timing signals were recorded on digital instrument recorders for off-line analysis. Action potentials of single neurons were converted to times of occurrence with spike-sorting software (DataWave Technologies). As previously described (see Fig. 1 in Ref. 27), up to eight waveform parameters were extracted to sort spikes with cluster analysis. The sorted waveforms were inspected to ensure that they did not change during vagotony and that sorting was not biased by movement or electrical artifacts such as those from blood pressure pulse or electrocardiogram. These data files were transferred to laboratory computers for subsequent processing and analyses. The signals of eff erent multunit nerve activities were mathematically integrated (time constant = 0.2 s). Stereotoxic coordinates of recording sites were mapped into the three-dimensional space of a computer-based brain stem atlas derived from The Brain Stem of the Cat: A Cytoarchitectonic Atlas With Stereotoxic Coordinates (8) with permission of the University of Wisconsin Press, as previously described (80).

Spike train analysis methods. Autocorrelograms were computed for each spike train as an aid in the interpretation of cross-correlograms as well as an additional measure to ensure that it represented the activity of one neuron (i.e., signals of multiple neurons would include short intervals not constrained by a refractory period). Two statistical tests were used to evaluate each spike train for the presence of respiratory-modulated activity (62, 70). A neuron was classified as respiratory...
modulated if either test rejected the null hypothesis ($P < 0.05$). A measure of respiratory modulation ($\eta^2$) and a similar measure of pulse pressure-modulated activity ($\rho^2$) were calculated before and after vagotomy (25, 73).

Neuron respiratory cycle-triggered histograms (rCTHs) were compared with phrenic multiunit cycle-triggered histograms (CTHs) to define the respiratory phase in which a respiratory-modulated neuron was more active. Neurons with peak firing rates in inspiration or expiration were classified as “I” or “E,” respectively (13). Those with peak average activities at the phase transitions were classified as either “IE” or “EI” phase-spanners. Respiratory cells that discharged in patterns not easily categorized were denoted as “others” (81).

CTHs calculated for each neuron during the baseline period, delayed-inflation tests, and after vagotomy were evaluated for changes in pattern. Spike trains were evaluated statistically for changes in discharge rates after vagotomy (82), and pairs of simultaneously recorded spike trains were assessed for nonrandom timing relationships using cross-correlation analysis (76) as detailed elsewhere (71, 80). Cross-correlation histograms (CCHs) were calculated for pairs of simultaneously recorded spike trains to detect and evaluate effective neuronal connectivity (2, 57) in five experiments that included both raphe and pons neurons that exhibited a variety of respiratory patterns as well as MWROs. Correlation linkage maps for groups of simultaneously monitored neurons were generated (e.g., see Fig. 6C).

Graphical representations of spike times and other digital and analog signals were generated with the utility program Xscope (49), which was also used to bandpass filter spike train data around the 0.1-Hz frequency of Mayer waves. Resulting displays aided detection and evaluation of the slow-wave oscillations. A waveform display showed the inverse of a fast Fourier transform (FFT) generated by
zeroing the frequencies outside a selected bandpass, usually five to seven cycles per minute, of the FFT of the original spike train. To evaluate consistency and signal strength of components in the frequency of interest for any selected data segment, usually 100–800 s, Xscope generated 20 surrogates for each displayed spike train. The surrogates were generated by zeroing coefficients of FFTs in the band of interest and generating a random spike train from the inverse FFT. The resultant spike train thus had the same firing rate characteristics as the original with the power in the band of interest conferred by randomness. A threshold was designated at the 95th percentile, or 1.65 SD above the mean of the surrogates. The logarithm of the ratio of the envelope of the original filtered spike train to the threshold was then mapped to a color display, with black designating periods in which the spike train had power that was <1.65 SD above the mean of the surrogates (see Fig. 1C).

Slow-wave CTHs were formed by using Xscope to choose the filtered spike train with the most consistent MWROs and manually inserting cycle triggers at the apex of displayed waveforms (Fig. 1C). Similarly, cycle triggers were inserted manually at peaks of integrated phrenic signals for production of delayed-inflation histograms (Fig. 1C). Power spectra and coherence analyses were performed in Octave running in a Linux operating system environment.

RESULTS

The results are based on an analysis of spike trains from pontine neurons monitored in nine animals (other results reported in Ref. 27), together with raphé neuron recordings from seven of those animals. Raphé neurons were also monitored in an additional animal in which the pons was not sectioned. A total of 158 pontine neurons were recorded at sites from 2 mm caudal to 0.3 mm rostral to the caudal border of the inferior colliculus, 3.2–5.5 mm lateral to midline, and 0.9–5.5 mm from the dorsal surface. Of the total, signals from 129 neurons were well sorted both before and after vagotomy. Raphé neurons (n = 95) were recorded within 0.4 mm of the brain stem midline, from obex to 12.0 mm rostral, and 0.2–4.7 mm from the dorsal surface. Most midline recordings were made in the regions of the obscurus and pallidus nuclei, with few in raphé magnus and the dorsal raphé. Figure 1A shows stereotaxic coordinates of recording sites in one experiment mapped in a three-dimensional cat brain stem atlas, together with examples of signals from single neurons monitored simultaneously at those sites.

Both inspiratory and expiratory durations increased in all experiments during delayed-inflation cycles (e.g., Fig. 1B) and after vagotomy (e.g., see Fig. 5), as described previously (27). The integrated phrenic nerve trace in Fig. 1B illustrates prolonged respiratory phase durations during two delayed-inflation trials (arrows). Firing rate histograms of three simultaneously recorded neurons are also shown in Fig. 1B. Slow oscillations in discharge rate are apparent, particularly in the top record from a pontine neuron. Such oscillatory modulations of firing rate were also observed in raphé neurons (Fig. 1C); three of four represented raphé neurons exhibited periods of significant MWROs, as documented in colored regions of corresponding band-passed frequency plots (see METHODS). These oscillations could occur during intervals with no apparent Mayer wave activity in blood pressure and are considered in more detail below.

Vagotomy alters respiratory-modulated raphé neuron discharge patterns. We have previously described changes in the respiratory-modulated firing rates of pontine neurons with vagotomy (27). Briefly, 56 (43.4%) spike trains displayed significant respiratory modulation when the vagi were intact; that number increased to 90 (69.8%) after vagotomy. The average strength of respiratory modulation as measured by $\eta^2$ increased significantly after vagotomy (0.08 ± 0.10 to 0.24 ± 0.22 SD; paired Student’s $t$-test, $P < 0.01$). Here, we report that the number of respiratory-modulated raphé neurons also changed with vagotomy, increasing from 53 (55.8%) to 63 (66.3%) of 95 neurons sampled along the brain stem midline. After vagotomy, the average $\eta^2$ increased (0.05 ± 0.10 to 0.12 ± 0.18 SD; Student’s paired $t$-test, $P < 0.01$).

Figure 2 provides dorsolateral views of stereotaxic coordinates of raphé neuron recording sites color-coded according to the magnitude of each neuron’s $\eta^2$ value before (top) and after (bottom) vagotomy. Raphé neurons with increased respiratory modulation were distributed throughout the midline region sample. Although individual neurons displayed changes, as a group there was no significant change in mean peak or overall average firing rates (paired $t$-test, $P > 0.05$). Neurons ($n = 19$, 20.0% of total) with a variety of other respiratory-modulated discharge patterns were transformed to inspiratory-modulated
patterns after vagotomy (e.g., compare the pre- and postvagotomy rCTHs in Figs. 3B and 5, A and B). Average firing rates and mean peak rates plotted against \( \eta^2 \) values for these neurons before and after vagotomy are shown in Fig. 3A. Other neurons \((n = 18, 18.9\%)\) with peak rates during inspiration or the phase transitions changed modulation with vagotomy (e.g., compare the pre- and post-vagotomy rCTHs in Fig. 5C).

Two distinct categories of altered firing rate modulation were observed during delayed-inflation tests before vagotomy in both pontine and raphé neurons. Activity acutely increased in 24% of pontine and 14% of raphé neurons or decreased in 8% of pontine and 13% of raphé neurons. Whether activity increased or decreased, the change in activity was confined to the enhanced phrenic cycle evoked by withholding inflation (e.g., Fig. 3B). Neurons with the evoked response confined to the delayed-inflation cycle were designated “C-type” cells (see DISCUSSION); they were similar to the “classic” responses described previously in the pons (15). In the other category, neurons displayed altered activity patterns that extended beyond the respiratory cycle with enhanced phrenic activity (Fig. 3C, middle). These neurons were designated “M-type” and exhibited MWROs (see below).

As groups, neither pontine nor raphé neurons exhibited a significantly changed arterial pulse pressure modulation after vagotomy as measured by \( \delta^2 \) (paired Student’s \( t \)-test, \( P > 0.05 \); Fig. 3D). Calculation of \( \delta^2 \) requires 2,500 cardiac cycles in which the spike train is active for optimal estimation (25); not all spike trains met this criterion before or after vagotomy. Before vagotomy, 26 (37%) of 71 raphé neurons were cardiac modulated, 22 of which were also respiratory modulated. Similarly, 33 (37%) of 90 pontine spike trains were cardiac modulated; 26 of these were respiratory modulated; 26 (34%) of 76 pontine neurons were cardiac modulated; 22 of these were respiratory modulated as well as cardiac modulated.

MWROs before and after vagotomy. As noted above (Fig. 1C), firing rate histograms and bandpass-filtered spike train
data revealed oscillatory discharge patterns with a frequency of \( \sim 0.1 \) Hz, a property characteristic of Mayer waves (40). Before vagotomy, such oscillations were apparent in 46 (48%) of the raphé neurons sampled and 41 (28%) of the pontine neurons. The amplitude of the oscillations fluctuated over time, and various phase relationships were observed among simultaneously monitored raphé and pontine neurons (Figs. 1C and 5). These MWROs in neuronal discharge patterns were found when no similar oscillations in blood pressure were detectable (Fig. 1C). However, when Mayer waves were present in blood pressure, both spectral and coherence analyses of a subset of neurons suggested that the 0.1-Hz spike train oscillations were correlated to the blood pressure oscillations (Fig. 4, B, C, and D). In 7 of 10 experiments, the phrenic nerve cycling frequency slowed after vagotomy to the Mayer wave frequency range and became synchronized with M-type neurons (e.g., Fig. 4A) recorded at sites distributed in the raphé and pons (Fig. 4E).

Prevagotomy rCTHs for three simultaneously recorded M-type neurons (Fig. 5, left column) document both pontine (Fig. 5A) and raphé neurons (Fig. 5B) that are more active during the expiratory phase, together with a second raphé neuron (Fig. 5C) that was inspiratory modulated. After vagotomy, the firing profiles of all three neurons changed (Fig. 5, right column). Figure 5, column 2, shows average firing rate histograms for the same three neurons (A–C), although now triggered on the MWROs identified in the bandpass-filtered spike train of one

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**Fig. 4.** MWROs intrinsic to the network before vagotomy synchronize with breathing rhythm after vagotomy. A: firing rate histograms of 7 pontine and 5 raphé cells, showing \( \text{Phr, TP, and BP} \). Top: before vagotomy, animal was ventilated using phrenic-triggered respirator; note slow oscillations in the neuronal activity. Bottom: after vagotomy, with ventilator on free run. Neuronal activity was synchronized with \( \text{Phr} \), which was entrained with \( \text{BP} \). We speculate that the intrinsic slow rhythm expressed in modulatory elements of the cardiorespiratory control networks contributed to the change in respiratory patterning. B: frequency domain analysis of selected 100-s samples supported the results of time domain analysis in these power spectra of pontine cell 554 (left) and BP (right). Before vagotomy, both the pontine cell and BP showed peaks at 0.07 Hz (within the frequency range of Mayer waves; see Ref. 40) and at 0.28 Hz (respiratory frequency); cell 554’s MWRO CTH clearly shows this 1-to-4 coupling (Fig. 5A). Postvagotomy, there is no longer a peak at 0.28 Hz in either power spectrum. C: recording was made just before vagotomy with the ventilator not triggered by \( \text{Phr} \) (30 breaths/min). The MWRO and Mayer wave-to-central breathing rhythm ratio was stable at 1:2. Filtered color and waveform plot (See Fig. 1 and text) of a pontine neuron in another recording show activity synchronous with Mayer waves identified from the BP trace (black dotted lines); respiratory-related Traube-Hering waves are indicated by red dotted lines. D: peaks (arrows) in the coherence plot (prevagotomy) suggest that the pontine neuron and BP express synchronized 0.1-Hz (Mayer wave) and 0.2-Hz (respiratory) oscillations. E: locations of neurons recorded in pons and raphé with (red) and without (gray) MWROs prevagotomy.
of the neurons (MWRO CTHs; see METHODS). In these prevagotomy MWRO CTHs, the peaks in the multiunit phrenic record (gray) suggested that four respiratory cycles were synchronized with one slow wave, a 4-to-1 coupling ratio. Seven of the 10 animals displayed such evidence for synchronization between respiratory cycles and slow waves before vagotomy, with a coupling ratio of either 4-to-1 (4 animals) or 3-to-1 (3 animals). In the other three animals, including one in which the respiratory rhythm did not slow to 0.1 Hz after vagotomy, time-series analyses were unable to determine whether the coupling ratio varied or whether the rhythms were not coupled.

Multicycle delayed-inflation CTHs, with intervals between delayed-inflation cycles normalized to the average interval, revealed that the period of MWROs was reset by the delayed-inflation perturbation. This resetting process resulted in a subsequent time-locked period of altered activity in neurons exhibiting this category of response (e.g., Fig. 5, A–C, column 3). We observed more complex responses to delayed inflations in 11.5% of the total number of neurons sampled in the pons and raphé. These responses had two apparent components: an acute response during the delayed-inflation cycle and a prolonged alteration in firing rate that persisted over several consequent cycles (see M-type example cell in Fig. 3C).

Short time scale correlations between neurons with MWROs in firing rate. As an initial step in assessing whether neurons with MWROs were coordinated, at least in part, through brainstem network interactions, we constructed CCHs to screen spike trains from five animals for nonrandom timing relationships indicative of paucisynaptic interactions among pairs of neurons. A goal of cross-correlation analysis is to build simple model circuits that can reproduce experimentally observed features (3); central peaks and troughs are considered indicative of shared influences of like and opposite sign, respectively. Peaks and troughs offset relative to the correlogram origin may be interpreted as signs of functional excitation and inhibition, respectively (see, e.g., Refs. 61, 76).

In these five animals, MWROs were detected in 22 pontine and 25 raphé cells. Overall, CCHs for 52 (14.4%) of the 360 pairs of neurons with MWROs displayed significant features indicative of mono- or paucisynaptic connectivity (Table 1). Primary features included 17 central peaks, 2 central troughs, 16 offset peaks, 5 offset troughs, and 12 instances of multiple peaks and troughs, suggesting that a variety of circuit properties existed within this data set. Results from one recording are shown in Fig. 6. MWRO CTHs from two pontine and two raphé neurons are shown in Fig. 6A; postvagotomy respiratory CTHs with cycle frequencies near 0.1 Hz. Note that the “beginning” of the MWRO was reset by the successive delayed-inflation tests (arrow). Column 4: postvagotomy respiratory CTHs with cycle frequencies near 0.1 Hz.  

Fig. 5. CTHs from the simultaneously recorded spike trains of 1 pontine neuron (A shows cell 554 from Fig. 4) and 2 raphé neurons (B and C). Column 1: respiratory CTHs were triggered by the onset of the expiratory phase and displayed neuronal activities (black lines) and multiunit phrenic activity (filled gray area). Column 2: histograms triggered on pulses derived from peak of bandpass-filtered waveform (5–7 cycles/min) of spike train in row B (darker filled area in foreground) demonstrate MWROs near 0.1 Hz. Note 4 peaks in multiunit phrenic activity for each MWRO. Column 3: average firing rates triggered on delayed-inflation test cycles generated every sixth respiratory cycle. Note that the “beginning” of the MWRO was reset by the successive delayed-inflation tests (arrow). Column 4: postvagotomy respiratory CTHs with cycle frequencies near 0.1 Hz.
neurons 923 and 936, which were most active in opposite phases of the MWROs. The central peak in CCH 2 (Fig. 6B, top right) is indicative of a shared paucisynaptic influence of like-sign acting on pontine neurons 558 and 557, whereas the offset peak in CCH 1 (Fig. 6B, top left) is consistent with either an excitatory action of neuron 923 on neuron 557 or a shared influence that affects both cells with different time delays. The trough in CCH 4 can be simply interpreted as a delayed shared input of opposite sign or evidence for an inhibition of neuron 557 by cell 936.

Table 1. Summary of results of cross-correlation analyses of 360 pairs of neurons with MWROs

<table>
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<th></th>
<th>Pons-Raphé 28/186 (15.1%)</th>
<th>Pons-Pons 13/67 (19.4%)</th>
<th>Raphé-Raphé 11/107 (10.3%)</th>
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<td></td>
<td>Central</td>
<td>Pons→Raphé Offset</td>
<td>Raphé→Pons Offset</td>
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<td>3</td>
<td>7</td>
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<tr>
<td>Troughs</td>
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<tr>
<td>Total</td>
<td>15</td>
<td>4</td>
<td>9</td>
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Results show significant correlations/total pairs (with percent in parentheses). Offset features involving pons-raphé pairs are separated according to the simplest interpretation of their cross-correlation histograms. MPT, multiple peaks and troughs; MWRO, Mayer wave-related oscillation.

![Fig. 6. Cross-correlation analysis results for a subset of neurons from 1 recording. A: prevagotomy MWRO CTHs of 2 pontine (blue) and 2 raphé (green) neurons and phrenic nerve activity (gray). B: cross-correlation histograms (CCHs) constructed from the entire record for pairs composed of the neurons in A. Gray lines in CCHs represent the mean activity ± 3 SD in shifted cycles. Numbers of spikes: for neuron 557, 3,248; for neuron 558, 6,734; for neuron 923, 5,451; for neuron 936, 27,088. Bin width for all CCHs = 20.5 ms. C: correlation linkage map shows feature and strength of extended correlational linkages among a subset of simultaneously monitored raphé and pontine respiratory group (PRG) neurons. Offset features are shown as a solid line linking the neurons; a central feature is denoted by a dashed line. Note that some represented linkages include a “secondary” feature; these features were juxtaposed on either side of the correlogram origin or more offset from the origin than primary features. Ovals and rectangles contain each neuron’s identification code and discharge pattern, which is also reflected by the color of the label. Cells with MWROs have an oval outline. The 4 neurons included in A and B are additionally highlighted in yellow. Encircled numbers correspond to CCH features shown in B.](http://jap.physiology.org/)
The correlation linkage map in Fig. 6C is a diagram of 40 short time scale correlations among the four cells shown in A and B (lines labeled with corresponding encircled numbers) and an additional 9 pontine and 13 raphé neurons. Line color and type reflect the feature (peak, trough, or multiple peaks and troughs) and its location (offset or central) in the CCH constructed for the corresponding pair of neurons. Note that detected correlations involving pontine 506 suggest that this neuron exerts influences of opposite sign on raphé 923 and 936; this complicated relationship would result in an increase in the firing rate of one cell (923) at approximately the same time that the activity of the other cell (936) decreased, leading to a central trough in the CCH constructed for the two target neurons (CCH 3).

**DISCUSSION**

We identified multiple rhythms in the spike trains of raphé and pontine neurons, including firing-rate oscillations modulated with the respiratory rhythm, the arterial pulse, and slower MWROs. Neurons with rate modulations confined to the single delayed-inflation cycles were designated C-type. Neurons with altered activity patterns that extended beyond the respiratory cycle with enhanced phrenic activity were designated M-type and exhibited MWROs. Although we anticipated identifying respiratory- and pulse-modulated activities, the prevalence of Mayer wave-related activity in pontine and raphé neurons is a novel finding and extends the cardiorespiratory coupling paradigm into slower rhythms. Furthermore, the coupling of the respiratory rhythm and MWROs was altered by both delayed lung inflation and vagotomy; the period of MWROs was reset by the delayed-inflation perturbation.

Respiratory modulation of raphé neuronal activity was similar to that of pontine neurons during delayed lung inflation tests and after vagotomy. After vagotomy, both the number of raphé neurons with respiratory-modulated activity and the average η of respiratory-modulated units increased. Similar to that shown in a subset of pontine neurons (27), the distribution of respiratory firing rate profiles of raphé neurons changed after vagotomy. The results of these reversible and irreversible perturbations, coupled with short time scale correlations indicative of paucisynaptic interactions between raphé and pontine neurons with MWROs, suggest that a distributed network participates in the generation of MWROs and the coordination of respiratory and vasmotor rhythms.

**Strengths and limitations of the approach.** The use of large arrays of electrodes with individual depth adjustments permitted the efficient acquisition of data and allowed direct comparison of many neurons recorded under the same pre- and postvagotomy conditions, a state change that could only be observed once in each animal. In addition, the simultaneous recording of many neurons in the pons and raphé allowed the calculation of MWRO CTHs. Although similar to respiratory or cardiac CTHs, MWRO CTHs differed in that activity of one neuron was used in each instance (see METHODS) to produce a trigger since Mayer waves in blood pressure were often not detectable. Differences in results obtained by vagotomy and the single-breath delayed-inflation technique may have arisen from consequences of the two approaches, including nonsteady-state firing profiles, short-term “memory” effects from previous breaths, and continuous and/or altered inputs from other vagal afferents (e.g., pulmonary rapidly adapting and C-fiber receptors, aortic baroreceptors, as well as those arising from the gastrointestinal tract).

In all protocols, the open chest preparation necessary for the stability of neuronal recordings may have resulted in pre vagotomy influences due to excess stimulation of lung receptors caused by regional changes in lung volume from normal. As noted above, in one experiment, the delayed-inflation tests stimulated the cough reflex. However, the remaining nine recordings agreed with previous experiments in more intact preparations (15, 83).

**Functional implications.** Pontine neurons are proposed to be affected by the inhibition or gating of an inspiratory efference copy by pulmonary stretch receptor (PSR) input (15). Under this classic model, removal of PSR input via delayed-inflation tests or vagotomy results in increased pontine inspiratory activity. The pontine respiratory group has been proposed to serve as an “internal vagus” because, in vagotomized animals with pontine lesions, breathing becomes apneustic, i.e., with prolonged inspiratory periods, whereas in those with an intact pons it does not (28, 68, 85). The altered patterns of pontine neuron activity after vagotomy would partially compensate for the loss of PSR effects and increase their influence on medul lary elements of an inspiratory “off switch” (15, 78). We extend this model to include cells in the raphé. Whether input from the pons is sufficient to maintain a nonapneustic respiratory rhythm and pattern in the absence of raphé influence after vagotomy is unknown; however, partial lesion of raphé nuclei in vagally intact cats can prolong inspiration and shorten expiration (38). Given the apneustic patterns observed with combined vagotomy and pontine lesions, it is likely that any putative contribution of the raphé as an internal vagus is not sufficient to compensate for the loss of both inputs. However, the pons is not sufficient to compensate for the loss of the caudal raphé in the production of rapid shallow breathing with increased temperature, which presupposes enhanced inspiratory and expiratory off switches (88).

The present results also support the hypothesis of a “common central oscillatory network,” coupling the cardiorespiratory functions at the brain stem level (44). Recent clinical research suggests that slow breathing is associated with an improvement of exercise tolerance in patients with congestive heart failure (9) and with a decrease in arterial pressure (39). Slow breathing may mimic vagotomy, in that it may modulate the firing pattern of brain stem neurons involved in respiratory control to synchronize with the slower MWROs. The synchronization of respiratory and slow-wave modulations of sympathetic and parasympathetic efferent activity may better match, for instance, ventilation and perfusion to contribute to the observed improvements.

A seeming paradox of the change in respiratory pattern with vagotomy is that the expiratory period and the inspiratory period are both prolonged (36, 79, 91, 92). The increases in activity of pontine and raphé neurons during the inspiratory-expiratory phase transition (e.g., rCTHs in Fig. 5B, see also Fig. 4 in Ref. 27) and phase shifts (e.g., Fig. 5, A and C) may feed back to the medulla to prolong expiration through excitation or disinhibition. This observation has been confirmed many times in paralyzed, ventilated animals and in anesthetized and unanesthetized freely breathing animals (21, 53, 91, 92). PSR input is excitatory to expiratory neuron activity and
prolongs the neural expiratory period (i.e., Hering-Breuer reflex). Removal of this input by vagotomy would be expected to decrease time in expiration. However, just the opposite occurs, possibly due to the elimination of information from other vagal lung afferents that are inhibitory to expiratory activity, in particular rapidly adapting and C-fiber receptors (21, 79). In contrast, the activity of these inputs is probably increased in delayed-inflation cycles, during which expiration was also paradoxically prolonged. Neurons in the present data set displayed prolonged activity in expiration after vagotomy (i.e., Fig. 5C). We therefore speculate that modulatory networks in the raphé and dl pons act to prolong the expiratory phase in the absence of vagal PSR inputs.

**Alternative models for effects of vagotomy on respiratory-modulated neuronal activity.** The activity profiles of C-type neurons in both raphé and pons during delayed-inflation tests and after vagotomy were generally similar and matched those of pontine neurons described by Cohen and colleagues (15, 83). C-type neurons change activity acutely during inspiration in delayed-inflation cycles and become more respiratory modulated after vagotomy. The present study shows that, although pontine and raphé neurons displayed altered respiratory modulation profiles after vagotomy, neither the average nor peak firing rates for either group changed significantly, suggesting that the long-standing model (15, 30, 83) of vagotomy resulting in simple removal of (possibly presynaptic) inhibition of inspiratory-modulated inputs is incomplete.

We recently proposed an alternative model for the generation of changes in C-type pontine neuron activity with vagotomy (27). The circuit (Fig. 7A), suggested by previous studies (50, 67, 71), includes a target population with direct inspiratory-modulated excitatory inputs from the ventrolateral medullary respiratory column and inhibitory inputs from a population that is driven by the same inspiratory population. The two sets of inputs to the tonic population are balanced so that the summed spike activity of the entire target population has no respiratory modulation. Given a random distribution of synapses, some individual neurons in the target population are not respiratory modulated, whereas others have peak firing rates during either inspiration or expiration. In this model, the response of the inhibitory population to a given increase in excitation is less than the response of the tonic target

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**Fig. 7.** Alternative model circuits and hypotheses for changes in tonic neuronal firing profiles and the respiratory motor pattern during withholding of lung inflation or vagotomy. **A:** ball and stick diagram of a feedforward inhibitory circuit module embedded in a larger recurrent inhibitory network. VRC I-Aug represents I cells in the medullary ventral respiratory column whose maximum firing rate occurred in the second half of the inspiratory phase. **B:** representative traces from simulated neurons before (left) and after (right) elimination of pulmonary stretch receptor feedback. Note lack of respiratory modulation of tonic neuron before vagotomy. Loss of PSR feedback generated inspiratory modulation of the previously nonrespiratory modulated tonic neuron. **C:** elaboration of the circuit to include reciprocal inhibition. **D:** illustration of hypothesis that, during delayed-inflation (left) and removal of vagal afferent feedback (middle), the MWROs of raphé and pontine neurons become synchronized with the respiratory rhythm (right). See text for details.
population; such differences in excitability have been observed in raphé neuron populations (e.g., Ref. 42). An increase in inspiratory activity, as would occur with vagotomy and disconnection of PSR feedback, would increase excitation of the tonic population more than the feedforward inhibition from the inhibitory population, thus shifting the percentage of tonic neurons with predominantly inspiratory or expiratory activity (27).

Computer simulations of neural circuit modules with the embedded feedforward inhibitory elements can reproduce features of C-type neuron responses to delayed-inflation and vagotomy (27). Figure 7B, left, shows traces from simulated neurons before elimination of PSR feedback; with vagotomy (Fig. 7B, right), a loss of PSR input produces increased inspiratory modulation in a tonic cell that did not exhibit a respiratory-related discharge pattern prior to vagotomy. Distinguishing between the classic gating hypothesis and the newer model is beyond the scope of the present study.

Our previous work suggested reciprocal inhibitory connections between raphé neuron pairs with shared inputs. These neurons responded to peripheral chemoreceptor stimulation and the induction of respiratory long-term facilitation with opposite changes in firing rates (63, 64, 67). We hypothesized a “ratchet” mechanism that reset the balance of reciprocal inhibition and contributed to the expression of the facilitation. In the present data set, central cross-correlogram features suggested the presence of shared inputs, whereas offset features were consistent with simple interpretations of excitatory, inhibitory, and reciprocal connections (Fig. 6, B and C). Elaboration and refinement of the inhibitory circuit into networks with reciprocal inhibition (e.g., Fig. 7C) should guide further model development and in vivo experiments aimed at integrating and testing hypotheses on gating and equilibrium-seeking or stabilizing influences in respiratory control and cardiorespiratory coordination.

The results of this study also suggest a model to account specifically for the changes found in M-type neurons during perturbations of PSR feedback and vagotomy. Slow rhythms of ~0.1 Hz have generally been associated with cardiovascular, not respiratory, regulation. Forty years ago, Cherniack et al. (12) proposed a model with mutual influences between central vasomotor and respiratory control networks based solely on simultaneously recorded phrenic nerve activity and blood pressure. A subset of the animals in their study had n-to-1 coupling between respiration and Mayer waves (see Fig. 4 in Ref. 12). When n-to-1 coupling occurred, inspiratory bursts were clustered during the rising phase of a blood pressure peak; prolonged expiratory phases occurred as blood pressure reached a nadir. They also established that Mayer waves can convert to the respiratory rhythm with vagotomy or that the respiratory rhythm can convert to the frequency of Mayer waves. These results, considered together with our present observations, suggest the hypothesis that the respiratory rhythm becomes synchronized (i.e., coupled 1-to-1) with the MWROs of raphé and pontine neurons after removal of vagal afferent feedback (Fig. 7D). This synchronization could contribute to the stability of the respiratory rhythm after vagotomy or other interventions that slow breathing, such as loading or narcosis.

The MWRO-triggered CTHs, the resetting of MWROs by the delayed-inflation cycles, and the postvagotomy synchronization of the respiratory cycle to the frequency of MWROs are consistent with the hypothesis that the coupling of the respiratory rhythm generator and MWROs is bidirectional (Fig. 7D, left). Figure 7D summarizes evidence for this hypothesis. Prevagotomy MWROs are coupled to the breathing rhythm in a 1-to-n ratio that is reset by delayed inflation (Fig. 7D, left). Postvagotomy coupling can become 1-to-1, stabilizing the breathing rhythm as suggested above (Fig. 7D, middle). Figure 7D, right, illustrates and summarizes results from the power spectra analyses in Fig. 4. Before vagotomy, the MWRO-to-breathing ratio was 1:4 (0.07 and 0.28 Hz, respectively); after vagotomy, the breathing rhythm slowed, whereas the MWRO frequency increased slightly to 0.1 Hz.

Before vagotomy, some M-type neurons showed no differences in acute activity during the delayed-inflation cycles beyond the resetting of their slow rhythm (36 of 46 raphé and 22 of 41 pontine M-type cells; for example, see Fig. 5B), suggesting that they did not directly receive efference copy or input from PSRs. The remaining 10 raphé and 19 pontine M-type neurons had clear acute differences in activity with delayed-inflation (see Figs. 3C and 5, A and C); this subset may be part of the substrate for respiratory-MWRO network interactions.

The present study is the first to extend MWROs to the pons. Three previous studies have recorded single neuron activity coherent to Mayer waves. One of the former studies recorded sympathetic preganglionic neurons (77), whereas the other two, both from the same laboratory, recorded raphé cells (58, 59). In agreement with those results, the 0.1-Hz activity recorded here in pons and raphé was present when there was no detectable blood pressure Mayer wave activity. Sympathetic nerve activity is also modulated coherent to Mayer waves (11, 41, 51, 60, 74, 94).

In addition to roles in the modulation of breathing, regions of the pons are thought to be involved in sympathetic control of heart rate and blood pressure (24, 34, 47). In contrast, raphé neural networks have been implicated in the control of sympathetic participation in thermoregulation and metabolic function (e.g., Ref. 29). These similar roles of raphé and pons in sympathetic-respiratory coordination may be reflected in the strikingly parallel responses of cells in both regions to lung inflation and vagotomy, as well as their cardiac cycle modulation and the expression of MWROs. These results are consistent with a new model of respiratory network architecture with reciprocal parallel signaling paths between the pons and ventral respiratory column through intervening raphé circuits (71, 80). Evidence for shared input and feedforward inhibition was also reported in that data set. Before and after vagotomy, we observed varying ratios of breathing to MWRO cycles. Varying the ratio of breaths to MWROs may serve to enhance the efficiency and maintain the stability of cardiorespiratory function while matching ventilation and perfusion to metabolic demand.

The involvement of pontine and raphé networks in the production of Mayer waves and the 0.1-Hz modulation of sympathetic nerve activity are yet to be determined. Although suggestive, the cross-correlation evidence here does not show causality of MWROs by the raphé and pontine networks. The neurons recorded may be followers of a 0.1-Hz rhythm generated elsewhere. However, the activities of neurons in both regions are appropriate to contribute to cardiorespiratory cou-
plunging; subsets are modulated with both breathing and blood pressure. Cardiac modulation of neurons as measured by $\beta^2$ did not change significantly in either the pons or raphé with vagotomy. This result is somewhat surprising in that vagotomy would have removed input from the aortic baroreceptors. However, inspection of arterial pulse-triggered histograms of many cells showed different profiles after vagotomy. Preliminary analyses of subsets of cells have revealed more subtle changes (22). Another possibility is that these “equilibrium-seeking” networks adapt to changes in system activity with efficient changes of spiking activity that act to reassert blood pressure control and modulation with minimal, neuronal metabolism-sparing overall changes in neuron firing rates.

Evidence presented here suggests that apparent short-term plasticity in spike trains pursuant to delayed-inflation tests (27) is accomplished by a resetting of MWROs. Increased MWRO activity during increased respiratory drive from hypoxia, hypercapnia, or many other stimuli would be expected to outlast the stimulus, given the 10-s periodicity of MWROs. Feedback from MWRO networks could then contribute to such respiratory memory effects as short-term potentiation or “after-discharge” (89). In rodent species, there is a posthypoxic frequency decline predominantly expressed as a prolongation of expiration. The increased expiratory modulation of cells in postulated reciprocal inhibitory networks and expiratory-phase MWROs seen with vagotomy is consistent with participation in posthypoxic frequency decline. Posthypoxic frequency decline is also abolished with pontine lesions (17, 18, 23).

Summary and conclusions. Both raphé and pontine neuronal activities vary with vagal input. The activities of neurons in both regions are appropriate to contribute to cardiorespiratory coupling; subsets of each are modulated with both breathing and blood pressure. Computational models (27) of a population of neurons with inspiratory efference copy input and feedforward inhibition produce firing rate profiles similar to those seen with vagotomy in vivo.

Mayer waves are thought to be associated with baroreceptor feedback regulation of blood pressure. Pontine and raphé neural networks are involved in both the respiratory rhythm and 0.1-Hz cardiovascular oscillation. Neuronal 0.1-Hz MWROs in the absence of detectable blood pressure Mayer waves suggest the presence of a central 0.1-Hz oscillator. The n-to-1 coupling of the respiratory rhythm with MWROs before vagotomy, the common synchronization of MWROs with the respiratory rhythm after vagotomy, the resetting of MWROs by delayed-inflation tests, and the conversion of Mayer wave activity to respiratory modulation of blood pressure all suggest a bidirectional coupling of 0.1 Hz and respiratory oscillators. However, it remains to be shown whether the raphé-pontine network is directly involved in production of the 0.1-Hz rhythm or receives an efference copy generated elsewhere.

This work suggests a functional coupling between the raphé nuclei and the pons in cardiorespiratory regulation. It also supports the conclusion of Cherniack et al. (12): “It is clear from these observations that any model of the regulation of ventilation or circulation, the purpose of which is to account fully for integrative mechanisms, must include provision for the interplay between respiratory and circulatory control mechanisms under different experimental conditions.”

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DISCLOSURES

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