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Effect of upper airway negative pressure and lung inflation on laryngeal motor unit activity in rabbit

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Ryan, Stephen, Walter T. McNicholas, Ronan G. O'Regan, and Philip Nolan. Effect of upper airway negative pressure and lung inflation on laryngeal motor unit activity in rabbit. *J Appl Physiol* 92: 269–278, 2002; 10.1152/jappphysiol.00413.2001.—Distortion of the upper airway by negative transmural pressure (UANP) causes reflex vagal bradycardia. This requires activation of cardiac vagal preganglionic neurons, which exhibit postinspiratory (PI) discharge. We hypothesized that UANP would also stimulate cranial respiratory motoneurons with PI activity. We recorded 32 respiratory modulated motor units from the recurrent laryngeal nerve of seven decerebrate paralyzed rabbits and recorded their responses to UANP and to withholding lung inflation using a phrenic-triggered ventilator. The phasic inspiratory ($n = 17$) and PI ($n = 5$) neurons detected were stimulated by -10 cmH₂O UANP and by withdrawal of lung inflation ($P < 0.05$, Friedman's ANOVA). Expiratory-inspiratory units ($n = 10$) were tonically active but transiently inhibited in postinspiration; this inhibition was more pronounced and prolonged during UANP stimuli and during no-inflation tests ($P < 0.05$). We conclude that, in addition to increasing inspiratory activity in the recurrent laryngeal nerve, UANP also stimulates units with PI activity.

larynx; laryngeal motor units; postinspiration; postinspiratory

THE PHARYNX IS A COMPLIANT structure that is susceptible to collapse, especially during sleep when upper airway (UA) muscles relax. When the pharynx narrows or obstructs, inspiratory efforts generate large negative transmural pressures across the wall of the extrathoracic airway caudal to the site of obstruction. When applied to the isolated UA of experimental animals, UA negative transmural pressure (UANP) evokes a reflex increase in UA muscle activity and a reflex reduction in motor drive to the diaphragm. The excitation of UA muscles is extensive and includes increases in phasic inspiratory (phasic-I) and tonic activity of nasal, palatal, pharyngeal, and laryngeal abductor muscles (31). The recruitment of UA muscles stabilizes the pharynx and reduces the series resistance to airflow through the nose and larynx. The reduction in thoracic pump mus-

cle activity limits the suction pressure generated by these muscles and transmitted to the UA during each inspiratory effort (1, 16, 17, 25, 31).

The responses to UANP are not confined to alterations in inspiratory activities or to the respiratory system. Our laboratory reported that UANP causes reflex vagal bradycardia (18, 19). This response must involve activation of cardiac vagal preganglionic neurons (CVPN). The excitability of CVPN is modulated by respiratory activity so that they discharge in postinspiration (5, 10). This suggests that UANP might also activate respiratory-related cranial motoneurons with postinspiratory (PI) activity. We have conducted a series of experiments recording from single motor axons in the recurrent laryngeal nerve (RLN) of the decerebrate rabbit to test the hypothesis that UANP, in addition to increasing the activity of laryngeal motor units with inspiratory activity, also recruits motor units with PI discharge. Given that the activity of laryngeal motoneurons is strongly modulated by afferent feedback from pulmonary slowly adapting receptors (26, 35) and that reflex responses to UANP are reduced by lung volume feedback (34), we also hypothesized that the laryngeal motor response to UANP would be modified by inflation of the lungs.

METHODS

General procedures. Experiments were performed in seven New Zealand White rabbits. Animals were sedated using a fentanyl-fluanisone mixture (Hypnorm, 0.2 ml/kg im, Janssen, Oxford, UK) and anesthetized with midazolam (Hypnovel, 1–2 mg/kg iv, Roche) in doses sufficient to suppress reflex withdrawal responses to paw pinch. Animals were placed in the supine position, and rectal temperature was maintained at 38–40°C with a thermostatically controlled heating blanket (Harvard homeothermic blanket system, Harvard Instruments, Kent, UK). Oxygen-enriched air [fresh gas flow = 5 l/min, inspired O₂ fraction (F_IO₂) = 0.37] was delivered to the animal through a face mask and a modified Jackson-Rees anesthetic circuit. The right femoral artery and vein were cannulated to record systemic arterial pressure (Statham P23Dd, Hato Rey, Puerto Rico) and for injec-

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tion of drugs, respectively. A lead II electrocardiogram was also recorded in four animals to monitor heart rate.

A midline ventral neck incision exposed the trachea, which was divided between the fifth and sixth cartilage rings, and a L-shaped polyethylene cannula was inserted into the caudal cut end of the trachea. The animals were allowed to breathe spontaneously through the tracheal cannula. Anesthesia was subsequently maintained with inhaled halothane delivered to a T piece attached to the tracheostomy (2% inhaled concentration, fresh gas flow: 3.5 l/min, $F_{I_{O_2}} = 0.37$). Tracheal pressure (Validyne DP45, Validyne, Northridge, CA) and end-tidal CO_2 (Engstrom Eliza, Gambro, Sweden) were continuously monitored.

The animals were fixed prone in a stereotaxic frame. Hypotension associated with the change in posture was prevented by infusion of epinephrine (1–2 $\mu\text{g}/\text{min}$ iv, Antigen Pharmaceuticals, Roscrea, Ireland). The skin over the dorsal surface of the skull was incised along the midline and reflected laterally, and a large frontoparietal craniotomy was performed using a dental-driven burr. The sagittal sinus was tied where it appeared at the edges of this craniotomy, with care being taken to ensure that the posterior margin of the craniotomy was sufficiently far forward to avoid damage to the confluence of the sinuses. The dura mater was cut and reflected to reveal the surface of the cortex. A thalamic decerebration was performed, removing neural tissue with a suction catheter, the metal tip of which also acted as an active diathermy electrode (Erbe Elektromedizin Erbotom T 300C) to minimize blood loss. The cortex, basal ganglia, and hippocampus were completely removed so that only the thalamus, hypothalamus, colliculi, and brain stem remained. Halothane was discontinued, and the epinephrine infusion was reduced and stopped. The animals were paralyzed with vecuronium (Norcuron, Organon, 0.2 mg/kg iv plus 0.3 mg·kg⁻¹·h⁻¹ iv) and artificially ventilated by means of a mechanical ventilator (CWE SAR-830/P, Charles-Ward, Chicago, IL) in standard, intermittent, positive-pressure ventilation mode ($F_{I_{O_2}} = 0.37$, inspiratory rate = 60 breaths/min, inspiratory time = 0.5 s, inspiratory flow rate = 2–2.5 l/min, tidal volume = 18–20 ml). After a stabilization period of 1 h, the animals were removed from the stereotaxic frame and returned to a supine position.

UA preparation. To isolate the UA, a second cannula was inserted in the trachea, pointing cranially with its tip lying ~5 mm below the vocal folds. Two short polyethylene cannulas (length: 30–35 mm, outer diameter: 4 mm, inner diameter: 3 mm) were advanced 3–5 mm into the external nares. The mouth and nose were sealed around these cannulas with cyanoacrylate adhesive and rapid-setting epoxy cement. These three cannulas were connected together and then led, via a solenoid valve, to a 200-liter pressure reservoir, which was evacuated to the required subatmospheric pressure using a vacuum pump. Remote activation of the solenoid valve applied negative pressure simultaneously to the nasal and laryngeal cannulas; thus the entire UA was exposed to the pressure applied. A small (2-liter) damping vessel between the solenoid valve and the UA controlled the rate of pressure change. Application of pressure stimuli occurred at the onset of inspiration (I) and was controlled by computer. Pressure within the UA fell to -10 cmH₂O within 400 ms. The total duration of each stimulus was 5 s. UA pressure was continuously recorded (Validyne DP-45, ±35 cmH₂O, Northridge, CA).

Nerve recording and phrenic trigger ventilation. Efferent activity was recorded from the left phrenic nerve, which was identified low in the neck and cut, and its desheathed central end was placed across a bipolar silver wire recording elec-

trode. The signal was amplified (Neurolog NL100K preamplifier and NL104 amplifier, Digitimer, Welwyn Garden City, UK), filtered (0.3–2 kHz, NL 125), rectified, and low-pass filtered (NL703, time constant: 100 ms). This signal was then processed by a custom-designed interface that detected the onset of phrenic activity and also its sharp decline at the beginning of the PI phase. The interface generated electronic pulses marking these events, and these pulses were used to control the ventilator. The system was set so that, when the onset of inspiratory phrenic activity was detected, the ventilator began delivery of a constant inspiratory airflow (2–2.5 l/min, $F_{I_{O_2}} = 0.37$) to the lungs, which was terminated on detection of the onset of PI. This mode of ventilation is referred to as phrenic-triggered ventilation.

The RLN was exposed along the length of the trachea and cut close to where it entered the larynx. The cut central end of the main trunk of the right RLN was placed into a small Perspex bath filled with phosphate-buffered saline solution (0.01 M phosphate buffer, Sigma Aldrich). A reference electrode (silver chloride) fixed in the bath was connected to the preamplifier (Neurolog NL100K). Efferent activity from single fibers in the right RLN was recorded using fire-polished glass suction electrodes, prepared from single-barrel glass-borosilicate glass tubing (PG61150-4, World Precision Instruments). Electrodes were pulled using a Narshige electrode puller (PC-10) and had a very small tip diameter (5–10 μm) so that single nerve fibers could be recorded by sucking on strands from the frayed end of the central stump of the RLN. The signal from the RLN was amplified (Neurolog NL100AK, NL104), filtered (0.3–2 kHz, NL 125), and fed to an audio monitor and an oscilloscope for monitoring purposes. We obtained some preparations with only one active motor unit but also collected data from preparations in which a few motor units were simultaneously active. We analyzed such records only if different motor units were clearly distinguishable from each other and from any background noise in terms of spike amplitude. Furthermore, the amplitude and shape of the recorded spike had to remain constant for the duration of the recording. Units discriminated from few-fiber recordings, according to these criteria, were then treated as separate motor units. Neural signals, systemic arterial pressure, tracheal pressure, and UA pressure were recorded and stored onto computer using a CED micro1401 interface and Spike2 software (CED, Cambridge, UK). RLN action potentials were recorded and stored at a sample rate of 25 kHz using the Spike2 WaveMark data-capture mode.

Experimental protocol. When a satisfactory single or few-unit recording from the RLN was obtained, three interventions were performed. First, the effect of -10 cmH₂O UANP delivered while the lungs inflated normally was examined. Second, -10 cmH₂O UANP were applied while artificial ventilation was simultaneously withdrawn to eliminate phasic lung volume feedback during the UANP stimulus. Third, for comparison, the effect of withholding lung inflation for 5 s was recorded. Where a preparation from the RLN did not discharge spontaneously, we tested for evoked activity in response to -10 cmH₂O UANP applied in the absence of lung inflation. Two minutes were allowed between successive interventions being performed.

Arterial blood-gas samples were drawn intermittently to monitor arterial P_{O_2} ($P_{a_{CO_2}}$), arterial P_{CO_2} ($P_{a_{CO_2}}$), and arterial pH (CIBA Corning 278 blood-gas systems). Sodium bicarbonate (1 M) was administered intravenously, as required, to correct any metabolic acidosis. At the end of each experiment, animals were killed by a lethal dose of pentobarbitone sodium (Sagatal, 200 mg/kg iv).

Data analysis. RLN motor unit activity was separately quantified for I and expiration (E) defined with reference to the efferent activity recorded from the phrenic nerve. I was defined as the period from the onset of inspiratory phrenic discharge to the beginning of its sharp decline. E was defined as the period from the sharp decline in inspiratory phrenic activity to the onset of the next inspiratory burst. We first examined the effect of withdrawing phasic lung volume feedback on the discharge of each unit. We compared unit discharge on the first breath for which lung inflation was withheld with discharge on the preceding control breath for which the lungs inflated normally. This procedure is comparable to a standard no-inflation test.

However, UANP often prematurely terminated the first inspiratory effort after stimulus application, as has been described in previous studies (33). As a result, we quantified unit discharge during the second breath of stimuli where UANP was applied and compared this with the activity on the breath immediately preceding the stimulus. When UANP was applied in the absence of lung inflation, discharge during the second breath of this stimulus was also compared with the activity recorded on the second breath when lung inflation was withheld but no pressure was applied to the UA.

These data were analyzed in two different ways. First, we obtained summary measures of the activity of each unit for each respiratory phase under each experimental condition. The summary measures used were the peak discharge frequency within the phase, the median discharge frequency across the phase, and the total number of action potentials within the phase. Some units were not spontaneously active (i.e., had zero discharge during a respiratory cycle); therefore, these measures were not normally distributed. Results are, therefore, presented as median and range, and statistical analysis was performed by using Friedman's test and a nonparametric calculation of the Student-Newman-Keuls test statistic (11), with $P < 0.05$ taken to indicate a statistically significant effect.

We found that units with respiratory-modulated activity fell into three distinct categories according to discharge pattern. Units within each category exhibited similar responses to UANP and withholding lung inflation (see RESULTS). We developed the following procedure to present the typical discharge pattern of all units within a category and their responses to different stimuli. The I and E phases of control and test breaths were each divided into 40 equal time bins. The actual width, in real time, of each bin depended on the duration of the respiratory phase. The discharge frequency within each bin was recorded for each RLN unit in the category. The median of the values recorded in corresponding bins for the different units within that category was calculated to summarize the behavior of all comparable units. This produced phase histograms, where the value for each bin was the median discharge frequency across all units for that bin. Given that the stimuli used in this study could alter the duration of the respiratory phases, the most faithful representation of discharge pattern is a plot of activity vs. time. Therefore, we also determined the median width across units of each bin in milliseconds and set the width of each bin to this value to produce plots of discharge frequency against time.

RESULTS

The values of arterial blood gases and pH for the recording period were as follows: PaO_2 , 150 ± 38 (SD) Torr; PaCO_2 , 40.1 ± 1.54 Torr; arterial pH, 7.385 ± 0.023 ($n = 7$). UANP evoked changes in heart rate and

respiratory timing. Baseline R-wave-R-wave interval was 286.8 ± 18.8 (SE) ms ($n = 4$). R-wave-R-wave interval was significantly prolonged by all three interventions performed ($P < 0.00001$, ANOVA and Student-Newman-Keuls test), increasing to 357.7 ± 35.3 ms in response to -10 cmH₂O applied during ongoing lung inflation, 640.3 ± 128.6 ms when lung inflation was withheld, and 695.8 ± 162.3 ms in response to -10 cmH₂O applied simultaneously with temporary cessation of lung inflation. Inspiratory time for control breaths [542 ± 30 (SE) ms, $n = 7$] significantly increased ($P < 0.003$) to 664 ± 76 ms in response to -10 cmH₂O delivered while normal lung inflation occurred, 623 ± 51 ms ($P < 0.0001$) when only lung inflation was withheld, and 798 ± 79 ms ($P < 0.001$) when -10 cmH₂O UANP were applied together with temporary withdrawal of lung inflation. Expiratory time for control breaths (849 ± 41 ms) significantly increased ($P < 0.003$) to $1,168 \pm 133$ ms only when -10 cmH₂O UANP were applied together with failure to inflate the lungs.

Recordings were obtained from 43 RLN units: 14 occurred as single-unit recordings, whereas the remainder were discriminated from few-unit preparations. Thirty-two units exhibited discharge patterns modulated by respiration under control conditions with ongoing lung inflation. Three distinct categories of the respiratory-modulated unit were observed. Seventeen units active only during I, typically with an augmenting discharge pattern, were called phasic-I units. Five units exhibited a burst of activity early in E, during the PI period, which decremented rapidly so that the unit was silent for most of E, and were classified as PI units. Ten units were active during both I and E with a short pause in activity in early E and are referred to as expiratory-inspiratory (E-I) units. The activities of 11 units displayed no relationship to the respiratory cycle and were called sporadic units. The statistical analysis reported here was conducted on units in which data were available for all of the three interventions. Complete data were available for 17 phasic-I units, 7 E-I units, and 5 PI units.

Fifteen out of seventeen phasic-I units were spontaneously active, and the remaining two units were recruited when lung inflation was withheld for one breath. Eleven phasic-I units commenced discharge within 50 ms of the onset of I (median: 29.5 ms, range: 14–42 ms). Their discharge augmented throughout I to reach a peak frequency of 64.7 Hz (18.5–93.4 Hz) at the end of I (Fig. 1). The remaining six units had much later onset times (median: 378 ms, range: 262–525 ms) and lower peak discharge frequencies (median: 18.8 Hz, range: 12.9–56.9 Hz). However, failure to inflate the lungs or UANP stimuli greatly advanced the onset times of four of these six units so that their discharge was similar to that of early onset units. It is, therefore, not clear whether there are two distinct subgroups of phasic-I motor units, and, given that all units exhibited similar responses to UANP stimuli, we analyzed all 17 units as a single group. Failure to inflate the lungs significantly increased the activity of all phasic-I units ($P < 0.02$, Friedman's ANOVA and Student-Newman-

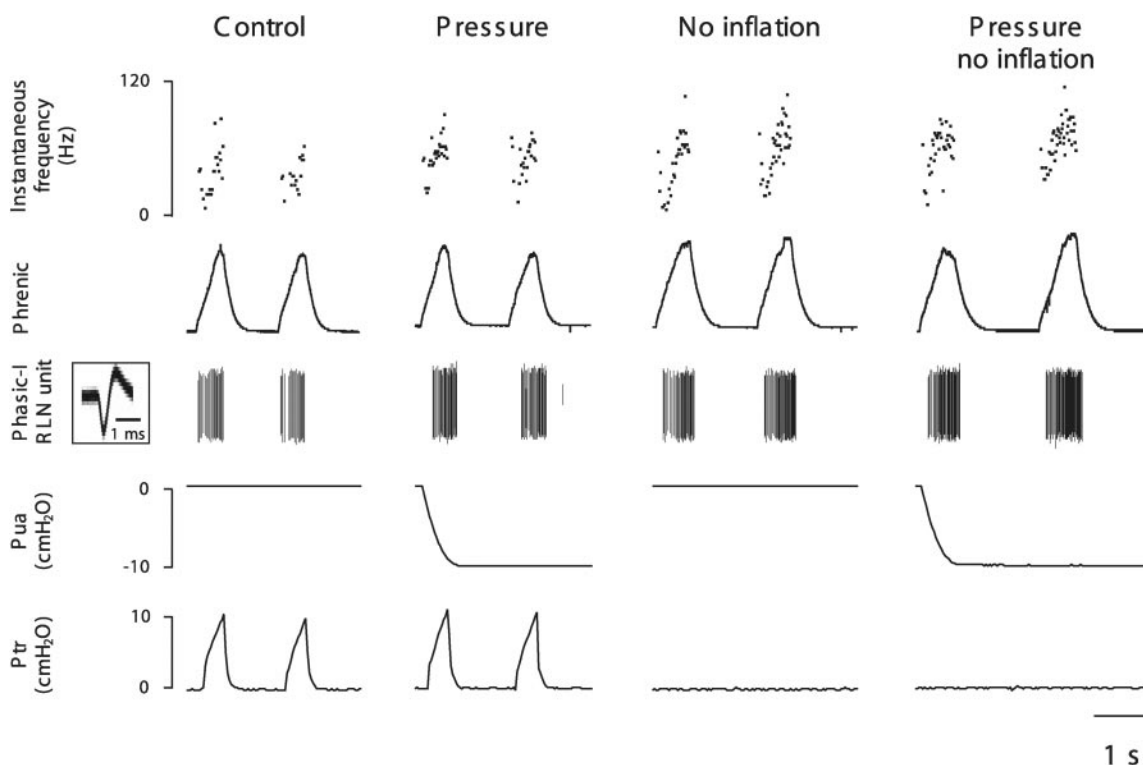


Fig. 1. Response of a phasic-inspiratory (phasic-I) recurrent laryngeal nerve (RLN) motor unit to upper airway negative pressure (UANP). The activity of the unit is shown during control breaths with normal lung inflation (Control), application of -10 cmH₂O UANP (Pressure), temporary cessation of lung inflation (No inflation), and -10 cmH₂O UANP applied while ventilation was temporarily withdrawn (Pressure, no inflation). RLN unit activity was stored as discriminated action potentials (WaveMark data, CED Spike2) so that only individual action potentials appear in the reproduced record. *Inset*: superimposed waveform for all phasic-I spikes shown in the record. Pua, upper airway pressure; Ptr, tracheal pressure.

Keuls; $n = 17$; Fig. 2A). UANP stimuli delivered while normal lung inflation continued also significantly increased unit discharge ($P < 0.02$; Figs. 1 and 3). A significantly greater increase in activity than that evoked either by negative pressure stimulation or withdrawal of lung inflation alone was observed when both stimuli were applied simultaneously ($P < 0.0001$; Fig. 3).

We recorded five PI units: two units were spontaneously active under normal conditions, two became active when lung inflation was withheld for one breath, and the remaining unit discharged only in response to -10 cmH₂O UANP. Withholding lung inflation for one breath significantly increased the activity of this group of units (Fig. 2C), with an increase in both the peak discharge frequency ($P < 0.025$, $n = 5$) and the number of action potentials recorded ($P < 0.03$). UANP applied alone also significantly increased activity ($P < 0.025$; Figs. 4 and 5) characterized by a marked increase in peak discharge frequency and longer burst duration (Figs. 4 and 5). UANP applied in the absence of vagal lung volume feedback also significantly increased PI activity ($P < 0.04$; Figs. 4 and 5). Failure to inflate the lungs did not significantly alter the effect of UANP on the discharge frequency of PI units (Fig. 5) but did significantly increase the total number of action potentials elicited (data not shown).

E-I units were all spontaneously active under control conditions. Discharge frequency was relatively constant throughout I, but activity was suddenly silenced or inhibited at the onset of the PI phase (Fig. 6). Units commenced firing again, with a gradually augmenting discharge, in midexpiration. Median discharge frequency during I was significantly greater than that recorded in E ($P < 0.003$; Fig. 7). There was no significant change in the inspiratory discharge of E-I units during any of the three interventions performed (Figs. 6 and 7). However, the PI inhibition of these units was more marked and prolonged in response to UANP (Figs. 6 and 7) and temporary cessation of lung inflation (Fig. 2B). This was reflected by a significant reduction in the median discharge frequency of these units during E ($P < 0.002$). Although UANP and withdrawal of lung inflation both decreased the expiratory activity of E-I units, there was no statistical evidence that withholding lung inflation altered the effect of UANP on E-I units.

DISCUSSION

This study is the first to describe the effects of UANP on the discharge of laryngeal motoneurons. We hypothesized that distortion of UA mechanoreceptors by negative transmural pressure would excite both inspira-

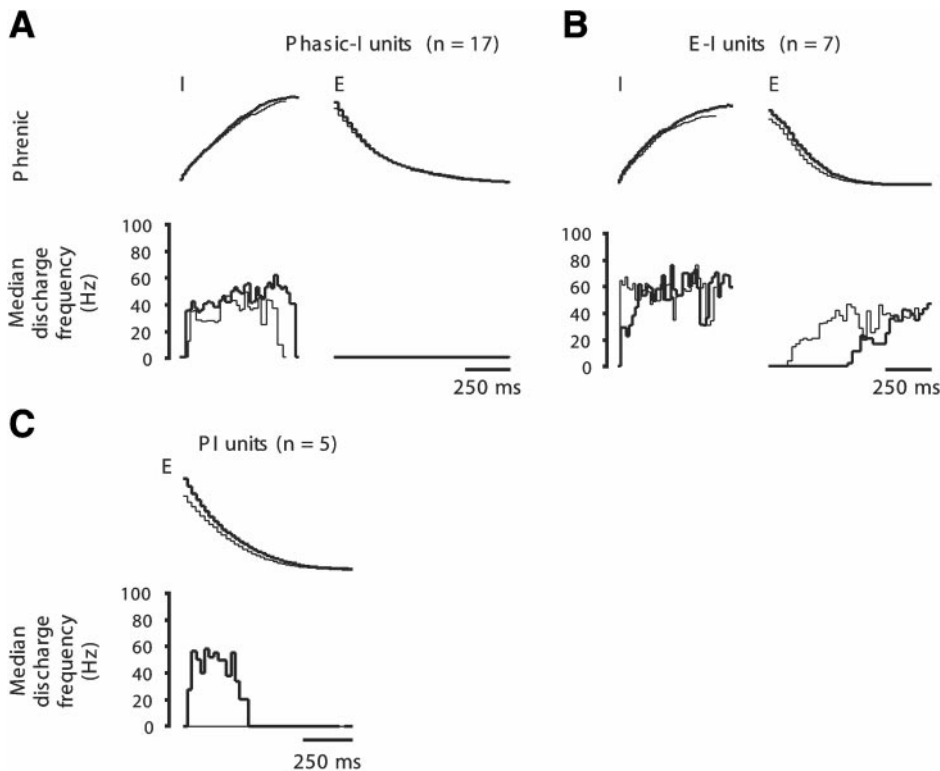


Fig. 2. Effect of lung inflation on group median discharge frequency of phasic-I (A), expiratory-inspiratory (E-I; B), and postinspiratory (PI; C) RLN motor units. The bin-median discharge frequency of phasic-I ($n = 17$; A) and E-I ($n = 7$; B) units, together with bin-average phrenic activity during inspiration (I) and expiration (E), and PI ($n = 5$; C) during expiration alone, before (thin line), and on the first breath (thick line) of withholding lung inflation are shown.

tory and PI motor activities in the RLN. We found that UANP does recruit laryngeal motoneurons with PI activity and stimulates a subpopulation of laryngeal inspiratory motoneurons.

We presume that the PI units recorded from the RLN in the present study are analogous to the early expiratory units reported in the decerebrate cat (26). However, the relative number of PI units detected in our study (16% of respiratory-modulated motor units) is less than that (35%) reported by Sica and co-workers (26) in the decerebrate cat. Furthermore, under our

experimental conditions, PI units tended to be silent or only sporadically active. This may have contributed to a sampling bias accounting for the paucity of PI neurons recorded in the present study. It is an important limitation of this study that only a small number of PI units were detected. It is possible that our sample is not representative of the entire population of laryngeal motoneurons with PI activity. Nonetheless, we found that the activity of all PI units detected increased markedly and significantly in response to UANP. Whereas this is a novel finding, it is consistent with

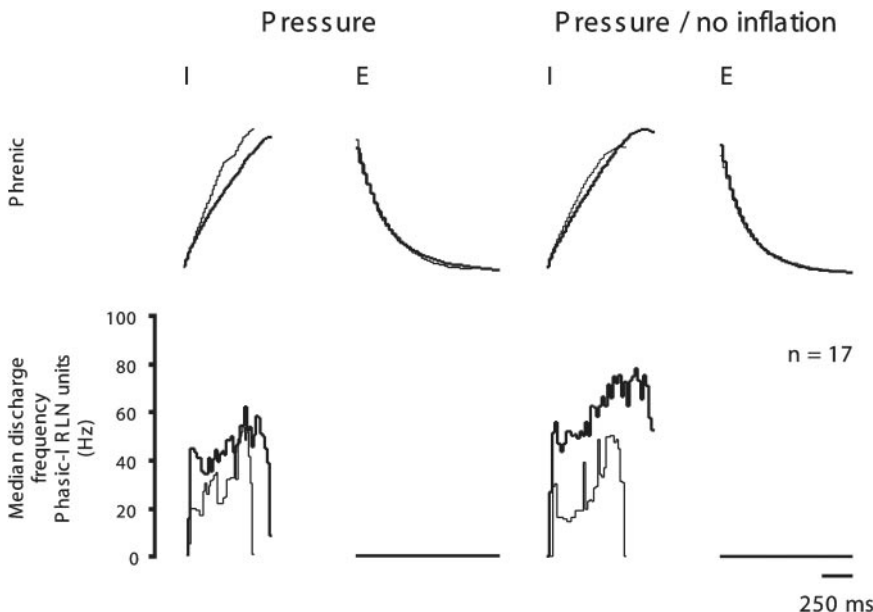


Fig. 3. Group median discharge frequency of phasic-I RLN motor units and their response to UANP. The bin-median discharge frequency of phasic-I RLN units ($n = 17$) and bin-average phrenic activity are shown during I and E. Unit activity during UANP stimuli (thick line) is compared with an appropriate control (thin line). *Left*: pressure. Activity on the second breath of -10 cmH₂O UANP delivered with ongoing lung inflation is compared with the discharge on the breath immediately preceding the stimulus. *Right*: pressure/no inflation. Activity on the second breath of -10 cmH₂O applied during temporary cessation of lung inflation is compared with the discharge on the second breath of an intervention where lung inflation was withheld but no pressure was applied to the upper airway.

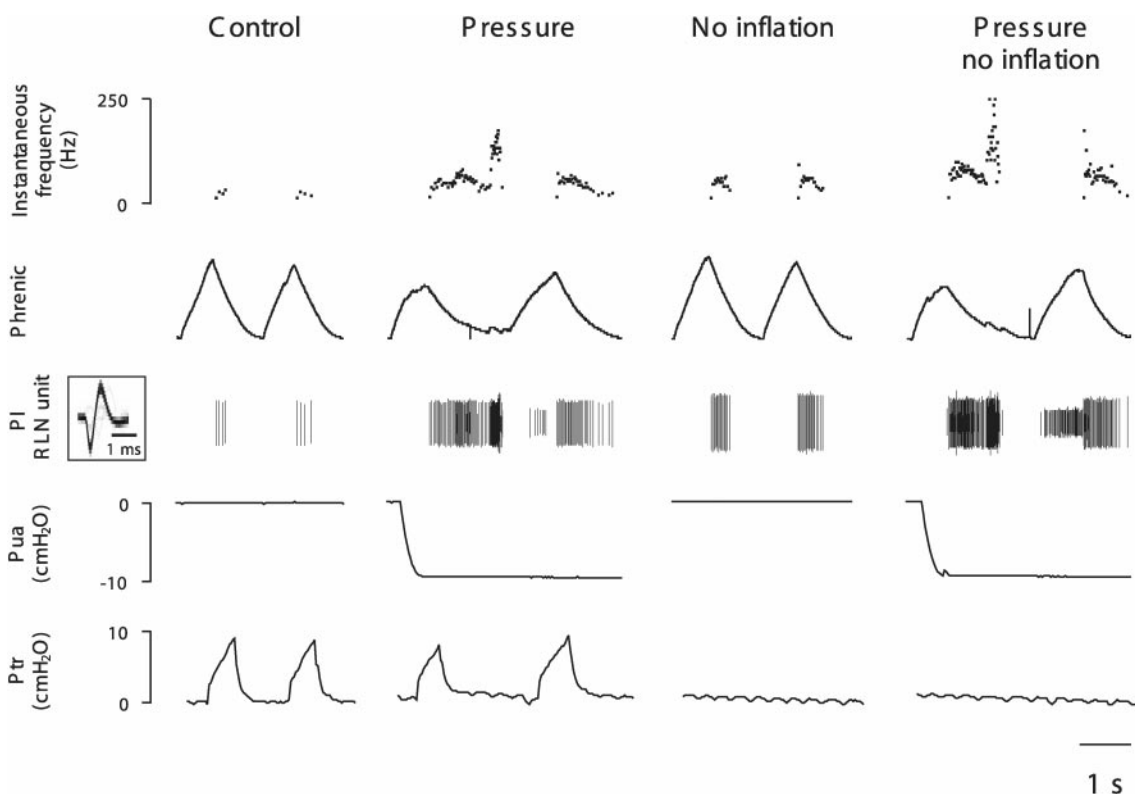
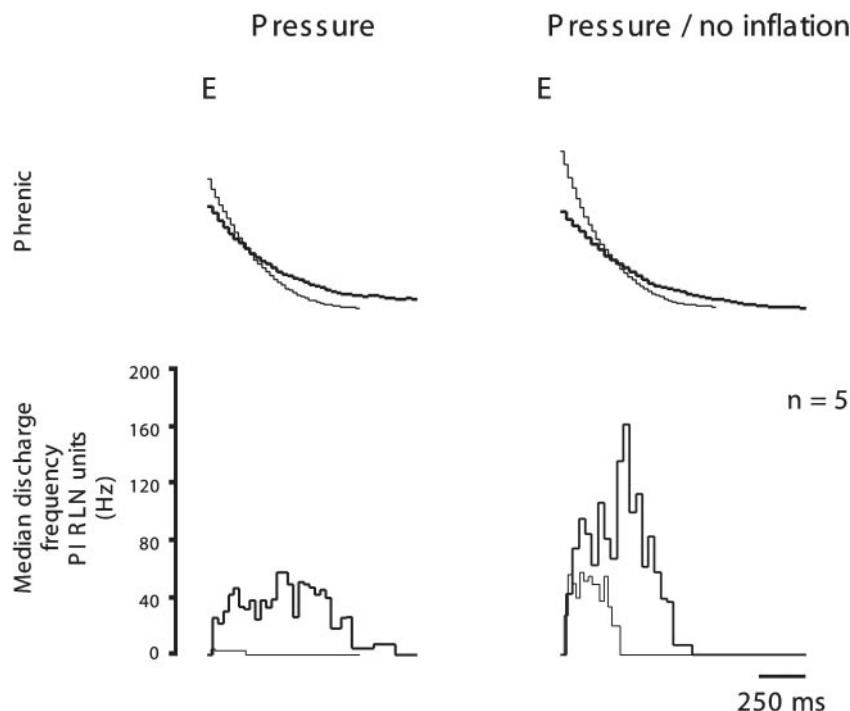


Fig. 4. Response of a PI RLN motor unit to UANP. The activity of the unit is shown during control breaths with normal lung inflation (Control), application of -10 cmH₂O UANP (Pressure), temporary cessation of lung inflation (No inflation), and -10 cmH₂O UANP applied while ventilation was temporarily withdrawn (Pressure, no inflation). RLN unit activity was stored as discriminated action potentials (WaveMark data, CED Spike2) so that only individual action potentials appear in the reproduced record. *Inset*: superimposed waveform for all PI spikes shown in the record.

other studies showing that medullary PI neurons are activated by electrical stimulation of the superior laryngeal nerve (3, 15) and by mechanical or chemical stimulation of the UA (19, 20).

Our prediction that UANP would evoke an increase in the activity of laryngeal motoneurons with PI activity arose from our observation that UANP causes a respiratory-modulated reflex vagal bradycardia (18,

Fig. 5. Group median discharge frequency of PI RLN motor units and their response to UANP. The bin-median discharge frequency of PI RLN units ($n = 5$) and bin-average phrenic activity are shown during E. Unit activity during UANP stimuli (thick line) is compared with an appropriate control (thin line). *Left*: pressure. Activity on the second breath of -10 cmH₂O UANP delivered with ongoing lung inflation is compared with the discharge on the breath immediately preceding the stimulus. *Right*: pressure/no inflation. Activity on the second breath of -10 cmH₂O applied during temporary cessation of lung inflation is compared with the discharge on the second breath of an intervention where lung inflation was withheld but no pressure was applied to the upper airway.



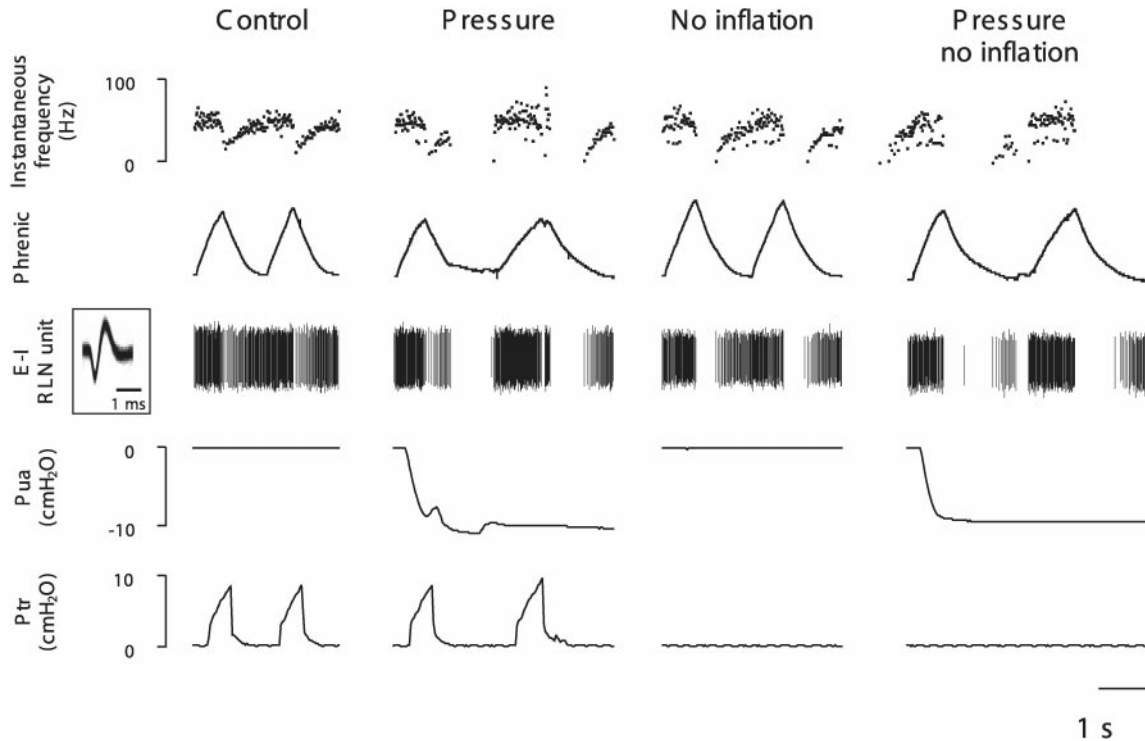


Fig. 6. Response of an E-I RLN motor unit to UANP. The activity of the unit is shown during control breaths with normal lung inflation (Control), application of -10 cmH₂O UANP (Pressure), temporary cessation of lung inflation (No inflation), and -10 cmH₂O UANP applied while ventilation was temporarily withdrawn (Pressure, no inflation). RLN unit activity was stored as discriminated action potentials (WaveMark data, CED Spike2) so that only individual action potentials appear in the reproduced record. *Inset*: superimposed waveform for all E-I spikes shown in the record.

19). This implies that this stimulus activates CVPN (5, 10), which can be thought of as a subpopulation of medullary PI neurons operating within a cardiorespiratory control system (21). This model suggests that activation of cardiovascular autonomic neurons with PI activity will be associated with increases in PI drive

to respiratory motoneurons. We found this to be the case, and our data support the idea that these functionally diverse PI motor outflows may share a pool of premotoneurons driven by a common cardiorespiratory controller. Our experiments do not directly demonstrate the nature or extent of coupling between cardio-

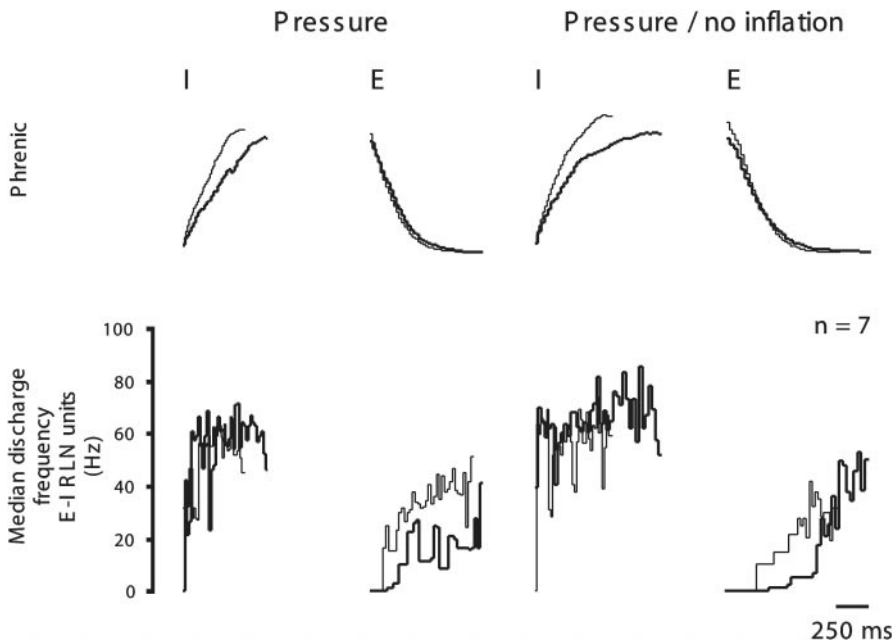


Fig. 7. Group median discharge frequency of E-I RLN motor units and their response to UANP. The bin-median discharge frequency of E-I RLN units ($n = 7$) and bin-average phrenic activity are shown during I and E. Unit activity during UANP stimuli (thick line) is compared with an appropriate control (thin line). *Left*: pressure. Activity on the second breath of -10 cmH₂O UANP delivered with ongoing lung inflation is compared with the discharge on the breath immediately preceding the stimulus. *Right*: pressure/no inflation. Activity on the second breath of -10 cmH₂O applied during temporary cessation of lung inflation is compared with the discharge on the second breath of an intervention where lung inflation was withheld but no pressure was applied to the upper airway.

vascular and respiratory control but do support the hypothesis that they are closely linked.

We recorded from two distinct types of RLN motor fiber with inspiratory discharge. Phasic-I neurons comprised 53% of respiratory-modulated units, were active only in I, and usually exhibited a progressive increase in discharge frequency as this phase proceeded. There was a bimodal distribution of onset times for the activity of these units to the onset of phrenic discharge. However, the time to discharge onset for "late" units could be greatly reduced by failure to inflate the lungs or UANP. Heterogeneity among inspiratory units has been reported for both phrenic motoneurons (13) and geniohyoid muscle motor units (30). Further work is required to determine whether phasic-I laryngeal motoneurons are a homogeneous or heterogeneous population.

E-I units accounted for 31% of respiratory-modulated units and are best described as tonically active but inhibited during PI so that their discharge frequency was relatively constant during I and had an augmenting pattern in E. Sica and colleagues (26) also distinguished these two categories of laryngeal motor fiber in the decerebrate cat and, in addition, described units on which a decrementing inspiratory burst of activity was superimposed on tonic background discharge (26). We did not record any motor fibers with this pattern of discharge.

Phasic-I neurons were recruited by UANP, whereas, in contrast, the inspiratory discharge of E-I neurons was not affected by this stimulus. We did observe that UANP markedly inhibits E-I neurons in PI. The fact that E-I units were inhibited and PI units excited during the PI phase supports the concept that the discharge of laryngeal motor units is determined, at least in part, by reciprocal inhibition between motoneurons or their premotor influences (26–28).

Our data show that there is a complex laryngeal motor response to distortion of the UA by negative transmural pressure. We do not know how these changes in motoneuron activity will affect glottic aperture and are cautious in interpreting the present results because the destination within the larynx of the motor axons from which we recorded is not certain. Neural recordings from the intralaryngeal branch of the RLN supplying the adductor muscles show exclusively PI or decrementing expiratory activity, whereas the branch to the sole abductor of the vocal folds, the posterior cricoarytenoid (PCA), exhibits mixed phasic inspiratory and tonic expiratory discharge (35). These data, along with electromyographic studies that show that the laryngeal adductor muscles are normally active only in E (9, 12, 14), strongly suggest that PI units in the main trunk of the RLN innervate laryngeal adductor muscles, whereas both the phasic-I and E-I units supply the PCA. We speculate that the laryngeal response to UANP has two components. Recruitment of phasic-I motoneurons innervating the PCA will dilate the glottis during I, whereas a combination of increased PI drive to laryngeal adductor muscles and

reduced activity of E-I units supplying the PCA will cause the glottis to narrow in early E.

The hypothesis that UANP causes inspiratory dilation of the glottis is supported by one previous study, which recorded an increase in the inspiratory electromyographic activity of the PCA when subatmospheric pressure was applied to the isolated UA (30). Inspiratory glottic dilation also fits well with the standard description of the reflex responses to UANP. Negative transmural pressures across the UA wall are a sensitive and specific signal that the airway is narrow or obstructed. The reflex responses to UANP represent a coordinated attempt to dilate and stabilize the airway and prevent further collapse. These responses include an increase in the activity of UA dilator muscles and a reduction in the drive to thoracic pump muscles (1, 16, 17, 31). Laryngeal abduction would reflect the global activation of UA dilator muscles and would also reduce total UA resistance during I.

The suggestion that the reflex responses to UANP may include glottic narrowing in early E is novel and requires experimental confirmation. Adduction of the vocal folds in E would brake expiratory airflow and help maintain or increase end-expiratory lung volume (EELV) (2, 29). It is known that an increase in EELV indirectly dilates the pharyngeal airway (29). Further work is required to determine whether the glottis narrows in early E in response to UANP and whether any resulting increase in EELV stabilizes the pharynx.

We detected two categories of units with inspiratory activity, which we presume both innervate the PCA. This agrees with prior studies showing a variety of patterns of discharge among RLN fibers with inspiratory activity (26) and motor units of the PCA muscle. There is considerable evidence that the PCA is a heterogeneous muscle with at least two anatomically, histochemically, and functionally distinct compartments (4, 7, 8, 22–24, 32). It is not known whether there is a relationship between the discharge properties of a motor unit and its location within the PCA, nor whether there is a correlation between discharge pattern and biomechanical function. We found that only one type of motor unit, the phasic-I, was activated by UANP. This is of interest because it suggests that the categories of PCA motor unit are functionally distinct, and those motor units with different discharge patterns may indeed have different functions.

We also examined the influence of lung inflation on laryngeal motoneuron activity, and in general our findings are in agreement with other published work (26). Lung inflation inhibited phasic-I units and PI units. Lung inflation did not alter the inspiratory discharge of E-I units, in contrast to the small inhibitory effect seen by Sica and co-workers (26). Nonetheless, we did find, in agreement with Sica and colleagues, that the inhibition of E-I unit discharge in PI was less pronounced when the lungs inflated normally during the prior I (26). The effects on RLN unit activity of failing to inflate the lungs are qualitatively similar to the effects of UANP, which is not surprising considering that both

UANP and loss of phasic lung volume feedback are signals that accompany UA obstruction. Failure to inflate the lungs also facilitated the excitatory effect of UANP on phasic-I units, which accords with other work demonstrating that reductions in lung volume feedback augment the effects of UANP on UA dilator muscle activity (34). Whereas UANP and withholding lung inflation also had qualitatively similar effects on the activity of PI and E-I units, the response to UANP applied simultaneously with temporary withdrawal of lung inflation was similar to or less than the algebraic sum of the responses to these interventions when performed alone. Given that we did not perform stimulus-response relationships for the effects of UANP and lung volume feedback, we cannot comment on the nature of the central interaction between these inputs in the control of these motor units. We must also be cautious in interpreting the effects of lung inflation in these experiments because there is some evidence that regions of the lung were atelectatic; given an $F_{I_{O_2}}$ of 0.37 and a P_{aCO_2} of 40.1 Torr, a P_{aO_2} of 150 Torr indicates a mean alveolar-arterial oxygen gradient of ~50–60 Torr.

The activity of a number of units from which we recorded was not modulated by respiration, nor did UANP or failure to inflate the lungs affect these units. Units with similar discharge properties have been reported before (6), and they may be motor fibers to skeletal muscle in the esophagus or autonomic fibers supplying the trachea or esophagus.

We conclude that motor fibers in the RLN with PI activity are recruited by UANP. This supports our hypothesis that UANP activates PI motor outflows whether they are involved in the control of the circulation or the regulation of respiration. These findings are consistent with the proposal that a common medullary cardiorespiratory network controls efferent neural activities to the cardiovascular and respiratory systems. The effect of UANP on subpopulations of RLN motor units suggests that this stimulus will dilate the glottis in I but adduct the vocal folds in early E. Further work is required to confirm that this is the case and to explore what function is served by adjusting glottic aperture as a reflex response to subatmospheric pressure in the UA.

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