Common drive to the upper airway muscle genioglossus during inspiratory loading

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Common drive to the upper airway muscle genioglossus (UAMs) such as genioglossus (GG) is matched by the complexity of their physiology. Thus GG motor neurons have a number of different respiratory-related discharge patterns (Nicholas et al. 2010; Saboisky et al. 2006; Wilkinson et al. 2008). Some neurons are tonically active with higher discharge rates during inspiration (Inspiratory Tonic) or expiration (Expiratory Tonic) or do not vary their discharge rate as a function of the respiratory cycle (Tonic). Other neurons are predominantly active during inspiration with a silent period during expiration (Inspiratory Phasic) or are active during expiration with a silent period during inspiration (Expiratory Phasic). Furthermore, within the respiratory system there are multiple premotor inputs, such as the respiratory pattern generator (RPG), mechanical and chemical stimuli, and sleep-wake state.

Despite, or perhaps because of, these complexities little is known about the motor control of UAMs, particularly the transmission of respiratory drive to the relevant motor neuron pool (Walls et al. 2013) or to neuromuscular compartments within that pool. Studies that have investigated the transmission of respiratory drive to hyperglossal motoneurons have identified a limited number of properties. For example, increases in respiratory drive are primarily reflected in recruitment of motor units rather than increases in the discharge rate of already active motor units (rate coding) (e.g., Nicholas et al. 2010; Richardson and Bailey 2010). This property of UAMs appears to be independent of the level of respiratory drive, as recruitment has been reported to be the dominant mechanism in response to relatively intense physical exercise (Walls et al. 2013). Indeed, in this study, while inspiratory discharge rates of motor units did not increase, expiratory discharge rates actually decreased, suggesting a central inhibitory component (Walls et al. 2013). Furthermore, a number of studies have indicated that inspiratory modulated (Inspiratory Phasic and Inspiratory Tonic) motor units are particularly sensitive to variations in respiratory drive (Trinder et al. 2014).

During a muscle contraction, a muscle’s active motor neurons tend to vary their firing frequency in unison. This activity pattern is thought to reflect a common, central, premotor input to the motor neuron pool, referred to as common drive. The process is thought to simplify motor control and increase efficiency, as the level of activity in the motor neuron pool is controlled by a single mechanism rather than each motor neuron being individually controlled (De Luca 1985). The strength of common drive in skeletal muscles has been shown to vary between muscles (De Luca et al. 1982; Patten et al. 2000) and as a function of a variety of conditions and manipulations. For example, common drive is lower in the first dorsal interosseous muscle in trained individuals (Semmler and Nordstrom 1998) and in the elderly compared with young adults (Erim et al. 1999).

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Mambrito 1987) or synergistic (De Luca and Erim 2002) coactivation.

Thus common drive may provide an additional mechanism by which a muscle may respond to increased drive. However, as far as we are aware, only two studies have investigated common drive in tongue or upper airway muscles. Laine and Bailey (2011) observed strong common drive in unilateral pairs of GG motor units during resting breathing. Furthermore, common drive was significantly higher during volitional tongue protrusion than during quiet breathing. Whether this difference represented stronger common drive to the same functional pool of motor neurons or the activation of a different pool of neurons could not be determined. Trinder et al. (2013) studied both GG and tensor palatini (TP) muscles and, consistent with the earlier study, observed significant common drive to motor units within each muscle but also between motor units from each of the two muscles, reflecting synergistic coactivation. Common drive was also higher in pairs of respiratory modulated motor units than in units without respiratory modulation (tonic units). It seems reasonable to assume that the strong common drive to inspiratory modulated motor units reflects premotor input from the RPG, particularly as inspiratory modulated motor units from different muscles shared common drive.

We are not aware of any study that has evaluated the effect of the level of respiratory drive to a UAM on the strength of its common drive and thus evaluated whether common drive is a mechanism used by UAMs to increase their effectiveness. In the present study we manipulated respiratory drive to the GG muscle by applying inspiratory loads. We hypothesized that common drive, as assessed by low-frequency coherence between pairs of motor units, would increase as a function of the demands placed on the muscle, such that increasing respiratory drive would increase common drive to the GG muscle. Furthermore, as inspiratory modulated motor units are more sensitive to variations in respiratory drive and show stronger common drive, we hypothesized that common drive to inspiratory modulated motor units would increase more than that to expiratory modulated or Tonic motor units.

METHODS

Subjects

The subjects were 20 men with a mean age of 32 yr (±2 yr), a mean height of 179 cm (±6 cm), and a mean weight of 79 kg (±12 kg). Data were collected from 17 subjects on one occasion, 2 subjects on two occasions, and 1 subject on three occasions, for a total of 24 recording sessions. The original study protocol included hyperventilation and inspiratory loading trials, the former data having been previously published (Nicholas et al. 2010). As elevated CO₂ may affect the fetus of a pregnant woman and pregnancy testing was not available within the University of Melbourne laboratory, women were excluded from the study. All subjects were healthy and were without sleep or respiratory complaints as determined by a questionnaire. The protocol conformed to the Declaration of Helsinki and had prior approval of the local Human Subjects Ethics Committee. Informed consent was obtained from each subject.

General Laboratory Procedures

Experimental sessions were conducted during the late morning or early afternoon. Subjects were requested to refrain from alcohol during the day before the experimental session and from food and caffeine for 4 h before the session. On arrival at the laboratory a local anesthetic cream was applied under the subject’s chin (lidocaine-prilocaine, Fogera, Melville, NY) in preparation for the insertion of the four EMG recording electrodes. Instrumentation of the subject commenced 30 min later. During data collection subjects remained quietly awake in the supine position on a bed in a recording room. The supine position was used as it is an easy position for subjects to sustain and standardize without moving and because it maximizes GG activity (Malhotra et al. 2004). Subjects were instructed to remain still and relaxed, to relax facial muscles as much as possible, to try to avoid swallowing, and to remain awake. Subjects breathed via the respiratory circuit for 5 min before data for analysis were collected so as to become accustomed to the conditions. All recordings were stored for subsequent off-line analysis.

During the first half of the session a series of six 3-min hyperventilative trials were presented, with an interval of ~2 min between trials, while in the second half a series of eight respiratory loading trials were presented. The present report presents data from the respiratory loading trials. Inspiratory loading trials consisted of a 30-s baseline period followed by a 60-s load and a 30-s recovery period. A trial was initiated by an experimenter entering the recording room and informing the subject that a trial was about to commence. The 30-s baseline period began when the subject was still and breathing regularly. The load (a resistor) was introduced into the inspiratory line during expiration via a three-way stopcock valve (Hans Rudolph 2110, Shawnee, KS) attached to Hans Rudolph 7100R series resistors and 60 s later removed during expiration. After the 30-s recovery period the experimenter informed the subject that the trial had been completed. The intertrial interval was ~2 min. Four load levels (resistors) were applied, 5, 10, 15, and 20 cmH₂O, with each load being presented twice in the sequence: 5, 10, 15, 20, 20, 15, 10, 5.

Measurements and Recordings

Ventilatory airflow was collected via a full face mask (type 431, Fisher and Paykel, Auckland, New Zealand) connected to a two-way breathing valve (2600 medium, Hans Rudolph). Rate of airflow was measured by a calibrated pneumotachometer (3719, Hans Rudolph), a differential pressure transducer (DP-45, Validyne, Northridge, CA), and a Validyne carrier demodulator (CD15). The pneumotachometer was placed between the mask and the two-way valve. P₆⁶ and O₂ were measured from the mask with Amatek CO₂ (CD-3A, Berwyn, PA) and O₂ (S-3A/I) analyzers. Pressures were measured in the mask with an open catheter attached to a pressure transducer (DP-45, Validyne) and in the airway at the level of the epiglottis with a pressure-tipped catheter (MPC-500, Millar, Houston, TX) inserted via a nostril.

Respiratory variables were recorded with Spike2 acquisition software (Cambridge Electronic Design, Cambridge, UK). Data were recorded as DC signals with Grass amplifiers (Grass Telefactor), with a 15-Hz low-pass filter. Signals were digitized at 100 Hz via a 1401 interface (Cambridge Electronic Design).

Muscle activity. The procedures described by Eastwood et al. (2003) were used to record GG EMG activity. Four monopolar intramuscular wire electrodes were inserted 2.4 cm from the surface of the skin with a percutaneous approach and referenced to a common surface electrode positioned over the bony mandible, with a large flexible ground strap placed on the left shoulder. Previous studies using ultrasound to identify the location of the GG indicate that at this depth the electrode consistently enters the GG near the lower margin of the muscle (Eastwood et al. 2003; Saboisky et al. 2006; Wilkinson et al. 2008). Location within the GG was also confirmed by standard maneuvers. Insertion was via a 25-gauge hypodermic needle with a single stainless steel, Teflon-coated 50-µm wire electrode (A-M Systems, Seattle, WA) in each of the needle insertions. The electrodes had 0.5 mm of the tip exposed. The four GG recordings were collected...
from electrodes placed in the left and right muscle bellies and in relatively anterior (closer to the mandible) and posterior (closer to the oropharyngeal airway) positions, ~3 mm each side of the midline and ~10 mm and ~20 mm from the inferior margin of the mandible. For the GG EMGs the filters were set at 0.03–3 kHz and the signal digitized and recorded at 10 kHz.

Data Reduction

Four groups of measurements were collected: respiration, GG EMG activity, the discharge rates of single motor units, and the coherence between pairs of motor units where the two units had the same discharge pattern (see below for a description of GG motor units’ discharge patterns).

Respiratory variables. Respiratory variables were measured on a breath-by-breath basis with software developed within the laboratory. The data for each trial were visually checked to ensure that the program’s breath detection algorithm was correctly identifying breaths. The variables analyzed were cycle duration, tidal volume, minute ventilation, $P_{ET}$CO$_2$, peak inspiratory flow, and nadir of epi-glottal pressure.

Multimodal GG activity. The overall GG EMG activity was quantified by determining the spike density on each electrode (Nicholas et al. 2010). Spike density was defined as the number of spikes per second that exceeded a threshold voltage. The threshold was defined as twice the voltage amplitude of the background noise. Background noise was assumed to primarily reflect activity of distant motor units and, at times, electrical interference. While a common threshold was used, spike density was determined independently for inspiration and expiration.

Identification of single motor units. The literature indicates that UAM motor units have six different discharge patterns (Saboisky et al. 2006): Inspiratory Phasic, units that show peak activity during inspiration and maintain a discharge rate of <2.0 Hz throughout the respiratory cycle; Inspiratory Tonic, units that show peak activity during inspiration and maintain a discharge rate > 2.0 Hz throughout the respiratory cycle; Expiratory Phasic, units that show peak activity during expiration and have a minimum firing frequency of <2.0 Hz during expiration (i.e., are silent for >500 ms); Inspiratory Tonic, units that show peak activity during inspiration and maintain a discharge rate > 2.0 Hz throughout the respiratory cycle; Expiratory Tonic, units that fire throughout the respiratory cycle and have no obvious respiratory or other modulation; and Tonic Other, units that fire throughout the respiratory cycle with modulation out of phase with respiration.

The data required to test the hypotheses were pairs of motor units where the two motor units had the same discharge pattern (e.g., both Inspiratory Tonic) and that were active over the same 2-min trial: the 30-s baseline, 1-min load, and 30 s after load. The two motor units could be recorded on the same or different electrodes during a trial. The decomposition of motor units from the raw EMG recordings involved five steps. First, the recordings during a trial were inspected and trials on which body movements and related events occurred were discarded. Second, the four GG electrodes were inspected visually and electrodes that could potentially yield motor units were subjected to formal decomposition. Third, the initial decomposition of motor units was conducted with Spike2 analysis software (Cambridge Electronic Design). Motor units were identified with a spike-triggered threshold voltage and sorted by the software into templates based on the spike’s detailed amplitude and shape. Fourth, additional software running as a script within Spike2 (software courtesy of Neuroscience Research Australia, Sydney, Australia) was then used to inspect and edit the initial classification on a spike-by-spike basis using amplitude, shape, and frequency information. This process allowed errors in the original sorting to be corrected and particular features to be identified. For example, electrode movement, which may occur during events such as coughs and swallows and which may have been missed in step 1, were identified by the loss of spike trains following the event. In such circumstances all affected electrodes were discarded. Slight movements, which occasionally occurred in association with respiratory activity, typically produce changes in spike amplitude, rather than spike frequency, and thus identified motor units were retained. Fifth, and finally, interspike interval histograms from the discharge times for each discriminated motor unit were obtained to further assess discrimination accuracy. A characteristic sign of discrimination error is the presence of abnormally long interspike intervals at multiples of the mean interspike interval, which occur because of superimposition with action potentials of other active motor units. Interspike intervals that were clearly outside the main distribution (because of discrimination error) were visually inspected and further edited on a spike-by-spike basis. This involved the insertion of a discharge time in an abnormally long interval where an action potential waveform was present (but not recognized because of superimposition errors). A motor unit was discarded if the proportion of uncorrected errors could not be reduced below 5% of the total number of intervals in the histogram distribution. Figure 1 illustrates the decomposition of two tonic motor units (Fig. 1A) and the resultant interspike histograms (Fig. 1B). The resulting discharge properties of the motor units were expressed as instantaneous frequency plots (Fig. 1A, top). Figure 1C illustrates the superimposition of spikes from two motor units and their resolution at the level of the instantaneous frequency plots.

The pairs of motor units identified showed four of the six discharge patterns originally identified by Saboisky et al. (2006): Inspiratory Phasic, Inspiratory Tonic, Expiratory Tonic, and Tonic. In a minority of instances motor units changed their discharge pattern in association with the load, in which case the motor unit was classified according to its preload pattern. In general the discharge pattern for motor units is readily apparent. However, in some cases the pattern is ambiguous, particularly between Inspiratory or Expiratory Tonic and Tonic patterns as this distinction depends on whether respiratory modulation of the unit can be identified. To determine a unit’s degree of respiratory modulation, instantaneous frequency values for the motor unit were cross-correlated with tidal volume, derived from an integrated flow signal, at the time of each spike, over a breath (Wilkinson et al. 2008). The value was calculated for each breath and averaged over breaths within the baseline period. The greater the cross-correlation value, the stronger the respiratory modulation. In accord with values published in earlier papers (Nicholas et al. 2010, 2012; Wilkinson et al. 2008, 2010), motor units with an average cross-correlation value of ≥0.49 were assigned to the Inspiratory Tonic or Expiratory Tonic category, depending on the phase of the discharge peak, while motor units with a cross-correlation value < 0.49 were classified as Tonic.

Discharge properties of motor units. The motor units’ discharge activities were then quantified (software developed by Neuroscience Research Australia). Three measures were produced: peak frequency (average for the 200 ms with the maximum average frequency), mean frequency (mean rate over phases without respiratory modulation), and tonic frequency (last 200 ms of any non-respiratory modulation phase).

Coherence analysis. Common drive for pairs of motor units was associated with a previously described frequency domain (coherence analysis) method (Semmler 2002; Semmler et al. 2004) implemented in MATLAB (MathWorks). Frequency domain coherence analysis was chosen because, compared with other methods such as time domain correlations, it provides a more complete description of all common oscillatory inputs and is better suited for quantifying narrow ranges of descending input into a single index (Myers et al. 2004). Instantaneous frequency plots were divided into contiguous, nonoverlapping epochs of 5.12 s. Within each epoch,
1,024 bins (width = 5 ms) were assigned a value of 1 if a discharge occurred within a selected bin or a value of 0 if no discharge was present. The new time series from each disjoint section was then subjected to fast Fourier transformation with a resolution of 0.2 Hz up to a limit of 50 Hz. Data < 30 Hz were used for analyses with frequencies between 0 and 5 Hz reflecting common drive. Auto- and cross-spectra were estimated by averaging over the disjoint sections to obtain the coherence data for each motor unit pair, which resulted in a measure of linear association with values between 0 (completely independent) and 1 (completely dependent) within each 0.2-Hz frequency bin (coherence coefficient). Two coherence plots, each for a single pair of motor units, are illustrated in Fig. 2. Figure 2, top, shows the coherence plot for a pair of motor units during the preload phase, indicating moderate common drive (maximum value of ~0.65), while Fig. 2, bottom, shows the coherence plot for the load phase, indicating strong common drive (maximum value of ~0.89). It should be noted that higher-frequency activity apparent in the profiles primarily reflects random noise that is removed when data are averaged over pairs of units, as illustrated in Fig. 3.

Common drive was quantified by a number of methods commonly used in the literature. The first was the maximum coherence coefficient value in the 0–5 Hz range. In the second method, the maximum coherence values were normalized with a standard score transform: $z = \sqrt{2L} \times \frac{1}{2} \cdot \text{arctanh}(\sqrt{r})$, where $L$ is the number of segments used, $r$ is the coherence value, and arctanh refers to the inverse hyperbolic function. The third method involved determining the probability with which coherence values within the 0.2-Hz-wide bins in the 0–5 Hz range were significantly greater than zero (Semmler 2000; Semmler et al. 2004). Finally, the frequency at which the maximum coherence coefficients occurred was analyzed.

To investigate coherence in higher frequencies, coherence profiles over the frequency range 0–30 Hz were constructed using coherence coefficient values and the probability of coherence values being greater than zero. The latter two analyses allowed coherence to be assessed at frequency ranges other than the range reflecting common drive (0–5 Hz).

**Statistical Analyses**

Pairs of units were not identified at all load levels for any subject, and a number of subjects had pairs at only one load level. An analysis of the available load level data showed a slight tendency for common drive to be larger during the higher loads; however, the effect was not significant [$F(1,75) = 1.66$, $P = 0.20$]. Thus load level was removed as a factor in the experiment.

Respiratory variables were averaged over breaths within phases of a load [the 30 s before the load (pre); the first 30 s of the load (first half); the second 30 s of the load (second half); and the 30 s after the load (post)] and then over trials within different load levels and over different loads within subjects. The effect of the inspiratory load on respiratory activity was assessed by one-way repeated-measures ANOVAs over the four load phases (pre, first half, second half, post). Analyses were conducted separately for each of the respiratory variables. Only trials that yielded pairs of motor units, and thus contributed data to the coherence analysis, were included in the respiratory analysis.

Spike density values were analyzed to determine the effect of the inspiratory loads on total inspiratory and expiratory GG activity. Only electrodes that yielded motor units were included in the analysis. Values
were averaged over breaths within load phases and over appropriate electrodes, trials, and loads within subjects. Values were compared over the four load phases (pre, first half, second half, post) and two respiratory phases (inspiration and expiration) in a 4 × 2 ANOVA with repeated measures on both variables.

The discharge characteristics (peak, mean, and tonic discharge rates) of the motor units used in the common drive analysis were averaged over breaths within load phases, over units with the same discharge pattern, and over trials and loads within subjects. Data were then compared over the four load phases (pre, first half, second half, post). Inspiratory Phasic motor units were combined with Inspiratory Tonic motor units to form an inspiratory category, while Expiratory Tonic motor units were combined with Tonic motor units to form an expiratory/tonic category. The rationale for this categorization was that previous studies have shown that motor units within each of these groupings respond similarly to changes in respiratory drive (Nicholas et al. 2010) or changes in sleep-wake state (Wilkinson et al. 2008, 2010). Thus the resulting analysis model consisted of a 4 phase of load × 2 discharge pattern category ANOVA averaged over subjects, with discharge patterns treated as independent conditions. Separate statistical analyses were conducted for each discharge measure (peak, mean, and tonic discharge rates).

For the analysis of common drive, only three subjects had both inspiratory and expiratory/tonic pairs. Thus for the three methods of evaluating common drive, the maximum coherence coefficient, the z score, and the probability of significant coherence values in bins, values were averaged over pairs within discharge pattern categories for each load phase. The data were analyzed in a 4 load phase (pre, first half, second half, post) × 2 discharge pattern category (inspiratory, expiratory/tonic) ANOVA with discharge patterns treated as independent groups. Additional analyses were conducted on the three subjects who had pairs with each discharge pattern to assess whether the results of the main analysis were an artifact of the way the data were grouped in the analysis based on pairs.

The average frequency of the maximum coherence coefficients was calculated for each phase of load and discharge pattern and statically averaged as for the measures of common drive.

Maximum coherence coefficients and probability values were plotted over the 0–30 Hz range for each phase of load and discharge pattern category.

RESULTS

The 20 subjects were presented a total of 160 inspiratory loading trials. Thus potentially as many as 640 electrode recordings were available, although ~25% of these were lost because of technical faults and events such as body movements. Within loading trials only electrodes that yielded motor units that contributed to pairs of units with the same discharge pattern were fully decomposed, i.e., a single motor unit that could not be matched with another unit with the same discharge pattern was discarded before decomposition was completed. Motor units that contributed to pairs of units could be on the same or different electrodes within a trial. Initially it was intended that only motor units that provided data throughout the trial (pre, first half, second half, post) would be included in analyses (see METHODS). However, in order to increase the number of pairs with the various discharge patterns, motor units with one section missing because of a swallow, and where it was obvious that the motor unit resumed its preswallow activity, were included.

Twelve of the 20 subjects produced trials with at least one pair of motor units (total of 29 trials, range 1–5 trials per subject). The 29 trials resulted in 84 motor units, yielding 77 pairs (range 1–21 pairs per subject). Two pairs were Inspiratory Phasic, 39 Inspiratory Tonic, 15 Expiratory Tonic, and 21 Tonic. Twenty of the 77 pairs had one segment of data missing, 4 before load and 16 after load. In summary, 11 of the 12 subjects with motor unit pairs provided respiratory data, 1 subject being discarded from this analysis because of unusually long cycle duration, while all 12 subjects provided EMG spike density values. For the analyses of motor unit discharge rates and common drive values, all 12 subjects provided motor units and motor unit pairs. However, only eight subjects provided inspiratory motor units or motor unit pairs, only seven provided expiratory/tonic motor units or motor unit pairs, and only three provided both inspiratory and expiratory/tonic motor units or motor unit pairs.

As indicated in Table 1, analyses of respiratory activity confirmed that the inspiratory loads had the expected effect on respiratory activity, with a reduction in respiratory output (a significant increase in cycle duration and significant falls in minute ventilation and peak inspiratory flow) and an increase
in respiratory drive (increased negative epiglottal pressure) during the loads. Each of these variables showed significant main effects of load and significant quadratic functions over the load phases, indicating that respiratory activity returned to the preload level after the load.

Spike density values, indicating the overall level of muscle activity recorded on electrodes that produced motor units, are illustrated in Fig. 4. As shown, GG activity during inspiration, but not GG activity during expiration, increased during the loads. Statistically there was a significant interaction effect \( F(3,33) = 6.06, P = 0.002 \) and a main effect of respiratory phase \( F(1,11) = 4.93, P = 0.048 \) but not a significant main effect of load phase \( F(3,33) = 1.69, P = 0.189 \). Post hoc tests indicated a significant quadratic function over load phase \( P < 0.05 \).

### Table 1. Respiratory activity over phases of inspiratory loads

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>1st half</th>
<th>2nd half</th>
<th>Post</th>
<th>Main effect</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ttot, s</td>
<td>4.4 (1.0)</td>
<td>4.8 (1.1)</td>
<td>4.7 (1.0)</td>
<td>4.4 (1.2)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VT, ml</td>
<td>0.62 (0.07)</td>
<td>0.56 (0.06)</td>
<td>0.60 (0.08)</td>
<td>0.64 (0.10)</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>VI, l/min</td>
<td>8.8 (2.2)</td>
<td>7.3 (1.8)</td>
<td>7.8 (1.6)</td>
<td>9.3 (2.5)</td>
<td>&lt;0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>P\textsubscript{ETCO}_2, mmHg</td>
<td>36.7 (3.4)</td>
<td>37.0 (2.9)</td>
<td>37.0 (3.9)</td>
<td>36.5 (3.5)</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>PIF, l/min</td>
<td>31.6 (6.7)</td>
<td>24.5 (5.8)</td>
<td>26.6 (6.2)</td>
<td>33.9 (8.0)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Pepi, cmH\textsubscript{2}O</td>
<td>-3.1 (1.3)</td>
<td>-6.1 (2.6)</td>
<td>-6.1 (2.6)</td>
<td>-3.4 (1.3)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values in parentheses are SDs. Ttot, cycle duration; VT, tidal volume; VI, minute ventilation; P\textsubscript{ETCO}_2, end-tidal CO\textsubscript{2}; PIF, peak inspiratory flow; Pepi, epiglottal negative pressure; ns, not significant.

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**Fig. 3.** Maximum coherence coefficient over the 0–30 Hz frequency range for the 4 phases of the load. *Top:* expiratory modulated and tonic motor units. *Bottom:* inspiratory modulated motor units. Shaded area indicates the frequencies reflecting common drive.
The discharge characteristics of motor units that were included in the coherence analysis are shown in Table 2. As can be seen, mean and peak discharge rates increased slightly, and significantly, during the load. In addition, the quadratic component over load phase was significant for these two measures, indicating that the increase in discharge rate was specifically associated with the load.

The onset of the inspiratory load increased all three measures of common drive in inspiratory modulated motor units compared with expiratory modulated or tonic motor units, with significant phase of load × discharge pattern interactions (statistical analyses are presented in Table 3, and the data for $z$ scores and the probability of significance are illustrated in Fig. 5). Post hoc analyses for all measures indicated that common drive to inspiratory modulated motor neurons was elevated above the preload level during and after removal of the load. There was a significant linear increase over load phase and significantly higher values than for expiratory/tonic neurons during the load and after the load for the maximum coherence coefficient and $z$ score and the second half of the load and after the load for the probability data (see Table 3 and Fig. 5). As indicated in Table 3, inspiratory motor units tended to show significant linear and quadratic functions, while expiratory and tonic motor units only showed significant quadratic functions. The main effect of discharge pattern was significant for the motor units only showed significant quadratic functions.

Table 2. Mean, phasic, and tonic discharge rates for single motor units with Inspiratory or Expiratory/Tonic discharge patterns as function of inspiratory load

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>1st half</th>
<th>2nd half</th>
<th>Post</th>
<th>Load</th>
<th>Pattern</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Insp</td>
<td>16.9 (5.0)</td>
<td>17.4 (5.4)</td>
<td>18.6 (4.5)</td>
<td>17.7 (4.6)</td>
<td>0.018*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Exp/Tonic</td>
<td>19.3 (3.4)</td>
<td>19.8 (3.1)</td>
<td>19.8 (3.5)</td>
<td>19.2 (3.1)</td>
<td>&lt;0.001*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Peak Insp</td>
<td>19.2 (4.8)</td>
<td>20.9 (4.8)</td>
<td>21.6 (4.9)</td>
<td>20.2 (4.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp/Tonic</td>
<td>21.7 (3.4)</td>
<td>22.5 (3.1)</td>
<td>22.6 (3.7)</td>
<td>21.7 (3.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonic Insp</td>
<td>14.7 (5.6)</td>
<td>14.9 (5.1)</td>
<td>16.1 (3.7)</td>
<td>16.3 (4.3)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Exp/Tonic</td>
<td>17.9 (2.7)</td>
<td>18.5 (2.7)</td>
<td>18.5 (3.5)</td>
<td>18.4 (3.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean, phasic, and tonic discharge rates in Hz. Insp, Inspiratory Phasic and Inspiratory Tonic motor units; Exp/Tonic, Expiratory Tonic and Tonic motor units. *Mean and peak frequencies also showed significant quadratic components over the phases of load.

DISCUSSION

Increasing respiratory drive by introducing inspiratory loads resulted in a strengthening of common drive that was more marked in inspiratory modulated GG motor neurons compared with Expiratory Tonic and Tonic motor neurons. In addition, the increase in the strength of common drive to inspiratory motor neurons persisted during the 30 s after load, while common drive to expiratory/tonic neurons returned to preload levels.

As was anticipated, inspiratory loads increased respiratory drive, as indicated by an increase in negative airway pressure (see nadir epiglottal pressure in Table 1), and decreased respiratory output, as indicated by increased cycle duration and decreased ventilation and peak inspiratory flow. While increases in both respiratory load and common drive (to inspiratory motor units) are compatible outcomes, it should be noted that the respiratory effects of the load were restricted to the period of load administration. Also as anticipated, GG activity rose as a function of the load, as indicated by both the spike density measure and the mean and peak discharge rates of single motor units. Again, in contrast to the changes in common drive, these effects were limited to the load period.

The pattern of results suggests that the introduction of the load caused a reorganization of the premotor control of the GG muscle,
particularly to inspiratory modulated motoneurons. As noted in the introduction, common drive is thought to represent a central premotor mechanism that subsumes motor control of a motor neuron pool increasing the pool’s efficiency. An increase in common drive in response to increased respiratory drive suggests

Table 3. Statistical analyses of common drive measures

<table>
<thead>
<tr>
<th></th>
<th>Maximum Coherence</th>
<th>z Score</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANOVA</td>
<td>F (df)</td>
<td>P</td>
</tr>
<tr>
<td>Phase of load</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.15 (3,225)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Discharge pattern</td>
<td></td>
<td>26.40 (1,75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phase × discharge pattern</td>
<td></td>
<td>3.68 (3,225)</td>
<td>0.013</td>
</tr>
<tr>
<td>Contrasts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inspiratory: linear</td>
<td></td>
<td>25.00 (1,40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quadratic</td>
<td></td>
<td>15.01 (1,40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exp/Tonic: linear</td>
<td></td>
<td>0.29 (1,35)</td>
<td>0.594</td>
</tr>
<tr>
<td>Quadratic</td>
<td></td>
<td>9.77 (1,35)</td>
<td>0.004</td>
</tr>
<tr>
<td>t-Tests between discharge patterns</td>
<td>t (df)</td>
<td>P</td>
<td>t (df)</td>
</tr>
<tr>
<td>Pre</td>
<td>2.19 (75)</td>
<td>0.032</td>
<td>1.06 (75)</td>
</tr>
<tr>
<td>1st half</td>
<td>4.15 (75)</td>
<td>&lt;0.001</td>
<td>3.96 (75)</td>
</tr>
<tr>
<td>2nd half</td>
<td>4.59 (75)</td>
<td>&lt;0.001</td>
<td>4.50 (75)</td>
</tr>
<tr>
<td>Post</td>
<td>6.02 (75)</td>
<td>&lt;0.001</td>
<td>6.31 (75)</td>
</tr>
</tbody>
</table>

Fig. 5. Top: maximum z score values. Bottom: probability of a frequency bin being significantly greater than zero in the 0–5 Hz range. Inspiratory modulated (Inspiratory Phasic and Inspiratory Tonic) and expiratory/tonic (Expiratory Tonic and Tonic) units as a function of the phase of the load are shown. Bars indicate SE. For both measures there were significant linear increases over load phase for inspiratory modulated motor units and significant differences between discharge pattern categories for the second half of the load and after the load. **p < 0.01, ***p < 0.001.

Fig. 6. Mean values for the 3 subjects who had both inspiratory and expiratory/tonic motor unit pairs. Top: maximum z score values. Bottom: probability of a frequency bin being significantly greater than zero in the 0–5 Hz range. Inspiratory modulated (Inspiratory Phasic and Inspiratory Tonic) and expiratory/tonic (Expiratory Tonic and Tonic) units as a function of the phase of the load are shown. Bars indicate SE.
an increase in the strength of this mechanism, presumably increasing the ability of the motor neuron pool to maintain airway patency in the face of increasing negative pressure, limiting the necessity for increases in motoneuron discharge rate and motor unit recruitment.

Nevertheless, in addition to the increase in common drive to inspiratory modulated motor neurons, inspiratory EMG activity (spike density) increased, as did the mean and peak, but not tonic, activity of single motor units. However, the changes in common drive did not appear to be a direct consequence of increased muscle activity, because while GG activity decreased during the postload recovery common drive to inspiratory modulated neurons remained elevated. Furthermore, common drive to Expiratory Tonic and Tonic motor neurons began to fall toward the end of the load, while GG activity was elevated. Thus common drive in UAMs appears to be a mechanism independent of, and in addition to, changes in discharge rate and recruitment. The selective effect of increased respiratory drive on common drive to inspiratory modulated motoneurons is consistent with previous literature that has demonstrated that Expiratory Tonic and Tonic motor unit activity is unchanged or reduced in other situations in which inspiratory GG activity is elevated, such as at arousal from sleep (Wilkinson et al. 2008), during hypercapnia (Nicholas et al. 2010), and during vigorous physical exercise (Walls et al. 2013).

The increase in the strength of common drive to inspiratory motor neurons persisted during the 30-s postload period. As noted above, the persistence of these changes in common drive was not due to direct effects of GG muscle activity, as respiratory activity, EMG spike density, and the discharge rates of single motor units returned to preload levels as soon as the load was removed. Similar “poststimulus activation” effects have been previously reported to occur in the respiratory system, most notably increased respiratory activity in response to intermittent hypoxia (Jordan et al. 2002). Such effects are generally considered beneficial, as the upper airway remains in a state of heightened preparedness should the load return. In the context of obstructive sleep apnea, responses of this type are thought to be protective of repetitive obstructions.
What cannot be determined from the present data is how long the elevation in common drive was sustained or what might be the nature of the mechanism.

Coherence between motor units at higher frequencies is considered to reflect processes such as tremor and short-term synchrony. However, as illustrated in Figs. 3 and 7, and consistent with previous studies of coherence between pairs of upper airway motor units (Laine and Bailey 2011; Trinder et al. 2013), we were not able to identify meaningful coherence at higher frequencies or any effect of loads on coherence at higher frequencies.

Load intensity was not formally analyzed, as the discharge patterns of motor unit pair units were not uniformly distributed over loads. Nevertheless, it would have been possible for the higher common drive observed in inspiratory modulated motor units to be due to them being elicited by higher loads. However, this was not the case, as inspiratory pairs occurred more frequently with lower loads and the effect of load intensity, although positive, was not significant.

Previous studies have been consistent in showing that the main mechanism by which UAMs change their level of activity is through recruitment/derecruitment of motor units. However, they have been inconsistent as to the role of discharge rate, with some studies showing small (1–2 Hz), but significant, changes in discharge rate in response to, for example, hypocapnia (Saboisky et al. 2010) while other studies have reported an absence of change in discharge rate in response to hypercapnia (Nicholas et al. 2010). The present results show a small rate coding effect.

A limitation of the present study, which is inherent in single-motor unit studies of UAMs, is that there was not, nor could there be, any experimental control over the nature of the discharge patterns recorded on any trial. Thus unequal representation of different discharge patterns within subjects was highly likely. Nevertheless, we have attempted to minimize this problem by analyzing the data using both motor unit pair units and subjects as replications in analyses. Within the limitations of statistical power the analyses were complementary.

The increase in GG activity in response to increasing respiratory drive has previously been shown to be due to the recruitment of inspiratory modulated motoneurons, with a smaller contribution from increases in motoneuron discharge rates (Trinder et al. 2014). The present study has identified a third mechanism contributing to the transmission of respiratory drive to the hypoglossal motoneurons, an increase in common drive to inspiratory modulated motor units. The independence of this mechanism is demonstrated by a poststimulus activation effect, such that the changes in common drive continued during the postload period when overall GG activity had returned to baseline levels.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: M.J.W., J.G.S., J.K.M.C., and J.T. analyzed data; M.J.W., J.G.S., A.S.J., and J.T. interpreted results of experiments; M.J.W. and C.L.N. prepared figures; M.J.W., C.L.N., J.G.S., A.S.J., and J.T. edited and revised manuscript; M.J.W., C.L.N., J.G.S., J.K.M.C., A.S.J., and J.T. approved final version of manuscript; C.L.N., A.S.J., and J.T. conception and design of research; C.L.N. and J.T. performed experiments; J.T. drafted manuscript.

REFERENCES