

# Discharge Patterns of Human Genioglossus Motor Units during Arousal from Sleep

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**Study Objectives:** Single motor unit recordings of the human genioglossus muscle reveal motor units with a variety of discharge patterns. Integrated multiunit electromyographic recordings of genioglossus have demonstrated an abrupt increase in the muscle's activity at arousal from sleep. The aim of the present study was to determine the effect of arousal from sleep on the activity of individual motor units as a function of their particular discharge pattern.

**Design:** Genioglossus activity was measured using intramuscular fine-wire electrodes inserted via a percutaneous approach. Arousals from sleep were identified using the ASDA criterion and the genioglossus electromyogram recordings analyzed for single motor unit activity.

**Setting:** Sleep research laboratory.

**Participants:** Sleep and respiratory data were collected in 8 healthy subjects (6 men).

**Measurements and Results:** 138 motor units were identified during prearousal sleep: 25% inspiratory phasic, 33% inspiratory tonic, 4% expiratory phasic, 3% expiratory tonic, and 35% tonic. At arousal from sleep inspiratory phasic units significantly increased the proportion of a breath over which they were active, but did not appreciably increase their rate of firing. 80 new units were identified at arousals, 75% were inspiratory, many of which were active for only 1 or 2 breaths. 22% of units active before arousal, particularly expiratory and tonic units, stopped at the arousal.

**Conclusions:** Increased genioglossus muscle activity at arousal from sleep is primarily due to recruitment of inspiratory phasic motor units. Further, activity within the genioglossus motoneuron pool is reorganized at arousal as, in addition to recruitment, ~20% of units active before arousals stopped firing.

**Keywords:** Motor control; upper airway; lung; single motor units; obstructive sleep apnea; motoneuron

**Citation:** Wilkinson V; Malhotra A; Nicholas CL; Worsnop C; Jordan AS; Butler JE; Saboisky JP; Gandevia SC; White DP; Trinder J. Discharge patterns of human genioglossus motor units during arousal from sleep. *SLEEP* 2010;33(3):379-387.

OBSTRUCTIVE SLEEP APNEA (OSA) IS A DISORDER OF SLEEP IN WHICH THE UPPER AIRWAY REPETITIVELY OBSTRUCTS CAUSING REDUCED AIRFLOW, OXYGEN desaturation and fragmented sleep.<sup>1</sup> The disorder is prevalent, occurring in over 4% of men and 2% of women,<sup>2</sup> and has major health consequences, contributing to a range of disorders, including cardiovascular disease and behavior problems such as tiredness-related road crashes.<sup>3</sup> Two general factors contribute to pharyngeal obstructions: sleep induced changes in upper airway muscle activity and an anatomically narrow upper airway.<sup>1</sup> The resolution of airway obstruction requires recruitment of upper airway dilator muscles, the recruitment often being associated with an arousal from sleep. Thus, sleep-wake-related changes in upper airway muscle activity are important for both the obstructions and their resolution. It follows that understanding upper airway muscle control during sleep and wakefulness remains crucial in understanding the pathophysiology of OSA.

The upper airway muscle most frequently studied in the context of OSA is the extrinsic protruder muscle of the tongue,

genioglossus. The genioglossus is accessible, is the major dilator of the upper airway during wakefulness,<sup>4</sup> has been shown to be particularly important in maintaining airway patency during sleep, and is thought to be representative of other upper airway muscles with respiratory phasic (inspiratory peak) activity patterns.<sup>1</sup> However, single motor unit recordings indicate that central control of the genioglossus is much more complex than has been suggested by multiunit recordings.<sup>5,6</sup> Thus, the phasic pattern of multiunit activity masks motor units that have a range of different discharge patterns, including units with peak activity during inspiration or expiration and units that fire tonically without respiratory modulation<sup>5</sup> (also see section on "Classification of Units" below). Although the precise distribution of the units with these patterns remains poorly defined, during relaxed wakefulness, approximately 60% of active units have peak activity during inspiration, 15% have peak activity during expiration, and roughly 25% have no respiratory modulation.

Multiunit studies have shown that the activity of respiratory muscles generally, and upper airway muscles in particular, falls abruptly at the transition into sleep, specifically at the  $\alpha$ - $\theta$  electroencephalographic transition,<sup>7</sup> exposing the airway to collapse. In a recent study,<sup>8</sup> we demonstrated that genioglossus motor units that had peak discharge rates during inspiration (inspiratory phasic and inspiratory tonic units) reduced their activity at the  $\alpha$ - $\theta$  transition, often becoming silent, whereas expiratory units and units that were purely tonic were unaffected by the transition. The pattern of change in the inspiratory units was such that their discharge rate remained constant so

Submitted for publication April, 2009

Submitted in final revised form August, 2009

Accepted for publication August, 2009

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long as they continued to fire, although they fired for a smaller proportion of each breath following the transition, and sometimes stopped firing completely. Consequently, with sleep, patency of the airway became more dependent on tonic muscle activity, a reorganization of motor unit activity that has been reported by others.<sup>9</sup>

Arousal from sleep is associated with increased genioglossus activity.<sup>7</sup> Hence, the aim of the current study was to determine the changes in the firing of single genioglossus motor units at arousal from non-rapid eye movement (NREM) sleep. Specifically, we first determined the effect of arousal from sleep on the distribution of motor units with different discharge patterns. Second, we determined whether the increased genioglossus activity reflected in multiunit recordings was due to recruitment of new motor units or to increased activity in units that were already active. Third, we determined the discharge pattern of units recruited at an arousal from sleep. We hypothesized that there would be different changes in the various classes of motor unit firing pattern at an arousal and that there would also be significant recruitment of new units.

## METHODS

### Subjects

Overnight data were collected from 6 men and 2 women. The subjects had an average age of  $27.6 \pm 7.3$  years and average body mass index of  $24.2 \pm 1.6$  kg/m<sup>2</sup>. The data were collected in the Brigham Sleep Disorders Research Program in Boston (6 subjects) and the sleep laboratory, Department of Psychology, The University of Melbourne (2 subjects), using identical methodology. Although these subjects participated in prior research studies,<sup>8</sup> none of the findings of the present study have been previously reported. All subjects were healthy and were without sleep complaints as determined by a questionnaire. No subject had repeated obstructive events during the recording night. The protocol conformed to the Declaration of Helsinki and had prior approval of the local Human Subjects Committees. Written informed consent was obtained from each subject.

### Equipment and Techniques

#### Sleep-wake state

Two channels of electroencephalography (C3-A2 and O1-A2), electrooculography, and a chin electromyogram (EMG) were recorded to determine sleep-wake state and to identify arousals from sleep. Spontaneous arousals from NREM sleep were defined according to the American Sleep Disorders Association criterion,<sup>10</sup> requiring more than 3 seconds of electroencephalographic  $\alpha$  or  $\beta$  activity, and were visually identified by an experimenter who was blinded to the genioglossus EMG recordings. The data analyzed were the 3 breaths of  $\theta$  activity preceding the arousal and the 3 breaths following the initiation of the arousal, irrespective of whether the subject returned to sleep. The first postarousal breath was defined by the breath for which at least 50% of inspiratory time followed the point of arousal. In addition, we required that the subject be in stable NREM sleep for at least 30 seconds before the periods analyzed. Arousals during which there was a body movement, swallow,

or other artifact were excluded. Body movements were identified by electrical interference or other artifact on any channel. Increased EMG activity on the submental EMG channel was not an exclusion criterion. Finally, with these restrictions in place, it was difficult to find arousals that had long prearousal and postarousal periods. Further, we specifically chose to study the immediate postarousal period. Thus the minimum duration requirement was set at 3 breaths.

#### Ventilation and airway mechanics

Respiratory airflow was measured by a calibrated pneumotachometer (Fleisch #2) and differential pressure transducer (DP-45, Validyne Corp., Northridge, CA.). Airflow was collected via a nasal mask (Respironics, Inc. Murrysville, PA) connected to a nonrebreathing valve. Thus, subjects breathed through their noses with their mouths taped shut during data collection. End tidal CO<sub>2</sub> (PETCO<sub>2</sub>) was monitored from the mask using an Ametek CO<sub>2</sub> (CD-3A) analyzer, while pressures were monitored in the mask with an open catheter attached to a pressure transducer (Validyne Corp) and in the airway at the level of the epiglottis using a pressure-tipped catheter (MPC-500, Millar, Houston, TX). To insert the Millar catheter, 1 nostril was decongested (oxymetazoline HCl) and anesthetized (lidocaine HCl), and the catheter was inserted through this nostril and located at the epiglottis.

#### Muscle activity

The procedures used to record genioglossal EMG activity followed those of Eastwood et al.<sup>11</sup> Genioglossus activity was recorded using 3 monopolar intramuscular wire electrodes referenced to a common surface electrode positioned over the bony mandible. A large flexible ground strap was placed on the right shoulder. The 3 electrodes were inserted into the muscle using a percutaneous approach.<sup>11</sup> For 6 of the subjects, the location and depth of the genioglossus muscle was determined individually for each subject using ultrasonography, whereas, for the remaining 2 subjects, electrodes were inserted 2.4 cm. This depth was within the range of values reported for subjects in 2 previous publications (Eastwood et al.<sup>11</sup> 1.8 to 3.0 cm for electrodes 1.0 cm and 1.8 to 3.1 cm for electrodes 2.0 cm from the inferior margin of the mandible, and Saboisky et al.<sup>5</sup> 2.2 to 3.0 cm). About an hour prior to electrode insertions, a local anesthetic cream (Lidocaine-Prilocaine, Fogera, Melville, NY) was applied to the skin. Insertion was via a 25-G needle, with 2 stainless-steel, Teflon-coated, 0.002"-diameter, wire electrodes in each needle. Each electrode had 0.5 mm of insulation removed from the recording surface. The insertion sites were distributed over left-right (3 mm each side of the midline) and relatively anterior-posterior positions (~10 and 20 mm from the inferior margin of the mandible respectively) such that, over the 8 subjects, all 4 sites were similarly sampled. Prior to data collection, the electrode from each pair of electrodes judged to have the clearest motor unit activity was selected for data acquisition and storage.

A methodologic consideration with intramuscular recordings is electrode migration. Thus, in the case of single motor unit recordings, it is possible that the onset or cessation of a motor unit's activity is an artifact of electrode movement. We have previously established that, in the absence of events such as

body movements or swallows, intramuscular recording electrodes are highly stable during sleep and do not move over the respiratory cycle or over sleep-wake transitions (see reference<sup>8</sup> and in particular Figure 1 from reference<sup>8</sup>). Accordingly, data were not analyzed over arousals in which there was a body movement or swallow.

### Data acquisition

Data were stored on computer using Spike 2 acquisition software with a 1401 interface (Cambridge Electronic Design, Cambridge, UK). All signals were amplified and filtered using GRASS amplifiers (Grass Telefactor, Warwick, RI) prior to analog-to-digital conversion by the 1401 (see our earlier paper for filtering, amplification and analog-to-digital conversion values<sup>8</sup>).

### General laboratory procedures

Subjects arrived at the laboratory 4 hours before their normal bedtime, and the recording equipment was attached. Subjects went to bed about an hour before their normal bedtime, and 20 minutes of wakefulness data were collected. Thereafter, the lights were turned off. To maximize the opportunity to collect data over sleep-wake state transitions, subjects were woken if they remained in stage 2 sleep or slow wave sleep for 5 minutes. Subjects were required to maintain a supine position throughout data collection.

### Data Reduction

#### Identification and quantification of respiratory variables

Breath-by-breath values for the pretransition and posttransition breaths were determined for cycle duration ( $T_{tot}$ ); tidal volume ( $V_T$ ), minute ventilation ( $V_V$ ), epiglottic pressure 200 ms into inspiration ( $Pepi_{200}$ ); minimum epiglottic pressure during inspiration ( $Pepi_{min}$ ), upper airway resistance (epiglottis to mask) 200 ms into inspiration ( $Rua_{200}$ ), and upper airway resistance (epiglottis to mask) at the minimum epiglottic pressure ( $Rua_{max}$ ) using customized software.

#### Quantification of overall genioglossus EMG activity

To confirm that overall genioglossus activity increased at arousals from sleep, the rate of action potentials was calculated for the 3 breaths before and after arousal as a function of respiratory phase. The calculation was performed on all electrodes that had technically valid recordings, whether or not it was possible to decompose the action potentials into motor unit activity. Thus, the number of action potentials/second (Hz) that exceeded a threshold was determined. The threshold was arbitrarily defined as twice the voltage amplitude of the background noise. Background noise primarily reflected activity of distant motor units and, at times, electrical interference. Spikes exceeding this threshold would normally be decomposable, except where this number was too great. It is recognized that multiunit measures underestimate EMG activity because of cancellation of positive and negative components of action potential waveforms<sup>12</sup>; nevertheless, an increase in action potential rate from before to after arousal would confirm that we had replicated the previously reported effect of arousal from sleep on genioglossus EMG activity.<sup>7</sup>

#### Identification of single motor units

The discharge properties of single motor units were determined using Spike 2 analysis software (Cambridge Electronic Design, Cambridge). The steps in the application of the software were as follows.

1. Spontaneous arousals from NREM sleep were identified according to criteria described above.
2. The EMG of each electrode was inspected for the presence of motor unit activity during the 30 breaths and also for units that were recruited at or subsequent to the arousal. If the electrode did not contain any motor unit activity, or if it contained too much activity for individual motor units to be identified, it was discarded from the initial data analysis but retained in analyses of action potential rate.
3. Motor units were extracted from the raw EMG signal based on a voltage threshold and then sorted into templates on the basis of their amplitude and shape.
4. Subsidiary software (written with Spike 2) was then used to inspect and edit the initial software classification.
5. The resulting action potential time series were displayed as instantaneous frequency plots and firing statistics were then generated.

To identify potential classification errors, we used a procedure analogous to error identification in heart rate data. A change of greater than  $\pm 40\%$  from 1 interspike interval to the next was used to identify classification errors. Such abrupt changes in discharge rate rarely occur in normal activity. Such errors persisted after editing because the unit had a poor signal-to-noise ratio, or because it was difficult to distinguish from other units on the same electrode. Thus, we defined a successful identification of the discharge pattern of a genioglossus motor unit as one in which fewer than 5% of consecutive interspike intervals varied by more than  $\pm 40\%$ .

#### Classification of units

Six different discharge patterns were initially identified visually according to procedures reported by Saboisky et al.<sup>5</sup>

*Inspiratory Phasic.* These were units that showed peak activity during inspiration and had a minimum firing frequency of less than 2.0 Hz (i.e., were silent for  $> 500$  ms in each respiratory cycle).

*Inspiratory Tonic.* These were units that showed peak activity during inspiration and maintained a discharge rate greater than 2.0 Hz throughout the respiratory cycle.

*Expiratory Phasic.* These were units that showed peak activity during expiration and had a minimum firing frequency of less than 2.0 Hz (i.e., were silent for  $> 500$  ms in each respiratory cycle).

*Expiratory Tonic.* These were units that showed peak activity during expiration and maintained a discharge rate greater than 2.0 Hz throughout the respiratory cycle.

*Tonic.* These were units that fired throughout the respiratory cycle but had no obvious respiratory or other modulation.

*Tonic Other.* These were units that fired throughout the respiratory cycle and showed variation in their discharge rates but not in phase with the respiratory cycle.

For tonic, inspiratory tonic, and expiratory tonic units, we also quantified the degree of respiratory modulation by comparing the within-breath variation in interspike interval to the

**Table 1**—Mean (SD) for respiratory variables and overall GG activity pre- and post-arousal from sleep.

Variables	Pre-Arousal	Post-Arousal
Cycle Duration (secs)	4.21 (0.70)	4.03 (0.62)
Tidal Volume (ml)	390 (140)	452 (165)
Minute Ventilation (l)	5.71 (2.34)	6.77 (2.48)*
Epiglottic Pressure 200 ms (cmH <sub>2</sub> O)	-0.8 (1.37)	-0.7 (1.24)
Epiglottic Pressure at Minimum (cmH <sub>2</sub> O)	-3.1 (1.71)	-2.6 (1.62)*
UA Resistance 200 ms (cmH <sub>2</sub> O/l/s)	2.38 (5.47)	1.83 (5.20)
UA Resistance at Minimum (cmH <sub>2</sub> O/l/s)	14.4 (7.33)	7.8 (3.97)*
Inspiratory Action Potential Rate (Hz)	11.7 (10.9)	23.0 (12.1)*
Expiratory Action Potential Rate (Hz)	7.6 (10.7)	17.5 (15.3)*

\*P < 0.05

variation in airflow over the breath using a cross-correlation procedure described in previous publications.<sup>5,8</sup> The final decision as to the classification of a unit was based on the correlation value, the value used being 0.49. This value was chosen because, in a previous study, it was shown to minimize the number of discrepancies between the cross-correlation and visual methods.<sup>8</sup>

#### Quantification of unit discharge patterns

The instantaneous frequency plots were quantified to characterize the discharge pattern of the motor units using the same measures used to quantify motor units during sleep onset.<sup>8</sup>

1. The mean frequency during either the inspiratory or expiratory phasic period, depending on the phase of the unit. For tonic units, the value was computed over inspiration.
2. The peak frequency during inspiration or expiration, depending on the phase of the unit (peak 200 ms average).
3. The tonic frequency, which was defined as the last 200 milliseconds before the onset of the inspiratory or expiratory phasic component. This measure did not apply to inspiratory or expiratory phasic units.
4. The time, with respect to the onset of inspiration, of the first inspiratory phasic action potential. A negative value indicated preactivation of the motor unit before inspiratory airflow. This measure did not apply to expiratory or tonic motor units.
5. The proportion of the breath during which the unit was active. This measure only applied to units that were inspiratory phasic or expiratory phasic at some point over the measurement period, as tonic units are by definition on 100% of the time.

In summary, 4 aspects of the arousal events were analyzed. First, estimates of the overall genioglossus EMG activity at arousals were determined. Second, the distribution of motor units with different discharge patterns was determined before and after arousal. Third, in motor units that were identified as being active during the prearousal period, the effect of the arousal on their postarousal discharge pattern was quantified as a function of their prearousal pattern. Fourth, motor units recruited at or after the arousal were identified, and their discharge pattern quantified.

#### Data Analyses

Respiratory variables were averaged over the 3 prearousal and postarousal breaths, and the values compared using paired t tests.

Estimates of the total EMG activity present before and after arousal, based on action potential rates, were calculated and analyzed as a function of respiratory phase (inspiration versus expiration) and prearousal and postarousal occurrence using a 2 × 2 analysis of variance.

Next, the distribution of the different motor unit discharge patterns was tabulated as a function of the prearousal and postarousal states, including changes from one discharge pattern to another following the arousal. In addition, motor units that stopped, or were recruited, following the arousal were identified. Differences in the distributions were tested using the  $\chi^2$  test.

The discharge characteristics, such as rate of discharge, of motor units present throughout the arousal period were assessed as a function of prearousal and postarousal states averaged over the 3 breaths in each state. Further, the discharge characteristics of recruited units were compared to postarousal values of units that were active before and after arousal. Two methods of statistical analysis were used. First, as is common for single motor units, motor units were treated as the unit of analyses. In addition, the data were analyzed using subjects as the unit of analysis.

## RESULTS

#### Preliminary Analyses

The 8 subjects provided 92 arousals, with a range from 2 to 19 per subject. Table 1 presents average respiratory activity before and after arousal, showing that the present data replicated that of previous studies in normal healthy individuals. Also shown in Table 1 are the frequency of action potentials before and after arousal, as a function of respiratory phase. Although the action potential rate showed the expected inspiratory modulated pattern, the difference between inspiration and expiration before arousal was not quite significant (P = 0.07). However, the action potential rate increased significantly (P < 0.05) after arousal during both inspiration and expiration, with an approximate doubling of motor unit activity. Thus, respiratory and overall genioglossus activity behaved much as would be anticipated on the basis of prior studies.<sup>7,13,14</sup>

#### Distribution of Motor Units

##### Motor units active pre-arousal

With 3 electrodes recorded simultaneously, the 92 arousals potentially yielded 276 events. However, only 104 events resulted in identifiable motor units, with 138 motor units being identified during prearousal  $\theta$ , with a range over subjects from 2 to 32. The remaining events either contained no detectable units or too many action potentials to identify individual motor units, or the recordings were of poor quality.

The pattern of active motor units during prearousal  $\theta$  is shown in Line 1 of Table 2. It indicates a high proportion of units with a phasic component during inspiration and of tonic units. Line 2 of Table 2 shows the postarousal distribution of the

units that were active during the prearousal period.  $\chi^2$  analysis indicated the 2 distributions were significantly different ( $P < 0.05$ ), with the major difference being a shift from inspiratory phasic to inspiratory tonic pattern. This is also shown in Table 3, which presents changes in discharge patterns of individual motor units from before to after arousal. It indicates that 40% (14 of 35) of inspiratory phasic units converted to inspiratory tonic. In total, 41 (30%) of the 138 prearousal units changed pattern (see Lines 3 and 6, Table 2), although there were no exchanges between inspiratory and expiratory patterns.

**Motor units that ceased activity following an arousal**

As shown in Line 4, Table 2, 30 (21.7%) motor units that were active before arousal ceased activity at some point over the first 3 postarousal breaths (5 on the first, 9 on the second, and 16 on the third). Expiratory and tonic units were more likely to stop than were inspiratory phasic or inspiratory tonic units (31% [18 of 58] vs 15% [12 of 80]), the difference being statistically significant ( $P < 0.05$ ).

**Motor units recruited following an arousal**

A total of 80 units with no discernable activity before arousal were recruited following an arousal. These were identified in 7 of the 8 subjects; 44.5% of recruited units were recruited in the first postarousal breath, 37.3% in breath 2, and 15% in breath 3. The distribution of the motor units over the different discharge patterns is shown in Line 7 of Table 2. As indicated, 46% were inspiratory phasic and 29% inspiratory tonic units (2 such units are illustrated in Figure 1), with relatively few tonic units being recruited. Indeed, the distribution of the recruited units was significantly different from both the prearousal ( $P < 0.02$ ) and postarousal ( $P < 0.001$ ) distributions of the motor units. The final data set in Table 2 (Line 10) shows the distribution of motor units that were active after arousal. The computation takes into consideration recruited units and units that stopped or changed discharge pattern. A  $\chi^2$  analysis indicated that this distribution was significantly different compared with that expected on the basis of the prearousal distribution ( $P = 0.0497$ ). Thus, there was a tendency for an increase in inspiratory phasic and inspiratory tonic units. These increased 58% from 80 to 126, whereas expiratory and tonic units remained relatively constant (58 to 62).

Motor units were also sorted according to how long they remained active: 55% of recruited units were active for only 1 or 2 breaths, with the remaining 45% remaining active for at least 3 breaths. Further, briefly active units tended to be phasic (75% were inspiratory phasic or expiratory phasic), whereas more enduring units were more likely to be tonic (78% were inspiratory tonic, expiratory tonic, or tonic).

**Distribution of motor units as a function of the anatomic location of the recording electrode**

During prearousal  $\theta$  activity, there was a possible tendency for relatively more anterior electrodes than posterior sites to

**Table 2**—The changing distribution of motor units as a function of arousals from sleep

	Discharge Pattern					Total
	IP	IT	EP	ET	TT	
<b>1. Pre-Arousal Motor Units</b>						
Number	35	45	6	4	48	138
% Distribution	25.4	32.6	4.3	2.9	34.8	
<b>2. Post-Arousal Distribution of Pre-arousal Units</b>						
Number	19	58	5	7	44	133
% Distribution	14.3	43.6	3.8	5.3	33.0	
<b>3. Units Converting to a Different Pattern</b>						
	17	9	-	1	14	41
<b>4. Units That Stopped by Breath 3</b>						
	5	7	6	2	10	30
<b>5. Units Lost to a Discharge Pattern (3+4)</b>						
	22	16	6	3	24	71
<b>6. Units Converting to this Pattern</b>						
	1	23	-	5	12	41
<b>7. Recruited Units</b>						
	37	23	4	2	14	80
<b>8. Units Gained By a Discharge Pattern (6+7)</b>						
	38	46	4	7	26	121
<b>9. Balance. Gained (8) – Lost (5)</b>						
	16	30	-2	4	2	50
<b>10. Total Post-Arousal Distribution</b>						
Number	51	75	4	8	50	188
% Distribution	27.1	39.9	2.1	4.2	26.6	

IP, inspiratory phasic; IT, inspiratory tonic; EP, expiratory phasic; ET, expiratory tonic; TT, tonic. There were no tonic other units. Left side numbers referred to in text.

record a proportionately greater number of inspiratory phasic units. The percentage distribution for IP, IT, EP, ET & TT units was 36, 38, 2, 2, 22, respectively, for anterior sites and 25, 36, 1, 3, 35, respectively, for posterior sites. However,  $\chi^2$  analysis was not significant ( $P > 0.05$ ). Recruited inspiratory phasic and inspiratory tonic units appeared more likely to be identified on posterior electrodes (with percentage distributions of 40, 23, 10, 7 20 and 54, 29, 2, 5, 10 for anterior and posterior sites, respectively). However, again, these differences were not significant ( $P > 0.05$ ). The absence of anterior-posterior differences is consistent with the observation that units with different discharge patterns often occur on the same electrode.

**Motor Unit Discharge Values**

**Motor units active before and after arousal**

Motor units were categorized according to their prearousal discharge pattern. As noted in the data-analysis section, the data were analyzed using 2 approaches, one using units and the other subjects as replications. The resulting mean values were similar for the 2 methods, although, as would be expected, using units as replications increased power and proved more sensitive in terms of statistical significance, although this method has the disadvantage that sampling of many units in a subset of individuals can bias the data set. The data are shown in Table 4, which presents the average values (both subject and unit averages) for inspiratory phasic, inspiratory tonic, and tonic units over the prearousal and postarousal phases averaged over the 3 breaths in each phase. Mean and peak discharge frequencies for the 3 discharge patterns over prearousal and postarousal breaths are shown in Figure 2. The mean and peak discharge frequencies of inspiratory phasic units increased slightly postarousal, although this was only significant for the inspiratory

**Table 3**—Effect of arousal from sleep on the distribution of discharge patterns in motor units active pre- and post-arousal

	Pre-Arousal		Post-Arousal				
	Total	IP	IT	EP	ET	TT	Stop *B1
IP	35	18	14	-	-	3	-
IT	45	1	35	-	-	8	1
EP	6	-	-	5	-	-	1
ET	4	-	-	-	2	1	1
TT	48	-	9	-	5	32	2
<b>Total</b>	<b>138</b>	<b>19</b>	<b>58</b>	<b>5</b>	<b>7</b>	<b>44</b>	<b>5</b>

IP, inspiratory phasic; IT, inspiratory tonic; EP, expiratory phasic; ET, expiratory tonic; TT, tonic.

\*Motor units that stopped on the first post-arousal breath (B1) could not be assigned a post-arousal discharge pattern.

phasic peak rate (increase of 2.3 Hz) and the inspiratory tonic mean rate (increase 0.8 Hz) when units were used as replications. However, consistent with the conversion of inspiratory phasic to inspiratory tonic units (see Table 3), the proportion of a breath in which inspiratory phasic units remained active increased and was significantly higher after arousal with both analysis procedures (increase of 25.4% and 18.8% for subjects and units, respectively). The response of a typical inspiratory phasic unit is shown in Figure 3. The increase in percentage duration was due to both increased prearousal activation and an extension into the expiratory phase of breaths (see Table 4). In contrast with inspiratory units, tonic units decreased their mean discharge rate following arousal from sleep (see Figure 4), although the effect was small (~1 Hz) and was only significant when the analysis was by units.

#### Motor units recruited following an arousal

The discharge characteristics of units recruited after arousal are illustrated in Figure 5. For comparison, the postarousal values of units active both before and after arousal, and classified according to their postarousal discharge pattern, are also shown. (The postarousal values of units active both before and after arousal, sorted according to their prearousal discharge pattern, are shown in Table 4.) There was a slight tendency of postarousal activity to be higher in motor units that were active before and after the arousal than in recruited motor units.

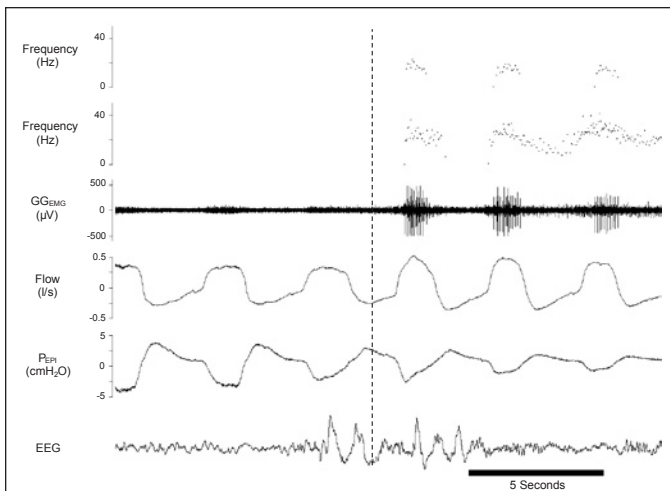
**Table 4**—Discharge characteristics of motor units active pre- and post-arousal from sleep

Variables	Discharge Pattern								
	Inspiratory Phasic (4 Ss, 35 Units)			Inspiratory Tonic (6 Ss, 44 Units)			Tonic (6 Ss, 46 Units)		
	Pre-	Post-	Sig.	Pre-	Post-	Sig.	Pre-	Post-	Sig.
Pre-activation (sec.)									
Ss	0.18 (0.25)	0.02 (0.09)		-0.17 (0.01)	-0.16 (0.02)		-	-	
Units	0.11 (0.24)	0.00 (0.16)		-0.16 (0.03)	-0.16 (0.05)		-	-	
Mean frequency (Hz)									
Ss	15.5 (3.2)	16.7 (2.1)		18.5 (4.2)	18.2 (3.9)		20.5 (3.8)	19.5 (2.8)	
Units	16.7 (3.6)	17.7 (3.1)		17.6 (3.3)	18.4 (3.3)	*	21.4 (4.8)	20.0 (4.8)	**
Peak frequency (Hz)									
Ss	18.7 (3.6)	21.4 (2.8)		23.9 (5.0)	23.3 (4.9)		-	-	
Units	20.1 (4.1)	22.4 (3.8)	**	23.1 (4.0)	23.9 (4.5)		-	-	
Tonic frequency (Hz)									
Ss	-	-		17.0 (5.5)	17.1 (4.9)		-	-	
Units	-	-		15.2 (4.2)	15.8 (4.0)		-	-	
% Duration									
Ss	47.1 (5.9)	72.5 (11.3)	*	-	-		-	-	
Units	47.2 (17.1)	66.0 (25.1)	**	-	-		-	-	

A dash indicates the variable was not relevant to that discharge pattern. Motor units were classified according to their pre-arousal discharge pattern and only units present both pre- and post-arousal were included. Subject (Ss) and Unit means presented. Standard deviations in brackets. \*P < 0.05; \*\*P < 0.01

#### DISCUSSION

In summary, the pattern of respiratory activity at arousals was consistent with the previous literature,<sup>7,13-15</sup> with an increase in ventilation, a reduction in negative airway pressure and airway resistance, and an increase in overall genioglossus activity. Consistent with our hypothesis, there were different changes in the classes of motor unit firing patterns, with the most prominent change being an increase in the activity of inspiratory modulated units. This was due to recruitment of new units, the extension of the proportion of a breath that inspiratory phasic units were active (earlier onset and extension into expiration), and, to a lesser extent, an increase in the discharge rate of inspiratory-modulated units. Tonic units slightly decreased their discharge rate and showed an intricate exchange of activity with approximately 20% of units stopping after the arousal and a similar number being recruited. The small number of recorded expiratory units behaved similarly to tonic units. Finally, a modest number of units changed from tonic to either inspiratory tonic or expiratory tonic, whereas a similar number changed in the opposite direction. This exchange may reflect units that were primarily tonic but that had some phasic activity that did not always exceed the criterion for identifying phasic activity.

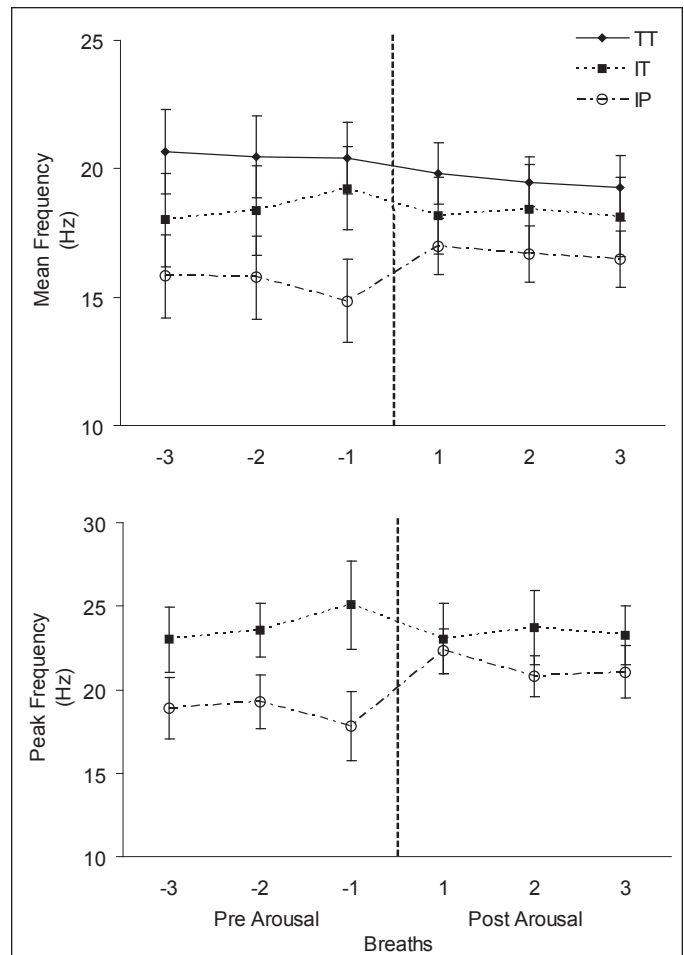


**Figure 1**—Instantaneous frequency distributions for 2 motor units captured on the same electrode. The top unit shows an inspiratory phasic pattern, whereas the bottom unit begins with an inspiratory phasic pattern and then extends to inspiratory tonic. Additional action potentials were present in the genioglossus electromyogram (EMG; GG<sub>EMG</sub>) but these could not be decomposed. The occasional large spikes were due to overlapping action potentials. Also shown are the raw EMG, airflow, epiglottic pressure (P<sub>EPI</sub>), and electroencephalographic (EEG) measures. The vertical dotted line indicates the arousal.

These results have implications for the nature of motor control of the genioglossus. Although it is widely agreed that arousal from sleep involves an increase in respiratory drive, the mechanisms for this increase are not agreed upon and may reflect a homeostatic response to elevated respiratory stimuli that are present at the point of arousal (e.g., CO<sub>2</sub> accumulation) or a reflex-like response elicited by the act of arousing.<sup>16-18</sup> Nevertheless, irrespective of the mechanism, the increase in respiratory drive at an arousal from sleep has its most pronounced effect on the genioglossus via inspiratory-modulated units. This pattern suggests that, after originating in arousal-related nuclei in the brain stem (e.g., reticular inputs), the drive component enters the hypoglossal motor nucleus directly or indirectly via the respiratory-pattern generator. Indeed, this conclusion may be extended to sleep-wake state changes in respiratory drive in general because, at sleep onset, the reduction in respiratory drive associated with the withdrawal of the wakefulness stimulus also has its primary effect on inspiratory-modulated motor units. Thus, at sleep onset, inspiratory units reduce the proportion of a breath over which they are active, frequently stopping entirely, but remain inspiratory phasic until the time at which they stop.<sup>8</sup>

The present data suggest that arousal from sleep preferentially affects inspiratory phasic motor units. It is of interest to know to what extent the effect may be generalized. Thus, does the phenomenon extend to muscle units that are primarily inspiratory, such as the upper external intercostals, as compared with muscle units that are primarily expiratory, such as the triangularis sterni?<sup>19</sup>

A limitation should be mentioned. To be confident that the recruitment and loss of units was not due to electrode displacement, we restricted arousals to those that did not include body movements. As such, our data are limited to “more tranquil” arousals, and, thus, our data might not be generalizable



**Figure 2**—Mean discharge frequency (Hz) for inspiratory phasic (IP), inspiratory tonic (IT), and tonic (TT) motor units, for the 3 breaths before and after arousal, averaged over subjects. Data are presented for motor units that were active before the arousal (data series from -3 to +3) and for units recruited at the arousal (data series from +1 to +3). Error bars are standard errors.

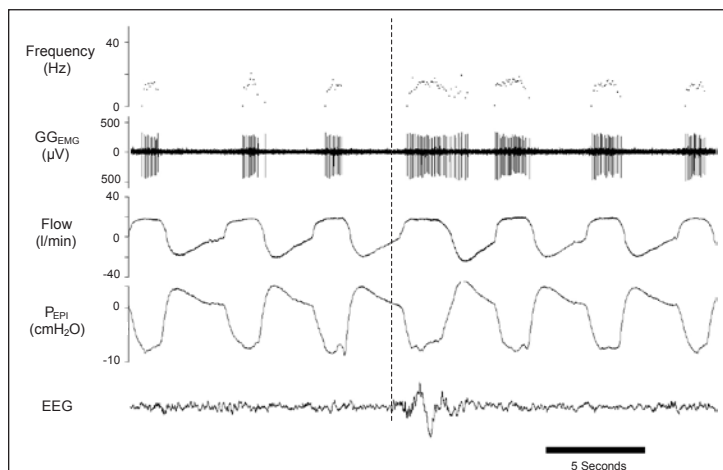
to the more vigorous arousals that often occur in patients with OSA.

In general, skeletal motor units modulate their discharge rate according to the level of drive and the state of the motoneuron pool.<sup>20</sup> This also applies to genioglossus motor units, as units with peak activity during either inspiration or expiration modulate their discharge rate within a breath in phase with the respiratory cycle, presumably as a consequence of changes in drive from the respiratory-pattern generator. However, unlike motor units in most muscles, genioglossus motor units do not appear to change their discharge rate appreciably over successive breaths as a function of sleep-wake state-dependent changes in respiratory drive (temporal recruitment). Thus, although new units were recruited (suggesting increased genioglossus activity), the rate of active units remained relatively constant, although active inspiratory phasic units did increase the amount of time within a breath that they were active. Consistent with this, we have previously reported that, during sleep onset, inspiratory-modulated units reduce the proportion of a breath over which they are active and often stop completely, but they do not reduce their firing rate before they cease activity.<sup>8</sup>

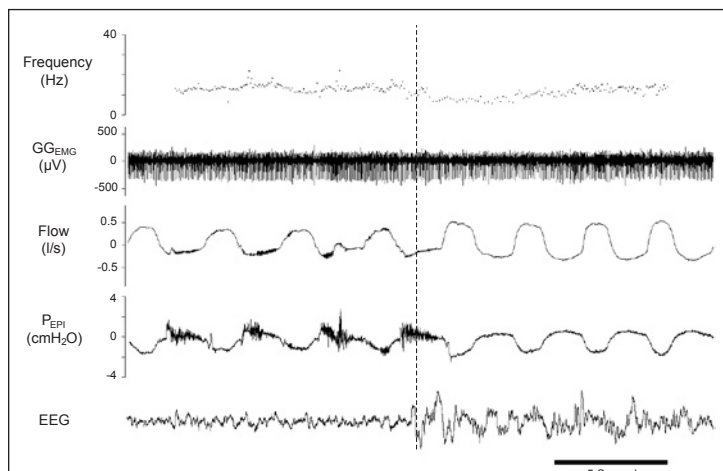
Traditionally, the motoneuron pool has been considered to follow a formal order of recruitment beginning with increases in discharge frequency and then recruitment of new units according to motoneuron size,<sup>21</sup> and, indeed, there are few major examples in which these principles are violated when applied to the discharge of human motor units. However, it is notable that such examples derive from respiratory motor neuron pools such as the diaphragm.<sup>22</sup> Furthermore, motor units in the intercostal muscles show a recruitment pattern that reflects their topographic and mechanical effectiveness.<sup>19</sup> One distinguishing feature about “respiratory” motoneuron pools is not only that they are activated for inspiratory or expiratory functions, but that they are also active in other motor activities (particularly postural tasks). In the case of the genioglossus, it also has essential nonrespiratory tasks, and this requirement

may contribute to the complexity of the neural drives that it receives during wakefulness, sleep, and the transitions between these states.

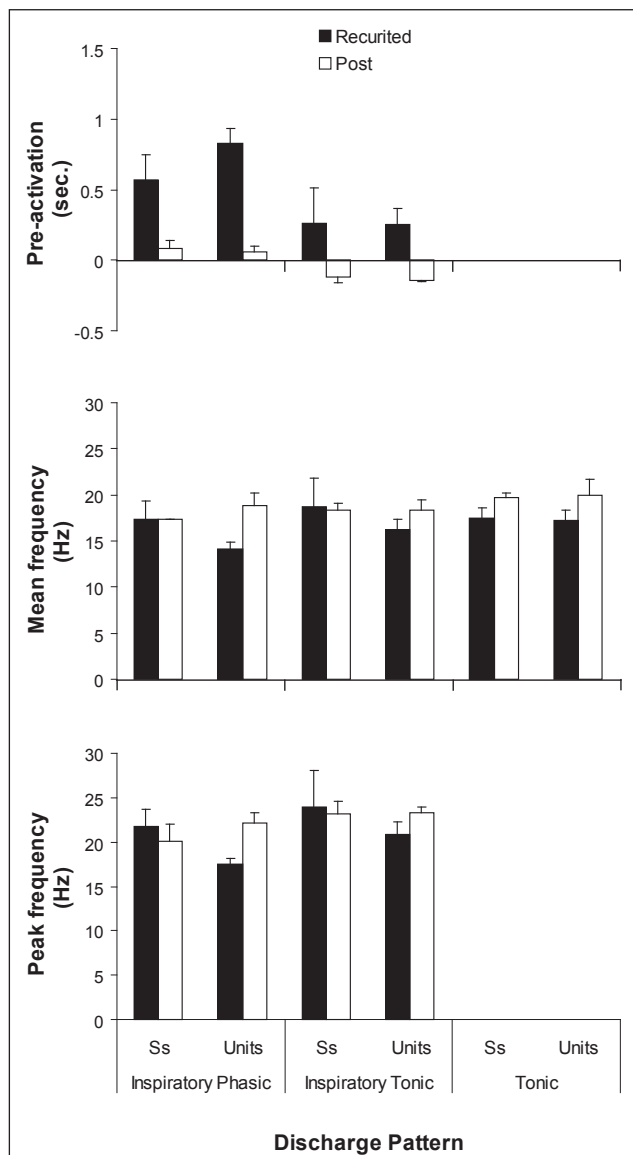
One of the potential benefits of single motor unit studies is the identification of the different prearousal motor inputs to the hypoglossal motor nucleus. From this perspective, our data suggest that the tonic component of inspiratory tonic motor units is not the same as the tonic component of tonic units, or of expiratory tonic units. Inspiratory units increased their tonic activity at an arousal from sleep, whereas tonic units and the tonic component of expiratory tonic units remained constant or showed a decrease in activity. Further, inspiratory tonic units were less likely than tonic or expiratory tonic units to stop firing following an arousal. This finding suggests that at least 2 different prearousal motor inputs influence tonic motor unit ac-



**Figure 3**—Instantaneous frequency distribution of an inspiratory phasic motor unit illustrating both the increase in the proportion of the breath that the unit fired and the consistency of the rate of firing, following an arousal from sleep. Also shown are the raw electromyogram (EMG), airflow, epiglottic pressure ( $P_{EPI}$ ), and electroencephalographic (EEG) measures. The vertical dotted line indicates the arousal.



**Figure 4**—Instantaneous frequency distribution of a tonic motor unit illustrating the slight dip in activity that characterized these units following an arousal from sleep. Also shown are the raw electromyogram (EMG), airflow, epiglottic pressure ( $P_{EPI}$ ), and electroencephalographic (EEG) measures. The vertical dotted line indicates the arousal.



**Figure 5**—Comparison of postarousal activity for motor units present before the arousal (sorted by postarousal pattern) and motor units recruited following the arousal. Preactivation and mean and peak frequency measures shown for inspiratory phasic, inspiratory tonic, and tonic units. Data has been averaged over subjects and motor units. Error bars are standard errors.



tivity. This must confound any simple interpretation of the tonic component of multiunit genioglossus EMG recordings.

Although total unit activity increased after arousal, 22% of motor units that were active before arousal stopped within the first 3 postarousal breaths. Expiratory and tonic units were more likely to stop, although around 15% of prearousal inspiratory units also stopped. Overall, there was a gain in inspiratory-modulated units but not in expiratory-modulated and tonic units. This finding suggests that the response of the genioglossus to an arousal from sleep is not just an increase in activity, but also a reorganization of the pattern of motor unit activity within the muscle.

The likely role of inspiratory-modulated motor units is to dilate the airway during inspiration and to reverse, at an arousal, any sleep-related closing tendency that had developed. The role of tonic and expiratory motor units is less obvious. It has generally been thought that the role of the tonic activity observed in multiunit recordings is to maintain patency by stiffening the airway.<sup>3</sup> It seems reasonable that this role is played by the tonic activity in tonic motor units and probably expiratory tonic units. In addition, we speculate that the role of tonic and expiratory motor units is more complex than merely stiffening the airway and that these motor units may fine tune genioglossus in the face of the unidimensional activity of the inspiratory motor units. Two aspects of the data suggested this view. The first was the small transient decrease in the discharge rate of tonic motor units at an arousal. The second was the reorganization of active motor units within the tonic and expiratory motor unit pool. If correct, this proposition suggests that, whereas there are antagonists balancing agonists in limb muscles, for a complex structure such as the tongue, this balance occurs partly within the genioglossus motoneuron pool.

A final question is whether the tonic component of inspiratory tonic units contributes to stiffening of the airway. We have earlier noted that the control of this component is probably different from the tonic component of expiratory tonic and tonic units. However, we remain uncertain as to whether the tonic component of inspiratory tonic units does act in a manner to similar that of the tonic component of tonic and expiratory tonic units.

In conclusion, arousal from sleep produced a particular pattern of change in genioglossus motor unit activity. The overall activity of the muscle increased as a consequence of increased activity in inspiratory-modulated motor units. The increase in inspiratory activity was primarily due to motor unit recruitment rather than an increase in the discharge rate of already active units and appeared to be a relatively undifferentiated transient burst of activity. This component probably arises in brain stem arousal-related nuclei and, as indicated by the current data, appears to be mediated via the respiratory-pattern generator. It would augment inspiratory-related airway dilation at the time of arousal from sleep. In addition, there were changes in the organization of motor units within the tonic and expiratory motor unit pools.

#### ACKNOWLEDGMENTS

This work was performed at the Division of Sleep Medicine, Brigham and Women's Hospital, Harvard Medical School and the Department of Psychology, University of Melbourne. Finan-

cial support for this study came from the National Health and Medical Research Council, Australia. Project Grant 454458. No off-label or investigational drugs were used.

#### DISCLOSURE STATEMENT

This was not an industry supported study. Dr. White is Chief Medical Officer for Respironics and has consulted for Itamar and Pavad. Dr. Malhotra has received research support and/or consulted for Respironics, NMT, Pfizer, Restore/Medtronic, Ethicon Division of Johnson & Johnson, Itamar, Apnex, Cephalon, and Sepracor. Dr. Jordan has received the use of a modified CPAP device from Respironics. The device was not used in this study. The other authors have indicated no financial conflicts of interest.

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