The Influence of Wakefulness on Pharyngeal Airway Muscle Activity

Yu-Lun Lo¹,²,³, Amy S Jordan¹,², Atul Malhotra¹,², Andrew Wellman¹,², Raphael A Heinzer⁴, Matthias Eikerman¹,²,⁵, Karen Schory¹, Louise Dover¹, David P, White¹,²

1. Sleep Disorders Research Program, Brigham and Women’s Hospital, Boston, MA, USA
2. Division of Sleep Medicine, Harvard Medical School, Boston, MA, USA
3. Department of Thoracic Medicine, Chang Gang Memorial Hospital, Chang Gang University College of Medicine, Taipei, Taiwan
4. Service de Pneumologie, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland.
5. Klinik für Anästhesiologie und Intensivmedizin, Universitätsklinikum Essen, Germany

Corresponding authors: Yu-Lun Lo, MD
Brigham and Women’s Hospital
Sleep disorder program @ BIDMC
75 Francis Street, Boston, MA, USA 02115
Phone: (617) 732-8450
Fax: (617) 732-7337
Email: lyulun@partners.org

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ABSTRACT

Introduction: Whether loss of wakefulness itself can influence pharyngeal dilator muscle activity and responsiveness is currently unknown. We, therefore, assess the isolated impact of sleep on upper airway muscle activity after minimizing respiratory/mechanical inputs. Methods: Ten healthy subjects were studied. Genioglossus (GG), tensor palatini (TP), and diaphragm (DIA) electromyography (EMG), ventilation, and sleep-wake status were recorded. Non-invasive positive pressure ventilation was applied. Expiratory pressure was adjusted to yield the lowest genioglossal EMG thereby minimizing airway negative pressure (mechanoreceptor) effects. Inspiratory pressure, respiratory rate, and inspiratory time were adjusted until the subjects ceased spontaneous ventilation, thereby minimizing central respiratory input. We evaluated muscle activity during wakefulness, wake-sleep transitions, stable non rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep in the supine position. Results: In transitions from wakefulness to sleep, we observed significant decrements in both average GG and TP EMG (1.6 (0.3) to 1.3 (0.4) % of maximal GGEMG; 4.3 (2.3) to 3.7 (2.1) % of maximal TPEMG; respectively). Compared with sleep onset, the activity of TP during stable NREM sleep and REM sleep demonstrated further decreases (3.7 (2.1) vs. 3.0 (2.0) vs. 3.0 (2.0) % of maximal EMG). However GGEMG was only further reduced during REM sleep (1.3 (0.4) vs. 1.1 (0.4) vs. 1.0 (0.3) % of maximal EMG). Conclusion: This study suggests that wakefulness per se, independent of respiratory/mechanical stimuli, can influence pharyngeal dilator muscle activity.

INTRODUCTION

Obstructive sleep apnea (OSA) is a common disorder characterized by recurrent collapse of the upper airway leading to repetitive episodes of hypoxemia, hypercapnia, and arousal from sleep. This disorder is associated with important consequences for afflicted individuals including decreased quality of life and likely adverse cardiovascular outcomes. The pathogenesis of OSA is multifactorial; however, an anatomically small pharyngeal airway in combination with decrements in pharyngeal dilator muscle
activity during sleep 6, 7 appear central to the disorder. Therefore, it is important to understand the effect of sleep on the mechanisms controlling the upper airway musculature.

Pharyngeal dilator muscle activity is influenced by inputs from the chemoreceptors8, the brainstem central respiratory pattern generator (CPG)9, intrapharyngeal negative pressure 10 and possibly vagal input from lung volume11, 12. Studies suggest that most of these mechanisms controlling the upper airway muscles are affected by sleep 13-15, with sleep generally decreasing the sensitivity or responsiveness of the control system. However, it is unclear whether wakefulness alone influences pharyngeal muscle activity, or whether its effect on muscle activation is mediated through brainstem respiratory neurons or other chemical/mechanical stimuli 16. At the transition from wakefulness to sleep, there is a decline in genioglossal and tensor palatini muscle activity accompanied by a reduction in ventilation and a rise in upper airway resistance (UAR)17, 18. This decrease in upper airway muscle activity at sleep onset could result from a withdrawal of direct wakefulness stimulation of upper airway muscles, decreased muscle responses to intrapharyngeal negative pressure and/or chemo-stimuli, or decreased inputs from brainstem respiratory neurons. Most evidence suggests that mechanical- (negative pressure) and chemo- (PO2, PCO2) responses are decreased during sleep 13, 14. Ventilation falls as well, suggesting decreased CPG output 18. However, whether loss of wakefulness has a direct input on muscle activity not mediated through other mechanisms has not been tested.

With the application of non-invasive positive pressure ventilation (NIPPV), subjects can be passively ventilated, therefore, achieving several goals. First, expiratory positive pressure has been shown to lead to a reduction in the negative pressure reflex thereby minimizing mechanoreceptor input to the upper airway muscle 6. Second, by adjusting inspiratory positive pressure and increasing respiratory rate to passively ventilate subjects, NIPPV can eliminate or minimize central respiratory drive to the upper airway muscles as well as to all respiratory muscles such as the diaphragm. NIPPV can also maintain ventilation and lung volume at constant levels. This should allow us to examine
upper airway muscle activation during wakefulness, wake-sleep (α-θ) transitions, stable non rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, without respiratory or mechanical inputs. Thus pure state (sleep) influences on muscle activity should become apparent.

METHODS

Subjects:
Nineteen healthy subjects without any cardio-respiratory or sleep disorder were enrolled in this study. Subjects were between 19 and 52 years old and had normal body weight (BMI < 27 kg/m²). Six subjects could not be passively ventilated and fall asleep. Three subjects chose to not finish the protocol. Therefore, only ten subjects could complete the entire study. There were no differences between subjects who completed the study and who could not in terms of age, sex, and BMI. The sample size was based on preliminary data obtained in the first three subjects in this study and a prior study from our laboratory. We determined that 9 subjects would be required, using the change at the α-θ transition with mechanical ventilation, to detect a 20% decrease in pharyngeal airway muscle activity with 80% power at the 5% significance level. Written informed consent was obtained from each subject, with the protocol having the prior approval of the Human Subjects’ Committee of the Brigham and Women’s Hospital. Females were studied during the follicular phase (day 5-11) of their menstrual cycle as determined by history.

Equipment and Techniques:
Subjects wore a nasal mask (Respirronics, Inc. Murrayville, PA, USA) connected to a heated pneumotachometer (Hans Rudolph Inc, Kansas City, MO, USA) and a differential pressure transducer (Validyne Corp., Northbridge, CA, USA), calibrated with a rotameter, for measurement of airflow. Inspiratory (Tᵢ) and expiratory (Tₑ) times were determined from this signal and it was electronically integrated for calculation of tidal volume (Vₜ). Minute ventilation (Vₑ) was calculated as the sum of all Vₜ per minute. The duty cycle (Tᵢ/TₜOT) was also calculated for each breath. End-tidal CO₂ (PETCO₂) was measured from expired air within the nostril using a calibrated infrared CO₂ analyzer.
(Capnograph/Oximeter Monitor, BCI, Waukesha, WI, USA) while arterial oxygen saturation (SaO₂) was measured using a pulse oximeter probe attached to the index finger (Capnograph/Oximeter Monitor). Pressure was monitored in the mask with an open catheter attached to a pressure transducer (Validyne Corp). The subjects were instructed to breathe exclusively through nose. This was ensured by taping the mouth and using of an infrared video camera to document that the mouth remained closed.

The genioglossal electromyogram (GGEMG) was measured with a pair of unipolar intramuscular electrodes referenced to a single ground, producing a bipolar recording. Two stainless steel, Teflon-coated, 30-gauge wire electrodes were inserted approximately 12-15 mm into the body of the genioglossal muscle 3 mm lateral to the frenulum on each side, using a 25-gauge needle. The needles were removed immediately, leaving the wires in place. The tensor palatini electrogram (TPEMG) was measured in a similar manner to that of the GG muscle, with a pair of unipolar intramuscular electrodes. The tip of the pterygoid hamulus was located at the junction of the hard and soft palate on each side. Using a 25-gauge needle, two stainless steel, Teflon-coated, 30-gauge wire electrodes were then inserted at a 45° angle along the lateral surface of the medial pterygoid plate, to a depth of ~10-15 mm into the palate. The needles were then removed, leaving the electrodes in place. This technique has been used previously in our laboratory.

Sucking, blowing, and swallowing, which have been shown previously to activate the TP muscle, were performed to confirm TP electrode placements. Diaphragm electromyogram (DIAEMG) was obtained from electrodes placed at the right sixth to eighth intercostal spaces adjacent to the costal margin. For all muscles the raw EMG was amplified (Grass Instrument, Quincy, MA, USA), band-pass filtered (between 30 and 1000 Hz), rectified, and electronically integrated on a moving time average (MTA) basis with a time constant of 100ms (CWE, Inc., Ardmore, PA, USA). Electrocardiographic (ECG) artifacts were removed from the raw Diaphragm EMG using an EKG blanker (CWE: Inc., Ardmore, PA, USA). To define maximal EMG for the muscles, subjects were asked to perform several different maneuvers. They were instructed to 1) swallow, 2) maximally protrude their tongue against the maxillary alveolar ridge, 3) inspire maximally against an occluded tube, 4) suck and blow, and 5) inspire as deeply as
possible. Each maneuver was performed several times, and the maximal EMG recorded during this calibration was designated as 100%. Electrical zero was then determined and each EMG was quantified as a percentage of maximal activation for that subject. The genioglossal and diaphragm muscles are inspiratory phasic muscles; therefore, their activation was assessed at two points during basal breathing. The tonic activation was defined as the lowest activation during expiration, the peak activation as the highest activation during inspiration (i.e. peak phasic), and the phasic activation as the difference between the peak and tonic activation. However, during mechanical ventilation without spontaneous breathing, no phasic activity was observed during inspiration. Hence, GGEMG and DIAEMG were reported as the average activation across each breath. As the tenor palatini is a tonic muscle without phasic activity, TPEMG was reported as the average activation across each breath during basal breathing and passive ventilation.

Two channels of electroencephalography (EEG: C3-A2, O2-A1), left and right electrooculograms (EOG), and the submental electromyogram (EMG) were recorded to document wakefulness or sleep and score the different sleep stages by a blinded technician. Subjects were maintained in the supine position throughout this study, verified by infrared video camera.

A microprocessor-controlled ventilator (Respironics, Inc., Murrysville, PA, USA) that operates in either a CPAP mode or a Spontaneous/Timed (S/T) mode was used in this study. In S/T mode, this ventilator allowed control of inspiratory positive airway pressure (IPAP), expiratory positive airway pressure (EPAP), respiratory rate, inspiratory time, and IPAP rise time. To yield the lowest GGEMG during wakefulness, CPAP mode was applied first and started at 4 cmH2O. This pressure level was increased until the minimal level of GGEMG was obtained or to a maximum of 8 cmH2O. If no obvious reduction in GGEMG was discernible, the subjects were studied on 6 cmH2O. The ventilator was then switched to S/T mode, and IPAP was adjusted to obtain the same or a slightly bigger tidal volume than observed during eupneic breathing. Respiratory rate was set to be the same as or slightly faster than during eupnic breathing, and inspiratory time was set based on respiratory timing in CPAP mode. IPAP rise time was set at 0.05 or 0.1
second. By adjusting respiratory rate, inspiratory time, IPAP rise time, and the pressure difference between IPAP and EPAP, subject passivity [assessed by (a) DIAEMG having no inspiratory phasic activity, (b) GGEMG demonstrating no inspiratory phasic activity or pre-activation prior to onset of airflow, (c) mask pressure showing no reduction before each ventilator-initiated breath, (d) airflow pattern showing a descending ramp shape, and (e) fixed inspiratory-expiratory time ratio] could be achieved (fig 1). Recordings were stopped when there was a departure from this passive pattern until adequate passivity could be achieved or the experiment was terminated.

Protocol:
Each subject reported to the laboratory at approximately 9:00 PM, having fasted for at least 4 hours. After informed consent was obtained, the sleep staging electrodes and intramuscular EMG wires were placed. Subjects then assumed the supine posture in bed and the nasal mask and pneumotachograph were attached. Subjects subsequently lay with their eyes open in this posture, and were allowed to acclimate to the equipment. Subjects were subsequently recorded during both basal breathing and passive ventilation during wakefulness for 5-10 min in each condition. After recording data during wakefulness, subjects were allowed to fall asleep (in the supine posture) with the mechanical ventilator in place. In order to obtain multiple sleep onsets, primarily transitions from quiet wakefulness to stage 1 sleep, subjects were awoken if they slept for 3-5 consecutive minutes without a spontaneous awakening, and then were allowed to fall asleep again. This procedure was repeated until adequate data (at least 3-5 transitions) had been collected. Thereafter, subjects were allowed to fall asleep without interruption. Subjects were subsequently recorded during passive ventilation during stable NREM and REM sleep for 5-10 minutes in each condition.

Data recording and analysis:
All signals (GGEMG, TPEMG, and DIAEMG (raw and MTA), Flow, VT, PETCO2, SaO2, EOG, EMG, ECG,) were recorded on a computer using an analog-to-digital converter (1401plus, Cambridge Electronic Design, Ltd, Cambridge, UK) and data acquisition software (Spike 2, version 5.03, Cambridge Electronic Design, Ltd). Certain signals
(GGEMG, TPEMG, and DIAEMG (MTA), Flow, VT, PETCO2, SaO2, EOG, EMG, ECG) were also recorded on a computer using Nihon Kohden software (Polysmith version 4.0, Neurotronic Inc., Tokyo, Japan). Sample rate varied from 125 Hz for respiratory signals to 1000 Hz for the raw EMG. For each of the 3 stable conditions [relaxed wakefulness, stable NREM sleep, and REM sleep (phasic and tonic)], during passive ventilation, the mean value for each variable over a 5-10 minutes recording time was determined.

For each subject each breath at α-θ transitions was assigned a value of α or θ by visual analysis of the occipital EEG signal, by two of the authors (Y.L.L., L.D.) independently (95% agreement) with any disagreements being resolved. A wake-sleep EEG transition required at least one minute of alpha activity followed by clear theta activity lasting for at least 10 seconds. The transition breaths were defined as alpha or theta breaths based on having greater or lesser alpha EEG activity. An adequate α-θ transition was defined as having at least three consecutive α breaths followed by at least two consecutive θ breaths. Each breath in the transition was then assigned a position relative to the transition from -5 to +5, as previously described by Worsnop. Thus, every transition had breaths -3 to +2, with fewer transitions having breaths in position -5 and -4, and +3, +4, and +5. All ventilation and EMGs parameters were calculated on a breath by breath basis. If the subject could not be passively ventilated within a defined α-θ transition, the data for the transition were discarded. Thus all ventilation and EMGs parameter were calculated as the mean α level (-5 to -1) and the mean θ level (+1 to +5). Only those subjects having at least 3-5 adequate transitions were used for this analysis.

All statistic analyses were performed with commercially available software (Excel 2000, Microsoft; and SigmaStat + Sigmaplot, SPSS, Chicago, IL, USA). A paired t-test was used to assess the effect of sleep onset on upper airway dilator muscle activity and ventilation. The Wilcoxon signed rank test was used if data were not normally distributed. Repeated-measures ANOVA with post hoc Student-Newman-Keuls testing was used to assess the effect of stable NREM and REM sleep on pharyngeal airway activity and ventilation. Whenever data were not normally distributed, Friedman’s non-
parametric repeated measures comparisons were used. All data are presented as means (SE) unless otherwise stated. P < 0.05 was considered statistically significance.

RESULTS
Full data sets were acquired in these 10 individuals under all conditions with one exception. In one subject, due to technical problems, no adequate TPEMG data are available. Thus for TPEMG, the data presented are from 9 individuals. For the ten subjects completing the entire study, the mean (SE) age was 33.0 (4.1) years and mean body mass index 24.0 (0.6) kg/m².

During basal breathing, inspiratory phasic diaphragm and genioglossus activation were observed in all subjects. Figure 1 shows an example of raw data in one individual during basal breathing and passive ventilation during wakefulness demonstrating no active breathing with NIPPV in place. To achieve passive ventilation, the following mechanical ventilator setting were required: EPAP pressure: 6.2 (0.1) cmH₂O, IPAP pressure: 12.7 (0.4) cmH₂O, inspiratory time: 1.2 (0.1) s, and respiratory rate: 17.7 (0.7) breaths/min. Muscle activity, respiratory data, ventilation, CO₂ level, and O₂ saturation data during basal breathing and passive ventilation during wakefulness are shown in Table 1.

Table 1: Muscles Activity and Respiratory Data during Basal Breathing and Passive Ventilation during Wakefulness

<table>
<thead>
<tr>
<th></th>
<th>Basal breathing</th>
<th>Passive ventilation</th>
<th>P value</th>
<th>Mean difference (95% Confident Intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIAEMG, % max</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td>12.0 (3.1)</td>
<td></td>
<td>6.8 (1.6)*</td>
<td></td>
</tr>
<tr>
<td>Tonic</td>
<td>5.7 (1.2)</td>
<td></td>
<td>6.8 (1.6)*</td>
<td></td>
</tr>
<tr>
<td>Phasic</td>
<td>6.2 (2.0)</td>
<td></td>
<td>6.8 (1.6)*</td>
<td></td>
</tr>
<tr>
<td>GGEMG, % max</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td>5.3 (1.4)</td>
<td></td>
<td>1.6 (0.4)*</td>
<td></td>
</tr>
<tr>
<td>Tonic</td>
<td>2.1 (0.5)</td>
<td></td>
<td>1.6 (0.4)*</td>
<td></td>
</tr>
<tr>
<td>Phasic</td>
<td>3.2 (1.0)</td>
<td></td>
<td>1.6 (0.4)*</td>
<td></td>
</tr>
</tbody>
</table>
Compared with basal breathing, there were significant increases during passive ventilation in minute ventilation and O₂ saturation and decreases in inspiratory time and duty cycle (TI/TTOT). No differences in tidal volume, ETCO₂ and respiratory rate were observed.

**Sleep onset effects**

Figure 2 shows an example of a transition from wakefulness to sleep under passive ventilation. For the group, the mean GGEMG and TPEMG during α breaths were significantly higher than θ breaths (Table 2). The individual results and group mean for the GGEMG and TPEMG during α-θ transitions, stable NREM and REM sleep are shown in figure 3A and 3B.

<table>
<thead>
<tr>
<th></th>
<th>Wakefulness (Alpha)</th>
<th>Sleep (Theta)</th>
<th>P value</th>
<th>Mean difference (95% Confidential Intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Muscles Activity, % max</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIAEMG</td>
<td>6.5 (1.5)</td>
<td>6.4 (1.6)</td>
<td>0.324</td>
<td>0.0 (0.0 to 0.1)</td>
</tr>
<tr>
<td>GGEMG</td>
<td>1.6 (0.5)</td>
<td>1.3 (0.4)</td>
<td>0.016</td>
<td>0.3 (0.1 to 0.5)</td>
</tr>
<tr>
<td>TPEMG</td>
<td>4.3 (2.3)</td>
<td>3.7 (2.1)</td>
<td>0.004#</td>
<td></td>
</tr>
</tbody>
</table>
\[ V_T, \text{ L} \quad 0.42 (0.0) \quad 0.41 (0.0) \quad 0.079 \quad 0.01 (0.00 \text{ to } 0.03) \]

\[ V_E, \text{ L/min} \quad 7.8 (0.7) \quad 7.7 (0.8) \quad 0.253 \quad 0.2 (-0.1 \text{ to } 0.4) \]

\[ \text{SaO}_2, \% \quad 97.2 (0.3) \quad 97.2 (0.3) \quad 0.755 \quad 0.0 (0.0 \text{ to } 0.3) \]

\[ \text{PETCO}_2, \text{ mm Hg} \quad 40.3 (0.8) \quad 40.6 (1.0) \quad 0.287 \quad 0.3 (-0.3 \text{ to } 0.9) \]

* Wilcoxon signed rank test

There was no difference in the mean DIAEMG during \( \alpha \) versus \( \theta \) breaths with NIPPV in place. There were also no significant changes in mean \text{SaO}_2, \text{PETCO}_2, \text{ tidal volume, or minute ventilation at } \alpha-\theta \text{ transitions.}*

**Sleep states effects**

Compared with relaxed wakefulness (Table 3), there were significant decreases in GGEMG and TPEMG during stable NREM sleep and REM sleep. However, post hoc testing showed that GGEMG as well as TPEMG were not statistically different between stable NREM and REM sleep. DIAEMG was not different during wakefulness, stable NREM sleep or REM sleep. Other respiratory variables are shown in Table 3.

**Table 3: Muscles Activity and Ventilation during Wakefulness, NREM sleep, and REM sleep during Passive Ventilation**

<table>
<thead>
<tr>
<th></th>
<th>Wakefulness</th>
<th>NREM Sleep</th>
<th>REM Sleep</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Averaged Muscles Activity, % max</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIAEMG</td>
<td>6.8 ± 1.6</td>
<td>6.4 ± 1.6</td>
<td>6.4 ± 1.6</td>
<td>0.273</td>
</tr>
<tr>
<td>GGEMG</td>
<td>1.6 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>1.0 ± 0.3</td>
<td>0.003</td>
</tr>
<tr>
<td>TPEMG</td>
<td>5.1 ± 2.2</td>
<td>3.0 ± 2.0</td>
<td>3.0 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( V_T, \text{ L} )</td>
<td>0.43 ± 0.0</td>
<td>0.41 ± 0.0</td>
<td>0.42 ± 0.0</td>
<td>0.278</td>
</tr>
<tr>
<td>( V_E, \text{ L/min} )</td>
<td>7.8 ± 0.6</td>
<td>7.5 ± 0.4</td>
<td>7.8 ± 0.5</td>
<td>0.569</td>
</tr>
<tr>
<td>\text{SaO}_2, %</td>
<td>97.4 ± 0.2</td>
<td>96.7 ± 0.3</td>
<td>96.9 ± 0.4</td>
<td>0.014</td>
</tr>
<tr>
<td>\text{PETCO}_2, \text{ mm Hg}</td>
<td>40.7 ± 0.5</td>
<td>41.4 ± 0.7</td>
<td>42.1 ± 0.9</td>
<td>0.055</td>
</tr>
</tbody>
</table>
When compared with sleep onset (θ breaths), GGEMG was significantly lower during REM sleep but not stable NREM sleep (1.3 (0.4) vs. 1.1 (0.4) vs. 1.0 (0.3) % of maximal EMG, p=0.019). However, TPEMG was significantly lower during stable NREM sleep and REM sleep when compared with sleep onset (3.7 (2.1) vs. 3.0 (2.0) vs. 3.0 (2.0) % of maximal EMG, p<0.001).

**DISCUSSION**

This is the first study, to our knowledge, to carefully assess the effect of sleep (loss of isolated wakefulness drive) as well as the different sleep states on upper airway dilator muscle activity while minimizing the influences of respiratory and mechanical inputs. The results indicate the following: First, at the wake-sleep transition, there were moderate reductions in both average GG and TP muscle activity. Second, during stable NREM and REM sleep, both GG and TP muscle activity were lower than relaxed wakefulness. When compared with sleep onset (θ breaths), there were further muscle activity decreases in TP during stable NREM and REM sleep, but only during REM sleep for the GG. Thus, wakefulness has an important independent effect on upper airway dilator muscle activity that is unlikely to be mediated through the respiratory or mechanical control systems.

**Immediate sleep effect on upper airway muscle activity**

At sleep onset (θ breaths), a consistent decrement in pharyngeal dilator muscle activity has been demonstrated previously by Mezzanotte and Worsnop, along with a reduction in minute ventilation and a rise in UAR\textsuperscript{17, 21}. However, in their observations, after the initial fall in GGEMG during the first 2 θ breaths, there was a subsequent recruitment in muscle activity. By the fifth breath after the transition, GGEMG had largely recovered to stable waking levels. They speculated that GG is recruited in response to increasing negative upper airway pressure and/ or rising CO\textsubscript{2}. By applying CPAP to diminish intrapharyngeal negative pressure, Fogel et al reported a similar reduction in GGEMG over the first two θ breaths following a transition, but CPAP prevented the rise in UAR at the transition, and eliminated the subsequent recruitment of GGEMG. Therefore, it was suggested that the initial reduction in upper airway muscle activity at sleep onset is due to loss of a 'wakefulness' stimulus, rather than to loss of responsiveness to negative pressure\textsuperscript{18}. 


However, the changes in the upper airway dilator muscle control that occur during sleep onset are complex. Shea et al reported that GGEMG responses to brief pulses of negative pressure were minimally reduced (not significantly) in the first five breaths after an $\alpha$-$\theta$ transition compared to stable wakefulness. Nonetheless, it is unclear how chemoreceptive inputs influence GGEMG at sleep onset, as such studies would be difficult to conduct and interpret. Thomson et al also reported that, at $\alpha$ to $\theta$ transitions, there are prolongations in breath duration and expiratory time in the absence of changes in airway resistance or fluctuations in ventilation and CO$_2$ tension. These observations suggest that there is an abrupt reduction in ventilatory motor output at the wake-sleep transition. As genioglossal muscle receives inputs from the CPG, it is possible that the change in CPG activity at sleep onset could influence genioglossal muscle activation. Taken together, it is likely that the decrement of GGEMG during sleep onset is a combination of a loss of the wakefulness drive, a mildly decreased negative pressure reflex, and a minimally reduced respiratory (CPG) input (fig 4).

In this study, with the application of NIPPV to eliminate or minimize inputs from local mechanical stimuli (negative pressure) and CPG, and to maintain ventilation and CO$_2$ levels across wake-sleep transitions, there was a moderate decrement in GGEMG, suggesting that the muscle is independently controlled by wake/sleep state. However, the baseline muscle activity was relatively low which might inflate the percent change. As a result, the clinical importance of this change in muscle activity is unclear. These observations are also consistent with the concept from Orem and colleagues’ work, suggesting that there is a wakefulness stimulus to breathing that is not the result of reflexes or sensory inputs. A number of neural systems play an important role in maintaining wakefulness, including noradrenergic, serotonergic, histaminergic, cholinergic, peptidergic, and dopaminergic neurons. All demonstrate decrements in the firing frequency from wakefulness to sleep. Our studies were designed to determine how sleep-induced changes in several of these neural systems might affect genioglossal activation. Evidence suggests that, during sleep, decrements in the firing frequency of noradrenergic neuron in the locus coeruleus and serotonergic neurons in the raphe could lead to disfacilitation of hypoglossal motoneurons, which innervate genioglossus.
motor units (fig 4). There is also evidence suggesting that active post-synaptic inhibition of these upper airway motoneurons during sleep via glycine and/or γ-aminobutyric acid may also contribute to decrements in muscle activity 27. Therefore, it is possible that the decrement in GGEMG across wake/sleep transitions observed in this study result from the disfacilitation and/or active post-synaptic inhibition of upper airway motoneurons.

Tensor palatini muscle activity consistently decreased at α-θ transition under passive ventilation confirming previous findings 17, 18. The tensor palatini, unlike the genioglossus, usually demonstrates primarily a tonic pattern of activity, without clear phasic respiratory modulation. Neither inspiratory resistive loading during wakefulness and sleep nor the addition of CO₂ during sleep 20, 28 could influence TPEMG. Therefore, the control of this muscle is substantially different from the genioglossus. However, these studies and ours would suggest that wakefulness has an important influence on the activity of this muscle that is gradually lost across progressively longer sleep times. It is likely that the tonic activity of TPEMG is important for maintaining upper airway patency 7, and the decrement of TPEMG at sleep onset could increase upper airway collapsibility, especially in the area behind the soft palate.

**Stable sleep effect on upper airway muscle activity**

Our findings that, with minimal respiratory and mechanical inputs, both GGEMG and TPEMG during stable NREM and REM sleep were lower than relaxed wakefulness further confirm that the wakefulness drive can modulate upper airway dilator muscle control. However, when compared with sleep onset, both stable NREM and REM sleep led to further TPEMG decrements, but only REM sleep was associated with further GGEMG reductions. This difference could result from the loss of wakefulness input having different influence on these two muscles. However, it is also possible that our inability to find a difference in GGEMG between sleep onsets and stable NREM may have been due to a relative lack of power given the small number of subjects studied. If such a difference exists, it is likely to be less robust than was seen for TPEMG.
The lack of observed muscle activity difference between stable NREM and REM sleep deserves comment. The majority of studies, including protocols in animals and humans, indicate that GGEMG is reduced more during REM than NREM sleep\textsuperscript{29-31}. However, one study by Wiegand and colleagues reported, in normal subjects, no difference in GGEMG between REM and NREM sleep when tonic REM sleep was considered\textsuperscript{32}. Nonetheless, none of these studies were designed to evaluate the isolated loss of wakefulness on upper airway dilator muscle activity. In this study, we observed that, after loosing the wakefulness drive to these upper airway dilator muscles, there is consistent tonic muscle activity in both the GG and TP across stable NREM and REM sleep and that this tonic muscle activity is not different between NREM sleep and REM sleep in these two muscles. However, this change in muscle activity may be complicated by a floor effect. Larger changes might have been observed during spontaneous breathing.

\textbf{Limitations}

Several methodological considerations must be examined when assessing our conclusions. First, during sleep, \textit{O}_2 saturation was lower and there was a trend towards increased ETCO\textsubscript{2} level suggesting that all subjects may not have been completely passively ventilated during non-invasive positive pressure ventilation at all times. However, we believe any active ventilation was minimal as our criteria for passive ventilation were strict. We demonstrated that, with NIPPV in place, there must be a loss of phasic genioglossal EMG plus loss of pre-activation (activation prior to the onset of airflow) in addition to a loss of phasic activation on the surface diaphragmatic EMG. The absence of reductions in mask pressure prior to each ventilator-initiated breath, the fixed inspiratory/expiratory time ratio, and descending ramp shape of the flow pattern also confirmed that there was no spontaneous breathing efforts. We speculate that the difference in lung and chest wall compliance during mechanical ventilation between wake-sleep states might account for this change in ventilation and blood gases. This would assume that the lung and chest wall (total respiratory system) becomes less compliance or stiffer during sleep than wakefulness, which has been carefully demonstrated during anesthesia\textsuperscript{33}, although this has not been studied during sleep. It is
also possible that subjects were simply entrained to the NIPPV rather than passive. However, we believe that the absence of phasic diaphragmatic EMG argues strongly against this. On the other hand, even if subjects were not completely passively ventilated; a very small decrease in respiratory drive cannot explain the large decrement in pharyngeal airway muscle activity from wake to sleep. Second, it is possible that the application of NIPPV may increase resting lung volume that in turn decreases upper airway muscle activity. However, the majority of data suggest the increased lung volume with increasing expiratory airway pressure is small and stable across wakefulness and sleep\textsuperscript{34}. Therefore, with NIPPV at fixed pressures across the different states, we believe lung volume would be maintained at a constant level. Third, the application of NIPPV could influence respiration in additional ways including a lowering of upper airway resistance and effects on hemodynamics\textsuperscript{35}. In addition, the application of positive pressure may directly stimulate positive pressure receptors that have been identified in the trachea although their function remains unclear. Thus the effects of NIPPV are likely more complex than the simplified view presented here. However, we maintained ventilation and EPAP at quite constant levels across our states. Thus we doubt these additional effects of NIPPV affected systemically our results or our conclusions. Finally, we only studied normal subjects; therefore, our observations don’t necessarily apply to patients with obstructive sleep apnea.

CONCLUSIONS

In conclusion, our observations suggest that the state of wakefulness has an important influence on pharyngeal dilator muscle activity that is not mediated through respiratory or mechanoreceptive mechanisms. Loss of this wakefulness input may have important consequences for upper airway patency.
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COMPETING INTERESTS
None of the authors have a competing interest(s).
REFERENCES


FIGURE LEGENDS

Figure 1: An example of raw data obtained in one individual during basal breathing and passive ventilation during wakefulness. As can be seen, no inspiratory phasic diaphragm and genioglossal activation as well as pre-activation of genioglossus prior to the onset of airflow are encountered during passive ventilation. Mask pressure demonstrated no reduction before the ventilator initiated a breath, flow pattern is shown with a descending ramp shape, and fixed inspiratory time/expiratory time ratio all confirm that the subject was passively ventilated without spontaneous active breathing. DIA, Diaphragm; EEG, Electroencephalography; GG, Genioglossus; MTA, Moving time average; PMASK, Mask pressure; TP, Tensor palatini; VT, Tidal volume.

Figure 2: An example of a transition from wakefulness to sleep under passive ventilation in one individual. There are substantial decreases in muscles activity in both genioglossus and tensor palatini across wake-sleep transition. DIA, Diaphragm; EEG, Electroencephalography; GG, Genioglossus; MTA, Moving time average; PMASK, Mask pressure; TP, Tensor palatini; VT, Tidal volume.

Figure 3: Mean GGEMG (A) and TPEMG (B) for each individual during the α-θ transitions, stable NREM and REM sleep. --●-- demonstrates the mean values for each condition. GGEMG, Genioglossus electromyogram; NREM, Non-rapid eye movement; REM, Rapid eye movement; TPEMG, Tensor palatini electromyogram.

Figure 4: A simplified schema illustrates three potential major sources of neural inputs involved in genioglossus muscle control across wake-sleep states. These are: 1) mechanoreceptor reflexes (negative pressure), 2) phasic respiratory inputs, and 3) wake-sleep sensitive neural systems. We believe 1 and 2 were eliminated by our NIPPV. Ach = acetylcholine; CPG = central pattern generator; 5HT = serotonin; Hist = histamine; NE = norepinephrine; NTS = nucleus solitary tract.
1. Mechanoreceptor Reflexes
   - Superior Laryngeal Nerve

2. Phasic Respiratory Inputs
   - Chemoreceptors
     - Central
     - Peripheral
   - Hypoglossal Motor Nucleus
   - NTS
   - CPG

3. Wake-Sleep Sensitive Neural Systems
   - NE, 5HT, Ach, Orexin, Hist

Pharyngeal Airway
Genioglossus Muscle