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Long-term facilitation of genioglossus activity is present in normal humans during NREM sleep

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Abstract

Episodic hypoxia (EH) is followed by increased ventilatory motor output in the recovery period indicative of long-term facilitation (LTF). We hypothesized that episodic hypoxia evokes LTF of genioglossus (GG) muscle activity in humans during non-rapid eye movement sleep (NREM) sleep. We studied 12 normal non-flow limited humans during stable NREM sleep. We induced 10 brief (3 minute) episodes of isocapnic hypoxia followed by 5 minutes of room air. Measurements were obtained during control, hypoxia, and at 5, 10, 20, 30 and 40 minutes of recovery, respectively, for minute ventilation ($\dot{V}_I$), supraglottic pressure (P_{SG}), upper airway resistance (R_{UA}) and phasic GG electromyogram (EMG_{GG}). In addition, sham studies were conducted on room air. During hypoxia there was a significant increase in phasic EMG_{GG} ($202.7 \pm 24.1\%$ of control, $p < 0.01$) and in $\dot{V}_I$ ($123.0 \pm 3.3\%$ of control, $p < 0.05$); however, only phasic EMG_{GG} demonstrated a significant persistent increase throughout recovery ($198.9 \pm 30.9\%$, $203.6 \pm 29.9\%$ and $205.4 \pm 26.4\%$ of control, at 5, 10, and 20 minutes of recovery, respectively, $p < 0.01$). In multivariate regression analysis, age and phasic EMG_{GG} activity during hypoxia were significant predictors of EMG_{GG} at recovery 20 minutes. No significant changes in any of the measured parameters were noted during sham studies.

Conclusion: 1) EH elicits LTF of GG in normal non-flow limited humans during NREM sleep, without ventilatory or mechanical LTF. 2) GG activity during the recovery period correlates with the magnitude of GG activation during hypoxia, and inversely with age.

Keywords

Long-term facilitation; sleep; episodic hypoxia; genioglossus; minute ventilation

1. INTRODUCTION

Exposure to intermittent hypoxia elicits a sustained increase in ventilatory motor output, referred to as respiratory long-term facilitation (LTF). The occurrence and expression of LTF varies across species, CNS arousal state, experimental paradigms and medullary motor nuclei. Episodic hypoxia elicits LTF in many (Maltais *et al.* 1991; Cao *et al.* 1992; Hayashi *et al.*

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1993; Mateika and Fregosi, 1997; Turner and Mitchell, 1997; Fregosi and Mitchell, 2000), but not all animal models (Janssen and Fregosi, 2000). Likewise, episodic hypoxia did not elicit LTF during wakefulness in humans (McEvoy *et al.* 1996, Jordan *et al.* 2002, Morris and Gozal, 2004). In contrast, studies from our laboratory have demonstrated LTF in a subset of sleeping humans, specifically, in individuals who snore and who demonstrate inspiratory airflow limitation during sleep, (Babcock and Badr, 1998, Shkroukani *et al.* 2002, Babcock *et al.* 2003), as well as in patients with obstructive sleep apnea (OSA) (Aboubakr *et al.* 2001). Experimental conditions also influence the development of LTF. An example is the occurrence of LTF following episodic hypoxia in awake human subjects, only in the presence of concomitant hypercapnia (Harris *et al.* 2006). Finally, LTF may manifest differently across different motor nuclei, manifesting in the phrenic motoneuron (Fuller *et al.* 2000) or hypoglossal motoneuron (Bach and Mitchell, 1996).

Respiratory LTF in sleeping humans is associated with decreased upper airway resistance and no change in the activity of thoracic pump muscles (Shkroukani *et al.* 2002) suggesting that episodic hypoxia elicits ventilatory LTF by preferentially activating upper airway dilating muscles. Findings from animal studies support this conclusion. However, it is unknown whether episodic hypoxia evokes LTF of the genioglossus muscle in sleeping humans. Thus, the purpose of this study was to test the hypothesis that episodic hypoxia elicits LTF of the genioglossus muscle activity during NREM sleep in normal humans, manifesting as increased genioglossus muscle activity after termination of the hypoxic exposure. Given the potential confounding effects of varying negative pressure with changing upper airway resistance (Malhotra *et al.* 2002), we chose to investigate this phenomenon in healthy subjects, free of snoring and flow limitation.

2. METHODS

2.1 Subjects

All the experimental protocols described were approved by the Human Investigation Committees of Wayne State University School of Medicine and Detroit Veterans Affairs Medical Center, and conformed to the standards set by the Declaration of Helsinki. Subjects were recruited via newspaper advertisements and local postings. Informed written consent was obtained from healthy participants free of daytime sleepiness, sleep-disordered breathing or other medical disorders. Twelve individuals successfully completed the experimental protocol. All participants were non-flow limited by definition. The absence of sleep-disordered breathing was confirmed by sham studies in seven of these participants.

2.2 Breathing Circuit

Each participant was connected to the breathing circuit with an airtight silicone rubber mask, strapped and glued to the face to prevent leaks. The mask was connected to a Plateau Exhalation Valve® (Respironics, Inc, Pittsburgh, PA), via a heated pneumotachometer. The valve, which provides a continuous leak path in the breathing circuit and serves as an exhaust vent, was connected on the inspiratory line. Three cylinders containing the following gases: 100% nitrogen (N₂), 8% oxygen (FiO₂ 0.08%, balanced with N₂), or 100% O₂ were connected to the inspiratory line. To maintain isocapnia, supplemental carbon dioxide (CO₂) (FiCO₂ 0.07%, balanced with N₂), was added to the inspiratory line from an external source to maintain end-tidal PCO₂ (P_{ETCO2}) at or near control levels.

2.3 Measurements

Electroencephalograms (EEG), electrooculograms (EOG), and chin electromyograms (EMG) were recorded using the International 10–20 system of electrode placement (EEG: C3-A2 and C4-A1; EOG F7-A2, F8-A2, O-A2). Inspiratory airflow was measured by a heated

pneumotachometer (Model 3700A, Hans Rudolph, Kansas City, MO) that was attached to a pressure transducer (Validyne, Northridge, CA). The tidal volume (V_T) was obtained from the electronic integration of the flow signal (model FV156 Integrator, Validyne, Northridge, CA). Supraglottic airway pressure was measured using a pressure transducer tipped catheter (Model TC-500XG, Millar Instruments, Houston, TX), with the tip positioned in the hypopharynx. The hypopharyngeal position was obtained by advancing the catheter tip for 2 cm after it disappeared behind the tongue. P_{ETCO_2} was measured using air sampled continuously from the nasal mask by an infrared analyzer (Model CD-3A, AEI Technologies, Pittsburgh, PA). Arterial O_2 saturation (SaO_2) was measured by a pulse oximeter (Biox 3700, Ohmeda). The signals were displayed on a polygraph recorder (model 7-D, Grass, West Warwick, RI) and recorded using Biobench data acquisition software (National Instruments, Austin, TX) for further analysis. The above technique has been used previously (Aboubakr *et al.* 2001).

Surface inspiratory muscle EMG (EMG_{dia}) was recorded using two surface electrodes (3M Red Dot, 3M Company, St. Paul, MN) placed 2–4 cm above the right costal margin in the anterior axillary line. One pair was positioned at the percussed dullness at total lung capacity and another pair was positioned at the point of percussed dullness at functional residual capacity. The electrode pair with the best signal-to-noise ratio was selected for analysis. The EMG_{GG} was measured with a pair of unipolar intramuscular electrodes referenced to a single ground, thus producing a bipolar recording. Two stainless steel Teflon-coated 36-gauge wire electrodes were inserted 15–20 mm into the body of the genioglossus muscle 3 mm lateral to the frenulum of the tongue on each side, using a 25-gauge needle, which was quickly removed, leaving the wires in place. This technique has been described previously (Vitti *et al.* 1975).

2.4 Protocol

Experimental study—Participants assumed the supine position for the entire experimental protocol that was conducted during stable NREM sleep. All trials were conducted while subjects were in stable stage 2 or stage 3 sleep (Night 1). Twelve subjects without flow limitation (non-flow limited, NIFL) participated in this protocol. Ten cycles of episodic hypoxia were induced in each subject (Fig. 1). The subjects breathed room air for 5 minutes (control period), followed by hypoxia that was rapidly induced by having the subject breathe 1 or 2 breaths of 100% N_2 , followed by continuous 8% O_2 for 3 minutes to maintain hypoxia (SaO_2 :80–85%). Supplemental CO_2 was titrated to maintain isocapnia during hypoxia guided by P_{ETCO_2} on a breath-by-breath basis. After 3 minutes, hypoxia was abruptly terminated with one to two breaths of 100% O_2 . This was followed by 5 minutes of room air. The breathing pattern was monitored for 40 minutes of recovery period following the tenth cycle of hypoxia.

Sham study—Sham studies (Night 2) were conducted to ascertain the presence of time dependent changes in ventilation and EMG_{GG} , independent of the experimental protocol. For this purpose, seven of the twelve subjects were restudied during stable NREM sleep. Of the remaining five subjects, who had participated in the experimental study, three could not be contacted to return for the sham study and two did not agree to undergo a repeat study. The subjects who underwent sham studies were connected to the same breathing circuit with identical instrumentation except that they breathed ambient air for a study time that was equivalent to the study time of the experimental night.

2.5 Data Analysis

All analyses were done during stable NREM sleep at different time points: baseline room air, during each of the ten hypoxic periods and during recovery, at five (R_5), ten (R_{10}), twenty (R_{20}), thirty (R_{30}) and forty (R_{40}) minutes after the tenth hypoxic episode (H_{10}). Wakefulness and sleep stages were scored according to standard criteria (Rechtschaffen and Kales, 1968) and transient arousals were scored using the ASDA criteria (Task Force. 1992). The subjects

were required to be in stable stage 2 or stage 3 sleep during control period, hypoxic exposures and recovery period data collection. Trials were eliminated from analysis if there was any arousal or change in sleep stage to a lighter stage.

Inspired tidal volume (V_T), inspiration time (T_I), total time for a breath (T_{TOT}), breathing frequency (fR), minute ventilation ($\dot{V}_I$), P_{ETCO_2} , and arterial O_2 saturation were calculated breath by breath during stable sleep during the first normoxic period ('control' period), during each of the hypoxic (H) periods and during recovery at R₅, R₁₀, R₂₀, R₃₀ and R₄₀. To ensure that changes in ventilation were not due to subtle changes in sleep state, an independent observer confirmed the stability of sleep state. For each variable, a mean value was computed from ten consecutive breaths during each of the hypoxia trials and at R₅, R₁₀, R₂₀, R₃₀, and R₄₀, respectively. For the control period two sets of ten consecutive breaths were analyzed. Shkroukani *et al.* (2002) have previously described this methodology. For graphical comparisons, $\dot{V}_I$ data was normalized to the control period data and reported as a percentage of baseline activity.

To ascertain changes in upper airway mechanics, a pressure-flow loop was plotted for every breath for 20 breaths of the control period, for 10 breaths at each of hypoxic periods and for 10 breaths each at R₅, R₁₀, R₂₀, R₃₀, and R₄₀. Upper airway resistance (R_{UA}) at a maximum linear flow (peakflow, $\dot{V}_{peakflow}$) was computed from each loop as a numeric representation of the slope of the linear part of the pressure-flow loop. To ascertain the presence or absence of inspiratory flow limitation in each subject, the pressure-flow loop of each breath was analyzed. Flow limitation was defined as a plateau in flow despite ≥ 1 cmH₂O decrease in the supraglottic pressure. Inspiratory flow limitation (IFL) has been previously described in detail by our laboratory (Rowley *et al.* 2001; Aboubakr *et al.* 2001; Shkroukani *et al.* 2002, Mansour *et al.* 2002).

To analyze GG, the raw EMG signal was amplified, filtered with a band pass filter of 50–10,000 Hz (Grass model 7-D Polygraph) and full wave rectified. EKG artifacts were "blanked" with an EKG blanker (CWE model SB-1). The processed signal was integrated with a moving-time averager with a time constant of 100 milliseconds. (CWE model MA-821 RES.) Phasic EMG activity was determined from the moving time average. Diaphragm EMG was analyzed similarly (Aboubakr *et al.* 2001). The GG EMG activity was quantified as a percent of maximal activation. To define maximal GG EMG activity, subjects performed three maneuvers: each subject maximally inspired against an occluded inspiratory line, maximally protruded the tongue against the back of the teeth, and swallowed (Mezzanotte *et al.* 1992). Each of these maneuvers was performed several times, with the maximal value recorded (from any maneuver) being called 100%. Electrical zero was then determined, with subsequent muscle activity being quantified as a percentage of maximal activation for that individual. Peak EMG_{GG} was determined from the peak integrated activity of the moving time average and expressed in arbitrary units (au) (Mezzanotte *et al.* 1992). Phasic EMG_{GG} was calculated as the difference between peak and tonic EMG_{GG} . Again measurements were made for every breath for 20 breaths of the baseline control period (prior to trial 1), for 10 breaths of the control period (room air) prior to the onset of each of hypoxia trials (trials 2 to 10), for 10 breaths at each of hypoxic periods, and for 10 breaths each at R₅, R₁₀, R₂₀, R₃₀, and R₄₀, respectively. To ensure that changes in EMG_{GG} were not due to changes in sleep state, the segments chosen for analysis were those during stable sleep. For comparison, EMG_{GG} data were normalized to the control period data and reported as a percentage of baseline activity.

For the sham study, data were sampled on ambient air at periods corresponding to the individual's control period and a hundred and twenty minutes of study time. The latter period corresponds to the total duration of the episodic hypoxia trials and the recovery period during the experimental protocol. Accordingly, sham 'recovery' periods were analyzed at 85 and 100

minutes from the beginning of the control period and represent R_5 and R_{20} , respectively. As noted earlier, conducted sham studies were conducted on seven of twelve subjects.

Statistical Analysis—For normally distributed data, comparisons between time-points were made using one-way ANOVA with repeated measures, followed by post-hoc analysis for all pairwise comparisons using the Holm-Sidak method. If the normality test failed, then ANOVA on Ranks was performed followed by pair-wise comparisons test using the Tukey method. To ascertain the time course of increased EMG_{GG} , we plotted the phasic EMG_{GG} during successive room air and hypoxia periods, respectively, for each individual subject. We then performed linear regression analyses to determine the slope of EMG_{GG} , for each subject during the trials, and used paired t-tests to determine if the slopes of the regressions were significantly different than zero. In addition, Pearson's correlation analyses evaluated correlations between the EMG_{GG} activity at R_{20} and multiple demographic and physiologic variables (see below). We used multivariate linear regression analyses to determine which independent variables predicted EMG_{GG} during the recovery period. Several independent variables were considered for inclusion in the linear regression models, on the basis of an *a priori* reasoning that they might predict EMG_{GG} activity at R_{20} (dependent variable). The following independent variables were tested: 1) age, 2) gender, 3) BMI, 3) $\dot{V}_I$ and R_{UA} (as a % of control) during hypoxia, 5) percent change in oxygen saturation during the hypoxia trials, and 6) phasic EMG_{GG} during hypoxia. The level of statistical significance was set at $p < 0.05$. All results are presented as mean $\pm$ standard error of mean (S.E.M).

3. RESULTS

The demographic data of the subjects are given in Table 1. Twelve subjects participated in the study with satisfactory ventilation data; satisfactory GG signal during stable sleep was available during the first 20 minutes of recovery in 10 subjects. All selected subjects were non-snorers and had no evidence of inspiratory flow limitation by an *a priori* definition as described above. Thus, in these 12 individuals, only $8 \pm 3\%$ of breaths were flow limited during the control period.

3.1 Hypoxia Periods

During the episodic hypoxia trials, oxyhemoglobin saturation decreased to $87.1 \pm 0.4\%$. The P_{ETCO_2} during hypoxia was 38.9 ± 1.0 mmHg compared to room air control values of 40.3 ± 0.9 mmHg (Table 2). Trials were eliminated from analyses if there was any arousal or change in sleep stage to a lighter stage; a mean of 8 ± 0.6 trials were averaged for each individual. Overall, there was a significant increase in $\dot{V}_I$, V_T and $\dot{V}_{peakflow}$ during the episodic hypoxia trials, $p < 0.05$, but no significant change in R_{UA} . Likewise, phasic EMG_{GG} increased significantly to $202.7 \pm 24.1\%$ of control period ($p < 0.01$) (Table 2, Fig. 3). The increase in phasic EMG_{GG} was related to a corresponding increase in peak EMG with no significant change in tonic EMG_{GG} , therefore only phasic activity is reported. Satisfactory EMG_{dia} signals were available in 6 subjects; EMG_{dia} increased during hypoxia ($131.5 \pm 19.1\%$ of control). While this was a numerically increased value, it failed to reach statistical significance owing to small number of subjects.

3.2 Recovery Period

Figure 2 shows a representative polygraph segment from a subject during NREM sleep. Results for the group during recovery (R_5 , R_{10} and R_{20}) are shown in Table 2. Table 2 shows that there was no significant change in $\dot{V}_I$, V_T , $\dot{V}_{peakflow}$ or R_{UA} during recovery compared to the control period. The aforementioned increase in the phasic EMG_{GG} activity persisted into the recovery period. Phasic genioglossus EMG at R_{20} was $205.4 \pm 26.4\%$ of RA control. In contrast, during recovery $\dot{V}_I$ returned to RA control levels; $\dot{V}_I$ was 7.0 ± 0.6 L/min at R_{20} vs. 6.8 ± 0.6 L/min during control, $p = ns$, where the statistical power of the test was > 0.90 . Likewise, EMG_{dia} ($n = 6$

subjects) returned to baseline RA control values in the recovery period (Fig. 4). There was no change in R_{UA} during the hypoxia or the recovery periods.

Furthermore, we determined the duration of GG LTF by analyzing all the stable sleep epochs in the recovery period. Stable NREM sleep was maintained during R_{30} and R_{40} in five individuals from the group. GG LTF was apparent during R_{30} and R_{40} , as phasic EMG_{GG} remained elevated at $207 \pm 56.4\%$ of control, Fig. 3 ($p=ns$ due to small number of subjects during R_{30} and R_{40}); there was no evidence of ventilatory LTF as $\dot{V}_I$ was only $98.7 \pm 7.4\%$ of control during this period. In addition, exposure to episodic hypoxia resulted in a gradual increase of phasic EMG_{GG} that occurred between the hypoxic episodes on room air as well as during the hypoxic periods, Fig. 5A and 5B. The phasic EMG_{GG} data were plotted for each individual subject, then least square regression lines were calculated for each individual (see *Statistical Analysis*). The slopes for the regression lines were significantly different than zero ($p < 0.05$).

To ascertain the determinants of phasic EMG_{GG} during the recovery period, we used regression analysis to test several variables that were deemed as potential determinants of genioglossus activity (see *Data analysis*). Two determinants were identified; the level of phasic EMG_{GG} during hypoxia ($r=0.88$, $p < 0.001$, Fig. 6) correlated with the genioglossus activity during the recovery period. In contrast, there was a negative correlation between the age of the participant and EMG_{GG} at R_{20} ($r=-0.64$, $p=0.04$, Fig. 7). There was no correlation between the EMG_{GG} at R_{20} and gender, percent change in oxygen saturation during hypoxia, or change in $\dot{V}_I$ or R_{UA} during either hypoxia or recovery (Table 3). In a multivariate regression model, both age and EMG_{GG} during hypoxia significantly predicted phasic EMG_{GG} (model $r^2=0.93$, $p=0.001$). In addition, in Fig. 6, after excluding the far right data point, the coefficient was 0.6, $p=0.06$; but in the multiple linear regression model, the coefficient for phasic EMG_{GG} during hypoxia was significant, (coefficient: 0.7, $p < 0.01$), i.e., after adjusting for age the EMG_{GG} during hypoxia is a significant independent predictor of EMG_{GG} at R_{20} . Similarly in Fig. 7, even after excluding the highest left point there remained a significant negative significant correlation between recovery EMG_{GG} and age, $r=-0.74$, $p=0.04$; age remained a significant independent predictor in the multiple regression model, $p < 0.01$. While there was no significant correlation between recovery genioglossus activity and the change in saturation during hypoxia, the phasic EMG_{GG} activity during hypoxia tended to be positively correlated with the degree of desaturation achieved during the hypoxia trials ($r=0.5$, $p=0.07$).

3.3 Sham study

Seven subjects underwent the sham protocol and met the criteria for stable NREM sleep. Fig. 8 is a polygraph segment from a representative sham study. Averaged results for the sham studies are tabulated in Table 4. There were no significant changes in $\dot{V}_I$, V_T , T_I , respiratory frequency, peak flow or P_{SG} during the designated 'recovery' period at R_{20} . In addition, there was no significant change in EMG_{GG} phasic activity during recovery.

4. DISCUSSION

4.1 Summary of findings

This study tested the effect of episodic hypoxic exposure on LTF of the genioglossus (GG) muscle in normal non-flow limited individuals during stable NREM sleep. Our major findings are: 1) Hypoxia results in increased activity of the genioglossus muscle, without a significant change in the magnitude of the supraglottic pressure. 2) GG LTF occurs following episodic hypoxia in normal sleeping humans; no such effect was noted in the sham studies. 3) The two independent predictors of GG LTF were the level of GG activity during hypoxia and the age of the participant. This is the first report concerning the direct assessment of respiratory LTF following episodic hypoxia in phasic activity of the genioglossus muscle in normal sleeping

subjects. The data provide important confirmation that episodic hypoxia induces LTF in upper airway motor output during sleep in humans as it does in several anesthetized rodent or cat preparations. The findings suggest that respiratory LTF in upper airway motor output has the potential to be an important regulator of upper airway patency during sleep.

4.2 LTF of Genioglossus

We noted that episodic hypoxia, per se, elicited increased activity of the genioglossus muscle, independent of changes in upper airway mechanics. We also noted that the EMG_{GG} was elevated during the recovery period relative to RA control; supporting our hypothesis that episodic hypoxia elicits LTF of GG during sleep. Our findings corroborate previous animal studies that have shown LTF of hypoglossal neural activity in male rats (Fuller 2005; Zabka *et al.* 2005); neonatal rats (McKay *et al.* 2004); anesthetized and vagotomized rats (Bach & Mitchell, 1996); and vagotomized cats (Mateika and Fregosi, 1997). We also observed an augmentation of phasic EMG_{GG} in the normoxic intervals between hypoxic episodes as well as in the successive hypoxic episodes (Fig. 5). This progressive augmentation is characteristic of some studies (Turner and Mitchell, 1997; Millhorn *et al.*, 1980), and not others (Bach *et al.*, 1996). Interestingly, GG LTF occurred without accompanying changes in minute ventilation or upper airway resistance during the recovery period. Genioglossus LTF was directly correlated with the magnitude of the phasic genioglossus response during hypoxia, and inversely correlated with age through a limited range. The occurrence of GG LTF was not explained by the degree of ventilatory response during hypoxia or by gender. Notably, GG LTF significantly declined with increasing age, despite the narrow age range of our subjects, and even after adjusting for EMG_{GG} during hypoxia. There is evidence that respiratory LTF is age dependent in male rats (McGuire and Ling, 2005; Zabka *et al.* 2005). The loss of LTF is potentially an important factor for the loss of upper airway patency noted with increasing age. This may be clinically relevant as a decline in LTF GG with increasing age might contribute to increased prevalence of sleep-disordered breathing in the elderly population (Bixler *et al.* 1998). However, in order to exactly determine the effect of age on LTF, a larger sample size with subjects in different age categories would be required. Our study was not designed to specifically investigate changes in LTF with aging, and while it raises an interesting correlation between age and LTF it does not establish this relationship. In addition, loss of LTF probably only partly explains the loss of upper airway patency with age. Other factors may include increased pharyngeal collapsibility (Eikerman *et al.* 2007) and changes in pharyngeal anatomy (Malhotra *et al.* 2006).

The occurrence of GG LTF in sleeping humans is in contrast to studies during wakefulness that have shown no evidence of GG LTF (McEvoy *et al.* 1996, Jordan *et al.* 2002), unless episodic hypoxia was accompanied by hypercapnia (Harris *et al.* 2006). The discrepancy between wakefulness and NREM sleep vis-à-vis GG LTF could be attributed to higher upper airway resistance during sleep, augmenting negative supraglottic pressure and the magnitude of GG activation. However, our subjects did not demonstrate high upper airway resistance during sleep and, by study design, did not have evidence of flow limitation. Moreover, they did not demonstrate a mechanical consequence for GG LTF (see below). Conversely, studies in unanaesthetized rats have demonstrated that LTF is most prominent during sleep. Unanaesthetized rats exhibit tidal volume LTF following episodic hypoxia while in documented NREM sleep, but not when awake (Mahamed and Mitchell, 2007). Thus, a possible explanation is that sleep may be more conducive to the development of LTF. Given that LTF of hypoglossal nerve activity recorded in rats has been noted to be dependent on serotonin pathways (Bach and Mitchell, 1996, Fuller *et al.* 2001), the occurrence of GG LTF in non-snoring, non-flow limited individuals suggests that sleep, per se, may be a determinant of LTF. This may relate to the fact that medullary serotonergic neuronal activity is decreased during NREM sleep, and discharges at higher, nearly maximal levels during wakefulness

(Veasey *et al.* 1995). Differences in the basal serotonergic neuronal activity may explain why the peak magnitude and duration of changes in ventilation in the awake goat (+70%, 40 min, Turner *et al.* 1997) and dog (+40%; Cao *et al.* 1992) are somewhat lower than that recorded in anaesthetized rats (+70 to +100%, >60 min; Hayashi *et al.* 1993; Bach and Mitchell, 1996). Similar findings are noted during sleep where the average firing rate of raphe neurons in cats decreased during slow wave and rapid eye movement sleep compared to alert waking state and correlated with increases in ventilation (Veasey *et al.* 1995). Thus, decreased firing rate of the raphe neurons during NREM sleep relative to wakefulness may permit a greater dynamic range of increased caudal raphe neuron activity with episodic hypoxic stimulation.

4.3 Absence of ventilatory or mechanical LTF

Our study demonstrated no evidence of ventilatory LTF, corroborating other studies in neonatal rats (McKay *et al.* 2004), where LTF was expressed primarily as an increase in GG EMG burst amplitude, without a significant change in either ventilatory or timing parameters. Likewise, our findings corroborate previous work from our laboratory demonstrating no evidence of diaphragmatic LTF (Babcock and Badr, 1998) and the study by Mateika and Fregosi (1997) demonstrating, LTF of the genioglossus and the alae nasae without diaphragmatic LTF in vagotomized cats.

The aforementioned studies suggest that upper airway neuromuscular LTF would lead to ventilatory LTF only if the electrical activity of the genioglossus is translated into a mechanical response, dilating the upper airway and reducing resistance to flow (Brennick *et al.* 2007). Thus, the manifestations of LTF depend upon the mechanical properties of the upper airway. Accordingly, the mechanical and ventilatory manifestations of increased upper airway neuromuscular activity are more pronounced in individuals with inspiratory flow limitation (IFL) relative to those who do not display IFL. Episodic hypoxia in *snoring* subjects is followed by decreased R_{UA} and an increased $\dot{V}_I$ during the recovery period (Shkroukani *et al.* 2002). In addition, ventilatory LTF was abolished when IFL was eliminated using nasal CPAP (Babcock *et al.* 2003). Conversely, in the current study, low upper airway resistance and the absence of flow limitation would explain the dissociation between the occurrence of GG LTF and a ventilatory or mechanical consequence. Likewise, Schnall *et al.* (1995) have demonstrated that electrical stimulation of the genioglossus results in reduced upper airway resistance only if the upper airway is narrowed. Our methodology for measuring upper airway resistance is quite accurate, sensitive and was previously validated (Mansour *et al.* 2002, Shkroukani *et al.* 2002). Clark *et al.* (1998), also have used the relationship between pressure and flow to calculate resistance as a “gold-standard” to study upper airway resistance and flow limitation. Nevertheless, upper airway muscle activity and upper airway resistance are two distinct constructs that may be altered independently (Schnall *et al.* 1995). Our subjects were non-snorers and have a favorable upper airway anatomy, as evidenced by the absence of flow limitation. Activation of the GG muscle is unlikely to lead to upper airway dilatation or decreased resistance. Likewise, Fuller *et al.* (1999) showed that co-activation of the upper airway muscles, in a rodent model, decreased pharyngeal collapsibility but did not dilate the pharyngeal airway. Accordingly, we speculate that the role of GG may be to stiffen the airways and prevent collapse, rather than dilate, in sleeping normal non-flow limited individuals.

4.4 Implications

Long-term facilitation of the GG EMG following episodic hypoxia may contribute to the maintenance of upper airway patency during sleep in normal humans. Although we have not studied GG LTF in sleep apnea patients, it is likely that upper airway dilating muscle (GG) activity was responsible for the decreased upper airway resistance observed in the aftermath of episodic hypoxia (Aboubakr *et al.* 2001) indicating an improvement in upper airway mechanics. The occurrence of GG LTF and the ensuing improvement in upper airway

mechanics may represent a protective mechanism mitigating upper airway narrowing and preserving alveolar ventilation following recurrent upper airway obstruction, and episodic hypoxia in sleep apnea. The LTF of upper airway dilating muscles in humans during sleep may restore upper airway patency following a series of apneas and hypopneas. Thus, we speculate that GG LTF may influence the expression of apneas during sleep.

The other implication of our findings is the observation that GG LTF inversely correlates with age, even within the narrow age range in our study sample. The decline in LTF with increasing age may render the aging individual more vulnerable to breathing instability during sleep explaining the increased prevalence of sleep-disordered breathing in the elderly (Bixler *et al.*, 1998). Studies with larger sample size are required to confirm this relationship.

4.5 Methodological considerations

Several methodological factors have to be considered for proper interpretation of our findings. First, we excluded trials and epochs with unstable sleep. Thus, the data reported here were from periods with stable sleep state with no change in sleep state. Second, although we attempted to carefully maintain precise isocapnia during hypoxia, a small reduction in P_{ETCO_2} did occur (Table 2). Mild hypocapnia might have dampened the magnitude of LTF. However, we were able to demonstrate GG LTF in these same individuals. Third, the degree of hypoxia may have been inadequate. However, a similar level of hypoxia has been attained in our prior studies with demonstration of LTF of $\dot{V}_I$, so the level of hypoxia alone does not explain the lack of rise in $\dot{V}_I$ during recovery. Fourth, we measured the activity of the genioglossus muscle as representative of phasic upper airway dilators. However, other upper airway muscles, such as the tensor palatini, have a different pattern of activation and different innervations. In addition, whether LTF can be elicited in other upper airway muscles, including the tensor palatini and tongue retractors, cannot be determined from our data. Likewise, increased drive to the GG does not necessarily result in shortening of the muscle, i.e. electrical activation does not necessarily correlate with mechanical activity or with shortening of muscle fibers (Brennick *et al.* 2001, Fuller *et al.* 1998). Fifth, sham studies could not be done in all subjects as planned (explained in *Methods*). However, we do not think that a persistent rise in phasic EMG_{GG} during the recovery period can be explained by time effect or by artifact alone. Sixth, we cannot be certain that we measured precisely EMG_{dia} signals given the surface location of the electrodes. In addition, satisfactory EMG_{dia} signals were available in only 6 subjects limiting meaningful statistical analysis for EMG_{dia} . Finally, although full polysomnography was not done beforehand, to determine the absence or presence of obstructive sleep apnea, subjects were recruited based on the absence of symptoms or risk factors for obstructive sleep apnea. In addition, the seven sham studies did not reveal evidence of sleep apnea and all 12 subjects were non-flow limited subjects by definition.

In summary, our study results have demonstrated that episodic hypoxia during sleep in normal subjects elicits long term facilitation of the GG muscle manifested by increase EMG_{GG} activity but without a change in ventilation or upper airway mechanics.

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Figure 1.
Algorithm of the study protocol.

Figure 2.

Representative polygraph segments from a subject during stable NREM sleep at different time points: **A.** Compressed, high-speed polygraph segments during room air control condition, hypoxia periods (**H1, H2, H3, H10**) and recovery (**R5, R10, R15** and **R20**). Note elevated phasic EMG_{GG} amplitude during isocapnic hypoxia that persists during recovery relative to control; V_T is increased during hypoxia alone. **B.** Polygraph at usual speed, for better visualization of EEG, EMG_{GG} moving time average and EMG_{dia}, clearly demonstrates increase in phasic EMG_{GG} in during recovery period relative to control.

Effect of episodic hypoxia on GG EMG and Ventilation

Figure 3.

Group data (all subjects) for phasic EMG_{GG} and $\dot{V}_I$ during hypoxia and recovery periods normalized to baseline as a percent of control. **H** represents averaged data of the hypoxia trials while **R₅, R₁₀, R₂₀, R₃₀, R₄₀** are averaged data as a percent of room air control during recovery 5, 10, 20, 30 and 40 minutes, respectively. Both $\dot{V}_I$ and phasic EMG_{GG} activity increased significantly during hypoxia vs room air control. However, only EMG_{GG} phasic activity was significantly increased during recovery periods **R₅, R₁₀, and R₂₀**, as a percent of room air control; ** $p < 0.05$, hypoxia vs control period; * $p < 0.01$, hypoxia, R₅, R₁₀, R₂₀ vs control period. While phasic EMG_{GG} remained elevated in **R₃₀** and **R₄₀**, the values did not reach statistical significance due to the small number of subjects with stable sleep during this period, see text for details.

Figure 4.

Group data for 6 subjects with satisfactory EMG_{dia} signals are presented. Data for phasic EMG_{GG} , $\dot{V}_I$, EMG_{dia} and V_T during hypoxia and recovery period (R_{20}) were normalized to baseline as a percent of control. Phasic EMG_{GG} , $\dot{V}_I$, and V_T , all increased significantly during hypoxia vs room air control. However, only phasic EMG_{GG} activity was significantly increased during R_{20} ; * $p < 0.05$.

Figure 5.

Least square regression analysis: **A.** There was a gradual increase in the phasic EMG_{GG} during the course of the room air control periods. The slope of the regression line was significantly different than zero ($p < 0.05$) and illustrates the augmentation of LTF in the normoxic intervals *between* hypoxic episodes (RA2 to RA10). **B.** There was also a progressive augmentation of the EMG_{GG} during successive hypoxia episodes (HX1 to HX10); slope of the regression line was significantly different than zero ($p < 0.05$).

Figure 6.

Demonstrates a significant positive relationship between phasic EMG_{GG} (% control) during recovery at 20 minutes and phasic EMG_{GG} (% control) during hypoxia. This correlation declined to $r=0.6$, $p=0.06$ after eliminating the highest right point (marked with X); but when adjusted for age in the multiple regression model, the coefficient of EMG_{GG} during hypoxia was significant, see text for explanation.

Figure 7.

Demonstrates a significant negative relationship between the phasic EMG_{GG} (% control) during recovery at 20 minutes and age (years). This correlation remained significant, $r=-0.74$, $p=0.044$ even after deleting the highest left point (marked with **X**).

Figure 8.

Representative polygraph segment from a sham protocol during stable NREM sleep. **A** represents room air control condition, while **B** represents 85 minutes and **C** represents 100 minutes, corresponding to the recovery periods at R₅ and R₂₀, respectively, of the subject's experimental study. Note unchanged V_T, P_{SG} and EMG_{GG} during **B** and **C** compared to segment **A** of the study.

Table 1

Subject demographics (n=12).

Variable	Results
Age (years)	25.8±2.0
Male/Female (n)	7/5
Neck circumference (cm)	35.7±1.1
BMI (kg/m ²)	22.4±1.3

Values are mean±SEM, BMI body mass index

Table 2
Group data for ventilation, upper airway mechanics and EMG during control, hypoxia and recovery periods.

Variables	Control	Hypoxia	R ₅	R ₁₀	R ₂₀
T _I , sec	1.7±0.1	1.6±0.1	1.7±0.1	1.6±0.1	1.6±0.1
T _E , sec	2.1±0.1	2.1±0.1	2.2±0.1	2.1±0.1	2.2±0.1
f _R , breath/min	16.4±0.9	16.6±0.8	16.1±0.8	16.4±0.9	16.5±0.6
P _{ETCO₂} (mmHg)	40.3±0.9	38.9±1.0	40.7±1.0	40.8±1.2	40.1±1.0
V _I , L/min	6.8±0.6	8.5±0.7**	6.7±0.5	6.9±0.6	7.0±0.6
V _T , L	0.42±0.31	0.51±0.32**	0.44±0.33	0.47±0.45	0.45±0.37
R _{UA} , cmH ₂ O/L/sec	5.8±1.0	4.9±0.7	5.9±0.8	5.5±1.1	6.3±0.9
P _{SG} cmH ₂ O	-2.6±0.3	-3.0±0.4	-2.5±0.4	-2.6±0.4	-2.8±0.4
V _{peakflow} , L/sec	0.32±0.03	0.40±0.03**	0.32±0.02	0.31±0.03	0.33±0.03
EMG _{GG} , % control	-	202.7±24.1*	198.9±30.9*	203.6±29.9*	205.4±26.4*

Values are mean±SEM

* p<0.01 recovery, hypoxia vs control period.

**

p<0.05 hypoxia vs control, T_I:inspiratory time, T_E:expiratory time, f_R:respiratory rate, P_{ETCO₂}:end-tidal CO₂ level, V_T:tidal volume, V_I:minute ventilation, V_T:tidal volume, R_{UA}:upper airway resistance, P_{SG}:nadir supraglottic pressure, V_{peakflow}:peak flow, EMG_{GG}:phasic EMG; R₅ R₁₀ R₂₀ recovery at 5, 10 and 20 minutes after hypoxia, respectively. EMG_{GG} is expressed as percent of the control values.

Table 3

Results of correlation analyses to determine relationship between recovery EMG_{GG} activity during NREM sleep and demographic and physiologic variables.

Variables	Correlation coefficient	p-value
EMG _{GG} during hypoxia (% control)	0.88	<0.001
Age (years)	-0.64	0.04
R _{UA} during hypoxia (% control)	-0.14	0.7
Gender	0.04	0.9
BMI (kg/m ²)	0.09	0.8
Percent change in SaO ₂	0.46	0.1
$\dot{V}_I$ during hypoxia (% control)	0.36	0.3
See text for explanation.		

Table 4

Ventilation, upper airway mechanics and EMG_{GG} during the control and recovery (R₂₀) periods of the sham protocols, n=7.

Variables	Control	Sham R ₂₀
T _I , sec	1.7±0.1	1.8±0.1
T _E , sec	2.1±0.2	2.0±0.1
f _R , breath/min	16.3±0.7	16.2±0.6
P _{ETCO₂} (mmHg)	38.5±1.7	38.6±1.6
$\dot{V}_T$, L/min	6.6±0.7	6.5±0.6
V _T , L	401.3±32.8	387.6±33.1
R _{UA} , cmH ₂ O/L/sec	6.7±1.0	7.1±1.3
P _{SG} , cmH ₂ O	-2.9±0.4	-2.8±0.5
$\dot{V}_{peakflow}$, L/sec	0.32±0.02	0.33±0.02
EMG _{GG} , % of control	--	111.4±4.2

Data are expressed as mean±SEM; no significant change in measured variables during sham R₂₀ compared to control period. See Table 2 for abbreviations.