

# Genioglossus reflex inhibition to upper-airway negative-pressure stimuli during wakefulness and sleep in healthy males

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During wakefulness, obstructive sleep apnoea patients appear to compensate for an anatomically narrow upper airway by increasing upper airway dilator muscle activity, e.g. genioglossus, at least partly via a negative-pressure reflex that may be diminished in sleep. Previous studies have assessed the negative-pressure reflex using multi-unit, rectified, moving-time-average EMG recordings during brief pulses of negative upper-airway pressure. However, moving-time averaging probably obscures the true time-related reflex morphology, potentially masking transient excitatory and inhibitory components. This study aimed to re-examine the genioglossus negative-pressure reflex in detail, without moving-time averaging. Bipolar fine-wire electrodes were inserted per orally into the genioglossus muscle in 17 healthy subjects. Two upper airway pressure catheters were inserted per nasally. Genioglossus EMG reflex responses were generated via negative-pressure stimuli ( $\sim -10$  cmH<sub>2</sub>O at the choanae, 250 ms duration) delivered during wakefulness and sleep. Ensemble-averaged, rectified, genioglossus EMG recordings demonstrated reflex activation (onset latency  $26 \pm 1$  ms; peak amplitude  $231 \pm 29\%$  of baseline) followed by a previously unreported suppression (peak latency  $71 \pm 4$  ms;  $67 \pm 8\%$  of baseline). Single-motor-unit activity, clearly identifiable in  $\sim 10\%$  of trials in six subjects, showed a concomitant increase in the interspike interval from baseline ( $26 \pm 9$  ms,  $P = 0.01$ ). Genioglossus negative-pressure reflex morphology and amplitude of the initial peak were maintained in non-rapid eye movement (NREM) sleep but suppression amplitude was more pronounced during NREM and declined further during REM sleep compared to wakefulness. These data indicate there are both excitatory and inhibitory components to the genioglossus negative-pressure reflex which are differentially affected by state.

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There are several protective respiratory reflexes that are activated during acute periods of increased respiratory load or airway occlusion. The genioglossus (gg) is the largest upper-airway dilator muscle and has been shown in animals and humans to be reflexively activated in response to negative upper-airway pressure to oppose upper-airway collapse (Mathew *et al.* 1982*a,b*; Horner *et al.* 1991*b*). Our current understanding of the basic reflex derived from multi-unit recordings suggests that pressure-sensitive nerve endings in the pharyngeal airway respond to rapid changes in negative upper-airway pressure. Sensory information is relayed via the superior laryngeal nerve and arrives at the nucleus tractus solitarius. The motor output to the muscle originates at the nearby

hypoglossal motor nucleus and is relayed to the muscle via the hypoglossal nerve (Mathew, 1984; Horner *et al.* 1991*a*; Ryan *et al.* 2001; White, 2005). In obstructive sleep apnoea, the pharyngeal airspace posterior to the gg is a common site for upper-airway collapse during sleep. Previous studies suggest that the gg negative-pressure reflex is largely attenuated during sleep, thereby contributing to the development of sleep-disordered breathing in individuals with an anatomically narrow upper airway (Wheatley *et al.* 1993; Horner *et al.* 1994; Shea *et al.* 1999). However, recent data demonstrating maintenance of, and in many individuals increased reflex activity to negative-pressure pulse stimuli in the supine position during non-rapid eye movement (NREM)

sleep, suggest that gg reflex attenuation may be position dependent (Malhotra *et al.* 2004).

Early quantification of the gg negative-pressure reflex in humans was performed using bipolar intraoral surface electrodes (Horner *et al.* 1991*b*; 1994). In these studies, data were expressed as a moving-time-average (10 ms) of the rectified, ensemble-averaged, electromyogram (EMG) to relatively few negative-pressure pulse stimuli ( $n = 6$ ). Surface EMG recordings have a relatively poor signal-to-noise ratio compared with intramuscular recordings (Bouisset & Maton, 1972; Perry *et al.* 1981) and the use of sliding-window averages distorts the details of the electromyographic response such as the relationship between excitatory and inhibitory responses, and particularly their latencies. Thus, elucidation of the precise morphology of the gg negative-pressure reflex was not possible from these early studies.

Other studies of the gg negative-pressure reflex have used intramuscular electrodes and increased the number of trials to improve the signal-to-noise ratio (Wheatley *et al.* 1993; Shea *et al.* 1999; Shea *et al.* 2000; Malhotra *et al.* 2004). However, in these studies, reflex characteristics were examined using even longer moving-time averaging windows (50–100 ms) of the rectified EMG. While moving-time averaging techniques are convenient for quantifying tonic and phasic respiratory muscle activity under the relatively slow time-constant conditions of tidal breathing, they inevitably blunt and distort more rapid respiratory reflex responses. Transient short-latency excitatory and/or inhibitory components of the negative-pressure reflex are likely to be beyond the resolution of these techniques. Thus, this study aimed to investigate the characteristics of the gg negative-pressure reflex using more sensitive neurophysiological techniques.

## Methods

### Subject selection

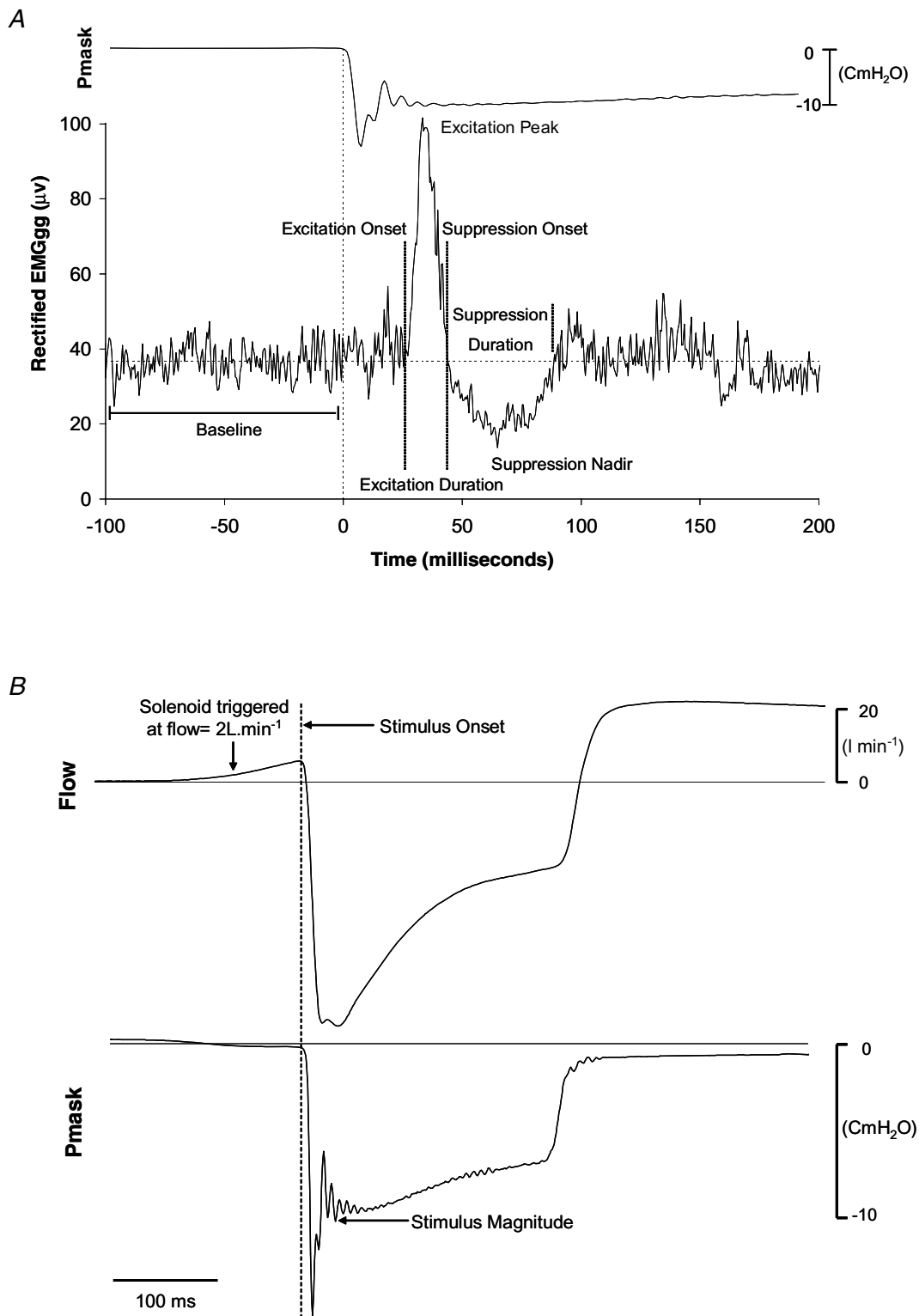
Eighteen healthy, young, non-smoking males, with no history of respiratory disease, sleep-disordered breathing, or regular medication use, and with baseline forced expiratory volume in 1 s (FEV<sub>1</sub>) and forced vital capacity (FVC) > 80% of predicted gave informed written consent to participate in the study. The study conformed to the standards set by the *Declaration of Helsinki* and was approved by the Daw Park Repatriation General Hospital and Adelaide University Human Research and Ethics Committees.

### Measurements and equipment

Subjects were instrumented with electroencephalograms (C3 and C4 referenced to linked ears), left and right electrooculograms and submental EMG for sleep staging

and arousal scoring. The nostrils were decongested with xylometazoline hydrochloride nasal spray (Otrivin, Novartis Australasia, Rowville, Victoria, Australia) and anaesthetized (2% lignocaine). Two custom-made air-perfused catheters (see Jordan *et al.* (2003) for further detail) were then inserted via the more patent nostril and attached to pressure transducers (MP45, Validyne Engineering, Northridge, CA, USA). One catheter was advanced 1–2 cm below the base of the tongue under direct visualization ( $P_{\text{epi}}$ ) and the other to the level of the choanae ( $P_{\text{cho}}$ ). Two fine-wire Teflon-coated intramuscular electrodes (316SS3T wire, Medwire, Mt Vernon, NY, USA) were inserted after surface anaesthesia (4% lignocaine) 4 mm either side of the frenulum to a depth of approximately 1–1.5 cm to measure gg EMG activity (EMG<sub>gg</sub>). These procedures were similar to those previously described (Mezzanotte *et al.* 1992; Eastwood *et al.* 2003). Subjects were fitted with a nasal mask (Gel mask, Respironics, Murraysville, PA, USA) with a two-way non-rebreathing valve attached (series 2600, Hans Rudolph, Kansas City, MO, USA). An additional pressure transducer was fitted to the mask to measure mask pressure ( $P_{\text{mask}}$ ). A pneumotachograph (PT36, Erich Jaeger, Germany) in series was used to monitor inspiratory flow. Upper airway negative-pressure pulses ( $P_{\text{cho}} \sim -10$  cmH<sub>2</sub>O, 250 ms duration) were delivered during early inspiration via a computer-controlled rapid-actuating solenoid valve system (Iso star, SXE9575-A70-00, Norgren, Switzerland). One arm of the solenoid was connected to a negative-pressure reservoir evacuated to  $\sim -100$  cmH<sub>2</sub>O via hospital suction, the other to air at atmospheric pressure. A pressure-limiting valve on the patient side of the solenoid valve was adjusted to achieve a  $P_{\text{cho}}$  pulse pressure of  $\sim 10$  cmH<sub>2</sub>O during wakefulness. The breathing circuit was similar to that previously described (Horner *et al.* 1991*b*). Negative-pressure pulse delivery was controlled via custom-written software that continuously monitored the inspiratory flow signal and triggered solenoid valve switching during early inspiration when flow reached  $2 \text{ l min}^{-1}$ . Pulses were delivered at random during stable breathing every 2–10 breaths. Figure 1*B* displays an example tracing of the inspiratory flow profile during negative pressure pulse delivery.

Data were acquired simultaneously on two separate recording systems. The first system (Compumedics E series, Abbotsford, Victoria, Australia) was used to determine sleep stage and to score arousals. All other data were acquired using the Windaq data acquisition system (DI-720 DATAQ Instruments Inc, OH, USA). In order to capture high-frequency reflex components and synchronize key stimulus magnitude parameters for event-related analysis, inspiratory flow, EMG<sub>gg</sub> and pressure channels were sampled at 2 kHz and filtered at 30–1000 Hz. The remaining channels that were not directly used for reflex-related synchronization purposes were



**Figure 1. EMG<sub>gg</sub> negative-Pressure reflex quantification procedures and stimulus characteristics.**  
 A, EMG<sub>gg</sub> reflex morphology and reflex quantification procedures. Ensemble-average of the rectified EMG<sub>gg</sub>, showing the reflex response of the genioglossus muscle and mask pressure tracing (P<sub>mask</sub>) to 70 negative-pressure pulses in a representative subject. B, the ensemble-average flow and mask pressure tracings to negative pressure-pulse stimuli in a representative subject. The vertical dashed line at time zero corresponding to the first rapid dip in mask pressure (P<sub>mask</sub>) was used to define stimulus onset. Note that the pressure pulse stimuli were delivered in early inspiration (flow tracing) and the point at which stimulus magnitude was quantified (P<sub>mask</sub> tracing); refer to the text for further detail.

sampled at 200 Hz. An event mark was simultaneously placed on both recording systems coincident with solenoid activation of each pulse, allowing both data acquisition systems to be accurately linked in time.

## Protocol

**Preliminary visit.** Subjects attended a preliminary visit during the day for familiarization with the testing environment, recording equipment and staff, and to provide informed consent. Spirometry was performed to ensure normal lung function (JLab software version 4.53; Compactlab, Jaeger, Wuerzburg, Germany).

**Main experimental visit.** Subjects arrived at the laboratory 2.5 h before their usual bedtime after abstaining from alcohol and caffeine for at least 12 h. Once all the recording equipment was applied, several negative-pressure pulses were delivered to familiarize subjects with the experimental intervention, after which the lights were switched off and subjects were allowed to sleep. During the night, in the event that the subject became uncomfortable maintaining the supine posture, they were given the opportunity to stretch before returning to sleep on their back.

After at least 20 min of stable, stage 2 NREM sleep, upper-airway negative-pressure pulses were delivered every 2–10 breaths. In the event of an arousal from sleep, pulses were not resumed until there was at least 1 min of arousal-free sleep. After subjects awoke the following morning, a total of approximately 60 pulses were delivered every 2–10 breaths to elicit EMG<sub>gg</sub> reflex responses during wakefulness.

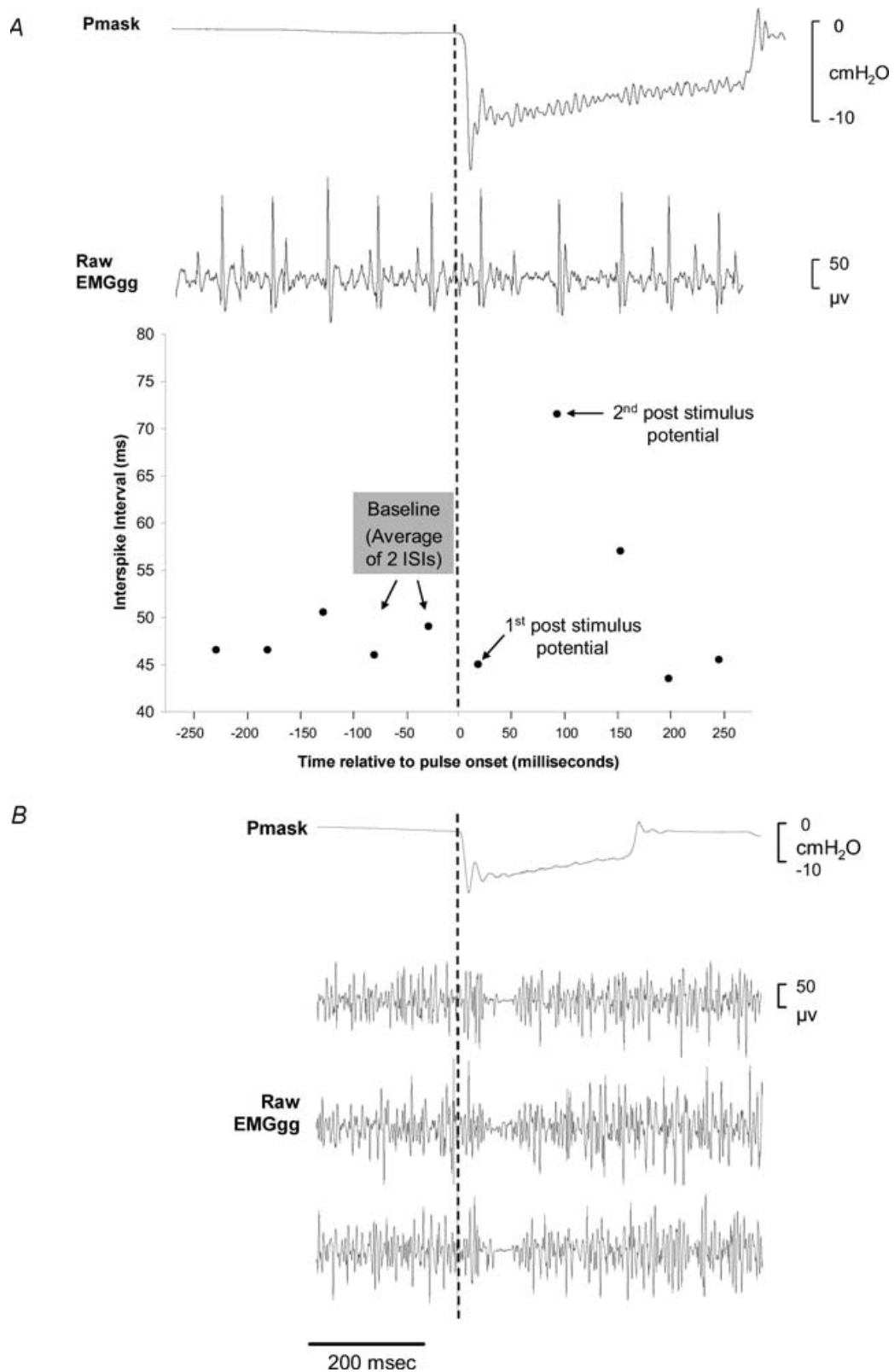
## Data analysis

A single trained sleep technician, blinded to the experimental manipulations, defined the presence of arousals and sleep stage according to standard criteria (Rechtschaffen & Kales, 1968; American Sleep Disorders Association, 1992). Custom-designed software to detect the most rapid change in  $P_{\text{mask}}$  during pulse presentation was employed to align each individual pulse to an accurately identifiable and highly reproducible reference point for EMG<sub>gg</sub> event-related analyses. Briefly, on breaths preidentified as having had a negative-pressure pulse presented, the software identified the point in  $P_{\text{mask}}$  at which the change in pressure with respect to time (i.e. slope) was most negative. This point was then used to time-align all replicate pulses for ensemble averaging. Stimulus onset (time zero) was defined in the conventional manner as the last point preceding

the sudden decrement in the ensemble-averaged  $P_{\text{mask}}$  following solenoid activation (Fig. 1). Negative-pressure pulse stimulus magnitude was calculated as the minimum pressure after the initial 'ringing' observed in the pressure channels (Fig. 1B) as previously described (Horner *et al.* 1994). Stimulus onset and rise times were quantified as the time from breath onset to the first sudden deflection in  $P_{\text{mask}}$ , and from this deflection point to the nadir of  $P_{\text{mask}}$ , respectively.

For each subject, all EMG<sub>gg</sub> trials free from movement artifact and arousal were grouped and ensemble-averaged according to sleep state. The various sleep states examined were (1) wakefulness; (2) stage 2 NREM sleep; (3) stages 3 and 4 NREM sleep combined (slow wave sleep (SWS)); (4) stages 2, 3 and 4 NREM sleep (combined NREM sleep); and (5) rapid eye movement sleep (REM). Raw EMG<sub>gg</sub> recordings were rectified without moving-time averaging for each subject. Individual subjects' ensemble-averaged, rectified EMG<sub>gg</sub> reflex responses were visually inspected to identify, using custom-designed semi-automated software, the presence, timing and amplitude of each positive and negative component of the EMG<sub>gg</sub> response. An example of a typical EMG<sub>gg</sub> reflex response and the criteria used to define the various reflex characteristics are displayed in Fig. 1. EMG<sub>gg</sub> reflex amplitude data were expressed as a percentage of baseline calculated as the average EMG<sub>gg</sub> activity for the 100 ms preceding pulse onset as previously described (Butler *et al.* 1997; Jeffery *et al.* 2006). Excitation onset was defined as the point at which the rectified EMG<sub>gg</sub> signal crossed baseline prior to the clearly defined positive EMG<sub>gg</sub> waveform. Suppression onset was defined as the first point at which the rectified EMG<sub>gg</sub> recording crossed the baseline level following the peak of the excitation response. The first point at which the rectified EMG<sub>gg</sub> returned to baseline levels after the suppression nadir was used to define the cessation of suppression.

Where available, EMG<sub>gg</sub> single-motor unit discharges (e.g. Figure 2A) were examined throughout the negative-pressure pulse application. The baseline activity of a motor unit was defined as the average of two prestimulus inter-spike intervals (ISIs) calculated from adjacent motor-unit action potentials (i.e. three ISIs) (see Fig. 2A for further detail). The ISI of the unit for the first and second potential after pulse onset was also calculated. In order to perform group comparisons, baseline, first and second potential ISIs of six negative-pressure pulses selected at random were averaged in each subject in whom single-motor-unit EMG<sub>gg</sub> activity was clearly identifiable. The average ISI during the 100 ms baseline period (early inspiration) was also compared to the adjacent preceding expiration period (100 ms average between 400 and 500 ms prior to pulse delivery) for each pulse in order to assist with single-unit discharge pattern classification.



**Figure 2. EMG<sub>gg</sub> Single motor unit and raw EMG<sub>gg</sub> multi-unit responses to negative-pressure pulse stimuli.**

*A*, example of mask pressure ( $P_{\text{mask}}$ ), raw EMG<sub>gg</sub> recording with a prominent single motor unit, and the interspike interval of the motor unit during a single pulse presentation in one subject. Note the clear increase in the interspike interval of the motor unit after the first post-stimulus potential. *B*, examples of the mean mask pressure ( $P_{\text{mask}}$ ) and raw multi-unit EMG<sub>gg</sub> recordings to three separate negative-pressure pulse stimuli in one subject.

## Statistical procedures

Repeated measures analysis of variance (ANOVA) was used to examine sleep state effects (awake, stage 2 NREM, SWS) for each EMG<sub>gg</sub> reflex peak amplitude component (i.e. the excitation and suppression peaks) and each EMG<sub>gg</sub> reflex latency component (i.e. excitation onset, excitation duration, suppression onset, suppression peak, suppression duration). Similarly, for comparisons incorporating REM data, repeated measures ANOVA was used to examine sleep state effects (awake, all NREM and REM) for EMG<sub>gg</sub> reflex inhibition amplitude and each EMG<sub>gg</sub> reflex latency component (i.e. inhibition onset, inhibition peak and inhibition duration). Repeated measures ANOVA was also used to examine the effect of negative-pressure pulse stimuli on EMG<sub>gg</sub> single-motor-unit discharges (baseline, first and second potentials). Where significant main effects were observed, *post hoc* comparisons were performed using Dunn–Sidak adjusted Student's paired *t* tests (Ludbrook, 1991). Statistical significance was inferred when  $P < 0.05$ . All data are reported as means  $\pm$  s.e.m.

## Results

### Anthropometric characteristics and sleep architecture

Reflex data were collected in 17 of the 18 subjects during sleep and 16 subjects during wakefulness. One subject demonstrated significant sleep-disordered breathing early in the night, and the study was stopped at that point. *Post hoc* sleep-stage analysis revealed that one subject spent the majority of the wakefulness period drifting in and out of stage 1 sleep such that there were too few replicate trials to generate an ensemble-averaged reflex response during wakefulness in this subject. The mean age and body mass index of the 17 subjects were  $24 \pm 1$  years and  $24 \pm 1 \text{ kg m}^{-2}$ , respectively. Subjects had normal lung function (Mean FEV<sub>1</sub>  $107 \pm 4\%$  and FVC  $108 \pm 3\%$  of predicted). Sleep architecture data are summarized in Table 1.

### Genioglossus negative-pressure reflex during wakefulness and NREM sleep

Inspiratory phasic EMG<sub>gg</sub> activity was observed in all subjects. There were no differences in the average EMG<sub>gg</sub> activity in the 100 ms prior to pulse onset during wakefulness compared to NREM sleep ( $19.7 \pm 5.3$  versus  $20.2 \pm 5.1 \mu\text{V}$ ,  $P = 0.954$ ). Negative-pressure pulse stimuli presented during wakefulness resulted in a short-latency peak followed by prolonged suppression of the rectified EMG<sub>gg</sub> in all subjects. The morphology of this reflex response was maintained during NREM sleep. Examples of the morphology of the EMG<sub>gg</sub> reflex response in two

**Table 1. Sleep architecture data**

Characteristic	
Sleep-onset latency (min)	$17 \pm 7$
Total sleep time (min)	$233 \pm 12$
Sleep efficiency (%)	$68 \pm 4$
Stage 1 (% total sleep time)	$15 \pm 3$
Stage 2 (% total sleep time)	$52 \pm 3$
SWS (% total sleep time)	$29 \pm 4$
REM (% total sleep time)	$5 \pm 1$
Arousal Index (arousals h <sup>-1</sup> )	$24 \pm 3$

Slow wave sleep (SWS), rapid eye movement sleep (REM). Values are means  $\pm$  s.e.m. ( $n = 17$  subjects).

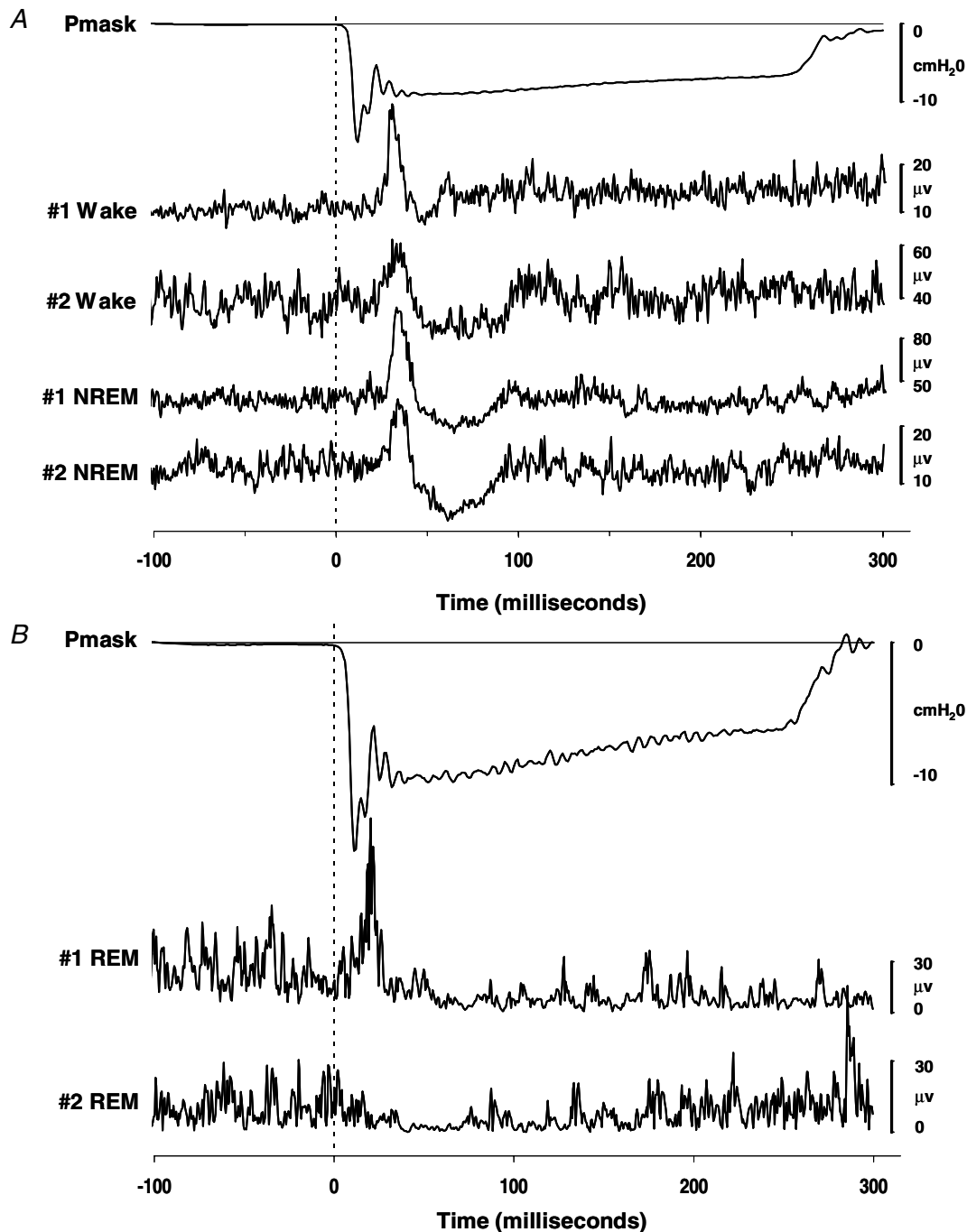
individual subjects during wakefulness and NREM sleep are displayed in Fig. 3A.

The number of stimulus presentations, peak reflex amplitudes, timing and stimulus properties during wakefulness, stage 2 NREM and SWS are summarized in Table 2. There were no differences in the number of artifact-free stimulus presentations, EMG<sub>gg</sub> reflex component amplitudes, latencies or stimulus properties between stage 2 NREM and SWS. Negative-pressure pulse stimuli resulted in a greater percentage of instances in which arousal from sleep occurred during stage 2 NREM sleep compared to SWS ( $31 \pm 7$  versus  $8 \pm 3\%$ ,  $P = 0.004$ ). The duration of the initial peak tended to be greater during wakefulness compared with stage 2 NREM and SWS ( $P = 0.050$ ). After the initial peak phase, there was a suppression of EMG<sub>gg</sub> amplitude below baseline that was significantly greater during stage 2 NREM and SWS compared with wakefulness (Table 2). Suppression onset tended to be earlier during stage 2 NREM and SWS compared to wakefulness, but this difference was not statistically significant ( $P = 0.060$ ). Mask and choanal pressure-stimulus magnitude was greater during stage 2 NREM and SWS compared to wakefulness (Table 2). However, the rise time from stimulus onset to  $P_{\text{mask}}$  minimum was not different between sleep states (Table 2). Epiglottic pressure catheters were prone to blockage and did not provide reliable recordings in most subjects. Of the limited data available in which reliable epiglottic recordings were available during wakefulness, stage 2 NREM and SWS, stimulus intensity at the level of the epiglottis was similar ( $-6.6 \pm 1.1$  versus  $-5.6 \pm 1.6$  versus  $-5.6 \pm 1.4 \text{ cmH}_2\text{O}$   $P = 0.267$ ;  $n = 5$  subjects). There were significantly fewer pulses presented during stage 2 NREM and SWS, respectively, compared with wakefulness (Table 2). However, for combined NREM sleep there were no differences in the number of artefact-free pulse presentations compared with wakefulness ( $68 \pm 7$  versus  $53 \pm 3$   $P = 0.089$ ). There were no other differences in EMG<sub>gg</sub> reflex component amplitudes or latencies between stage 2, SWS and wakefulness, respectively.

Negative-pressure pulse delivery produced inspiratory time prolongation compared to the preceding breath ( $2.4 \pm 0.1$  versus  $2.0 \pm 0.1$  s,  $P < 0.01$ ), but no change in subsequent expiratory time ( $2.6 \pm 0.1$  versus  $2.5 \pm 0.1$  s,  $P = 0.38$ ). Inspiratory time and expiratory time for the breath prior to pulse delivery compared to the breath immediately after pulse delivery was not different

( $2.0 \pm 0.1$  versus  $2.0 \pm 0.1$  s,  $P = 0.999$  and  $2.6 \pm 0.1$  versus  $2.8 \pm 0.1$  s,  $P = 0.072$ , respectively), indicating that pulse-related breath timing effects were short lasting.

Clearly identifiable single-motor-unit activity was observed in six subjects. An example of a prominent single EMG<sub>gg</sub> motor unit during and immediately prior to a single negative-pressure pulse and the ISI of the



**Figure 3.** Ensemble-averaged mask pressure ( $P_{\text{mask}}$ ) and ensemble-averaged rectified EMG<sub>gg</sub> reflex responses to brief negative-pressure pulse stimuli in two subjects

*A*, traces during wakefulness and NREM sleep, and *B*, traces during REM sleep.

**Table 2. Genioglossus reflex characteristics to negative-pressure pulse stimuli during wakefulness and NREM sleep**

	Wake	Stage 2	SWS
<b>Excitation phase</b>			
Onset latency (ms)	26 ± 1	23 ± 1	25 ± 2
Peak amplitude (% baseline)	231 ± 29	249 ± 16	218 ± 11
Peak latency (ms)	38 ± 3	32 ± 2	34 ± 2
Duration (ms)	23 ± 2	16 ± 1	17 ± 2
<b>Suppression phase</b>			
Onset latency (ms)	47 ± 2	39 ± 2	42 ± 2
Nadir amplitude (% baseline)	67 ± 8	39 ± 4*	46 ± 4*
Nadir latency (ms)	71 ± 4	63 ± 3	67 ± 2
Duration (ms)	42 ± 6	39 ± 4	40 ± 3
<b>Stimulus properties</b>			
Breath onset to pulse onset (ms)	92 ± 3	93 ± 3	91 ± 3
$P_{\text{mask}}$ magnitude (cmH <sub>2</sub> O)	-9.5 ± 0.3	-10.8 ± 0.3*	-10.7 ± 0.3*
$P_{\text{mask}}$ rise time (ms)	11.3 ± 0.2	10.9 ± 0.4	11.1 ± 0.4
$P_{\text{cho}}$ magnitude (cmH <sub>2</sub> O)	-8.3 ± 0.5	-10.0 ± 0.4*	-9.8 ± 0.6*
Number of artefact-free pulse presentations	53 ± 3	27 ± 5*	40 ± 6*

Slow wave sleep (SWS), mask ( $P_{\text{mask}}$ ) and choanal pressures ( $P_{\text{cho}}$ ). Reflex timing data are quoted in milliseconds (ms). Reflex amplitude data are quoted as percentage baseline of the average EMG<sub>gg</sub> activity 100 ms prior to pulse onset (% baseline). \*Significant difference compared to wakefulness. Values are means ± s.e.m. (Data presented are from  $n = 16$  subjects in whom complete data were available during wakefulness, stage 2 NREM and SWS).

**Table 3. Genioglossus single-motor-unit interspike intervals during negative-pressure pulse stimuli**

Subject number and discharge pattern	Pre-stimulus baseline (ms)	First post-stimulus potential (ms)	Second post-stimulus potential (ms)
1 (IT)	57 ± 3	54 ± 5	79 ± 3*†
2 (ET)	51 ± 4	50 ± 3	60 ± 5*
3 (T)	55 ± 2	50 ± 5	84 ± 5*†
4 (IT)	99 ± 3	120 ± 23	112 ± 11*
5 (IT)	44 ± 2	45 ± 2	60 ± 3*†
6 (T)	40 ± 3	41 ± 4	108 ± 7*†
Mean	58 ± 9	60 ± 12	84 ± 9*†

The average interspike interval (ISI) of genioglossus single motor units during six pulse presentations per subject immediately prior to and the first and second potentials after pulse onset, respectively. (See Fig. 2A for an example tracing in one subject). Inspiratory tonic (IT), expiratory tonic (ET) and tonic (T) discharge patterns according the definitions described by Saboisky *et al.* (2006); see text for further details. \*Significant difference compared to baseline, †significant difference compared to first post-stimulus potential.

unit is displayed in Fig. 2A. The average ISI for the baseline, first and second potentials after stimulus onset for six separate pulses per subject and the motor-unit discharge patterns are displayed in Table 3. While detailed descriptive analyses as described by Saboisky *et al.* (2006) were not available in this study, of the 36 motor units examined all discharged during inspiration and expiration. Upon visual examination and according to the classification categories described by Saboisky *et al.* (2006), 18 appeared to increase their firing frequency with inspiration (inspiratory tonic), 12 did not appear to change their firing frequency throughout the respiratory

cycle (tonic) and 6 units appeared to fire more rapidly during expiration (expiratory tonic). Accordingly, there was a decrease in the ISI during inspiration for motor units classified as inspiratory tonic ( $\Delta\text{ISI} -67 \pm 25$  ms,  $P = 0.016$ ). The ISI during inspiration for motor units classified as expiratory tonic significantly increased ( $\Delta\text{ISI} 8 \pm 3$  ms,  $P = 0.032$ ). The ISI for units classified as tonic did not differ between the expiratory period and the inspiratory period ( $45 \pm 4$  versus  $47 \pm 3$  ms,  $P = 0.532$ ). The ISI of the first potential after negative-pressure pulse onset did not change from baseline ( $P = 0.525$ ), whereas the ISI of the second potential significantly increased



(Table 3). In addition, a clear reflex-mediated reduction in EMG<sub>gg</sub> activity was, in many instances, observed during single negative-pressure pulse trials on the raw multi-unit EMG<sub>gg</sub> recording (Fig. 2B).

### Genioglossus negative-pressure reflex during REM sleep

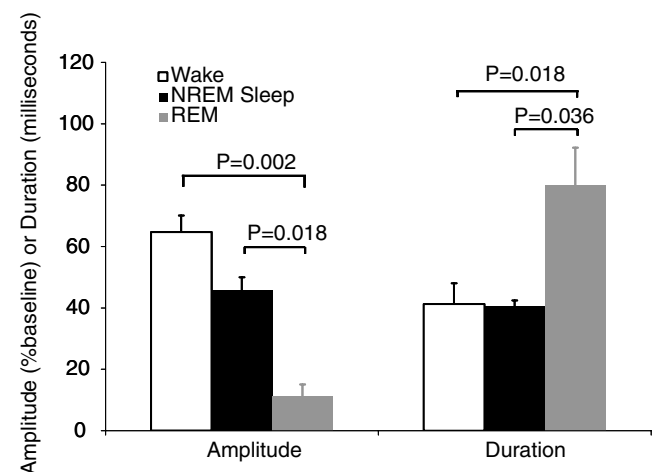
Sufficient REM sleep to present negative-pressure pulse stimuli and generate an ensemble-averaged reflex response was achieved in five subjects. There were no differences in the average EMG<sub>gg</sub> activity in the 100 ms prior to pulse onset during NREM compared to REM sleep in these subjects ( $12.6 \pm 4.4$  versus  $17.9 \pm 3.8 \mu\text{V}$ ,  $P = 0.143$ ). While replicate trials were limited ( $7 \pm 2$ ), the predominant reflex response of a prolonged period of suppression of muscle activity with (2/5) or without (3/5) any preceding excitation was still quite clear. Indeed, the suppression often approached complete silencing of the EMG<sub>gg</sub>. The rectified EMG<sub>gg</sub> reflex responses in two individual subjects during REM sleep are displayed in Fig. 3B. ANOVA for repeated measures exploring state (wake, NREM and REM) effects revealed significantly greater EMG<sub>gg</sub> amplitude suppression during NREM and REM sleep compared with wakefulness, with particularly marked suppression during REM sleep in these five subjects (Fig. 4). In addition, the duration of the suppression phase was significantly longer during REM sleep compared to wakefulness and NREM sleep. There were no significant state effects in reflex peak amplitudes or latencies for comparisons incorporating REM data.

### Discussion

The ensemble-averaged rectified EMG<sub>gg</sub> response to brief pulses of negative airway pressure revealed a previously unknown pattern, namely, an initial increase followed by a decrease from baseline EMG<sub>gg</sub> activity. This may not have been observed in earlier studies for several reasons. Previous studies of the gg negative-pressure reflex in humans have been conducted using a combination of surface and intramuscular electrodes for multiple-unit recordings (Horner *et al.* 1991a, 1991b, 1994; Wheatley *et al.* 1993; Shea *et al.* 1999, 2000; Berry *et al.* 2003; Malhotra *et al.* 2004). The relatively poor signal-to-noise ratio arising from surface EMG electrodes and the small number of replicate trials (Horner *et al.* 1991a, 1991b, 1994) may have obscured these reflex characteristics in previous studies. Subsequent studies that utilized intramuscular electrodes with increased replicate trial numbers probably improved the signal-to-noise ratio characteristics (Wheatley *et al.* 1993; Shea *et al.* 1999, 2000; Malhotra *et al.* 2004) but analysis using moving-time averaging may have obscured a short-latency inhibitory response.

Where a reflex response consisting of an excitation phase followed by suppression is present on the rectified EMG, it is possible that the suppression component is an epiphenomenon of motor-unit synchrony rather than reflex inhibition (Kasser & Cheney, 1985). Briefly, the excitation phase can cause otherwise out-of-phase motor-unit action potentials to become temporally aligned, as asynchronous units within the motor neuron population are near-simultaneously brought to firing threshold as a result of the stimulus. This temporal alignment can cause a subsequent suppression phase characterized by a relative lack of EMG activity where previously asynchronous motor units are refractory and do not discharge until their membrane potentials return to firing threshold and asynchronous firing patterns begin to return (Miles *et al.* 1989). In the current study, consistent with suppression due to reflex inhibition rather than purely a phenomenon of synchronous firing, there was a consistent marked increase in the ISI of single motor units in the second post-stimulus interval. Further, the inhibition phase was also evident on the raw multi-unit recordings during single trials.

While it is difficult to quantify definitively using the sampling frequencies and techniques in the current study, the lack of change from baseline in the ISI of the first post-stimulus discharge for the motor units examined suggests that the excitation phase of the gg negative-pressure reflex probably occurs primarily as a result of additional motor-unit recruitment rather than an increase in the firing frequency of individual motor units. Recent human studies in which detailed quantification of gg single-motor-unit activity was undertaken during normal respiration has highlighted the heterogeneous nature of the gg muscle and its neural inputs (Saboisky *et al.*



**Figure 4. Ensemble-averaged, rectified EMG<sub>gg</sub> reflex suppression amplitude and suppression duration during REM sleep compared to wakefulness and NREM sleep**  
Values are means  $\pm$  s.e.m. ( $n = 5$  subjects).

2006). To determine definitively the neural mechanisms mediating the initial peak of the gg negative-pressure reflex and the precise role of negative pressure in modulating gg activity would require a similar systematic exploration of the behaviour of single motor units within the muscle.

Functionally, these data suggest that the reflex response of the gg muscle to transient negative pressure is not simply an excitatory response resulting in tongue protrusion or perhaps stiffening as previously thought (Malhotra *et al.* 2000, 2002; Akahoshi *et al.* 2001; Fogel *et al.* 2001; Malhotra & White, 2002). While moment-to-moment excitatory modulation of upper-airway gg activity may be an important protective mechanism in maintaining upper-airway patency during normal inspiration (Malhotra *et al.* 2000, 2002; Akahoshi *et al.* 2001; Fogel *et al.* 2001; Malhotra & White, 2002), the findings of the present study raise the possibility that this response may be threshold dependent. For example, the response to relatively small negative pressures in the upper airway, as would occur during normal tidal breathing, may be largely excitatory whereas the predominant response to large negative pressures may be inhibitory. The relatively long stimulus duration in the present study (250 ms) combined with the substantial stimulus amplitude may be more akin to rapid airway occlusion than within-breath fluctuation in airway pressure to which gg reflexes may normally be able to respond effectively. Consequently it is possible that shorter-duration and less-negative airway pressure stimuli would show a more pronounced excitatory response compared with the dominant inhibitory response observed in this study.

The precise functional role of gg reflex inhibition is unclear and would appear to be counterproductive to respiratory homeostasis. However, it is possible that reflex inhibition in itself may be protective. The initial response appears to be excitatory, which would tend to dilate the upper airway. However, if this initial response was not sufficient to overcome the impediment to respiration (whether it be obstruction by the tongue or inhalation of a foreign object) it would be counterproductive, and perhaps deleterious to the maintenance of inspiratory flow (e.g. tending to cause worsening flow limitation), to continue making sustained inspiratory effort. Thus, the reflex inhibition observed may be a result of inhibition of the respiratory pattern-generator inputs to this reflex arc in the same manner that respiratory drive muscles have been shown to be inhibited by brief respiratory load stimuli (Davis & Sears, 1970; Butler *et al.* 1995, 1996, 1997; Jeffery *et al.* 2006). In support of this hypothesis, the latency of the onset of the gg reflex inhibition phase to negative-pressure pulse stimuli was remarkably similar to those reported in other respiratory drive muscles in these previous studies. Thus, reflex gg inhibition could simply reflect the upper-airway component of a more global inhibitory

respiratory reflex serving to maintain an appropriate balance of upper-airway *versus* respiratory pump muscle drive to achieve non-flow-limited inspiratory airflow.

An alternate explanation is that the gg negative-pressure reflex to a negative-pressure pulse may inhibit normal respiratory inputs to the hypoglossal motor nucleus, and this inhibitory effect may outlast the direct excitatory effect and be manifest as gg suppression. A systematic exploration of single-motor-unit behaviour similar to that reported by Saboisky *et al.* (2006) would be helpful in delineating which types of motor units are involved in the excitatory and inhibitory reflex responses.

### **The effect of sleep on reflex responses to negative-pressure pulse stimuli**

Earlier studies investigating the effect of sleep on gg reflex responsiveness to negative pressure either did not control for posture, or studied subjects in the lateral position (Wheatley *et al.* 1993; Horner *et al.* 1994). These studies found delayed latency and attenuation of the excitatory gg negative-pressure reflex response during NREM sleep. The present finding that the excitatory component of the gg negative-pressure reflex in the supine posture was unaffected by sleep is different, but is in agreement with the recent findings of Malhotra *et al.* (2004). This provides strong support for the concept that gg reflex activity to negative pressure is posture dependent.

In contrast to earlier studies (Wheatley *et al.* 1993; Horner *et al.* 1994), we found that the latencies of the various gg reflex characteristics were similar during NREM sleep compared to wakefulness. However, consistent with the general concept of more pronounced excitation during wakefulness, there was a trend for prolongation of the duration of excitation and delay of onset of suppression during wakefulness. While the precise reasons for the discrepancies between this and previous studies are unclear, they may relate to postural and other methodological differences. However, this is difficult to evaluate given that latency data were not reported in the only other study to examine systematically the gg negative-pressure reflex in the supine position (Malhotra *et al.* 2004).

The finding of marked reflex inhibition during REM sleep, often in the absence of any preceding excitation, is in agreement with previous gg negative-pressure reflex findings during REM sleep (Shea *et al.* 1999) and is consistent with earlier reports demonstrating reduced gg activation to occlusive stimuli during REM sleep (Kuna & Smickley, 1988; Okabe *et al.* 1994). The lack of excitatory responsiveness of the largest upper-airway dilating muscle to negative pressure during REM sleep is likely to be an important contributing factor to the increased severity of sleep-disordered breathing observed in this sleep stage.

In contrast to the previous concept that sleep caused a suppression of the excitatory response to negative-upper-airway pressure (Malhotra & White, 2002; White, 2005, 2006), thereby increasing the likelihood of upper-airway collapse, our data suggest that the secondary inhibition phase of the gg negative-pressure reflex may also play a key role. While the gg negative-pressure reflex has not been studied in this manner previously, it is possible that greater reflex inhibition of the gg negative-pressure reflex during sleep may be mediated by changes in sleep-specific neuromodulators such as serotonin (Jevlev *et al.* 2001; Sood *et al.* 2005).

### Possible relevance to sleep disordered breathing

A diminished excitatory gg negative-pressure reflex during sleep has been postulated to contribute to the development of obstructive sleep apnoea in patients with an anatomically narrow upper airway. The finding that the amplitude of the initial excitatory component of the reflex measured in the supine posture was not different between wake and NREM sleep does not support this hypothesis. However, more pronounced gg reflex inhibition following the initial excitation phase to negative pressure during NREM sleep could render the upper airway more prone to collapse. In the same way that inspiratory pump muscle reflex responses to respiratory occlusion are altered in obstructive sleep apnoea patients (Jeffery *et al.* 2006), it will be important to investigate if the inhibition component of the gg negative-pressure reflex is similarly affected in obstructive sleep apnoea patients.

### Methodological considerations

There are several important methodological considerations with regard to this study. Firstly, we elected to study only male subjects due to the known influence of changes in respiratory stimulant hormones that occur throughout the menstrual cycle and their associated effects on ventilation and gg muscle activation (Popovic & White, 1998). Further, the prevalence of sleep-disordered breathing is greater in men than women (Young *et al.* 1993). While we believe that the effects observed in this study in men would probably be similar in women if the stage of menstrual cycle was controlled, this has yet to be determined. Thus, future carefully designed studies are required to systematically explore this important unresolved issue.

Although we hypothesize that more pronounced gg reflex inhibition observed during NREM sleep compared to wakefulness occurs due to changes in sleep-specific neuromodulators, it is possible that this difference may be explained by the greater stimulus presented during NREM sleep. However, we feel that this is unlikely, given that differences in stimulus magnitude were small

( $\sim 1\text{--}2\text{ cmH}_2\text{O}$ ) and other components of the reflex were not different between states. In addition, the precise stimuli required to activate the gg negative-pressure reflex are not known. It may be that the rate of change in negative pressure, which was not different between states, may be more important than the absolute change in pressure in eliciting the gg negative-pressure reflex. However, the possibility that differences in stimulus magnitude between NREM sleep and wakefulness may contribute to the greater reflex inhibition observed in NREM sleep can not be dismissed.

Another potential limitation of the study is that negative pressure was applied nasally rather than being generated distal to the upper airway as occurs in obstructive sleep apnoea. While this approach may stimulate additional receptor systems not implicated in upper-airway collapse in obstructive sleep apnoea, available evidence suggests nasal and laryngeal receptors respond in a similar manner (Horner *et al.* 1991a; Malhotra *et al.* 2004). While the present findings suggest that this is unlikely, it is possible that this type of stimulus stimulates additional proprioceptive afferents that are known to cause suppression of gg activity in animal models (Sauerland & Mizuno, 1970).

Finally, subjects were heavily instrumented during the study and it is possible that reflex responses may have been altered as a result. The inspiratory circuit added a small ( $\sim 2\text{ cmH}_2\text{O l}^{-1}\text{ s}$ ) resistance to inspiration, and all subjects breathed nasally. However, given that all subjects experienced the same conditions, these factors are unlikely to account for the sleep-state-related change observed in this within-subjects study design.

### Summary

This study describes a previously unreported reflex suppression of  $\text{EMG}_{\text{gg}}$  in response to brief pulses of negative upper-airway pressure that is consistent with reflex inhibition of the gg muscle. This reflex inhibition was more pronounced during NREM and particularly during REM sleep compared to wakefulness. Greater gg reflex inhibition to negative-pressure stimuli during sleep raises the possibility that this mechanism may contribute to upper-airway collapse in individuals with an anatomically narrow airway.

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