REGULATION OF UPPER AIRWAY MUSCLE ACTIVITY ACROSS THE LUNG VOLUME RANGE IN HEALTHY YOUNG ADULTS

By

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A Thesis Submitted to The Honors College
In Partial Fulfillment of the Bachelors degree With Honors in
Physiology
THE UNIVERSITY OF ARIZONA

MAY 2017

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Abstract
Speech is a voluntary task that requires fine motor control of ~50 muscles, including muscles of the tongue. One tongue muscle, the genioglossus (GG), has been well studied as an airway dilator that defends the airway in sleep (Remmers 1980). The results of this work highlights the GG’s role in airway defense and factors that augment (PaCO$_2$) or inhibit (pulmonary stretch receptor feedback) GG activity (Bailey et al. 2001).

We recorded GG electromyographic (EMG) activity across the lung volume range testing the hypothesis that tongue muscle activation declines with lung volume in respiratory and volitional movements. We measured EMG activity across voluntary e.g., meaningful speech (phrases), speech sound devoid of meaning (sustained vowels) and respiratory tasks performed across the vital capacity range (0-80%VC).

We show that GG EMG activity is variable across adults but is greatest for tasks performed at the lowest lung volumes. Our results are consistent with results in the rodent that show greatest GG EMG in the absence of lung inflation attributed to release from vagal inhibition (Bailey et al. 2001) and provide additional new insights into airway muscle control during high airflow (speech) relative to low airflow (controlled breathing) tasks.
**Introduction**

The tongue comprises 7 muscles. These include three muscles that originate outside of the tongue - the so-called “extrinsic tongue muscles” (styloglossus, hyoglossus and genioglossus muscles), and muscles that originate and terminate in the tongue referred to as “intrinsic muscles” (inferior and superior longitudinalis, transverus and verticalis muscles). All seven muscles are innervated by cranial nerve XII -- the hypoglossal nerve.

![Schematic representation of the human tongue showing extrinsic (styloglossus, hyoglossus and genioglossus) and intrinsic (inferior and superior longitudinalis, verticalis and transversus) tongue muscles (from Takemoto, H 2001)](image)

The genioglossus (GG) has been the focus of attention for decades as GG dysfunction during sleep leads to airway collapse and is thought to underlie Obstructive Sleep Apnea (OSA), one of the most prevalent sleep related breathing disorders (Remmers 1980). Airway collapse and OSA are due to a disorder in the automatic (i.e., respiratory) control of tongue muscle activation that originates in the respiratory Central Pattern Generator (CPG) in the medulla and which is relayed to the tongue via the hypoglossal nerve. Action potentials in the hypoglossal nerve result in a contraction of the tongue muscles, and GG activation pulls the tongue anteriorly in early inspiration,
thereby preserving airway patency. Because the respiratory regulation from the CPG is present even during sleep, a failure of this regulation/drive may result in the tongue repeatedly obstructing the airway throughout the night.

In view of the significance of the GG’s role in determining airway patency, much of the previous research has focused on the control of the GG, and the magnitude of GG EMG during the inspiratory phase of each respiratory cycle especially during sleep. And, we know from previous work in the rodent and human airways (Mateika et al. 1999) that inspiratory drive to the GG of the tongue muscle more broadly is regulated by afferent feedback from pulmonary stretch receptors that respond to lung inflation (Bailey et al. 2006). Such lung inflation stimulates mechanoreceptors (pulmonary stretch receptors) which lie in the distal airways and which discharge action potentials that terminate in the respiratory nuclei (nucleus of the solitary tract) in the medulla and inhibit upper airway muscle activation, including activation of the GG (Bailey et al. 2001) (Walls et al. 2013).

In addition to its regulation by the medullary respiratory CPG, the GG is also under voluntary control. Voluntary control originates in the cerebral motor cortex and is critical for the control of tongue muscles for complex functions such as chewing, swallowing and speech. Speech is of interest because it is a highly skilled motor act performed on expiration and which requires respiratory support. Accordingly, we are interested in how both these drives are regulated and how much each contributes to tongue muscle activity during speech production versus sound production and as compared to controlled exhalation across the vital capacity range. If afferent (PSR) feedback that regulates tongue muscle activity during breathing also regulates tongue muscle activity during speech, then we would predict that the GG EMG will be profoundly inhibited
during speech/sound production at highest lung volumes and exhibit least inhibition
during speech and sound production at the lowest lung volumes.

**Methods**

We recruited five healthy adult female volunteers. All subjects were students at The University of Arizona between the ages of 19 and 23 years. Before testing, each subject gave written informed consent. All procedures were approved by the University of Arizona and the Human Subjects' Committee (Project #1300000263).

*Electromyography.* Before insertion of the electrodes into the GG, we first determined the appropriate insertion depth. We did this by using ultrasonography of the muscles under the chin, immediately posterior to the mandible. Subjects were seated with their head in a neutral position while the area underneath the chin was imaged (Aloka ProSound SSO-3500 Plus) to locate the genioglossus and the depth to the belly of the GG muscle belly -- usually ~1.0-1.5cm (Bailey et al. 2015).

GG activities were recorded using bipolar intramuscular tungsten microelectrodes (30mm, 1-5µm tip diameter, 250µm shaft diameter) inserted bilaterally into the muscle through the skin, the microelectrodes lacked insulation on the last 5.0mm. A surface electrode placed on the clavicle served as a ground. Subjects were seated in a dental chair and reclined to a supine position for electrode insertion. Microelectrodes were inserted to the depth determined by ultrasound. Electrode placement was confirmed by activation on tongue protrusion their tongues and in connected speech. GG EMG signals were pre-amplified (1000x) and band-pass filtered (30 - 3,000Hz) using CED
1902 amplifiers and head stages (Cambridge Electronic Design, Cambridge, UK) and sampled at 10 kHz.

**Respiration-related signals.** Chest wall motions were recorded using a Respitrace Calibrator (Ambulatory Monitoring, Inc., NY). Respitrace bands were placed around each subject’s thorax at the level of the axilla. The chest wall signal was digitally sampled at 500Hz. Subjects were instructed in performance of a forced vital capacity (VC) maneuver (maximal inhalation followed by maximal exhalation) to determine peak inspiratory (100%VC) and end-expiratory (0%VC) lung volumes. Once the individual’s vital capacity was determined it was subsequently divided into ranges as follows: 80-60%VC; 60-40%VC; 40-20%VC and 20-0%VC and within which each of the ranges subjects performed speech production, sound production, and controlled breathing tasks (see following).

**Speech signals.** Audio signals were recorded using a microphone placed approximately 6 inches from the subject’s mouth. The signals were preamplified (Sound Devices MixPre) and digitally sampled (42 kHz). All signals (EMG, respiratory-related and speech) were digitized and stored using a CED 1401 unit and Spike2 (version 7.0) software (Cambridge Electronic Design, UK).

**Experimental Procedures**

Individual chest wall traces and the location of each range within the vital capacity were displayed via digital projection onto a wall directly in front of each subject. The projection provided subjects the necessary information regarding each of the lung volume ranges that assisted them in monitoring the lung volume events required in the
performance of each of the tasks. With this information subjects were instructed in completing the tasks as follows: maximal tongue protrusion out of the mouth, maximal protrusive force against the hard palate, sustained vowel, controlled exhalation and spoken phrases (heed is word, hod is a word, who'd is a word, had is word). Each of these tasks were repeated 3-5 times within each of the lung volume ranges (% vital capacity) encompassing: 80-60 %VC, 60-40 %VC, 40-20%VC and 0-20 %VC.

Data Analysis
All data were analyzed using Spike2 and custom-designed software (CED). EMG signals were rectified and integrated and expressed as a percentage of maximum EMG (% max) for that lung volume range. Maximum EMG was based on the magnitude of EMG in either two forceful protrusions or force exerted against palate.

Results
We recorded data from five healthy female subjects, ages 19-23 years mean BMI 25.9 +/- 3.15. Demographic data for each of our five healthy subjects are provided in Table 1.

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<thead>
<tr>
<th>Subject</th>
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Representative recordings obtained from one subject obtained during each of four tasks, are shown in Figure 2. Proceeding from top to bottom, the audio signal, rectified GG electromyography signal (GG EMG) expressed as a percentage of the maximal maneuver (%max), untreated GG EMG, and lung volume shown as percentage of the
vital capacity range (%VC). In Panel A, the subject performed a controlled exhalation within their 40-20%VC range. Note the slow exhalation over 2.6 seconds within the range and the low amplitudes of GG EMG in that task. Note that there is no audio signal in this condition because controlled exhalations do not entail voice/sound production. In Panel B, the subject sustained the vowel /ee/ also within the 40-20%VC range. Note a steady amplitude audio signal consistent with production of a sustained vowel. Note too, the GG EMG amplitudes are greater in this task relative to the EMG amplitudes in the controlled exhalation, as seen in both the untreated EMG and rectified EMG signals. In Panels C and D, the subject performed the meaningful speech task ("Heed, is a word"). In panel C, the task is performed within their 80-60%VC range. Note increased GG EMG amplitude relative to the sustained vowel task and that the duration of the task is shorter due to higher airflow required for speech compared to isolated vowels or controlled breathing. In panel D, the subject performed the same speech task but within their 20-0%VC range. As shown, EMG amplitudes are greater relative to those recorded during the same task within the 80-60%VC range.

It should be noted that each subject was instructed to perform both the speech and sound production tasks at their normal conversational loudness level (compare audio traces in Panels C and D). Importantly, for all tasks, protrusion of the tongue out of the mouth served as the maximal GG activation to which all other GG EMG activity was normalized (%max). A representative EMG recording obtained during maximal protrusion is shown in Panel E. As anticipated, there is no audio signal for tongue protrusion.
For this subject, the greatest amplitude GG EMG was in the context of meaningful speech produced within the lowest lung volume range (20-0%VC). Tasks performed within the 80-60, 60-40 and 40-20%VC ranges resulted in comparable levels of activation but were consistently lower than EMG amplitudes recorded in the lowest lung volume range.

Figure 2: Representative recordings from subject 1 during A. controlled exhalation, B. Sustained isolated vowel /ee/, C. Meaningful speech produced within 80-60% of the vital capacity range (%VC), D. Meaningful speech task produced within 20-0% of the vital capacity range (%VC), and E. Maximal effort via protrusion task.

Figure 3 displays individual GG EMG averages in performance of controlled exhalation tasks within each of the four lung volume ranges. The GG EMG signals here are of very low magnitude, and there is not much variance between subjects. Whereas GG EMG for Subjects 1 and 5 exhibited slight increases in amplitude in the 20-0%VC range, the same trend was not evident in Subjects 2, 3 or 4.
Figure 3: Average (±SE) GG EMG for each subject during controlled breaths performed within each of the lung volume ranges. Muscle activation did not display distinct changes over lung volumes.

Figure 4 shows GG EMG averages for each subject performing the sustained vowel task. As shown, there is considerable inter-individual variability in the EMG amplitude although for all subjects, GG EMG amplitudes were greater during this voice production task than for controlled breathing task. Thus, Subjects 3, 4 and 5 exhibit slight increases in GG EMG within the lower %VC ranges, whereas the increase in Subject 1 was much greater. EMG activity in each of the four lung volume ranges (80-60, 60-40 and 40-20% and 20-0%VC) encompassed, 4.38-47.42 (%max), 4.10-46.02 (%max), 4.00-45.12 (%max) and 7.04-71.65 (%max).
Figure 4: Average (±SE) GG EMG over lung volumes of the five individual subjects during sustained vowel /ee/ task. Note the large variation between subjects.

Figure 5 shows GG EMG averages for each subject during performance of meaningful speech phrases within each of the four lung volume ranges (80-60, 60-40, 40-20 and 20-0%VC). Whereas subjects 2, 3, 4 and 5 exhibited small increases in GG EMG amplitudes within the 20-0%VC range, there was considerable between subject variability. Subject 1 exhibited the greatest GG EMG amplitude and the greatest increase in amplitude in the 20 to 0%VC range. For speech or sound production tasks, there was no evidence of a difference in GG EMG amplitude for meaningful versus non-meaningful speech, although GG EMG amplitudes were consistently greater in meaningful speech tasks performed within the lowest lung volume range (20-0%VC).
Figure 5: Average (±SE) GG EMG during meaningful speech tasks performed within each lung volume range.

Figure 6 shows averaged GG EMG data for all subjects in each of the three tasks (controlled breathing, sustained vowel, meaningful speech). Consistent with the individual data shown in Figure 1, group data also show greater GG EMG activation within the lowest lung volume range. Although the averaged data for isolated vowel tasks showed a trend toward greater GG EMG than for meaningful speech tasks, this difference is attributed to results obtained from one subject in whom GG EMG activation was much greater in this task. The controlled breathing task on average also showed a small increase in the 20-0%VC range but for any given lung volume range, GG EMG amplitude during controlled breathing tasks was lower relative to all other tasks.
**Conclusion**

We assessed GG electromyographic activity during speech production, sound production and controlled breathing tasks. Our results indicate that GG EMG amplitudes during speech (meaningful and non-meaningful) are greater relative to EMG amplitudes in non-speech production. Second, EMG amplitudes in speech production tasks are greatest at the smallest lung volumes i.e., within the 20-0%VC range in all subjects. The latter finding within the context of speech production performed one expiration is consistent with previous research that showed that vagal (pulmonary stretch receptors, PSRs) afferents inhibit hypoglossal motoneurons and genioglossus muscle activity more specifically (Bailey et al. 2001) (Bailey et al. 2006). Thus, lung volume expansion is associated with increased pulmonary stretch that stimulates PSR feedback that
inhibits GG motoneuron activation. Conversely, at smaller lung volumes GG motoneurons are released from inhibition and GG EMG amplitude increases. Therefore, the increase in GG EMG amplitude evidenced by the present subjects performing tasks within the lowest lung volume range are, in part attributed to disinhibition of GG EMG secondary to a decline in PSR feedback.

The increase in GG EMG during speech tasks also may be attributed to increases in drive to the hypoglossal motoneuron pool and to GG motoneurons more specifically. Speech differs from controlled breathing and sustained vowel production in that it requires much higher expiratory airflows and more precise configuration of the upper airway in order to shape the vocal tract for the target utterances. We have shown previously that shaping of the upper airway together with the increased breath support required for speech is associated with significantly higher firing rates of GG motor units (Lacross et al. 2017). Because this study did not entail recordings from single motor units it is not possible for us to determine how the increases in EMG occurred. That is, whether the increase is due to removal of PSR inhibition or due to increases in volitional and/or respiratory (CPG) inputs onto the hypoglossal motoneuron pool. Certainly, the convergence of two or more drives onto the genioglossus motoneuron pool likely would result in greater EMG activation either via increases in discharge rates of already active motor units (rate coding) or by bringing previously silent motor units to threshold (recruitment) (MacIntosh, Gardiner and McComas 2005).

**Limitations and future directions**

Previous research has shown that increased volitional modulation of the GG muscle results in increased EMG activity (Bailey and Vranish 2015). However, the current study
focused on whole muscle GG EMG activity. While the increase in whole muscle EMG at low lung volumes was anticipated we cannot be certain that the larger amplitude GG EMG is attributable to disinhibition secondary to removal of PSR feedback or due to increases in drive onto the hypoglossal motoneuron pool that increased the firing rates of already active motoneurons or the recruitment of previously quiescent motoneurons. Although our preliminary results are encouraging, additional studies will be needed to determine if the effects of lung volume on upper airway EMG are robust and consistent across a larger number of subject participants. Importantly, because previously we had reported that GG EMG amplitude is modulated in parallel with laryngeal airway aperture and that the effect differed between men and women (LaCross et al. 2017), it will be important to recruit male subjects to determine if the effects of lung volume on GG EMG activity are similar for men and women.
References


