Single motor unit recordings in human geniohyoid reveal minimal respiratory activity during quiet breathing


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Brown EC, Hudson AL, Butler JE, McKenzie DK, Bilston LE, Gandevia SC. Single motor unit recordings in human geniohyoid reveal minimal respiratory activity during quiet breathing. J Appl Physiol 110: 1054–1059, 2011. First published February 17, 2011; doi:10.1152/japplphysiol.00454.2010.—Maintenance of airway patency during breathing involves complex interactions between pharyngeal dilator muscles. The few previous studies of geniohyoid activity using multunit electromyography (EMG) have suggested that geniohyoid shows predominantly inspiratory phasic activity. This study aimed to quantify geniohyoid respiration-related activity with single motor unit (SMU) EMG recordings. Six healthy subjects of normal body mass index were studied. Intramuscular EMG recordings of geniohyoid activity were made with a monopolar needle with subjects in supine and seated positions. The depth of the geniohyoid was identified by ultrasound, and the electrode position was confirmed with maneuvers to isolate activity in geniohyoid and genioglossus. Activity was recorded at 85 sites in the geniohyoid during quiet breathing (45 supine and 40 seated). When subjects were supine, 33 sites (73%) showed no activity during breathing and 10 (22%) showed tonic activity. In addition, one site showed a tonic SMU with increased expiratory discharge, and one site in another subject had one unit with expiratory phasic activity. When subjects were seated, 27 sites (68%) in the geniohyoid showed no activity, 12 sites (30%) showed tonic activity that was not respiration related, and one unit at one site showed phasic expiratory activity. The average peak discharge frequency of geniohyoid motor units was 16.2 ± 3.1 impulses/s during the “geniohyoid maneuver,” which was the first part of a swallow. In contrast to previous findings, the geniohyoid shows some tonic activity but minimal respiration-related activity in healthy subjects in quiet breathing. The geniohyoid has little active role in airway stability under these conditions.

The pharynx is a muscular tube with numerous functions including swallowing, phonation, and maintenance of airway patency. To perform these tasks, complex muscular interactions occur in a coordinated manner. The muscles need to maintain airway stability in the face of negative intraluminal pressures generated by inspiration, while protecting the lower airway from foreign matter contamination. The genioglossus is the largest, most-studied airway dilator muscle. It appears to dilate the upper airway even during quiet breathing (24). It maintains airway stability (12, 13, 17), and single motor units (SMUs) in the genioglossus have phasic and/or tonic activity during the respiratory cycle (2, 24, 25, 29, 39). It appears to show modified electromyographic (EMG) activity in OSA subjects during wakefulness (19, 25), and activity is reduced more during sleep in OSA subjects than in healthy subjects (8, 15, 18). Dynamic imaging studies in quiet breathing have shown that the genioglossus dilates the airway during inspiration by anterior movement, and it appears to slide over a stationary geniohyoid with minimal surrounding movement (5).

The geniohyoid muscle has two small parts, one on each side of the midline, that arise from the inferior mental spine of the mandible and run infero-posteriorly to insert on the anterior body of the hyoid bone. Compared with the genioglossus, the geniohyoid has a smaller muscle mass and less mechanical advantage to dilate the airway (3). The geniohyoid displaces the hyoid anteriorly, which has been hypothesized to dilate the airway (3, 4, 14) and also has a role in the initiation of swallowing (14, 27), stabilization of the hyoid arch (33), and mouth opening (21). It shows increased EMG activity during inspiratory resistive loading in some animal models using intramuscular recordings but with associated muscle lengthening (20). Hypercapnia produced no change in activity from baseline (40). In humans, two studies using intramuscular electrodes have reported inspiratory phasic activity in geniohyoid during quiet breathing in the supine position (28, 38). However, in some instances these recordings may have been measuring the electrical activity of the nearby genioglossus. Therefore the role of geniohyoid during respiration and in airway patency remains to be elucidated.

We hypothesized that there is no activity in geniohyoid during quiet breathing, contrary to previous reports in humans that showed respiratory modulation (28, 38). The hypothesis was based on apparent lack of movement of the geniohyoid on imaging (5) and on our observations of the lack of activity in studies in which intramuscular genioglossus EMG was recorded when the electrode moved through geniohyoid into genioglossus (24, 25). A number of animal studies have shown no activity in the geniohyoid in eupneic conditions (30, 33, 40). Our aim in the present study was to examine the activity of the
geniohyoid during quiet breathing, using intramuscular recordings of SMU activity to quantify any respiratory activity.

METHODS

EMG studies were conducted on six healthy subjects (4 men, 2 women). The average age was 40 yr (range 28–57 yr). Subjects for this study were selected in the normal weight range of body mass index (BMI) < 25 kg/m² (mean 22 ± 2 kg/m²). Informed written consent was obtained from all subjects. The study was approved by the Human Research Ethics Committee of the University of New South Wales and conducted according to the Declaration of Helsinki.

EMG recordings were made from the geniohyoid under two conditions, with subjects seated upright and lying supine. Subjects were seated comfortably with back support and a pillow behind the head. In a second session the same subjects (n = 5) lay supine on a bed with their head supported by a pillow. One subject was unavailable for the second session.

Ultrasonography

Ultrasonography (model 128XP/4; Acuson, Mountain View, CA) was used to determine the depth of the genioglossus and geniohyoid, and these depths were used to guide electrode placement, as previously described by Saboisky et al. (24). Depth of the geniohyoid was confirmed in two subjects with a second ultrasound system (Phillips iU22, Bothwell, WA) (Fig. 1). The depth of the midpoint of the geniohyoid muscle for the electrode insertion site and the depth of the border of the genioglossus were recorded. The geniohyoid is easily visualized by ultrasound, and care was taken that minimal pressure was placed on the skin with the transducer in order to gain accurate measurements of muscle depth. Measurements were performed in both the supine and seated positions just before electrode insertion in each position.

EMG Recordings

The monopolar electrode (Teflon-coated, 50 mm in length with a recording area of 0.34 mm²; Teca) was inserted into the geniohyoid muscle to the predetermined depth, 10–20 mm posterior to the posterior bony edge of the mental protuberance, 2–3 mm left of the midline. Topical local anesthetic (lidocaine-prilocaine, EMLA 5%) was applied 40 min before EMG recordings to anesthetize the skin (5 of 6 subjects). Recordings were referenced to a surface electrode placed above the ipsilateral angle of mandible with a large surface ground electrode placed on the ipsilateral shoulder. The experimenter inserting the needle electrode had visual feedback of EMG activity throughout the experiment and auditory feedback during electrode insertion. The subject had no visual or auditory feedback during recordings of EMG activity. The respiratory cycle was monitored with an inductance band (Respiritrace, Ardsley, NY) placed around the abdomen.

At each recording site baseline measurements of stable quiet nasal breathing for 30-s blocks were obtained. The position of the electrode in the geniohyoid was verified with the “geniohyoid maneuver” (see below) repeated three times. A further three stable breaths were recorded, and then the subject was asked to perform three tongue protrusions. All maneuvers were performed at the end of expiration. Activity was recorded from 4–11 (median 9) sites in each subject in the seated position and 5–11 (median 10) sites in the supine position. In one subject in the seated position the protocol was terminated after five recording sites had been studied because of pain radiating to the jaw, with one of these sites having incomplete data. In the supine position this subject again had pain, but the needle electrode was relocated and the study completed without discomfort. Data were collected only when subjects reported minimal or no discomfort. EMG activity was amplified (5,000×), band-pass filtered (53 Hz to 3 kHz), and sampled at 10 kHz. Data were stored for analysis with Spike2 software (CED 1401 interface, Cambridge, UK).

Maneuvers and Recording Site

The subjects were taught a “geniohyoid maneuver” before electrode insertion with the aim of the maneuver being to activate the geniohyoid muscle. This consisted of the first part of a swallow or an attempt to move the hyoid bone forward (3, 4, 14, 27), with the maneuver demonstrated by the instructor. Subjects were given time to practice until they could confidently perform the maneuver on command. The second maneuver was a tongue protrusion, in which subjects were instructed to gently press their tongue against the back of their bottom teeth, which should preferentially activate the genioglossus (11, 21, 34). The maneuvers were used as a confirmatory test of placement and to establish that the electrode was able to detect activity at sites that were silent during quiet breathing.

The site of recording was categorized as being in the geniohyoid muscle based on the depth of the electrode at the time of recording with reference to the ultrasound recordings. The maneuvers were performed as a secondary check of electrode site. Recordings were designated to be from the geniohyoid if the depth of the electrode was consistent with the ultrasound-determined depth of the muscle and activity accompanied the geniohyoid maneuver (Fig. 2). Occasionally in the seated position the electrode was inserted more deeply than intended, with subsequent recordings made from the genioglossus. Recordings that were made at a depth consistent with genioglossus were excluded from the analysis (9 of 49 sites).

Analysis of EMG

Recordings were scrutinized as multiunit recordings, and then those same periods of EMG were used to extract SMUs if such activity was present. EMG activity during three consecutive stable breaths was assessed. The breaths selected were after the geniohyoid maneuver demonstrated by the instructor. Subjects were given time to practice until they could confidently perform the maneuver on command. The second maneuver was a tongue protrusion, in which subjects were instructed to gently press their tongue against the back of their bottom teeth, which should preferentially activate the genioglossus (11, 21, 34). The maneuvers were used as a confirmatory test of placement and to establish that the electrode was able to detect activity at sites that were silent during quiet breathing.

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Analysis of EMG

Recordings were scrutinized as multiunit recordings, and then those same periods of EMG were used to extract SMUs if such activity was present. EMG activity during three consecutive stable breaths was assessed. The breaths selected were after the geniohyoid maneuver and before the tongue protrusions. If breathing was not stable between maneuvers, then three breaths before the initial maneuver but at the same recording site were analyzed. To classify activity, the EMG was visually inspected during quiet breathing. The EMG was then passed through a leaky integrator (decay time constant 100 ms), and the output was inspected to confirm whether or not phasic activity was present. For each site, recordings were classified according to whether activity was present and whether it was phasic or tonic.

The recording was determined to have no activity if the baseline was <40 μV amplitude in the absence of any phasic activity. Analysis was performed by a single observer and then checked by the other experimenters.
Activity in the geniohyoid and tongue protrusion maneuvers was further quantified by the measurement of the firing rates of SMUs. The procedures have been previously described in detail (6, 7, 10). The three repetitions of both maneuvers were analyzed, and triggers were set manually to capture all spikes with an appropriate signal-to-noise ratio. Spikes were subsequently recalled and sorted into templates based on their size and detailed morphology (Fig. 3). Peak firing frequencies were measured for each unit during the geniohyoid maneuver and for active units during quiet breathing. Unless otherwise indicated, results are expressed as means ± SD. Seated and supine discharge frequencies were compared with a paired Student’s t-test (no. of subjects = 5) using SPSS software (Chicago, IL). Significance was defined as a P value < 0.05.

RESULTS

Geniohyoid EMG activity was recorded from 85 sites in total, 45 sites with the subject supine and 40 sites with the subject seated. An average of 15 ± 2.7 (mean ± SD; median 14) sites were recorded per subject. The mean depth of the midpoint of geniohyoid was 15 ± 2 mm, and the border with genioglossus was 19 ± 3 mm.

Task-Related Classification of EMG Activity and Single Motor Unit Activity

Electrode placement in the geniohyoid as determined by ultrasound depth was confirmed for 85 of 85 (100%) sites by EMG with the geniohyoid maneuver. Tongue protrusion elicited activity in 43 of 85 (51%) geniohyoid sites.

Supine. Table 1 shows the data for each subject in the supine position. When subjects were lying down, 33 (73%) sites showed no EMG activity during quiet breathing and 10 (22%) sites showed tonic multiunit activity. Two (4%) recordings showed phasic activity. In one subject one motor unit fired tonically with an increase in discharge during expiration (Fig. 4). Another subject showed one unit firing only during expiration in one site only. This unit discharged during the geniohyoid maneuver before recording the period of quiet breathing between maneuvers.

Seated. Table 1 shows the data for each subject while seated. During quiet breathing, 27 (68%) recording sites in the geniohyoid showed no EMG activity and 12 (30%) showed tonic
multiunit activity. In 1 of the 40 sites in the seated position, there was a SMU that showed a phasic increase in activity during expiration. No other units recorded at this site were active during quiet breathing.

Increased Respiratory Drive

In the 85 recordings there were 6 (spontaneously occurring) augmented breaths. There was no change to the EMG activity during these breaths. In further recording sessions (2 subjects), subjects were asked to voluntarily increase their tidal volume almost to total lung capacity at the usual inspiratory flow rate. Again, there was no evidence of any change in activity or muscle recruitment in the geniohyoid EMG recordings.

Single Motor Unit Firing Patterns

The average peak firing frequency of SMUs during the geniohyoid maneuver for each subject is given in Table 1. The average firing frequency of all units was 16.2 ± 3.1 impulses per second (ips). There was no difference between the firing frequencies in the geniohyoid maneuver produced in the seated and supine postures (P = 0.64). Few units could be discriminated in tongue protrusion, so they were not analyzed further. Although activity was present, it was from distant units that were difficult to discriminate from baseline.

### Supine

The activity of 49 units was analyzed in the geniohyoid maneuver. Their average discharge frequency was 15.7 ± 2.7 ips. During quiet breathing, seven motor units discharged tonically without respiratory modulation. Their average firing frequency was 9.6 ± 5.9 ips. There was one expiratory phasic unit recorded in the supine position that discharged at a peak of 24.8 ips during the geniohyoid maneuver and 11.3 ips during the three subsequent breaths. In another subject at one site there was one unit (Fig. 4) that showed an increase of 3.0 ips during expiration from a tonic background rate of 10.0 ips. Other units at this site showed no EMG activity during quiet breathing.

### Seated

The activity of 56 SMUs was analyzed in relation to the maneuvers tested (Table 1, Fig. 3). The peak discharge rate of the one unit active in expiration in quiet breathing was 18.5 ips. It also discharged during the geniohyoid maneuver. Three tonic units discharged in quiet breathing in the seated position, with an average firing frequency of 15.6 ± 6.0 ips.

### DISCUSSION

The geniohyoid is an anterior pharyngeal muscle that has a role in swallowing (14, 27) and is able to dilate the airway (3, 4, 33). This study showed minimal respiration-related activity in healthy subjects in the geniohyoid during quiet breathing in both the supine and seated positions. Tonic activity occurred rarely, with most recordings showing no activity. We were careful to ensure that the intramuscular electrode recorded the

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**Table 1. Geniohyoid muscle EMG activity during quiet breathing and geniohyoid maneuver**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Supine Sites</th>
<th>Supine Units</th>
<th>Seated Sites</th>
<th>Seated Units</th>
<th>Total No. of Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>E</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>F</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Mean</td>
<td>33</td>
<td>10</td>
<td>27</td>
<td>12</td>
<td>85</td>
</tr>
</tbody>
</table>

**Table 1. Geniohyoid muscle EMG activity during quiet breathing and geniohyoid maneuver**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Frequency, ips</th>
<th>No. of units</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13.5 (1.3)</td>
<td>8</td>
</tr>
<tr>
<td>B</td>
<td>18.9 (3.8)</td>
<td>8</td>
</tr>
<tr>
<td>C</td>
<td>14.6 (4.3)</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>18.4 (3.0)</td>
<td>11</td>
</tr>
<tr>
<td>E</td>
<td>13.1 (2.1)</td>
<td>17</td>
</tr>
<tr>
<td>F</td>
<td>15.7 (2.7)</td>
<td>9.8</td>
</tr>
<tr>
<td>Mean</td>
<td>16.5 (3.7)</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Frequency, ips</th>
<th>No. of units</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.0 (3.4)</td>
<td>22</td>
</tr>
<tr>
<td>B</td>
<td>16.1 (3.1)</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>14.6 (1.6)</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>14.9 (7.9)</td>
<td>4</td>
</tr>
<tr>
<td>E</td>
<td>23.7 (4.3)</td>
<td>8</td>
</tr>
<tr>
<td>F</td>
<td>13.6 (3.2)</td>
<td>14</td>
</tr>
<tr>
<td>Mean</td>
<td>16.6 (3.7)</td>
<td>6</td>
</tr>
</tbody>
</table>

Number of sites for each subject, type of activity recorded during quiet breathing in the supine and seated positions, number of single motor units (SMUs) studied during the geniohyoid maneuver for each subject, and mean (SD) peak firing frequencies [impulses per second (ips)] of the units are shown. The majority of sites were silent, with some sites showing tonic activity. The 3 phasic sites showed expiratory increase in discharge. *Site that was tonically active with an expiratory increase in discharge; there was activity present only during expiration at the other phasic sites.
geniohyoid and was not contaminated by activity from the genioglossus. Although EMG activity has been shown in the geniohyoid without resulting movement in some animal studies (33, 40), the EMG results presented here show no phasic inspiratory activity and are consistent with a recent study using dynamic MRI that showed antero-posterior movement of the genioglossus over a stationary geniohyoid during respiration in young normal subjects (5).

Two previous multiunit EMG studies in humans have shown phasic activity in the geniohyoid. Wiegand et al. (38) observed inspiratory phasic activity both in wakefulness and in sleep in seven healthy males, using a submental intramuscular wire electrode. The depth of the geniohyoid was extrapolated from measurements in cadavers. According to our ultrasound measurements in vivo, it is probable that in that study (38) recordings were made from the genioglossus. Problems with the use of cadaver-derived measurements to determine electrode placement include anatomic variability between subjects and post-mortem changes to muscle and surrounding tissues. Takahashi et al. (28) reported inspiratory phasic activity in 7 of 10 recordings of the geniohyoid, but they did not demonstrate whether the electrode was recording from geniohyoid or inferior genioglossus. They noted that supine or upright position affected genioglossus but not geniohyoid activity. The most likely explanation of our findings is that geniohyoid exhibits minimal respiratory activity under nonloaded conditions. Subjects were carefully selected as being unlikely to have any extra load on their airway due to adipose tissue to avoid confounding factors, hence the relatively low average BMI. It may be that in a population with much higher BMI there is activity in the geniohyoid during quiet breathing to compensate for higher extraluminal pressures (35). It is unlikely that there were changes to respiratory drive due to instrumentation that affected geniohyoid activity because subjects were relaxed and had minimal discomfort and respiration remained stable throughout the experimental procedure. Subjects were seated initially to decrease collapsibility of the airway, but minimal activity was also found in the supine position, a condition associated with increased airway resistance (9).

Investigations of geniohyoid activity in animals have provided mixed results both across and within species. Inspiratory activity during quiet breathing has been shown in rats (20) and rabbits (23), although another study of rats has shown only occasional respiratory activity (16). No activity has been shown in cats during normal breathing, either in the geniohyoid or the branch of the hypoglossal nerve supplying it (30). Shown in cats during normal breathing, either in the geniohyoid or the branch of the hypoglossal nerve supplying it (30).

A challenge with the measurement of EMG activity from the geniohyoid is that the adjacent genioglossus exhibits phasic (both inspiratory and expiratory) and tonic activity (2, 24, 25, 29, 39). It is possible that the occasional tonic and expiratory phasic activity recorded in our study was from the genioglossus muscle, although we excluded recordings with distant activity. It may be that the geniohyoid muscle exhibits activity in some subjects. Because of the care taken to verify electrode placement and because there was always activity recorded during the geniohyoid maneuver, it is unlikely that our finding of minimal activity in geniohyoid during quiet breathing is due to incorrect electrode placement. In this study two checks were used to verify electrode position—an in vivo anatomic check at the time of the experiment and an electrophysiological check at each recording site. Also, in two subjects the electrode location was verified by ultrasound. Thus, in each subject, the depth of the geniohyoid was imaged with ultrasound before electrode recording in each posture (supine and seated), and physiological maneuvers such as the first part of a swallow were used to assess voluntary activation of the muscle. Little is known about the activity of SMUs in the geniohyoid during the experimental maneuvers used here, as previous EMG studies of swallowing and tongue protrusion have used multiunit recordings. Voluntary anterior displacement of the hyoid bone was used as a maneuver to confirm geniohyoid electrode placement based on the known actions of the muscle (3, 4, 14, 27). The geniohyoid is the most consistently activated muscle in swallowing (27), but there is no specific action for the geniohyoid as the suprahyoid muscles act in concert (21), and the intrinsic and extrinsic tongue muscles are also involved in swallowing. Hrycyszyn and Basmajian (14) found significant intersubject variation in geniohyoid recruitment in swallowing. It has been postulated that the inferior and posterior fibers of the genioglossus are activated with tongue protrusion, with the anterior and superior fibers having a lesser role (21). Thus tongue protrusion was used to differentiate between the lower genioglossus and geniohyoid. However, this action is effort dependent and not completely specific to genioglossus (11, 34), so in our study activity during tongue protrusion did not rule out the recording being from the geniohyoid. Hence we relied primarily on depth of recording site to verify anatomic position.

Minimal respiration-related activity in the geniohyoid contrasts with the activity in other upper airway muscles in close proximity. Upper airway mechanoreceptors provide within-breath reflex activation of airway dilators to protect the airway (1), but our results suggest that the geniohyoid is not involved in this airway regulation during quiet breathing in healthy subjects. Thus genioglossus and geniohyoid behave differently during quiet breathing. SMU studies of the genioglossus have previously shown that the genioglossus has a complex neural drive including inspiratory and expiratory phasic and tonic discharges (2, 24, 25, 29, 39). Future studies are needed to look at the role of the geniohyoid under conditions of increased respiratory load including whether it is recruited in subjects with OSA due to increased airway collapsibility. The results of this study suggest that the genioglossus and geniohyoid receive different euean respiratory and voluntary drives, and although the geniohyoid may have a role in stabilizing the airway it is not a principal airway dilator, as it is not usually active on a breath-by-breath basis. Although sharing a common
innervation from nearby brain stem nuclei and close anatomic proximity, the two muscles have different patterns of activity.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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