Effects of Aging on Genioglossus Motor Units in Humans

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Effects of Aging on Genioglossus Motor Units in Humans

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Abstract

The genioglossus is a major upper airway dilator muscle thought to be important in obstructive sleep apnea pathogenesis. Aging is a risk factor for obstructive sleep apnea although the mechanisms are unclear and the effects of aging on motor unit remodeling in the genioglossus remains unknown. To assess possible changes associated with aging we compared quantitative parameters related to motor unit potential morphology derived from EMG signals in a sample of older (n = 11; >55 years) versus younger (n = 29; <55 years) adults. All data were recorded during quiet breathing with the subjects awake. Diagnostic sleep studies (Apnea Hypopnea Index) confirmed the presence or absence of obstructive sleep apnea. Genioglossus EMG signals were analyzed offline by automated software (DQEMG), which estimated a MUP template from each extracted motor unit potential train (MUPT) for both the selective concentric needle and concentric needle macro (CNMACRO) recorded EMG signals. 2074 MUPTs from 40 subjects (mean±95% CI; older AHI 19.6±9.9 events/hr versus younger AHI 30.1±6.1 events/hr) were extracted. MUPs detected in older adults were 32% longer in duration (14.7±0.5 ms versus 11.1±0.2 ms; P = 0.05), with similar amplitudes (395.2±25.1 µV versus 394.6±13.7 µV). Amplitudes of CNMACRO MUPs detected in older adults were larger by 22% (62.7±6.5 µV versus 51.3±3.0 µV; P<0.05), with areas 24% larger (160.6±18.6 µV.ms versus 130.0±7.4 µV.ms; P<0.05) than those detected in younger adults. These results confirm that remodeled motor units are present in the genioglossus muscle of individuals above 55 years, which may have implications for OSA pathogenesis and aging related upper airway collapsibility.


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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

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Introduction

Obstructive sleep apnea is a common disorder that increases in prevalence with age, although the mechanisms are unclear. The genioglossus is a major upper airway dilator muscle whose activity is thought to be representative of muscles critical for maintaining pharyngeal patency. Thus, research into the motor control of the genioglossus is likely to provide insights into sleep apnea pathogenesis. Motor unit potential (MUP) analysis provides insight into the normal function of skeletal muscle and aids in the assessment of neuromuscular disorders [1]. For example, skeletal muscle remodeling is associated with physiological factors that can change the characteristics of MUPs [2–4]. MUPs with increased durations can be detected in many skeletal muscles, reflected as remodeled motor unit territories as a result of denervation, collateral sprouting and reinnervation [5–7].

Anatomically the genioglossus muscle is one of the largest extrinsic muscles of the tongue [8–10]. The hypoglossal nerve branches that innervate the genioglossus muscle are much denser in humans compared to other species [11–15], likely reflecting small motor unit territories required for the high level of fine motor control required for speech. The complex innervation of the muscles of the tongue may indicate they are less prone to aging effects than is seen in other skeletal muscles [16]. Structural remodeling changes previously reported in the tongue musculature of obstructive sleep apnea patients [17] may not be characterized by a proximal weakness, such as, overt dysphagia, but, may nevertheless predispose the pharyngeal airway to collapse with increasing age.

The activity of the human genioglossus is complex, with activity in phase with both inspiration and expiration [18–21]. Anatomically the position of the genioglossus muscle and its role in dilating the airway is of great interest in understanding the pathogenesis of obstructive sleep apnea [17,22–24]. The tongue also plays a critical role in swallowing and speech pathologies [25]; therefore, understanding how the neuromuscular innervation may be remodeled with aging is clinically important and may provide insight for therapy or treatment based on age.
Changes that occur within the pharyngeal musculature with aging remain incompletely understood. Despite the fact that the genioglossus muscle is known to contain a high proportion of Type II muscle fibers and aging effects are pronounced for Type II fibers, [26,27], we recently found no evidence for increased MUP durations associated with age in humans [17]. While some investigators have found a continuous increase of MUP durations in skeletal muscles from 1 year of life up to 80 years [28] other investigators have demonstrated that the aging effect on MUP duration occurs predominantly after 35 years of age [29]. Previously, we reported neural injury associated with obstructive sleep apnea patients, observed by an increase in the durations of MUPs of the genioglossus muscle of humans [17,22]. However, these investigations were not specifically targeted to investigate aging, as we included primarily younger adults.

Limited information exists about changes in the morphology of MUPs with aging. Based on the aging literature compensatory adaptation with muscle fiber hypertrophy or neurogenic changes such as collateral sprouting of motor axons may occur indicating a remodelling of the motor units [2,30,31]. We aimed to investigate the effect of aging, in genioglossus muscle, with a range of conventional and specialized electromyographic techniques to obtain features of EMG signals that relate to one or more aspects of normal and pathologic function. Thus, we measured the activity from the genioglossus in younger and older adults (both with and without obstructive sleep apnea) while they were awake and breathing quietly. Based on the MUP literature we hypothesized that MUPs detected in older adults (>55 years) would show signs for greater degrees of reinnervation (collateral axonal sprouting).

Methods

The present investigation was based on new analyses of motor units obtained during awake, quiet breathing from a previous experiment [17], together with newly acquired data from nine older subjects. The previous investigation was designed to confirm neurogenic changes in patients with obstructive sleep apnea versus healthy control participants. This current reanalysis allowed us to compare a large number of MUPs in a group of participants spanning a large age range who were all screened for obstructive sleep apnea. Demographic details of the older and younger group are given in Table 1. Control subjects were defined with an AHI of less than 10 events/hr. Newly acquired data included: four controls [65±2.8 years, age range: 61–67 years, apnea hypopnea index (AHI): 4.5±2.1 events/hr, AHI range: 0.6–9.3 events/hr] and five OSA [67±5.6 years, age range: 58–74 years, AHI: 36.3±12.1 events/hr, AHI range: 24–56 events/hr]. The demographic details for a detailed sub-analysis on control subjects are given in Table 2. All subjects gave written, informed consent before participation in this study, which had been approved by the Partners’ Human Research Committee and conformed to the Declaration of Helsinki.

**Table 1. Subject demographics for study populations.**

<table>
<thead>
<tr>
<th>Group subject characteristics</th>
<th>Older</th>
<th>Younger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>11</td>
<td>29</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.2±4.6</td>
<td>36.8±2.9</td>
</tr>
<tr>
<td>(Age range: 74–58)</td>
<td>(Age range: 53–19)</td>
<td></td>
</tr>
<tr>
<td>Female/male</td>
<td>2/9</td>
<td>8/21</td>
</tr>
<tr>
<td>Apnea hypopnea index (events per hour of sleep)</td>
<td>19.6±9.9</td>
<td>30.1±6.1</td>
</tr>
<tr>
<td>(AHI range: 56–0.6)</td>
<td>(AHI range: 93.6–0.6)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.2±6.1</td>
<td>173.7±4.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.9±11.4</td>
<td>94.0±9.1</td>
</tr>
<tr>
<td>Body mass index (BMI, kg/m²)</td>
<td>27.5±3.1</td>
<td>30.9±2.4*</td>
</tr>
<tr>
<td>Neck size (cm)</td>
<td>38.9±2.6</td>
<td>40.3±1.5</td>
</tr>
<tr>
<td>Number of units per subject</td>
<td>49.7±15.0</td>
<td>52.7±7.9</td>
</tr>
<tr>
<td>Inferior margin of geniohyoid muscle (mm)</td>
<td>15.9±2.6</td>
<td>15.1±1.5</td>
</tr>
<tr>
<td>Inferior margin of genioglossus muscle (mm)</td>
<td>24.8±2.4</td>
<td>25.3±1.5</td>
</tr>
<tr>
<td>Genioglossus width (mm)</td>
<td>16.3±0.8</td>
<td>18.0±0.8</td>
</tr>
<tr>
<td>Modified borg scale (0–10)</td>
<td>2.8±0.8</td>
<td>2.4±0.5</td>
</tr>
</tbody>
</table>

The modified Borg Scale was used to measure the “worst level of pain” experienced by each subject during the recording with 0 = Nothing at all and 10 = Maximal. Values are Mean ± CI. *indicates P<0.05. 

doi:10.1371/journal.pone.0104572.t001
To determine the depth and location of the needle electrodes in the genioglossus the anatomy of the pharyngeal musculature was examined with ultrasonography (12 L high-frequency linear array transducer, Vivid i GE Healthcare Chalfont St. Giles, Bucks, UK) [18,19,22,32]. The distance from the surface of the skin to the inferior margin of the genioglossus and geniohyoid muscles and the lateral width of the genioglossus were recorded using an electronic caliper [32] (see Table 1 for values).

**Table 2.** Demographics for older versus younger control subjects.

<table>
<thead>
<tr>
<th>Group subject characteristics</th>
<th>Older</th>
<th>Younger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.8±3.2</td>
<td>32.5±4.5</td>
</tr>
<tr>
<td>(Age range: 67–59)</td>
<td>(Age range: 42–19)</td>
<td></td>
</tr>
<tr>
<td>Female/male</td>
<td>7/4</td>
<td>6/7</td>
</tr>
<tr>
<td>Apnea hypopnea index (events per hour of sleep)</td>
<td>5.1±3.3</td>
<td>3.4±1.4</td>
</tr>
<tr>
<td>(AHI range: 9.3–0.6)</td>
<td>(AHI range: 8.7–0.6)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.4±9.3</td>
<td>169.3±6.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.9±13.1</td>
<td>85.4±15.9</td>
</tr>
<tr>
<td>Body mass index (BMI, kg/m²)</td>
<td>27.3±4.8</td>
<td>29.3±3.9</td>
</tr>
<tr>
<td>Neck size (cm)</td>
<td>38.1±3.2</td>
<td>38.3±2.6</td>
</tr>
<tr>
<td>Number of units per subject</td>
<td>61.2±28.7</td>
<td>47.9±9.3</td>
</tr>
<tr>
<td>Inferior margin of geniohyoid muscle (mm)</td>
<td>15.2±0.4</td>
<td>14.3±0.2</td>
</tr>
<tr>
<td>Inferior margin of genioglossus muscle (mm)</td>
<td>23.8±0.3</td>
<td>23.6±0.2</td>
</tr>
<tr>
<td>Genioglossus width (mm)</td>
<td>16.3±0.2</td>
<td>17.7±0.1</td>
</tr>
<tr>
<td>Modified borg scale (0–10)</td>
<td>3.2±1.1</td>
<td>2.7±0.9</td>
</tr>
</tbody>
</table>

Control subjects were defined with an AHI of less than 10 events/hr. doi:10.1371/journal.pone.0104572.t002

**RECORDINGS**

To determine the depth and location of the needle electrodes in the genioglossus the anatomy of the pharyngeal musculature was examined with ultrasonography (12 L high-frequency linear array transducer, Vivid i GE Healthcare Chalfont St. Giles, Bucks, UK) [18,19,22,32]. The distance from the surface of the skin to the inferior margin of the genioglossus and geniohyoid muscles and the lateral width of the genioglossus were recorded using an electronic caliper [32] (see Table 1 for values).

Topical anesthetic cream (Emla, AstraZeneca) was placed on the surface of the skin under the chin for a minimum of 30 minutes. The chin was then thoroughly cleaned using disposable topical antiseptic wipes and a small reference location was drawn 10 mm posterior to genial tubercle in the midline under the chin. With the subjects relaxed and lying supine two concentric needle electrodes (26G, 50 mm, recording area 0.07 mm²; TECA ELITE) were inserted lateral to the midline at 90° to the skin surface and advanced through underlying muscle

**Figure 1. Typical example of genioglossus data.** Shown is a typical example of two selective concentric MUP (left) and CNMACRO MUP (right) templates from a recording from an older (top panel; 71 years, AHI 2.8) and younger (lower panel; 38 years, AHI 9.3) subject. Calibrations of the selective concentric needle MUP and CNMACRO MUP, 500 µV and 2 ms. The duration of the MUP as measured between the onset of the first and the offset of the last deviation from the baseline of the MUP are indicated by the vertical dotted lines. The duration is marked relative to the timing of the young MUPs to indicate the lengthening of the MUPs in older subjects.

doi:10.1371/journal.pone.0104572.g001
Figure 2. Mean feature values of selective concentric needle MUPs and concentric needle macro (CNMACRO) MUPs. Each panel represents mean values of selected features from all the individual MUPs and CNMACRO MUPs. The solid columns depict values from older subjects and the shaded columns represent values from younger subjects. Significance is given where appropriate in the respective panels. doi:10.1371/journal.pone.0104572.g002
Aging Effects Genioglossus Motor Units in Humans

- **Duration**
  - Younger: 10.8 ms
  - Older: 13.6 ms
  - P < 0.05

- **Amplitude**
  - Younger: 314.8 µV
  - Older: 320.8 µV

- **Area**
  - Younger: 479.2 (µV.ms)
  - Older: 487.2 (µV.ms)

- **Size Index**
  - Younger: 4.01
  - Older: 4.08

- **Area to Amplitude Ratio**
  - Younger: 1.46
  - Older: 1.52
  - P < 0.05

- **Relative Irregularity Coefficient**
  - Younger: 13.9
  - Older: 17.2
  - P < 0.05

- **CNMACRO Duration**
  - Younger: 12.9 ms
  - Older: 12.5 ms

- **CNMACRO Amplitude**
  - Younger: 37.8 µV
  - Older: 44.9 µV
  - P < 0.05

- **CNMACRO AREA**
  - Younger: 98.3 (µV.ms)
  - Older: 112.2 (µV.ms)
  - P < 0.05
Figure 3. Histogram display with the featured values of selective concentric needle MUPs and concentric needle macro (CNMACRO) MUPs from all 40 subjects. The solid filled columns depict values from younger subjects and the white columns represent values from younger subjects. Medians are indicated by arrows in each panel and in each circumstance the filled arrow indicates the median of younger subjects and the unfilled arrow depicts the median of older subjects. Significance is given where appropriate in the respective panels. doi:10.1371/journal.pone.0104572.g003

Table 3. Key results from all 40 subjects.

<table>
<thead>
<tr>
<th></th>
<th>Older</th>
<th>Younger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of MUP templates</td>
<td>547</td>
<td>1527</td>
</tr>
<tr>
<td>Number of MUPTs/recording</td>
<td>2.7±0.3</td>
<td>3.2±0.2*</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>14.8±0.4</td>
<td>11.0±0.2*</td>
</tr>
<tr>
<td>Number of phases</td>
<td>2.8±0.1</td>
<td>2.9±0.0*</td>
</tr>
<tr>
<td>Number of turns</td>
<td>3.7±0.2</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>Amplitude (μV)</td>
<td>400.3±23.6</td>
<td>393.1±14.3</td>
</tr>
<tr>
<td>Area (μV.ms)</td>
<td>578.7±31.2</td>
<td>560.4±18.8</td>
</tr>
<tr>
<td>Thickness</td>
<td>1.6±0.0</td>
<td>1.5±0.0*</td>
</tr>
<tr>
<td>Area/Phase</td>
<td>208.6±11.0</td>
<td>196.3±6.6</td>
</tr>
<tr>
<td>RIR</td>
<td>19.0±1.8</td>
<td>22.3±1.1*</td>
</tr>
<tr>
<td>Size index</td>
<td>4.1±0.0</td>
<td>4.0±0.0</td>
</tr>
<tr>
<td>Number of CNMACRO MUP templates</td>
<td>383</td>
<td>1033</td>
</tr>
<tr>
<td>MACRO area (μV.ms)</td>
<td>167.7±14.2</td>
<td>125.1±8.8*</td>
</tr>
<tr>
<td>MACRO amplitude (μV)</td>
<td>65.4±5.4</td>
<td>492.3±3.4*</td>
</tr>
<tr>
<td>MACRO duration (ms)</td>
<td>14.9±0.9</td>
<td>14.1±0.5</td>
</tr>
</tbody>
</table>

Values are Mean ± CI. *indicates a significant difference P<0.05. doi:10.1371/journal.pone.0104572.t003
fibers closest to the electrode. Area was measured as the sum of the rectified MUP sample values over the duration (in μVs) times the sampling interval (in ms). Area is less susceptible to measurement miscalculation than amplitude and is able to characterize abnormal potentials. The number of MUP phases was defined as the number of baseline crossings minus 1. A turn of a MUP was defined as a change in the direction of the MUP waveform with a magnitude of at least 25 μV. The complexity of a MUP was quantified with a number of indices, including the resulting: area/phase ratio, which is better able to distinguish myopathic MUPs from normal MUPs. The table below summarizes the key results from the 18 control subjects.

**Table 4. Key results for the 18 control subjects.**

<table>
<thead>
<tr>
<th></th>
<th>Older</th>
<th>Younger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of MUP templates</td>
<td>306</td>
<td>623</td>
</tr>
<tr>
<td>Number of MUPTs/recording</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>14.1 ± 0.7</td>
<td>10.5 ± 0.3*</td>
</tr>
<tr>
<td>Amplitude (μV)</td>
<td>358.3 ± 29.1</td>
<td>385.1 ± 20.2</td>
</tr>
<tr>
<td>Number of phases</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± 0.1*</td>
</tr>
<tr>
<td>Number of turns</td>
<td>3.7 ± 0.2</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Area (μV.ms)</td>
<td>520.3 ± 38.8</td>
<td>529.2 ± 27.7</td>
</tr>
<tr>
<td>Thickness</td>
<td>1.6 ± 0.1</td>
<td>1.4 ± 0.0*</td>
</tr>
<tr>
<td>Area/Phase</td>
<td>188.4 ± 13.3</td>
<td>188.8 ± 9.6</td>
</tr>
<tr>
<td>RIR</td>
<td>19.6 ± 2.2</td>
<td>18.7 ± 1.3</td>
</tr>
<tr>
<td>Number of CNMACRO MUP templates</td>
<td>228</td>
<td>389</td>
</tr>
<tr>
<td>MACRO amplitude (μV)</td>
<td>51.2 ± 6.2</td>
<td>40.7 ± 3.4*</td>
</tr>
<tr>
<td>MACRO area (μV.ms)</td>
<td>130.2 ± 15.2</td>
<td>105.5 ± 8.4*</td>
</tr>
<tr>
<td>MACRO duration (ms)</td>
<td>14.3 ± 1.1</td>
<td>13.2 ± 0.6</td>
</tr>
</tbody>
</table>

**Figure 4. Duration of MUPs plotted versus subject age.** Mean MUP duration (ms) versus age (years). The MUPs with longer durations are associated with increased age. The Pearson correlation coefficient was calculated from the mean value of all 40 subjects (r = 0.660; P = 0.00000359). The open black circles depict values from older control subjects while solid circles depict values from older obstructive sleep apnea subjects. The open grey circles depict values from younger control subjects while solid circles depict values from younger obstructive sleep apnea subjects. doi:10.1371/journal.pone.0104572.g004
from normal MUPs with greater accuracy than area alone. The size index and area/amplitude ratio \([38]\) are parameters that assist in distinguishing between neurogenic and regular MUPs and control for the effect of needle position \([39]\). The relative irregularity coefficient \([40,41]\) encapsulates the complexity of the MUP waveform and is calculated as: \(\text{RIR} = \left(\frac{S-2A}{2A}\right) \times 100\) (where \(A\) is the amplitude, and \(S\) is calculated as the sum of the absolute values of the intersample changes seen in the MUP waveform template, i.e., the irregularity in the MUP template). Thus, with partial denervation, denervated muscle fibers are reinnervated by peripheral sprouting thereby the territory the remaining motor units occupy is increased \([42]\). These new axonal branches conduct impulses with a lower velocity over extended distances, reflected as longer duration and more complex MUP waveforms. The maintenance of muscle size and force generating ability effectively conceals the decreased numbers of surviving motoneurons that have regenerated their axons \([43]\). All parameter values were manually inspected by a single investigator using DQEMG who was blinded to subject OSA status. For each valid concentric needle MUPT extracted a CNMACRO MUP template was extracted from the raw cannula signal using ensemble averaging. The structures of the concentric needle macro (CNMACRO) MUPs were also measured, using previously established methods \([17]\).

DATA ANALYSIS

Statistical differences for the dependent MUP feature values were assessed using two-way analyses of variance (ANOVA) to determine the effects of older and younger adults, with the interaction of age with obstructive sleep apnea status (patients and control subjects). Remaining comparisons between older and younger adults were performed using one-way ANOVA. Comparisons utilized the Student-Newman-Keuls post-hoc analysis. As data were not normally distributed a Kruskal-Wallis one way ANOVA on ranks was performed with Dunn’s method post-hoc test applied. The non-parametric statistics are reported in the Figures (SigmaPlot 11). Statistical significance was set at \(P<0.05\). Values are given as the mean ± 95% CI.

Results

TOTAL ANALYSIS

Electromyographic data were recorded from forty subjects yielding a total of 671 successful unitary recordings from the genioglossus muscle with selective concentric needle and concentric needle macro (CNMACRO) electrodes. The DQEMG algorithms provided a total of 2074 selective concentric needle MUP templates (older 547 versus younger 1527), and 1526 CNMACRO MUP templates (older 493 versus younger 1033). From these recordings 929 of the motor units sampled were from control subjects (306 older [4.4±0.4 events/hr] versus 623 younger [3.6±0.2 events/hr]). On average, the number of MUPs extracted per recording was lower for older adults compared to younger adults (2.7±0.3 versus 3.2±0.2; \(P<0.05\), Dunn’s Method). Data underlying the findings reported herein can be located in the File S1. “DQEMG_Data_Aging”.

Figure 5. Mean relative irregularity coefficient versus minimal oxygen saturation. More complex motor unit potentials (MUPs), as calculated with the relative irregularity, are associated with minimal oxygen saturation detected from the overnight polysomnography. The Pearson correlation coefficient was calculated from the mean value of 37 subjects \((r=0.633; P=0.0000267)\). The three squares on the X-axis represent relative irregularity coefficient data from subjects whose minimal oxygen data were not obtained (not included in the correlation).
doi:10.1371/journal.pone.0104572.g005
MOTOR UNIT POTENTIALS

The compound effects of aging together with the influence of obstructive sleep apnea effects were investigated with two approaches; utilizing a two-way ANOVA and a detailed sub-analysis (see Detailed sub-analysis below).

The selective concentric needle MUPs detected in older adults had 34% longer duration \( P < 0.001 \), with greater thickness \( P < 0.05 \), but did not have greater amplitude, area, number of phases or RIR coefficient, than those detected in younger adults (see Figures 2, 3 & 4; Table 3).

For the OSA patients, MUPs with increased amplitudes were detected in older patients compared to younger patients \( 442.2 \pm 35.3 \mu V \ versus \ 401.2 \pm 18.1 \mu V; P < 0.05 \). However, for the OSA patients, more complex MUPs, as quantified using the RIR coefficient, were detected in younger patients compared to older patients \( 25.9 \pm 1.4 \ versus \ 18.3 \pm 2.7; P < 0.05 \; (Figure \ 2) \). We found that the complexity of the motor unit waveform as calculated with the RIR coefficient to correlate with the minimal oxygen saturations \( P < 0.001 \).

CONCENTRIC NEEDLE MACRO MOTOR UNIT POTENTIALS

CNMACRO MUPs reflect the overall number of muscle fibers in a motor unit \([16]\). We estimated a CNMACRO MUP template by ensemble averaging the cannula recorded EMG signal using the discharge times of the respective individual motor unit during normal eupnic breathing. As not all sites that recorded a MUP had an adequately measureable CNMACRO MUP the total number was 1415. The CNMACRO MUPs detected in older adults had larger amplitudes \( (32\%\); \( P < 0.001 \), and areas \( (34\%\) than those detected in younger adults \( P < 0.001 \; (see \ Table \ 3 \ and \ Figure \ 2 \ & \ 3) \).

The CNMACRO MUP area was larger in OSA patients compared to healthy controls by, 37.2% in the younger subjects \( P < 0.05 \), and areas \( (34\%\) than those detected in younger adults \( P < 0.05 \; (see \ Table \ 3 \ and \ Figure \ 2 \ & \ 3) \).

Detailed sub-analysis

To determine the effect of aging independent of the presence of obstructive sleep apnea (a known contributor of neurogenic changes in the genioglossus muscle \([17]\)) a sub-analysis was performed on the eighteen control subjects. The older adults \( n = 5 \) AHI \( = 5.1 \pm 3.3 \ events/ hr \ versus \ younger \ adults \( n = 13 \) \( 3.4 \pm 1.4 \ events/hr \ (range \ 0.6-9.3 \ events/hr; \ Table \ 2 \ for \ demographic \ properties \). This analysis included 306 MUP templates and 228 CNMACRO MUP templates from older adults, and 623 MUP templates and 389 CNMACRO MUP templates from younger adults (for results see Table 4). The selective concentric needle MUPs detected in older adults had longer durations \( (34\%\), see Figure 4\), and greater thickness \( (14\%\) compared to those detected in younger adults \( P < 0.05 \). CNMACRO MUPs detected in the older adults had greater area \( (23\%\) and amplitude \( (25\%\) compared to those detected in younger adults \( P < 0.05 \).

Discussion

This study clearly demonstrates age-related changes in motor unit potentials (MUPs) detected in the genioglossus muscle. We found evidence for remodeling suggesting denervation, collateral sprouting and reinnervation of orphaned muscle fibers leading to increased motor unit size, spatial dispersion of motor unit territories and, size and temporal dispersion of motor unit potential (MUP) components in older adults. The functional consequences of upper airway remodeling remain inconclusive; however, the age related changes are likely to involve an increased predisposition to obstructive sleep apnea (OSA).

Aging is a major factor contributing to the risk of obstructive sleep apnea, although the exact mechanisms as to how aging affects OSA risk remains incompletely understood (for review see: \([44]\)). Multiple investigators have found increased prevalence of sleep apnea in older individuals from cross sectional studies \( [45–47] \). Despite a few pathophysiological traits having been explored the proportion of key anatomic and neuro-physiological variables that may indeed contribute to the worsening of OSA are not clearly defined. Some factors linked to OSA, such as the ventilatory control system \([48]\), parasympathetic-nervous activity \([49–51]\), arousal threshold \( [52]\) and cardiovascular responses to arousal \( [53,54]\) remain stable in older adults. However, there have been a number of factors documented that may predispose individuals to obstructive events and which are age related \([55,56]\).

Anatomically the volume of the parapharyngeal fat pads has been documented to increase with age \([57]\) independent of overall body fat and neck circumference \([58]\). However, aging effects on upper airway anatomy are variable in different studies, perhaps reflecting the complexity of sleep apnea pathogenesis \([57–62]\). Furthermore, sensory impairments may be linked to a decrement in the genioglossus reflexes during wakefulness \([57]\), although effects of aging on overall pharyngeal motor control are also complex \([63–66]\). These physiologic traits may increase the susceptibility for upper airway collapse that occurs in older adults \([52]\).

Our study supports the hypothesis that older individuals have neurogenic changes in the genioglossus muscle. We found MUPs with increased durations and numbers of phases suggesting that peripheral axons have sprouted to compensate for a loss of motor axons. These data indicate that the presence of ‘neurogenic’ changes is associated with the physiologic aging processes in the genioglossus muscle. In addition, as we detected a lower number of motor unit potential trains per recording in older subjects, this finding may indicate fewer motor neurons and thus a lower level of motor unit recruitment in older adults. Importantly, these data were collected during wakefulness. In young adults approximately 50% of inspiratory modulated genioglossus motor units become inactive at sleep onset \([67]\). While there is no data on older adults, if the proportional fall were the same as in young adults, we speculate that at sleep onset fewer motoneurons would be active in older adults leading to repetitive airway collapse. Interestingly, the more complex MUPs, were detected in younger patients indicating ongoing reinnervation. The smoothed less complex MUPs in the older adults may indicate a reduced level of ongoing, as compared to completed, reinnervation in the older adults.

We sampled motor units using two established electromyographic techniques both selective concentric needle electrodes, and non-selective concentric needle macro (CNMACRO) electrodes. These two methods complement each other such that the fiber density and fiber distribution of the whole cross-section of a motor unit can be explored \([68,69]\). While a selective concentric needle MUP with prolonged duration can be an indicator of localized peripheral neuropathy, the size of a CNMACRO MUP is an established parameter related to the number of fibers belonging to its respective motor unit. Specifically, the area of a CNMACRO MUP has been linked to the twitch force generated by its motor unit \([33,70]\). CNMACRO MUPs therefore can provide unique insight into the contribution of a motor unit to the force produced by a muscle. Our evidence of increased amplitude and area of CNMACRO MUPs demonstrates that older adults may have increased force generation per motor unit activation. Accordingly, our results suggest a greater percentage of muscle fibers will be
recruited with each depolarization of a motoneuron for older subjects; thereby, changes are partially concealed through the increased force produced of reinnervated orphaned fibers [71]. This finding suggests that the upper airway in older adults may be compromised due to an overall loss of motoneurons. The airway is therefore, particularly more likely to collapse due to fewer motor units available to be recruited during quiet breathing (despite the compensatory changes in force output of remodeled units). Overall, a smaller reserve of motor units remains available for dilating the airway due to the remodeling processes within the muscle.

Our study has a number of strengths. In contrast to prior studies we used ultrasound guidance to ensure electrodes were placed in the genioglossus [72,73]. In addition, we studied the genioglossus muscle during normal eupnic breathing, as opposed to studies using volitional output that may target different non-respiratory muscles: II. Compartmentalization of the canine genioglossus muscle. Anatomical Record 246: 378–386.


variable. However, as clearly observable in the results of the sub-analysis reported in Table 4, independent of sleep apnea there is an age related increase in a number of key parameters. Finally, the data in this study showed a very clear, highly significant main effect of age in the two-way ANOVA. Therefore, despite these acknowledged limitations, we believe our findings provide an important advance in the literature.

In summary, the results of this study indicate that neurogenic changes occur in the genioglossus muscle of older adults. While it is unlikely a single mechanism can explain all the data concerning the alterations in the MUFs observed, we believe these changes reflect the ongoing influence of age [76]. The results indicate, structural neurogenic changes are present in older adults.

Supporting Information

File S1 “DQEMG_Data_Aging”. (XLSX)

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Author Contributions

Conceived and designed the experiments: JPS DWS AH-W SN JT AM. Performed the experiments: JPS. Analyzed the data: JPS DWS AH-W SN. Contributed reagents/materials/analysis tools: JPS DWS AH-W. Contributed to the writing of the manuscript: JPS DWS AH-W SN JT AM.

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