1. Title: Parameters affecting pharyngeal response to genioglossus stimulation in sleep apnoea.

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4. Key words:
   1) Critical pressure
   2) Electrical stimulation
   3) Genioglossus
   4) Sleep apnoea syndrome
   5) Velopharynx
ABSTRACT

Chronic stimulation of the hypoglossus nerve may provide a new treatment modality for obstructive sleep apnoea (OSA). In previous studies we observed large differences in response to stimulation of the genioglossus (GG). We hypothesized that both individual patient characteristics and the area of the GG stimulated are responsible for these differences.

In the present study we compared the response to GG stimulation at the anterior area (GGa-ES), that activates the whole GG, and the posterior area (GGp-ES), that activates preferentially the longitudinal fibers. Studies were performed in 14 propofol-sedated OSA patients. The parameters evaluated included cephalometry, pressure-flow relationship, and pharyngeal shape and compliance assessed by pharyngoscopy.

Compared to GGa-ES, GGp-ES resulted in significantly larger decreases in Pcrit (from 3.8±2.2 to 2.9±3.3 and to -2.0±3.9 cmH2O, respectively (p<0.001)). Both tongue size and velopharyngeal shape (antero-posterior to lateral ratio) correlated significantly with the decrease in Pcrit during GGp-ES (R=0.53 and -0.66, respectively, p<0.05). In the patients with the larger tongue size (n=7), the decrease in Pcrit reached 8.0±2.2 cmH2O during GGp-ES.

We conclude that directing stimulation to longitudinal fibers of the GG improves the flow-mechanical effect. In addition, patients with large tongues and narrow pharynx tend to respond better to GGp-ES.
INTRODUCTION

Obstructive sleep apnoea (OSA) is a highly prevalent syndrome with multiple clinical implications [1]. It is estimated that OSA affects 4% of men and 2% of women, with continuously increasing incidence due to the obesity epidemic. The current treatment of OSA is the continuous positive airway pressure (CPAP) device, a simple yet very efficient modality, but unfortunately also uncomfortable, resulting in poor adherence. The obvious need for new treatment modalities is directed toward two lines of approach: anatomic (such as surgical procedures), and functional, i.e., electrical stimulation of the upper airway dilator muscles [2,3]. The genioglossus muscle, which is the main tongue protrusor, has been shown to reduce pharyngeal resistance and collapsibility by far more than other upper airway dilator muscles [4,5], and has become, therefore, the main target for functional stimulation for therapeutic purposes [2,3].

In previous studies we found that genioglossus contraction improves pharyngeal stability in OSA patients during sleep and anaesthesia [6,7], characterized by reduction of the pressure at which the pharynx collapses and occludes (the critical pressure, Pcrit). However, large inter-individual differences in both the magnitude of decrease in Pcrit (i.e., improvement in stability) and enlargement of the pharynx were observed [7]. We hypothesized that both individual patient-characteristics and the mechanical function of the area of the genioglossus where stimulation was applied may be responsible for these differences. As the genioglossus fibers are arranged as a hand held fan, the anterior, vertical fibers cause primarily depression of the tongue, and only the dorsally-oriented horizontal fibers protrude the tongue and can enlarge the pharynx [8]. Accordingly, directing stimulation to the protrusive part of the genioglossus is likely to improve the flow-mechanical response. In addition, we expected that in subjects with a relatively large protrusive part of the genioglossus, contraction of this muscle will have a larger dilatory effect on the pharynx.

Identification of parameters that improve the response to functional stimulation of the tongue may help selecting patients most appropriate for this approach as a treatment modality. Accordingly, the present study was designed to compare the effect of electrical stimulation of the anterior versus the posterior parts of the tongue on pharyngeal stability and size in OSA patients. In addition, we evaluated the relationship between individual relevant cephalometric characteristics of our patients and the response to electrical stimulation of the genioglossus.
METHODS

Subjects: Letters were sent to all patients who had undertaken a full sleep study in the Technion Sleep Laboratory (Technion, Haifa, Israel) during the year prior to the present study and found to have an apnoea-hypopnoea index > 20, requesting them to participate in this research. Patients with any disease that could pose a risk during anesthesia, including ischemic heart disease, any lung disease, severe or uncontrolled hypertension, and a body mass index (BMI) more than 35, as well as subjects with known side-effects to any previous anesthesia, were excluded. All studies were performed in the respiratory research laboratory of Bnai Zion Medical Center (Technion). The aims and potential risks of the study were explained, and informed consent was obtained from all subjects. The study was approved by the Human Investigations Review Board of Bnai Zion Medical Center.

Cephalometry: Lateral cephalometric radiographs were performed with the subjects erect. The patients were directed to gaze forward, holding their heads in a natural position with the jaw closed, and digital head x-ray was taken. Exposure parameters were chosen to enable visualization of both bony and soft tissue landmarks. Out of a large number of published cephalometric parameters [9-11], we chose for analysis seven variables considered to have the potential to affect the response to genioglossus contraction (figure 1). These parameters included mental position in relationships to the maxilla (angle between the nose base to the maxilla and the nose base to the chin), distance of the hyoid bone from the mandible, and sagittal cross-sectional area of the velo-and oropharynx and soft palate. In addition, the sagital cross-sectional area of the tongue, divided into the protrusor and depressor part of the genioglossus (by drawing a line between the mid-point of mandibular insertion to the posterior edge of the hard palate) was also measured. In addition, the "mouth-box" was measured, based on Tsuiki et al. description of the lower face cage [11] with minor modifications, as the area enclosed by the front teeth, hard palate, lower margin of the mandible and the anterior boarder of the vertebral column. The cross-sectional size of the tongue was calculated also as a ratio of this area.

Ultrasound: In order to direct intra-muscular electrodes placement, submental soft tissue anatomy was examined using an ultrasound linear probe [12]. Coronal scans were obtained with the transducer directed vertically under the chin in the midline and axial scans obtained by turning the transducer 90° from this position. During each scan, care was taken to minimize compression of the skin under the chin by the probe, and scanning sessions were recorded. The surface of the tongue, including the anterior wall of the pharynx, was visualized to assess the distance and direction for genioglossus electrodes placement, and Doppler was used to delineate the position of local blood vessels, to prevent their injury.

Recording procedures: Standard polysomnographic techniques, including submental surface electromyography (EMG), C3/O1 and C3/A2 electroencephalography (EEG), ECG and oxygen saturation measurement, were employed in order to monitor the patient during anaesthesia and exclude arousal. Subjects breathed through a tight-fitting nasal mask and pneumotachometer, connected to a Validyne ±2 cmH2O pressure transducer (Validyne, Los Angeles, CA, USA), with the mouth carefully and tightly sealed. The pneumotachometer was connected to a digitized variable pressure source at the inflow port, enabling variation of nasal pressure (Pn) in the range of 20 – -10
cmH2O. Pn was monitored with a catheter connected to a side port of the mask. Intrathoracic pressure was measured with an esophageal balloon catheter (Ackrad Laboratories, Cranford, NJ, USA), and used to identify flow limitation, as well as to distinguish between inspiration and expiration during complete apneas. Analogue-to-digital acquisition of all parameters was performed at 1,000 Hz for monitoring and data storage on a digital polygraphic data acquisition system (LabVIEW; National Instruments, Austin, TX, USA).

**Anaesthesia:** Propofol anaesthesia was delivered by an anesthesiologist, using a loading dose of 2.5 mg/kg body weight and continuous drip of 6–12 mg/kg body weight/hr. Using positive levels of Pn (i.e., continuous positive airway pressure, CPAP) that enabled breathing without flow limitation, patients were maintained under stable anaesthesia that eliminated any reaction to pain and electrical stimulation, while maintaining adequate ventilation, as monitored by the pneumotachometer and pulse-oximetry.

**Pharyngoscopy:** A flexible fiberoptic endoscope (Olympus BF-3C40; Olympus, Tokyo, Japan; outside diameter 3.3 mm) was inserted through an adequately sealed port in the nose mask and positioned above the site of collapse of the pharynx. The image was recorded on videotape, accompanied by audio explanations.

**Electrical stimulation:** Genioglossus stimulation was applied via Teflon-coated 0.2 mm diameter hook wire electrodes with bared 0.13 mm diameter ends. The anterior electrodes were inserted sublingually, trans-mucosally, 10–15 mm deep into the anterior retromandibular body of the genioglossus, as previously described [6,7]. The posterior electrodes were inserted transcutaneously through the submandibular area, directed by ultrasound guidance and the lateral head x-ray for angle and depth of insertion, to be positioned 2-3 cm ventral to the posterior surface of the tongue, at the level of the lower border of the soft palate (figure 1). Four to six electrodes were inserted into the genioglossus in each subject. Using a neuromuscular stimulator (Dynex III; Medtronic, Inc., Minneapolis, MN, USA), 40 Hz bursts of 6-8 sec, with biphasic pulses of 100-ms width, were applied. Pharyngoscopic observation enabled selection of the electrodes and stimulation intensity that provided the best pharyngeal dilatory response. The intensity of stimulation was limited to levels that were well tolerated during wakefulness in previous and preliminary experiments.

**Experimental procedure:** After obtaining head x-ray and mouth-base sonography, patients were prepared with EEG, submental EMG and venous access, and placed in the supine position. Following induction of anesthesia, CPAP was applied via nose mask, and raised to the level that abolished flow limitation (holding pressure). Thereafter, the endoscope, esophageal balloon and both pairs of genioglossus electrodes were positioned and the mouth was sealed. The primary site of collapse, determined visually during gradual reduction of Pn and verified by the concomitant cessation of airflow, was at the level of the velopharynx in all our subjects. Therefore, the endoscope was placed above the area of velopharyngeal collapse. Thereafter, flow/Pn and pharyngeal area/Pn relationships before and during genioglossus stimulation were determined quasi-simultaneously, as previously described [6,7]. With the patient maintained at the holding pressure, Pn was lowered randomly for few breaths, encompassing four to six levels associated with inspiratory flow limitation and the level below which airflow ceased. At each Pn, after the fourth breath, stimulation was performed for two or three
consecutive breaths, and, after an additional two or three unstimulated breaths, the Pn was raised back to the holding pressure until stable baseline ventilation was observed. The same protocol was followed for each patient once for GGp-ES and once for GGa-ES, in random order. The same intensity of stimulation was used for both stimulation sites, after determining the stimulation intensity that provided the best response in both sites.

Data analysis: The flow/Pn relationship data were analyzed using digital software and determined using least-squares linear regression. Maximal inspiratory flow was measured at the level at which inspiratory flow was maximal and plateaued while esophageal pressure fell progressively, indicating the presence of flow limitation. This relationship was used to calculate the Perit as the Pn below which airflow became zero, as well as the flow/Pn slope. ΔPcrit (baseline Pcrit minus Perit during genioglossus stimulation) was used to quantify the mechanical effect of GGa-ES and GGp-ES. Cephalometric areas of the digital head x-ray were outlined manually and calculated digitally using computer software. The video images of the pharyngeal lumen, recorded during evaluation of the flow/Pn relationship before and during stimulation, were digitized and viewed, and single images from the end-expiratory pause were captured and stored. The respiratory frequency of all of the patients was relatively low (always<20 breaths/min) due to the state of anaesthesia and high holding Pn used to prevent flow limitation, resulting in a sufficiently long end expiratory pause (always>0.5 s) during which intra-pharyngeal pressure could become equal to Pn. The velopharyngeal cross-sectional area in each digitized frame was calculated digitally as described for the cephalometric areas. The esophageal pressure tube, marked at regular levels, was used as a landmark, in addition to pharyngeal structures, to enable measurement of the area perpendicular to the pharyngeal axis and at the same distance from the endoscope before and during ES, and was used as a calibration reference for calculating the area in absolute units, as previously described [13]. The area/Pn relationship (i.e. pharyngeal compliance) was determined for the close-to-linear portion of this relationship only (i.e, below the Pn of the bending point of the exponential relationship that characterizes the tube law of collapsible tubes), using least-squares linear regression. In addition, the shape of the velopharynx was assessed by measuring the antero-posterior and bilateral distance at each Pn level, before and during genioglossus stimulation. As the magnitude of the diameters depended on Pn, we used for each patient the diameters at the middle of the area/Pn range used for calculation of the slope, and expressed the shape as the ratio of these diameters.

Data are presented as mean±SD. The effects of GGa-ES and GGp-ES, as well as comparisons between groups (cephalometric parameters) and between the sites of stimulation, were compared using ANOVA for repeated measures. Correlations were assessed by the least-squares method. Stepwise hierarchical regression analysis (SPSS) was used to test for independent effects of inter-dependent variables. p<0.05 was considered as statistically significant.
RESULTS

**Anthropometric and polysomnographic characteristics:** The anthropometric and polysomnographic characteristics of the study subjects (n=14; all male) are given in table 1. Patients were predominantly middle-aged overweight men (11/14 BMI>30), and all but one had severe OSA (AHI>40 events/h).

Table 1: anthropometric and polysomnographic characteristics of the study subjects

<table>
<thead>
<tr>
<th>patients (n=14)</th>
<th>mean±SD</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI (events/h)</td>
<td>54.1±15.9</td>
<td>21-78</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>53.4±10.5</td>
<td>34-66</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.3±2.5</td>
<td>28-35</td>
</tr>
<tr>
<td>Apnoeas/total (%)</td>
<td>64.7±2.7</td>
<td>17-100</td>
</tr>
<tr>
<td>Lowest SO₂ (%)</td>
<td>70.1±13.4</td>
<td>55-91</td>
</tr>
</tbody>
</table>

AHI – apnoea-hypopnoea index. Apnoeas/total – ratio of apneas of all events. Lowest O₂ – lowest oxygen saturation value recorded during the sleep study.

**Cephalometry:** The results of the cephalometric measurements, as defined in the methods section, are presented in table 2. Based on the ratio between the genioglossus and the mouth-box cross-sectional area in the sagittal plane, the patients could be divided arbitrarily into two equal (n=7) groups, namely patients with a relatively high (>0.75) and low (≤0.75) ratio. It can be seen that the higher ratio was due primarily to the larger size of the tongue of these patients, as the area of the mouth-box, the angle reflecting the position of the mandible in relationship to the maxilla, the distance between the hyoid bone to the mandible, as well as the size of the pharynx and soft palate, were similar in both groups. Also, the caudal-posterior part of the genioglossus, likely to exert the main protrusive force, tended to be larger in these patients.
Table 2. Cephalometric data used for the present study. Patients were divided into two equal groups (n=7) with higher (>0.75) and lower (≤0.75) tongue to mouth-box ratio. Data presented as mean±SD.

<table>
<thead>
<tr>
<th>Data Presented as Mean±SD</th>
<th>Tongue/mouth-box ratio &gt;0.75</th>
<th>Tongue/mouth-box ratio ≤0.75</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth box, sagittal area (cm²)</td>
<td>98.2±23.3</td>
<td>84.7±16.7</td>
<td>NS (&gt;0.2)</td>
</tr>
<tr>
<td>Tongue, sagittal area (cm²)</td>
<td>77.0±18.1</td>
<td>58.9±12.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ratio, tongue/mouth box</td>
<td>0.76±0.04</td>
<td>0.69±0.04</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Protrusive part of the tongue (cm²)</td>
<td>48.9±10.5</td>
<td>33.7±8.9</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Ratio, protrusive part/whole tongue</td>
<td>0.61±0.02</td>
<td>0.55±0.06</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Velopharynx, sagittal area (cm²)</td>
<td>5.7±1.3</td>
<td>5.6±2.2</td>
<td>NS (&gt;0.9)</td>
</tr>
<tr>
<td>Oropharynx, sagittal area (cm²)</td>
<td>9.2±3.3</td>
<td>9.3±3.5</td>
<td>NS (&gt;0.9)</td>
</tr>
<tr>
<td>Soft palate, sagittal area (cm²)</td>
<td>5.7±1.6</td>
<td>5.0±2.2</td>
<td>NS (&gt;0.4)</td>
</tr>
<tr>
<td>Hyoid to mandible distance (cm)</td>
<td>3.4±1.1</td>
<td>2.7±0.7</td>
<td>NS (&gt;0.1)</td>
</tr>
<tr>
<td>Angle maxilla-nose-chin</td>
<td>4.1±2.5</td>
<td>2.7±1.7</td>
<td>NS (&gt;0.2)</td>
</tr>
</tbody>
</table>

Flow: Figure 2 depicts the effect of genioglossus stimulation in a representative patient. It can be seen that 1. Electrical stimulation shifted the flow/Pn curve to the left, toward lower pressures, as a result of higher flow rates at any given Pn. 2. The slope of the curves remained nearly unchanged. 3. The effect of GGp-ES was substantially larger than that from GGa-ES: In this patient Perit decreased from baseline of 5.4 to 2.8 and -5.6 cmH₂O during GGa-ES and GGp-ES, respectively. Mean data for the whole group are shown in figure 3. It can be seen that the response to GGa-ES was variable, decreasing Perit in the majority but not all patients. In the mean, Perit decreased during GGa-ES insignificantly from baseline of 3.7±2.3 to 2.9±3.5 cmH₂O. GGp-ES, on the other hand, lowered Perit in all subjects, and in the mean to -2.4±3.7 cmH₂O (p<0.001). Additional data derived from the flow/Pn relationship measurements are given in table 3. It can be seen that the change in Perit during stimulation was significantly larger with GGp-ES. Similarly, when assessing flow at atmospheric pressure (i.e., without CPAP, Pn=0), only GGp-ES produced a significant increase in flow rate. This effect occurred because the number of patients with Perit <0 increased from 1 to 10 patients. Both modes of genioglossus stimulation did not affect the slope of flow/Pn, and therefore it's reciprocal, upstream resistance, remained unchanged.
Table 3: Data derived from the pressure: flow relationship before and during electrical stimulation at the anterior (GGa-ES) and posterior (GGp-ES) areas of the genioglossus.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>GGa-ES</th>
<th>GGp-ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pcrit (cmH2O)</td>
<td>3.7 ± 2.3</td>
<td>2.9 ± 3.5</td>
<td>-2.4 ± 3.7*</td>
</tr>
<tr>
<td>∆ Pcrit (cmH2O)</td>
<td></td>
<td>0.82 ± 2.8</td>
<td>6.1 ± 2.9*</td>
</tr>
<tr>
<td>Rus (cmH2O/l/sec)</td>
<td>28.5 ± 12.2</td>
<td>27.2 ± 13.6</td>
<td>28.4 ± 16.0</td>
</tr>
<tr>
<td>Flow at Pn=0 (l/min)</td>
<td>0.02 ± 0.07</td>
<td>1.1 ± 2.4</td>
<td>6.0 ± 5.6*</td>
</tr>
</tbody>
</table>

Pcrit = critical pressure. Rus = upstream resistance (Pn/flow). Pn = nasal pressure
*p<0.01 compared to baseline and GGaES

Pharyngoscopy: Pharyngoscopy was performed in 11 of the patients. Figure 4 illustrates the effect of electrical stimulation on velopharyngeal cross-sectional area in one of the patients. It can be seen that 1. The velopharynx enlarged by electrical stimulation applied at both sites. 2. The effect of GGp-ES was larger than that of GGa-ES at both levels of Pn, and only GGp-ES opened the occluded velopharynx at Pn=0. 3. GGp-ES enlarged the velopharynx both in the anterior-posterior and lateral direction, while the GGa-ES affected mainly the anterior-posterior direction. For the whole group, the baseline area/Pn slope (i.e., compliance of the site of collapse, 17.8±9.8 mm²/cmH2O), remained nearly unchanged during GGa-ES and GGp-ES (19.2±8.5 and 15.9±9.6 mm²/cmH2O, respectively). Velopharyngeal end-expiratory area, measured at the middle of the area/Pn slope, was 74.5±38.0 mm², and increased to 88.2±40.1 (p>0.1) and to 130.0±68.7 mm² (p<0.001) during GGa-ES and GGp-ES, respectively (p<0.05 for the comparison of the two sites of stimulation). The difference between the two sites of stimulation was due mainly to the difference in their effect on the lateral diameter. As illustrated graphically in figure 5, both GGa-ES and GGp-ES tended to enlarge the antero-posterior (sagittal) diameter of the velopharynx (p=0.078 and p<0.01, respectively). However, while the lateral diameter remained unchanged with a tendency to decrease during GGa-ES (p>0.2 for comparison with baseline), it increased significantly during GGp-ES (p<0.01).

Relationship between baseline parameters and the response to electrical stimulation: The individual response to electrical stimulation was defined as ∆Pcrit. The response to genioglossus stimulation was independent of body weight, age or polysomnographic parameters.

Relationship between cephalometric parameters and the response to electrical stimulation: Out of the large number of cephalometric parameters described in previous studies [9-11], including the 7 parameters we considered as likely to affect the response to contraction of the genioglossus (figure 1), only the sagittal area of the tongue (corrected for the size of the bony walls of the mouth cavity) correlated significantly (R=0.63, p<0.02) with ∆Pcrit during GGp-ES (figure 6). Patients with
large tongue relative to the oral cavity (tongue/"mouth-box" ratio >0.75, n=7) had in the mean a ΔPcrit of 8.0±2.2 cmH2O, compared to patients with smaller tongue/mouth-box ratio (ΔPcrit 3.8±2.1 cmH2O, p<0.005). On the other hand, we found no correlation between ΔPcrit during GGa-ES and the above parameters.

**Relationship between flow parameters and the response to electrical stimulation:** Baseline Pcrit and upstream resistance did not correlate with Pcrit during GGp-ES, and the latter was determined primarily by ΔPcrit (R=0.78, p<0.01). Therefore, although one may expect low Pcrit to contribute to the magnitude of Vmax at atmospheric pressure obtained during electrical stimulation, the correlation between these variables was not significant (R=-0.31). On the other hand, Vmax at atmospheric pressure during GGp-ES correlated significantly with both Pcrit during stimulation and ΔPcrit (R=-0.73 and R=0.69, respectively, p<0.01 for both).

**Relationship between velopharynx shape and the response to electrical stimulation:** ΔPcrit was affected also by the baseline shape of the velopharynx (figure 7): a flat-elliptical shape of the velopharyngeal orifice with low anterior-posterior to lateral diameter ratio tended to have a larger response to GGp-ES (R=-0.74, p<0.01). However, the antero-posterior to lateral diameter ratio was also closely and inversely related to the sagital size of the tongue (R=-0.61, p<0.05), with large tongues associated with narrow pharynx. On the other hand, we found no correlation between ΔPcrit during GGa-ES and the above parameters. Also, no correlation was found between ΔPcrit and the compliance at the site of collapse.
DISCUSSION

The present study evaluated the effects of electrically-induced contraction of the main pharyngeal dilator, the genioglossus, on flow-mechanics and pharyngeal patency in patients with obstructive sleep apnoea. In continuation to our previous work [7] we now assessed the effects of site of stimulation and the importance of cephalometric parameters. We found that both the specific area of the genioglossus recruited and specific patients' characteristics are important: Stimulation of the posterior part of the genioglossus stabilized the pharynx more than the anterior part, and the best results were obtained in patients with large tongue and narrow pharynx.

Previous studies that used intramuscular stimulating electrodes to assess the effect of electrical stimulation of the genioglossus on pharyngeal patency usually addressed this muscle as acting mechanically as a single functional unit, and used one stimulation site [6,7,14,15]. However, the genioglossus fibers are arranged as a hand held fan, and the effects of contraction of the genioglossi may be understood by considering the direction of their fibers [16]: the horizontal-longitudinal fibers are the actual tongue protrusors that draw the tongue forward, while the anterior fibers are oriented vertically, and their contraction, also when acting together with the longitudinal fibers, act to depress and draw down the tongue [8]. We previously observed that contraction of the depressor part of the genioglossus may sometimes obstruct the pharynx, by causing posterior bulging of the dorsal part of the tongue (like squeezing a ball) [16]. Therefore, we hypothesized that stimulation of different areas of the genioglossus affects its vector of contraction, and targeting the main body of the protrusive part of the genioglossus may have a larger effect on pharyngeal patency, diminishing recruitment of vertical genioglossal fibers co-activated by anteriorly placed electrodes (figure 1). The present findings seem to confirm this assumption, indicating that stimulation of specific areas of the tongue is needed to obtain an optimal flow-mechanical effect.

An additional finding of the present study was the interrelationship between velopharyngeal shape, tongue size and the response to genioglossus contraction. It has been previously shown by several (although not all) studies, that patients with OSA tend to have upper airway with a high antero-posterior / lateral diameter ratio, suggesting that the shape of the pharynx may have an independent pathophysiologica significance [17-20]. It has been postulated that an antero-posteriorly oriented elliptical shape may be disadvantageous and predispose the pharynx to collapse, as the genioglossus, that acts primarily to enlarge the pharynx anteriorly, is likely to be less effective if the pharyngeal shape is oriented in the antero-posterior direction [18]. This intuitive hypothesis was confirmed by the significant correlation between shape and response to genioglossus stimulation observed in our patients. However, the explanation for this finding seems to be complex. We found that the antero-posterior / lateral diameter-ratio was inversely related to the size of the tongue, probably because a large tongue is likely to compress the pharynx. Although we corrected the size of tongue to that of the bony structure of the mouth cavity, a similar correlation with the shape was found also with the uncorrected measured size of the tongue. Accordingly, the larger mechanical effect could be related to either the size of the tongue and/or to the shape of the velopharynx. We found a difference in change in shape produced by GGp-ES and GGa-ES: both sites of stimulation increased the antero-posterior diameter of the velopharynx, but the superiority of GGp-ES appeared to be due to its additional enlarging effect on the lateral diameter (figure 5).

Most likely, the effects of genioglossus contraction (and probably additionally co-activated tongue muscles) on the velopharynx includes forces additive to unloading the weight of the tongue [21]. Electrical stimulation of the genioglossus appears to affect also the lateral pharyngeal walls, probably involving mechanical coupling of the base of tongue and the soft palate via the fauces [22]. Using MRI with a novel spatial modulation of magnetization (SPAMM) technique, a significant stretch of lateral tissue was observed during medial hypoglossus branch stimulation [23]. Although in this study, performed in rats, lateral enlargement was observed only in the oropharyngeal region, significant enlargement in the lateral diameter was observed also at the level of the velopharynx during stimulation of the medial, lateral and whole hypoglossus in cats [24]. As soft tissue is not compressible, contraction of the genioglossus probably enabled enlargement of the pharynx by caudal displacement of soft tissue outside the maxillo-mandibular bony enclosure, as described by Tsuiki et al. [11]. While compliance may decrease at the level of the oropharynx during genioglossus stimulation [15], we did not find a change in compliance at the level of the velopharynx, confirming previous observations in humans [7, 15], as well as in animals studied with MRI [14,25]. This finding supports the notion that genioglossus stimulation alters velopharyngeal patency primarily by its effect on the surrounding external pressure [26].
Several limitations of this study and potential confounders have to be acknowledged. First, this is a laboratory based study, evaluating physiological parameters under Propofol anaesthesia. We choose to use anaesthesia to assess the mechanical effect of electrical stimulation and to enable endoscopic evaluation over a wide range of Pn levels, a task that could not be performed during normal sleep. Both propofol and isoflurane are being used to evaluate pharyngeal mechanics during anesthesia, and were found to cause a dose dependent increase in pharyngeal collapsibility [27]. As during sleep, both anesthetics abolish genioglossus reflex activation, while enabling spontaneous breathing, and their use was often referred to as "drug-induced sleep" [28]. Nevertheless, the use of propofol poses limitations to extrapolation of our findings to conditions occurring during sleep. Propofol may produce more muscle relaxation than sleep, rendering the upper airway more passive and more collapsible [29]. However, this only means that we may expect baseline Pcrit of our patients to be lower (i.e., the velopharynx more stable) during sleep. Propofol does not influence involuntary isometric skeletal muscle strength [30,31], and the magnitude of stimulation-induced change in Pcrit during sleep and propofol anaesthesia is similar [32]. Therefore, we believe that the experimental conditions actually underestimated the expected flow response to genioglossus stimulation at atmospheric pressure. In our patients, 13/14 were completely obstructed at atmospheric pressure, and GGp-ES enabled flow at Pn=0 in 10 patients. With lower baseline Pcrit expected during sleep, and equal ΔPcrit, a larger clinically-relevant effect of GGp-ES may be anticipated. Although reductions in Pcrit were found to be similar with genioglossus and hypoglossus stimulation during sleep [6], our results indicate that specific areas of the tongue should be stimulated to improve pharyngeal dilatation. As intramuscular electrodes stimulate also sensory nerve fibers and causes arousal, hypoglossus nerve stimulation may enable painless tongue muscle contraction. However, it is unclear to what extend is selective stimulation feasible with neural stimulation. The hypoglossus can be divided into medial and lateral branches, with the former innervating the genioglossus, as well as the geniohyoid and intrinsic tongue muscle [13,14,23]. The intramuscular branching of the hypoglossus has not been evaluated. Therefore, correlation between responses to genioglossus and hypoglossus stimulation can be assessed only after an implantable device producing effective hypoglossus nerve stimulation is developed. The line drown in figure 1 to separate the predominantly protrusor from the predominantly depressor part of the genioglossus is rather schematic, as the genioglossus is not divided into distinct functional units. This may be the cause for the close relationship between the depressor, protrusor and total size of the tongue that precluded demonstration of an independent relationship between the protrusor part of the tongue and the response to genioglossus stimulation. Therefore, the improved response observed could be attributed to either unloading a heavier tongue, or contraction of a larger protrusive muscle (table 2), or both. It should be noted that in the present study, as in our previous study performed under Propofol sedation, the primary site of collapse was the velopharynx (or combined velo- and oropharynx) in all patients [7]. Accordingly, as we have previously found that the effect of genioglossus stimulation on the oropharynx is larger than on the velopharynx [7], a larger effect is expected in patients with oropharyngeal primary site of obstruction. The difference in response to the two sites of stimulation could be due also to differences in co-activation of retractor tongue muscles, but retractor recruitment is unlikely to produce an important change in response [16]. We did not evaluate systematically the reproducibility of our results, but current and previous
experience based on insertion of additional electrodes in the same area of stimulation suggests that the effects of stimulation are closely reproducible. Small changes in the level of anaesthesia, as well as head or mandibular position known to affect the response to genioglossus contraction [33], could occur between the evaluations of the two sites of stimulation, despite our efforts to maintain stable conditions. However, the two sites were evaluated in random order, to prevent systematic error. Measurement of the velopharyngeal area could be distorted by axial movements of the pharynx during stimulation, changing the distance between the pharyngoscope and the measured region [13]. However, no axial movements occurred during stimulation, as opposed to substantial axial shift during inspiration, as clearly evident from the relationships between the endoscope, pharyngeal structures, and the esophageal tube that was marked at regular intervals. It should be noted that the site of collapse was determined endoscopically in conjunction with flow monitoring during gradual reduction of CPAP, enabling accurate assessment of the primary area responsible for pharyngeal occlusion. We used the cross-sectional area to pressure relationship as an estimate of the compliance of the velopharyngeal site of collapse, although compliance is, per definition, the relationship between volume and pressure. This approach was used in most studies evaluating regional compliance of the pharynx [7,14-16,23], and may be more relevant for the assessment of pharyngeal flow mechanics than the compliance of the whole upper airway. Finally, we limited baseline anatomic evaluation to simple cephalometry, considered sufficient for the information need for the present study. Several new and more sophisticated modalities based on 3D reconstruction of CT and MRI imaging of the upper airway have been recently developed [34,35], that enable much more accurate anatomic and morphologic characterization of the pharynx and peripharyngeal structures, and may be also useful for the evaluation of muscle stimulation [36].

The need for specific site of genioglossus activation combined with specific anatomic characteristics to achieve effective pharyngeal dilatation reflects the well known interaction between neuromuscular and structural/anatomic factors in the pathogenesis of OSA [37]. Functional electrical stimulation of striated muscles provides an important tool to assess their physiological effect and has been largely employed for the study of the mechanical effects of upper airway dilator muscles both in animals and humans. In addition, however, evaluation of the effect of stimulation of the main tongue protrusor, the genioglossus, provides information that may be useful for future therapeutic interventions. The main treatment modality for OSA is the application of CPAP, a remedy that is poorly tolerated, leading to low compliance or frank refusal by many patients. Considering the obvious role of neuromuscular mechanisms in the pathogenesis of OSA, since apnoeas occur only during sleep in association with a decline in genioglossus activity [38], it is reasonable to assume that adequately applied electrical activation of this muscle may prevent pharyngeal collapse. Therefore, attempts to stimulate the genioglossus in OSA patients for therapeutic purpose have been undertaken ever since the physiological importance of this muscles’ action began to be appreciated, with variable results [2,36,39,40]. A major advance has been achieved recently when a group of OSA patients has been implanted with chronic hypoglossus nerve stimulators, using cuff or sleeve-like electrodes implanted unilaterally around a hypoglossus nerve, with stimulation triggered by inspiration [3,41]. Although technical malfunctions precluded prolonged follow-up, large decreases in apnoeas and hypopnoeas were observed in most patients. These encouraging findings triggered ongoing attempts to improve the new
device. Consequently, the pathophysiological questions evaluated in the present study gain clinical significance and relevance, as their answers may be useful for the development of this new treatment modality and characterize OSA patients most likely to benefit from it. If response to genioglossus stimulation will be found, in prospective studies, to correlate with the response to hypoglossus stimulation, the former may be used for screening OSA patients expected to have a good response to hypoglossus stimulator. It should be noted that no complication occurred during our studies from electrodes insertion. Considering the instability of pharyngeal shape, which is highly dependent on the level of Pn, and the relative difficulty obtaining this parameter, measuring the size of the tongue by x-ray (or CT) and relating it to the size of the bony cage of the mouth cavity may be a simpler alternative. Our findings suggest that the best response to electrical stimulation of the tongue is expected to be obtained in OSA patients with large tongues, during preferential activation of the longitudinal (protrusive) fibers of the genioglossus. However, considering the scatter observed in figures 6 and 7, additional parameters that will improve patients' selection are desirable.

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REFERENCES


FIGURES

Figure 1: Cephalometric parameters.

1. The genioglossus part that advances the tongue. 2. The genioglossus part that depresses and draws down the tongue. 3. Oropharynx. 4. Velopharynx  5. Soft palate.

6. Distance between the hyoid bone and the mandible. 7. Angle of the chin-nosebase-maxilla. The heavy black quadrangle delineates the "mouth box". The circles indicate the areas where the electrodes were positions at the posterior (GGp) and anterior (GGa) sites of stimulation.
**Figure 2:** Pressure-flow relationship in one study subject at baseline and during electrical stimulation at the anterior (retro-gnataal, GGa-ES) and the posterior (pre-pharyngeal, GGp-ES) sides of the genioglossus. 

$V_{\text{max}}$ – maximal inspiratory airflow. $P_n$ – pressure at the nose
Figure 3: Individual and mean±SD Pcrit values for all patients at baseline, and during electrical stimulation of the genioglossus at the anterior (GGa-ES, left) and posterior (GGp-ES, right) area of the genioglossus.

* - p<0.01 for comparison of GGp-ES with baseline and GGa-ES.
Figure 4: Genioglossus stimulation effect on the velopharyngeal area of collapse at two levels of Pn (CPAP), during anterior (GGa-ES) and posterior (GGp-ES) stimulation. Ant – anterior. VP – velopharynx. EB – esophageal balloon tube.
Figure 5: Schematic presentation of mean changes of the antero-posterior (sagittal) and lateral (transversal) diameters of the velopharynx of all patients. Shadowed ellipse - baseline. Thick-line ellipses – shape during electrical stimulation of the genioglossus at the anterior (GGa-ES) and posterior (GGp-ES) sites. * p<0.01 for comparison of diameters between electrical stimulation and baseline. The increase in sagittal diameter with GGaES was of borderline significance (p=0.078).
**Figure 6:** Relationship between the size of the genioglossus assessed as its sagittal cross-sectional area and given as a ratio of the sagittal cross-sectional area of the mouth-box, and the change in $P_{cr}$ ($\Delta P_{cr}$, baseline $P_{cr}$ –$P_{cr}$ during genioglossus contraction). The response to electrical stimulation was larger with increasing size of the tongue.

![Graph showing relationship between genioglossus size and $\Delta P_{cr}$](image)

$R=0.632$, $p<0.02$

**Figure 7:** Relationship between the shape of the velopharynx and the response to electrical stimulation of the genioglossus (posterior site). The shape is characterized by the ratio of antero-posterior to lateral (AP/L) diameters. The response to stimulation is given by the change in $\Delta P_{cr}$ during stimulation. The response to electrical stimulation was larger in patients with narrow antero-posterior and large lateral diameter.

![Graph showing relationship between AP/L diameter and $\Delta P_{cr}$](image)

$R=0.738$, $p<0.01$