Fiberoptic imaging was performed in six decerebrate, tracheotomized cats to determine the effect of pharyngeal muscle activation on the pharyngeal airway. The fiberoptic scope was advanced through the rostral trachea into the pharynx. Computer-based planimetry was used to measure airway area and maximum anteroposterior and lateral diameters in the rostral oropharynx, velopharynx, and caudal oropharynx. Cuff electrodes stimulated the bilateral distal cut ends of the following nerves: medial hypoglossus (MHG), lateral hypoglossus (LHG), glossopharyngeus, and pharyngeal branch of vagus (PBV). The velopharyngeal area increased with stimulation of the MHG, MHG plus LHG, and glossopharyngeus. The velopharyngeal area decreased with PBV stimulation. Similar effects occurred in the caudal oropharynx. The percent increase in velopharyngeal area with combined MHG and LHG stimulation was greater than the sum of the increases stimulating either branch alone. In the rostral oropharynx, airway area increased with individual and combined stimulation of the MHG and LHG. Changes in airway area at the different levels were concentric with the HG stimulations, but glossopharyngeal stimulation resulted in a greater increase in lateral than anteroposterior wall movement. The results indicate that the mechanical effects of pharyngeal muscle contraction depend on the airway level and the specific muscles that are activated.

**Keywords:** hypoglossus nerve; glossopharyngeus nerve; vagus nerve; velopharynx; oropharynx

The muscles surrounding the pharyngeal airway are generally felt to have a role in the pathogenesis of obstructive sleep apnea (OSA), a respiratory disorder characterized by the repetitive closure or abnormal narrowing of the collapsible pharyngeal airway during sleep (1, 2). Contraction of pharyngeal muscles can dilate and stiffer the pharyngeal airway (3–5). Pharyngeal muscle activation can also constrict and shorten the airway (11). Loss of motor output to pharyngeal dilating muscles during sleep is felt to make the airway more susceptible to collapse by increasing airway compliance. Newly proposed implantable medical devices and pharmacological treatments for OSA are aimed at maintaining pharyngeal patency during sleep by stimulating upper airway muscles that dilate and stiffen the airway (12–16). Further development of these approaches depends, in part, on gaining a better understanding of the mechanical effects of pharyngeal muscle activation on the pharyngeal airway.

The pharyngeal airway muscles have complex anatomic relationships and receive their motor output from several different nerves (17). The hypoglossus (HG) nerve provides motor output to the intrinsic and extrinsic tongue muscles. The medial branch of the HG nerve innervates the tongue protrudors: genioglossus and geniohyoid. The lateral branch of the HG innervates the tongue retractors: styloglossus and hyoglossus. The pharyngeal branch of the vagus innervates the pharyngeal constrictor muscles. The glossopharyngeal nerve provides motor output to the stylopharyngeus muscle (17). In addition, recent studies indicate that the glossopharyngeus also supplies the levator veli palatini, pharyngeal constrictors, and criopharyngeus muscles via its inputs to the pharyngeal plexus (18, 19).

Previous studies have examined the effects of respiratory-related pharyngeal muscle activation in response to a systemic stimulus such as hypercapnia on the global function of the pharyngeal airway (3–5). Other studies have focused on the effects of activating a particular muscle or set of muscles on the entire pharyngeal airway (6–11). Although these latter studies indicate that the mechanical effects of pharyngeal muscle activation depend on the muscle that is activated and the conditions of the airway at the time of activation, little is known about the effects of activating specific muscles or muscle groups on different regions of the pharyngeal airway. Such studies are of importance as they relate to the pathogenesis and treatment of obstructive sleep apnea. In addition, further knowledge about the regional mechanical effects of pharyngeal airway muscle activation may help improve surgical techniques that transpose upper airway muscles and nerves to repair and restore upper airway function.

The purpose of the current study was to determine the regional effects of pharyngeal muscle activation on airway area and configuration. Fiberoptic imaging of the airway was performed in cats with and without stimulation of nerves supplying motor output to various pharyngeal muscles. The study tested the hypothesis that pharyngeal muscle contraction has characteristic regional effects on the pharyngeal airway.

**METHODS**

**Animal Experiments**

The protocol was performed in six decerebrate, tracheotomized adult cats as approved by the Animal Care Committee. Anesthesia and general surgical preparation were described previously (11). The animals were mechanically ventilated (Harvard Apparatus). A rostral tracheal cannula immobilized the upper airway at resting length.

Cuff electrodes stimulated the bilateral distal cut ends of the following nerves: medial HG branch, lateral HG branch, glossopharyngeus, and pharyngeal branch of vagus. Each cuff electrode was attached to a stimulator (Grass). Voltage threshold to induce muscle contraction was determined visually for each nerve (5 Hz frequency). Throughout the recordings, individual nerves were stimulated at their particular voltage threshold (30 Hz frequency, 0.2 ms duration) to produce a summated contraction. For all trials, stimulation periods of 3–5 s were separated by at least 2 min. Trials were performed at atmospheric pressure with bilateral stimulation of the individual nerves and simultaneous bilateral stimulation of both medial and lateral HG branches. Following each trial, the freely moving tongue was restored to its resting position using a loose suture secured to its tip.

A fiberoptic scope (Pentax) was advanced through the rostral trachea into the pharyngeal airway. The fiberoptic image was displayed...
on a video monitor and recorded on VCR tape (Sony). Recordings were obtained at two levels of the pharyngeal airway: the edge of the soft palate and the rostral oropharynx. Due to the retrograde approach of the scope, the image at the edge of the soft palate revealed the orifices to the velopharynx and caudal oropharynx (Figure 1).

Data Analysis
Video images were analyzed off-line on a personal computer (Dell) using a frame grabber and planimetry software (Snappy). At each airway level, the following measurements were obtained with and without nerve stimulation: area and maximum anteroposterior and lateral diameters. Measurements were expressed in metric units. Calibration was performed by positioning a catheter tip of known diameter at the airway level of interest.

In each cat, the values in metric units for each of the three planimetry outcome parameters at a given level under passive conditions were expressed as a mean. One-way repeated measures ANOVA was used to compare (1) area, maximum anteroposterior diameter, and lateral diameter under passive conditions across levels, (2) percent increase in area produced by stimulating the different nerves at a given airway level, and (3) percent change in area produced at different airway levels by stimulating a given nerve. When data were not normally distributed, a Kruskal–Wallis one-way ANOVA on ranks was performed and the Student–Newman–Keuls method was used to make pairwise comparisons. Values of $p < 0.05$ were considered significant. Paired $t$ tests were performed to determine if significant differences were present in area, anteroposterior diameter, and lateral diameter with and without stimulation of a given nerve(s) at a particular airway level. Paired $t$ tests were also performed to determine if any differences existed between the change or percent change in anteroposterior versus lateral diameter with stimulation of a given nerve(s) at a given airway level.

RESULTS
Under passive conditions, there were no significant differences in airway area ($p = 0.74$), maximum anteroposterior diameter ($p = 0.50$), or maximum lateral diameter ($p = 0.06$) across the three measurement sites: velopharynx, caudal oropharynx, and rostral oropharynx (Table 1). Figure 1 shows the fiberoptic images at the soft palate level with and without stimulation of the glossopharyngeal nerve in one cat. Nerve stimulation enlarged the orifices to both the velopharynx and oropharynx. As shown in the same cat, stimulation of the pharyngeal branch of the vagus constricted the two orifices with the most prominent effect in the velopharynx (Figure 2). Figure 3 shows the increase in airway area in the rostral oropharynx with stimulation of the medial branch of the HG.

The upper panel of Figure 4 shows the percent change in area and maximum anteroposterior and lateral diameters with nerve stimulation at the velopharynx in all six animals. Stimulation of the pharyngeal branch of the vagus resulted in a significant decrease in airway area ($p = 0.013$). Stimulation of the medial branch of the HG, medial plus lateral branches of the HG, and glossopharyngeal nerves significantly increased velopharyngeal area (all $p$ values $\leq 0.025$). Paired $t$ test revealed that combined stimulation of the medial and lateral HG branches had a synergistic effect on velopharyngeal area, that is, the increase with combined stimulation was greater than the sum of the increases that occurred with stimulation of either branch alone ($p = 0.020$). The percent increase in velopharyngeal area with stimulation of the glossopharyngeal nerve was similar to that with stimulation of the medial HG and medial plus lateral branches of the HG. Increases in velopharyngeal area with nerve stimulation were frequently associated with a thinning of the rim of the soft palate.

Maximum anteroposterior and lateral velopharyngeal diameters increased with stimulation of the medial and medial plus lateral branches of the HG, and decreased with stimulation of the pharyngeal branch of the vagus ($p$ values $\leq 0.031$). Stimulation of either the lateral branch of the HG or glossopharyngeal nerve increased velopharyngeal lateral ($p$ values $\leq 0.038$) but not anteroposterior diameter ($p$ values $< 0.061$). The change and percent change in lateral diameter were significantly greater than comparable results in anteroposterior diameter with stimulation of the glossopharyngeal nerve or pharyngeal branch of the vagus ($p$ values $< 0.035$). No differences between the change or percent change in anteroposterior and lateral diameters with individual or com-

<table>
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<th>Rostral Oropharynx</th>
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<td>7.9 (1.0)</td>
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Figure 1. The fiberoptic images at the soft palate level with (right panel) and without (left panel) bilateral stimulation of the glossopharyngeal nerve in one cat. In this retrograde fiberoptic view of the pharyngeal airway, the orifice to the velopharynx (black semicircular shape) is above that to the caudal oropharynx. The two orifices are separated by the rim of the soft palate. The epiglottis forms the lower border of the orifice to the caudal oropharyngeal airway. Nerve stimulation enlarges both the velopharynx and caudal oropharynx.
combined stimulation of a HG branches were detected at the velopharynx (p values < 0.122).

As shown in the lower panel of Figure 4, results in the caudal oropharyngeal airway were similar to those in the velopharynx. Stimulation of the pharyngeal branch of the vagus resulted in a significant decrease in caudal oropharyngeal area (p = 0.031). Stimulation of the medial branch of the HG and the glossopharyngeal nerve increased airway area (p values < 0.031). The increase in caudal oropharyngeal area with glossopharyngeal stimulation was greater than that with stimulation of the medial and/or lateral branches of the HG (p = 0.004). In contrast to the results at the velopharynx, the increase in caudal oropharyngeal area with stimulation of the medial plus lateral branches of the HG nerve did not achieve significance (p = 0.059). Glossopharyngeus stimulation was associated with a significant increase in both anteroposterior and lateral diameter (p values < 0.001). Stimulation of the pharyngeal branch of the vagus was associated with a significant decrease in both anteroposterior and lateral diameters (p values < 0.031). The medial branch of the HG stimulation resulted in an increase in anteroposterior (p = 0.017) but not lateral diameter (p = 0.230). Stimulation of the glossopharyngeal nerve and pharyngeal branch of the vagus produced greater changes in lateral than anteroposterior diameter (p values < 0.035).

Due to the elliptical shape of the caudal oropharynx (Figure 1), the percent change in anteroposterior diameter was greater than that in the lateral diameter with glossopharyngeal stimulation. No other differences between the change or percent change in anteroposterior and lateral diameters with stimulation of a given nerve were detected at the caudal oropharynx (p values > 0.094).

Figure 5 shows the effects of nerve stimulation in the rostral oropharyngeal airway. Significant increases in area occurred with stimulation of the medial or medial plus lateral branches of the HG (p values < 0.050). The increases in area with stimulation of the medial or medial plus lateral branches of the HG were greater than that resulting from stimulation of the lateral branch alone. These increases in airway area were accompanied by significant increases in both anteroposterior and lateral diameters (p values < 0.041). In the rostral oropharynx, the increase in airway area with stimulation of the glossopharyngeal nerve approached significance (p = 0.055), but no differences in area were apparent with and without stimulation of the pharyngeal branch of the vagus (p = 0.270). The change in lateral diameter in the rostral oropharynx with glossopharyngeal stimulation was greater than that in the antero-
posterior dimension ($p = 0.031$). No other differences between the change or percent change in anteroposterior and lateral diameters with stimulation of a given nerve were detected in the rostral oropharynx ($p$ values $\geq 0.136$).

One-way repeated measures ANOVA of percent changes in area at the different levels of the pharyngeal airway with stimulation of a given nerve revealed that stimulation of the medial, lateral, and medial plus lateral branches of the HG produced greater changes in the rostral oropharynx than in the caudal oropharynx or velopharynx. With stimulation of the pharyngeal branch of the vagus, the decrease in velopharyngeal area was greater than that in the rostral or caudal oropharynx. Comparison of the percent changes in area at the different levels with glossopharyngeal nerve stimulation approached significance ($p = 0.050$).

**DISCUSSION**

The results of the study indicate that the effect of pharyngeal muscle contraction on airway area depends on the specific muscles that are contracting and the particular level of the airway that is being examined. Individual and combined stimulation of the HG nerve branches, supplying motor output to the tongue protrudor and retractor muscles, caused the greatest increase in airway area in the rostral oropharyngeal airway, though area also increased significantly in the caudal oropharynx. Stimulation of the glossopharyngeal nerve, supplying motor output to several pharyngeal muscles including the stylopharyneus and levator veli palatini, dilated the velopharynx and oropharynx at the rim of the soft palate. Stimulation of the pharyngeal branch of the vagus, supplying motor output to the pharyngeal constrictor muscles, constricted the airway at the soft palate level but caused no change in area in the rostral oropharynx.

Brennich and coworkers (20) performed magnetic resonance images of the pharyngeal airway in cats and found regional differences in pharyngeal airway mechanics under passive conditions, that is, without muscle activation. The current study extends these findings by showing the selective activation of pharyngeal muscles has characteristic regional effects on the pharyngeal airway. Other investigators have shown that pharyngeal airway closure during sleep in patients with obstructive sleep apnea can occur at different locations in the pharyngeal airway (21, 22). A better understanding of the regional mechanical effects of selective pharyngeal muscle stimulation on the heterogeneous pharyngeal airway may help the development of implantable stimulators to stiffen and dilate the pharyngeal airway during sleep in patients with obstructive sleep apnea (15, 16).

One of the limitations of fiberoptic imaging is the inability to evaluate depth on the images. When imaging a tube-like structure such as the pharyngeal airway, the lack of depth perception makes it difficult to make measurements at a particular level. To help circumvent this problem, the analysis was restricted to areas of the pharyngeal airway that had anatomic landmarks that could be used to identify depth in the planimetry analysis. Retrograde insertion of the fiberoptic scope into the pharyngeal airway through the rostral tracheal cannula allowed visualization of the rim of the soft palate, a distinct edge that facilitated measurements. Use of a catheter to calibrate the images also helped to overcome the depth perception problem associated with fiberoptic imaging.

It was visually evident that stimulation of the various nerves changed airway size and configuration both within and outside the cross-sectional plane selected for measurements. It was also evident that nerve stimulation caused structures to move not only in the radial but also in the axial plane, despite the

![Figure 4. Mean ± SD of percent change in area (open bars), and maximum anteroposterior (slanted bars) and lateral (hatched bars) diameters at the soft palate level with nerve stimulation in the six animals. The upper panel shows the results from the velopharynx. The lower panel shows the results from the caudal oropharynx. Med HG = medial branch of the hypoglossal (HG) nerve; Lat HG = lateral branch of the HG; Med + Lat HG = combined stimulation of the medial and lateral branches of the HG; GP = glossopharyngeus; PBX = pharyngeal branch of the vagus.]

![Figure 5. Mean ± SD of percent change in area (open bars), and maximum anteroposterior (slanted bars) and lateral (hatched bars) diameters in the rostral oropharynx with nerve stimulation in the six animals. Abbreviations as in Figure 4.]
fact that the rostral tracheal cannula was secured in a fixed position. For example, stimulation of the lateral HG branches caused a noticeable retraction of the base of the tongue, and stimulation of the pharyngeal branch of the vagus caused rostral movement of the rim of the soft palate into a “V”-shaped structure. These axial movements are of potential importance because axial tension may play an important role in maintaining airflow patency (23). Although the axial movements could not be controlled or quantified, it is felt that they did not significantly affect the cross-sectional measurements. To determine whether axial movements associated with stimulation of the HG branches or glossopharyngeal nerve may have changed the distance between the fiberoptic scope and the rim of the soft palate, a catheter was advanced through the service channel of the fiberoptic scope and advanced to the airway level of interest. The distance between the tip of the catheter and the fiberoptic scope did not change with nerve stimulation.

Another limitation of fiberoptic imaging is the inability to determine the effects of the experimental intervention on pharyngeal soft tissue and bony structures. The concentric narrowing of the velopharynx and caudal oropharynx with stimulation of the pharyngeal branch of the vagus is consistent with the circumferential anatomic orientation of the pharyngeal constrictor muscles at that level of the pharyngeal airway. The lateral widening of the velopharynx with GP nerve stimulation may be explained by activation of the anatomic orientation of the stylopharyngeus muscle. On visual inspection of the ventral outer surface of the exposed neck, glossopharyngeal nerve stimulation was associated with a prominent lateral movement of the rotating hyoid bone. The otherwise concentric changes in airway dimension with dilator muscle activation do not provide insight concerning the predominant direction of forces exerted by stimulating these muscles. Other imaging techniques such as computerized tomography or magnetic resonance imaging might be able to overcome some of the above limitations by tracking changes in bony and soft tissue structures.

Evidence from previous investigators indicates that the pharyngeal airway at a given level is more compliant in the lateral than in the anteroposterior direction (24). In the current study, significant changes in airway area with nerve stimulation were associated with significant unidirectional changes in both maximum anteroposterior and lateral diameters. Stimulation of the medial, lateral, and medial plus lateral HG branches produced similar changes in anteroposterior and lateral dimensions at a given airway level. These results indicate that the changes in airway area in response to contraction of these pharyngeal muscles were concentric. In contrast, stimulation of the glossopharyngeal nerve resulted in a greater increase in lateral diameter than anteroposterior diameter at the velopharynx and both oropharyngeal levels. Greater lateral than anteroposterior wall movement was also observed with stimulation of the pharyngeal branch of the vagus, but in this case, both lateral and anteroposterior diameters decreased with stimulation.

Combined stimulation of the medial and lateral branches of the HG nerve had a synergistic effect in the velopharyngeal airway, that is, the increase in airway area with combined stimulation was greater than that with stimulation of either nerve alone. These findings are of importance given the recent studies of Fregosi and colleagues in anesthetized rats (25–27) showing that conditions causing an increase in respiratory motor output, such as hypercapnia and hypoxia, are associated with increases in phasic inspiratory activation of both the tongue protruder and retractor muscles. Combined stimulation of the medial and lateral branches caused a greater decrease in critical airway pressure, an index of airway collapsibility, and a greater increase in maximum inspiratory flow, an index of airway patency, than occurred with stimulation of the medial HG branch alone (27). The current results extend these findings by examining these effects at specific regions of the pharyngeal airway and support the observation that simultaneous activation of these seemingly antagonistic extrinsic tongue muscles may have a beneficial effect in helping to maintain pharyngeal airway patency.

Three anatomic differences in the pharyngeal airway in humans compared with that in felines and other mammals may explain why the human airway is at greater risk of developing upper airway closure during sleep. The abrupt turn of the human pharyngeal airway at the nasopharynx gives it an “L” shape whereas the feline pharynx is a straight tube. In humans, there is an anatomic uncoupling of the epiglottis and soft palate. The tip of the epiglottis is separated from the rim of the soft palate whereas these two structures are at the same cross-sectional level in the felines and actually overlap in porcines. Finally, the hyoid bone has no bony attachments in humans. This floating structure could lead to greater instability of the anterior pharyngeal wall than exists in other mammals in which the hyoid bone articulates with the vertebral column. Despite these anatomic differences, the feline airway was chosen for investigation in the current study as it has a neuromuscular anatomy that is otherwise very similar to that in humans.

Studies indicate that airflow obstruction in humans during sleep and in the isolated upper airway of animals often occurs near the palatal rim (21, 27). The recordings in the current study were performed at atmospheric pressure and did not explore pressure–area relationships in the airway under static or dynamic conditions. However, one can speculate that airflow collapse at the velopharynx under passive condition, that is, no muscle stimulation, is due to a relatively greater compliance at this airway level than at other regions of the pharyngeal airway. Activation of muscles that stiffen and dilate the velopharyngeal airway could be of particular importance in helping to preserve airway patency. It is important to note that even muscles that are not directly surrounding the velopharynx may be affecting its size and stiffness. For example, in the current study, stimulation of the tongue muscles that lie on the ventral surface of the oropharynx had significant dilating effects in the velopharynx. This may have been due to the tethering effects of tongue movement pulling on soft tissue structures attached to the tongue and soft palate. Alternatively, tongue movement may have altered the position of the hyoid bone causing a secondary enlargement of the velopharynx via other fibromuscular attachments to the hyoid.

An unexpected finding of the study was the relatively large increase in airway area at the soft palate level with glossopharyngeal nerve stimulation. Although it is widely recognized that the glossopharyngeal nerve supplies motor output to the stylopharyngeus muscle (17), recent reports by other investigators indicate that this nerve also innervates the levator veli palatini, cricopharyngeus, and pharyngeal constrictor muscles (18, 19). Stimulation of the pharyngeal branch of the vagus, which also provides motor innervation to the pharyngeal constrictors, decreased the area in the velopharynx and caudal oropharynx. In contrast, stimulation of the glossopharyngeus increased these airway areas. These results were likely to have been influenced by the need to sever the branch from the glossopharyngeal to the pharyngeal branch of the vagus during dissection of the nerves in order to free up enough of the glossopharyngeal nerve to place it in the cuff electrode. Kogo and coworkers (28) performed fiberoptic imaging of the pharyngeal airway to study the effect of selective levator veli palatini
muscle stimulation on pharyngeal airway size and found only a small amount of lateral wall movement. Their results suggest that the increase in maximum lateral diameter glossopharyngeal stimulation found in the current study was due to the activation of the stylopharyngeus muscle.

In summary, the study shows that the effect of pharyngeal muscle contraction on airway area depends on the specific muscles that are contracting and the particular level of the airway that is being examined. The results indicate that the changes in airway area in response to pharyngeal muscle contraction are due primarily to eccentric changes in the airway. The results also show that combined stimulation of the medial and lateral branches of the HG nerve have a synergistic effect in the velopharyngeal airway, that is, the increase in airway area with combined stimulation is greater than that with stimulation of either nerve alone. All of these measurements were performed at a fixed airway length and with the airway lumen at atmospheric pressure. Varying the length and radius of the pharyngeal airway is likely to alter their effects on airway size and configuration.

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References