

MRI study of pharyngeal airway changes during stimulation of the hypoglossal nerve branches in rats

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Brennick, Michael J., Theodore P. Trouard, Arthur F. Gmitro, and Ralph F. Fregosi. MRI study of pharyngeal airway changes during stimulation of the hypoglossal nerve branches in rats. *J Appl Physiol* 90: 1373–1384, 2001.—The medial branch (Med) of the hypoglossal nerve innervates the tongue protruder muscles, whereas the lateral branch (Lat) innervates tongue retractor muscles. Our previous finding that pharyngeal airflow increased during either selective Med stimulation or whole hypoglossal nerve (WHL) stimulation (coactivation of protruder and retractor muscles) led us to examine how WHL, Med, or Lat stimulation affected tongue movements and nasopharyngeal (NP) and oropharyngeal (OP) airway volume. Electrical stimulation of either WHL, Med, or Lat nerves was performed in anesthetized, tracheotomized rats while magnetic resonance images of the NP and OP were acquired (slice thickness 0.5 mm, in-plane resolution 0.25 mm). NP and OP volume was greater during WHL and Med stimulation vs. no stimulation ($P < 0.05$). Ventral tongue depression (measured in the midsagittal images) and OP volume were greater during Med stimulation than during WHL stimulation ($P < 0.05$). Lat stimulation did not alter NP volume ($P = 0.39$). Our finding that either WHL or Med stimulation dilates the NP and OP airways sheds new light on the control of pharyngeal airway caliber by extrinsic tongue muscles and may lead to new treatments for patients with obstructive sleep apnea.

pharyngeal airway geometry; magnetic resonance imaging; nerve stimulation

THE EXTRINSIC TONGUE MUSCLES originate outside of the tongue and insert into the tongue body. The principal actions of these muscles include protrusion, retraction, depression, and elevation of the tongue. In mammals, including humans, the genioglossus muscle protrudes and depresses the tongue, the hyoglossus causes retraction and depression, and the styloglossus retracts the tongue and elevates its lateral borders (1). The tongue muscles are innervated by the hypoglossal nerve, which sends a clearly definable medial branch to the genioglossus and a lateral branch to the hyoglossus and styloglossus (12). The base of the tongue serves as the ventral wall of the oropharyngeal (OP) airway.

However, because it is mobile, its position can have a large impact on the patency of the pharyngeal lumen. Indeed, posterior displacement of the tongue base can partially or completely occlude the pharyngeal airway, resulting in snoring or obstruction (21). These phenomena, which are referred to collectively as the obstructive sleep apnea/hypopnea syndrome, occur during sleep in 24% of middle-aged males and in 9% of adult females (26). Thus understanding how the nervous system controls the extrinsic tongue muscles and therefore the position of the tongue during breathing is clinically significant.

The great majority of the work on the respiratory-related control and behavior of the extrinsic tongue muscles has focused on the genioglossus muscle. This line of investigation was a logical one, given the strong protrusive actions of unopposed genioglossus muscle activation (9, 11). However, recent studies from our laboratory showed that the genioglossus and the hyoglossus were activated in parallel during inspiration in the rat and that the tongue retracts slightly in phase with this protruder/retractor muscle coactivation (9). Subsequent studies using an isolated pharyngeal airway preparation showed that stimulation of protruder and retractor muscles together (coactivation) stiffened the pharynx considerably and still allowed increases in flow (10). In contrast, independent stimulation of the genioglossus muscle increased flow but did not alter airway stiffness, whereas independent retractor muscle stimulation reduced flow and did not change stiffness significantly (10). These findings indicated that either coactivation of protruder and retractor muscles or independent activation of protruder muscles can defend the pharyngeal airway, but by different mechanisms. Interestingly, a recent study in human subjects with obstructive sleep apnea also showed that coactivation of the protruder and retractor muscles caused tongue retraction and an increase in airflow (7). It is clear that delineating how the extrinsic tongue muscles interact in defending the pharyngeal airway is important, especially in light of recent attempts (7, 23)

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to develop implantable hypoglossal nerve stimulators in patients with obstructive sleep apnea: should the electrodes be placed on the medial branch (protruder stimulation) or on the whole nerve, proximal to the bifurcation (coactivation of protruder and retractor muscles)?

Nevertheless, a somewhat perplexing aspect of previous studies in rats and humans was the tongue retraction that accompanied coactivation of the protruder and retractor muscles (6, 9). It is difficult to envision how tongue retraction and increased airflow are compatible. We hypothesized that tongue depression was responsible for widening and stiffening the airway, because tongue depression is an action that is common to both genioglossus and hyoglossus muscle contraction (9). However, our simple method of measuring tongue movements allowed measurements in the axial plane only, so that only protrusion and retraction could be quantified. Moreover, our biomechanical studies did not provide us with insight into how pharyngeal geometry changed with stimulation of the different tongue muscles.

Accordingly, the experiments presented herein were designed to address these issues using magnetic resonance imaging (MRI) techniques in anesthetized rats, with the following specific aims: 1) To measure changes in pharyngeal cross sectional area while motor nerves to the protruder and retractor muscles are stimulated independently or simultaneously; 2) to examine the three-dimensional geometrical changes under these conditions, by measuring changes in airway volume with stimulation; and 3) to estimate the magnitude of tongue depression evoked by independent and simultaneous stimulation of protruder and retractor muscles. Our major hypothesis is that either coactivation of protruder and retractor muscles or the independent stimulation of protruder muscles will cause similar changes in airway volume and cross-sectional area (CSA). We also hypothesized that coactivation will result in stronger tongue depression than does independent protruder muscle activation.

METHODS

The University of Arizona Institutional Animal Care and Use Committee approved all procedures reported herein. Studies were carried out in 21 male Sprague-Dawley rats whereas results of complete studies were obtained in 13 rats. Their mean weight was 395 ± 4 g, and all were surgically prepared under urethane anesthesia (2.5 g/kg ip, supplemented as needed with 0.25 g/kg, ip). After observation that there was no response to strong pressure on the paw with a hemostat, surgery was performed with the rats supine on a water-heated pad that was controlled so that rectal temperature was maintained at 37°C. A ventral midline cut along the submental surface from the genu to the sternum allowed for placement of a tracheal cannula and bilateral ventral exposure of the hypoglossal nerve and its branches. The ligaments connecting the anterior and posterior portions of the digastric muscle were cut bilaterally, and the mylohyoid muscle was dissected along the midline. Care was taken to avoid sectioning or damage to the remaining inferior and superior hyoid muscles. The hypoglossal nerve was exposed

ventrolaterally and was cut proximal to the bifurcation into lateral and medial branches. The cut end of the muscle nerve (~1.0–1.5 cm) was separated from surrounding tissue (not desheathed) and installed in the nerve-cuff electrodes for the whole nerve stimulation trials (see *Stimulation Parameters*, below). For medial branch stimulation, the medially directed hypoglossal nerve branches were left intact whereas the laterally directed branches were cut so that stimulation of the main nerve trunk activated only the medial branches. Conversely, for lateral branch stimulation, the medial branches were cut (7).

The nerve-cuff electrode design (provided through correspondence with Victor Fenik, M.D., at the University of Pennsylvania) utilized two Teflon-coated 0.005-in. diameter platinum wires with ~1.0 mm exposure at the tips. The wires were threaded through a 1.5-cm length of polyethylene tubing (0.58 mm ID), and on the end of one wire a small hook (diameter <0.4 mm) was fashioned. The cut nerve was inserted into the hook and drawn into the tube so that the interelectrode distance was ~1 cm. The platinum electrode wires were connected to 28-gauge shielded copper wires. The shielded leads were then connected to a stimulus isolation unit (see *Stimulation Parameters*, below).

During surgery and MRI experiments, the rat was placed supine on a 4.5×60 cm Plexiglas platen. On this platen were two upright laterally positioned posts, 3 cm high and 3 cm apart, which were used to align and secure the head and shoulders in the same fashion for all rats. An anteriorly placed clamp was used to secure the superior incisors of the upper jaw. These procedures ensured that all rats were positioned in a uniform manner and that the three-dimensional geometry was constant during all imaging protocols.

Stimulation Parameters

A two-channel stimulator (Grass Model 88, Quincy, MA) was used in combination with two constant-current stimulus isolation units (Grass, PSIU 8). In this way, differences in impedance between the right and left hypoglossal nerve-cuff electrodes could be accounted for by independently setting the current for each electrode. Except for the independent current levels, the other stimulator settings were the same for both right and left electrodes. Pilot studies showed that, at stimulation frequencies below 80 Hz, tongue fluctuations resulting from nonfused contractions caused blurred images. Thus, to achieve a fused, tetanic contraction, 0.1-ms pulses were delivered at 90 Hz throughout the testing. This is just above the tetanic fusion frequency for rodent tongue muscles (11).

To accommodate for nerve-cuff electrode impedance variations among rats, a method was employed to determine the stimulus current “threshold” and “maximum” in each preparation before the rat was placed in the magnet (2). For this, the tongue was placed at the front teeth and the low current threshold was defined as the lowest stimulus current that caused visible tongue movement. The maximum current level was the current above which tongue protrusion or retraction appeared to be maximal. Threshold and maximum levels were determined separately for the right and left electrodes. Threshold values ranged from 0.1 to 50.0 μ A, whereas maximum values ranged from 14.0 μ A to 4.0 mA. A stimulus level of one-third to one-half maximum was used for the initial testing in the MRI protocol. Before placement into the magnet, the jaw was taped closed while the head was maintained in its secure position. Some fine adjustments were made to the stimulus levels according to the MRI setup protocol.

MRI Protocol

Overview. Two different MRI sequences were used. Gradient echo fast imaging (GEFI) was used first to acquire a time series of midsagittal images that would show whether retraction or protrusion of the tongue was evident during stimulation. The GEFI sequence was used to rapidly acquire images that showed whether or not the tongue was moving appropriately during stimulation of the hypoglossal nerve and its branches. If the GEFI images showed no movement, or inappropriate movements, the animal was removed from the magnet and the stimulating electrodes were adjusted. After the GEFI protocol, a high-resolution spin echo sequence [rapid acquisition with relaxation enhancement (RARE) technique] was used in concert with a stimulus-gated acquisition protocol. Spin echo sequences (such as RARE) are associated with reduced susceptibility artifact because the 180° radio frequency (RF) pulses used in the sequence refocus the phase errors due to magnetic field inhomogeneity and prevent signal dephasing at the center of each data acquisition period. Air and tissue have different magnetic susceptibilities, and this means that the magnetic field is distorted near air-tissue interfaces. In the GEFI sequence, this field distortion leads to signal loss due to dephasing of the spins.

The RARE protocol was used to acquire axial images (transversely sliced) encompassing the pharyngeal airway during nerve stimulation (Stim) and with no stimulation (No Stim). After acquisition of these data, the GEFI protocol was repeated to check the viability of the nerve-muscle preparation. Finally, an additional RARE stimulus-gated sequence was performed to acquire sagittal images during Stim and No Stim conditions.

Initial setup. An Avance Bruker Biospec MRI instrument (Bruker Instruments, Billerica, MA) was used for all imaging studies reported herein. This instrument was equipped with a 40-cm-bore 4.7-T superconducting magnet, an actively shielded gradient coil capable of 140 mT/m, and a 72-mm-diameter volume RF coil. The platen, which supported the rat, rested inside the RF coil and was positioned so that the volume of interest was at the iso-center of the gradient coil. Leads from the nerve-cuff electrodes were connected to the stimulator, which was set to deliver trains of stimulation to both right and left electrodes simultaneously.

GEFI protocol. Longitudinal relaxation time weighted single-slice sagittal images were acquired with the GEFI sequence. Imaging parameters were: Repetition Time (TR) = 20 ms, Echo Time (TE) = 6.8 ms, flip angle = 30° , number of averages or excitations (NEX) = 1, field of view (FOV) = 8×8 cm, and a 128×128 data matrix. A temporal sequence of 12 images was acquired, with each image requiring ~ 4 s. Nerve stimulation was delivered during a discrete, 16-s interval while data were acquired. This protocol provided four serial images without stimulation, four with stimulation, and four after stimulation. These images were used to visualize tongue movements, to optimize stimulus current levels, and to check the viability of the nerve preparation before and after each set of RARE images was obtained. In cases in which the images showed no tongue movement, the preceding axial or sagittal series were excluded from further analysis.

Stimulus-gated RARE protocol. The stimulus-gated RARE MRI protocol is shown schematically in Fig. 1. The stimulus-gated protocol was designed to acquire data in a way that allowed us to deliver stimuli to the muscle in short trains that were separated by periods of no stimulation (2). A 1.7-s stimulus train was delivered every 5 s, resulting in a duty cycle (stimulus on/stimulus off) of 34%. Magnetic resonance

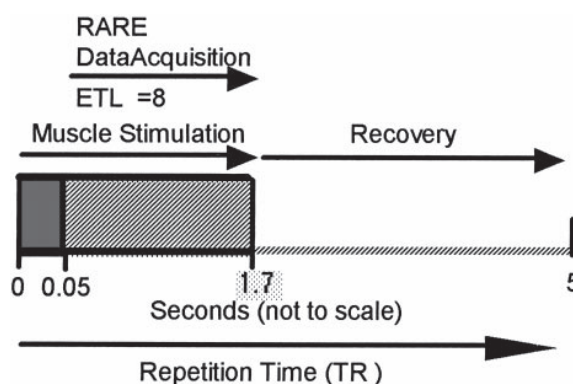


Fig. 1. Schematic representation of the stimulus-gated rapidly acquired refocused spin echo (RARE) magnetic resonance imaging (MRI) protocol used to acquire axial and sagittal images during stimulation of the hypoglossal nerves. The duty cycle was 34% (1.7-s stimulation period, 3.3-s recovery period). Repetition time (TR) was 5 s. The excitation train length (ETL) indicates that 8 data lines were acquired in each TR period. A trigger signal from the stimulator initiated the RARE data acquisition sequence 50 ms after the onset of nerve stimulation. (See METHODS for a more detailed description of the stimulation protocol.)

image data were collected during the stimulus period by triggering the acquisition software with the stimulator output pulse. Acquisition began 50 ms after the onset of stimulation to allow the muscle to shorten before image acquisition (3). The same protocol was used to obtain baseline images, except that in this case the electrode wires were disconnected from the stimulus isolation unit and therefore no current was delivered to the muscle nerves.

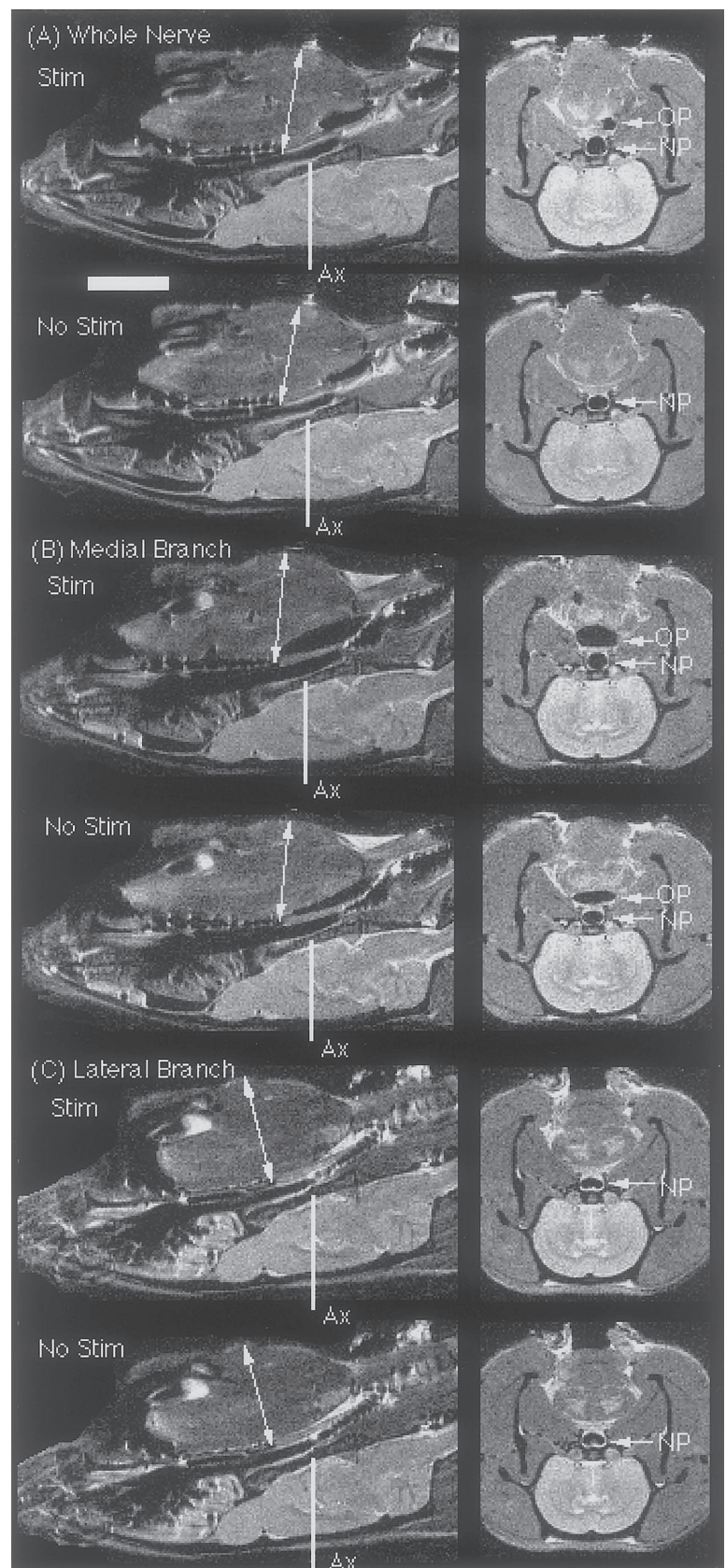
Parameters for the RARE protocol were: TE = 6.8 ms, TR = 5 s, excitation train length = 8, and FOV was 3.2×3.2 cm on a 128×128 matrix. Thus 8 of 128 lines of data could be collected in one TR period, and the total data collection time for each volume acquisition with four averages (NEX = 4) was $(128 \times 4 \times 5 \text{ s})/8$, or 320 s. To achieve thin slice resolution with minimal artifact, two series of 18 0.5-mm-thick axial images were acquired at 1.0-mm spacing, with the first series starting 0.5 mm rostral to the second.

Each rat was used for a single stimulus condition (either whole nerve, medial branch, or lateral branch stimulation), except in one case in which a single rat was studied during whole nerve stimulation and also after section of the lateral nerve branches (see Fig. 2). If the GEFI time series obtained after the first stimulation protocol showed no decline in the magnitude of tongue movements, a series of sagittal images were acquired during Stim and No Stim conditions, using the same stimulus-gated protocol described above. The sagittal images were used for qualitative analysis and for measurements of tongue depression during stimulation (see *Measurement of ventral tongue movements*, below).

Data Analysis

Analysis of CSA and ventral tongue movements. Image analysis was performed on a Macintosh computer using the NIH Image program (v.1.61/ppc, developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>). NIH Image computes the minimum and maximum pixel values of 16-bit raw images and uses this information to linearly scale data to 8 bits. It uses an inverted contrast scale in which a pixel value of 0 corresponds to white and a pixel value of 255 corresponds to black.

Fig. 2. Six pairs of sagittal (*left*) and axial (*right*) views obtained during stimulation (Stim) and control conditions (No Stim) for whole nerve (A), medial branch (B), or lateral branch (C) stimulation. Horizontal calibration bar (in A) is equal to 1.0 cm. Sagittal views show the rat in the supine position, and the line marked Ax in each sagittal midline view indicates the location of the transverse plane where the accompanying axial image was obtained. A double-headed arrow in each sagittal view denotes the location from which the measurement of ventral depression of the tongue was made. The arrow originates at the junction of the soft and hard palate and radiates to the furthest measured ventral point of the tongue. Single headed arrows on the axial images indicate the nasopharyngeal (NP) and oropharyngeal (OP) airways in these axial slices (see text for further description).



A threshold method was employed to differentiate the airway (the air-filled cavities are black) from the surrounding tissues. Each series of axial images was analyzed by using a threshold value determined in the following manner. From a representative sample (typically 8) of axial images, the minimum, maximum and standard deviation of the mean $\pm$ SD pixel values were measured from a region of the black space at the outer boundary of the image. The SD of a homogenous region, such as the black space surrounding the image, can be considered an estimate of the noise in the image (18). Thus, to account for noise in the airway region, the sample mean SD for the black spaces was subtracted from the sample mean minimum pixel value measured for the black spaces, and this value was used as the threshold value to differentiate black space (airways) from tissue (tissue surrounding the airway). For a typical set of images, the average minimum black space pixel value was 220, the mean SD was 5, and, therefore, the threshold value used was 215. Airway regions were usually well differentiated from the surrounding tissues by use of this threshold technique. In some cases, there were airway boundaries missing because of susceptibility artifact, typically near the sphenoid sinus, which forms the dorsal boundary of the retropalatal airway. If the airway could not be isolated using the threshold values, a boundary was inscribed manually to connect the established edges along the curvature of the airway.

Alignment of axial slices and airway regions of interest. To register the axial slices in a consistent manner in all experiments, we first identified an "alignment slice" that was anatomically reproducible in all rats. We chose to use a slice that was just caudal to the olfactory bulb of the brain. This location was chosen because it is easily seen as a "notch" between the olfactory bulb and the cerebral hemisphere in sagittal section (Fig. 3). In addition, this slice is very close to the junction of the hard to soft palate in all rats. Once the alignment slice was located, we began measuring the CSA in 0.5-mm slices starting 2 mm distal to the alignment slice and extending for 7.5 mm along the rostral-caudal axis of the

airway. The alignment slice and the next three adjacent slices (total 2 mm) were excluded from analyses because they were more rostral to the junction of the hard to soft palate. We chose to limit the analysis of the pharyngeal region to 7.5 mm because beyond this length the nasopharyngeal (NP) and OP airways could not be clearly differentiated.

The NP airway was defined for our analyses as the retropalatal portion of the airway, from the junction of the hard and soft palate to the distal tip of the soft palate. The OP airway was defined as the portion of the airway on the ventral side of the soft palate and included the oral airway between the surface of the tongue and the soft palate. The location of these regions is noted in Fig. 3. To examine the effects of whole, medial, or lateral nerve branch stimulation on regional airway dimensions, the 7.5-mm region from which slices were obtained was arbitrarily divided into three equally-sized regions called high, mid, and low (Fig. 3). These regions do not necessarily relate to any particular anatomical landmarks but instead divide the retropalatal airway into subsegments along its rostral-caudal axis.

The airway volume of each of these three regions was calculated as the volume of each cylindrical slice ($CSA \times 0.5$ mm), summed over the 2.5-mm boundary in the Z-axis. Thus, for each condition, high, mid, and low regional airway volumes were calculated and expressed as "estimated airway volume." Total airway volume was then calculated by summing the volume of each region.

Measurement of ventral tongue movements. Tongue movement in the ventral direction was measured in the midsagittal plane from sagittal images taken during Stim and No Stim conditions in four rats of each preparation (whole nerve and medial and lateral nerve branch stimulation). A line was extended from the junction of the hard and soft palate to the furthest point on the ventral surface of the tongue in images acquired during both stimulation and control conditions (see Fig. 2). The length of the line reflects the hard palate-to-submental distance and provides an index of tongue depression during nerve stimulation.

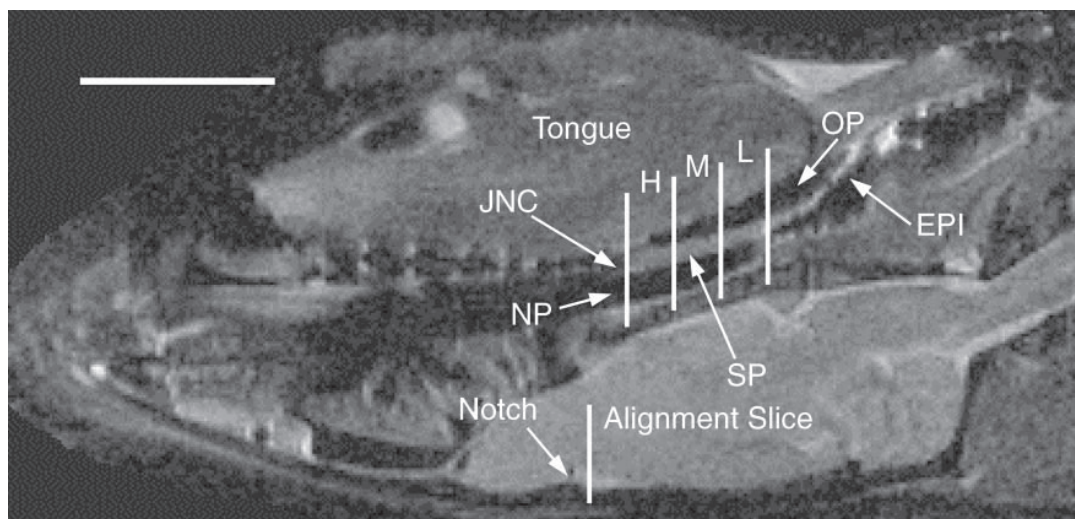


Fig. 3. MRI image of the midsagittal view of the rat, in the supine position, prepared for medial branch stimulation imaged during control, No Stim conditions. Horizontal calibration bar is equal to 1.0 cm. An arrow denotes the "notch" caudal to the olfactory bulb that was used to locate the (noted) alignment slice (see METHODS). Vertical lines, directed in the dorsoventral direction of the rat are drawn in at (scaled) 2.5-mm spacings along the Z-axis of the magnet and delineate the high (H), mid (M), and low (L) regions in both the NP and OP airways. SP, soft palate; JNC, junction of the soft to hard palate; EPI, epiglottis.

Statistical Analyses

The influence of whole nerve ($n = 5$) or medial branch ($n = 5$) stimulation on total airway volume was assessed with two-way repeated-measures (RM) ANOVA (SigmaStat, Jandel Corp, San Rafael, CA). The effects of lateral branch stimulation ($n = 4$) on total airway volume were tested separately by using one-way RM ANOVA. Student's t -test was used to compare the magnitude of the increase in total OP airway volume between whole nerve and medial branch stimulation. The effect of whole nerve and nerve branch stimulation on regional airway volume was tested in each discrete airway region (high, mid, and low) with one-way RM ANOVA. The hard palate-to-submental distance under both stimulated and control conditions was compared in each animal with a paired, two-tailed t -test. Differences in the magnitude of the change in the hard palate-to-submental distance evoked by stimulation of the whole nerve or of the medial or lateral nerve branches were assessed with one-way ANOVA. Significance for all analyses was assumed if $P < 0.05$.

RESULTS

Effects of Hypoglossal Nerve Branch Stimulation on Pharyngeal Airway Geometry

Imaging results from 14 complete studies (5 whole nerve, 5 medial nerve, and 4 lateral nerve conditions) were used for the analyses. The images in Fig. 2 represent three pairs of sagittal and axial views obtained under control conditions and during stimulation of the whole hypoglossal nerve (Fig. 2A), or of the medial (Fig. 2B) and lateral branches (Fig. 2C). In this example, the same rat used for the whole nerve experiment was restudied during medial nerve stimulation (Fig. 2, A and B). This was accomplished by removing the rat from the magnet, sectioning the lateral nerve branches, and repeating the protocol.

Inspection of the axial images in Fig. 2A shows that whole nerve stimulation widened the NP and opened the OP airway. During whole nerve stimulation, the mean hard palate-to-submental distance was also increased, and the data are given in Table 1. Medial branch stimulation caused profound enlargement of the OP in this rat, as well as dilation of the NP and tongue depression (Fig. 2B and Table 1). Lateral branch stimulation reduced the CSA of the NP and OP airways, which is best seen by comparing the sagittal images at the level of the slice bar in the top and bottom panels of Fig. 2C. Lateral branch stimulation had no significant effect on the hard palate-to-submental distance (Table 1).

Table 1. Hard palate to submental distance

	No Stim, mm	Stim, mm	t -Test (two-tailed)
Whole nerve	12.8 $\pm$ 0.6	13.8 $\pm$ 0.6	$P < 0.03$
Medial branch	12.9 $\pm$ 0.3	14.1 $\pm$ 0.2	$P < 0.005$
Lateral branch	13.2 $\pm$ 0.2	13.8 $\pm$ 0.2	Not significant ($P = 0.06$)

Values are means $\pm$ SD; $n = 4$ rats. Stim and No Stim, imaging with and without nerve stimulation, respectively.

Changes in NP and OP CSA in the high, mid, and low regions of the pharyngeal airway for all animals are shown in Figs. 4, 5, and 6 for whole, medial, and lateral nerve branch stimulation, respectively. Whole nerve stimulation increased NP CSA at the mid and low levels of the airway in rats A, B, and C, but no changes were noted in rats D and E (Fig. 4, triangles). The OP CSA was increased by whole nerve stimulation in all five rats (Fig. 4, circles). The OP airway was typically not evident in the high pharyngeal regions, and in three rats (A, D, and E), only the low pharyngeal region had a detectable OP airway. Medial branch stimulation increased NP CSA, especially in the mid and low regions in rats A, C, and D, but no differences were noted in rats B and E (Fig. 5, triangles). During medial branch stimulation, the OP airway showed marked dilation in all rats, with the greatest effect in the mid and low regions (Fig. 5, circles).

The OP airway was not detected during lateral branch stimulation experiments, and it remained closed during stimulation. This was because preexperimental stimulation of the lateral branches always caused strong retractions of the tongue. Apparently the tongue did not return to its baseline position after stimulation. As a result, the OP airway was not visible; this issue is discussed in more detail in the DISCUSSION. In contrast, the NP airway was always visible in these animals. Lateral branch stimulation reduced the CSA of the NP airway in rats A, B, and C, but for the group the changes were not statistically significant ($P = 0.39$, see Fig. 6).

The average data for the regional airway volume measurements with and without stimulation of the whole hypoglossal nerve or of the medial and lateral branches are shown in Fig. 7. Whole nerve stimulation caused significant increases in the OP airway in the mid and low regions of the pharynx, with no change noted in the high region (Fig. 7A). The influence of whole nerve stimulation on NP airway volume was more modest and not significant in any specific region. Medial branch stimulation widened both OP and NP airways significantly in all regions, but the effects on the OP were much larger (Fig. 7B). Although lateral branch stimulation tended to reduce airway volume in all regions, the changes were not significant (Fig. 7C).

Figure 8 shows the influence of nerve stimulation on total airway volume, which was obtained by summation of the regional airway volumes shown in Fig. 7. The bars in the left-hand panel show that total NP airway volume increased significantly in response to both whole and medial nerve branch stimulation, and that lateral branch stimulation had little effect. The bars in the right-hand panel show changes in total OP volume with stimulation of the whole hypoglossal nerve and the medial branches. Stimulation of either the whole nerve or the medial branch caused large increases in OP airway volume, although total OP airway volume was significantly greater during medial branch compared with whole nerve stimulation.

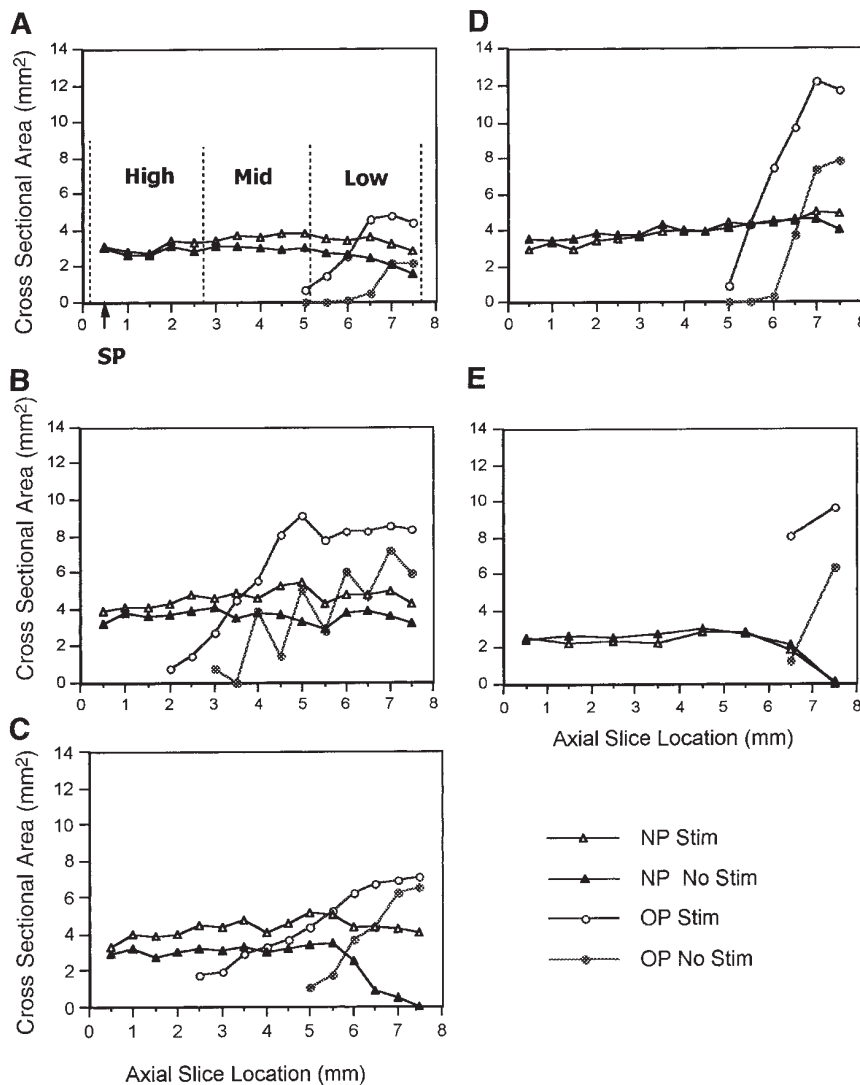


Fig. 4. Airway cross-sectional area (CSA) measurements for 5 individual rats (A–E) prepared for whole nerve stimulation. X-axis represents the location of the axial slice (along the magnet Z-axis) where the CSA was measured, from most rostral (0 mm) to most caudal levels (8 mm). Arrow labeled SP in A indicates the axial slice just proximal to the junction of the soft palate. Inset dashed lines in A show the boundaries used to define the high, mid, and low regions of the rat pharyngeal airway (see METHODS).

DISCUSSION

Summary

The major findings of this study were threefold. First, we found that pharyngeal airway CSA and volume increased during both whole nerve and medial branch stimulation, whereas lateral branch stimulation was associated with a reduction in NP CSA and volume and lower airway occlusion in some rats. Second, both whole nerve and selective medial branch stimulation caused similar increases in NP airway volume, but medial branch stimulation enlarged the OP airways significantly more than whole nerve stimulation. Third, measurement of hard palate-to-submental distance in the midsagittal plane showed that the increases in NP and OP airway volume during either whole nerve or medial branch stimulation were coincident with a significant degree of ventral depression of the tongue, with no significant difference between conditions. These results provide important in-

sight into the influence of tongue movement on pharyngeal airway geometry. Moreover, given certain methodological considerations, the data verify previous findings from this laboratory (8–10) and are consistent with the findings of other investigators (6, 7, 11) who have examined the influence of selective stimulation of the hypoglossal nerve branches on pharyngeal airway physiology.

Critique of Methods

Although event-gated MRI is not new (19), the use of event-gated acquisition during MRI has only recently been described for genioglossal muscle stimulation by Brennick et al. (2). The present study has employed similar MRI techniques to acquire, noninvasively, three-dimensional information on the rat pharyngeal airway during electrical stimulation of the hypoglossal nerve and its branches. We chose to study the pharyngeal airway in rats because the rat is an established model for respiratory studies (8–11, 24). Although the

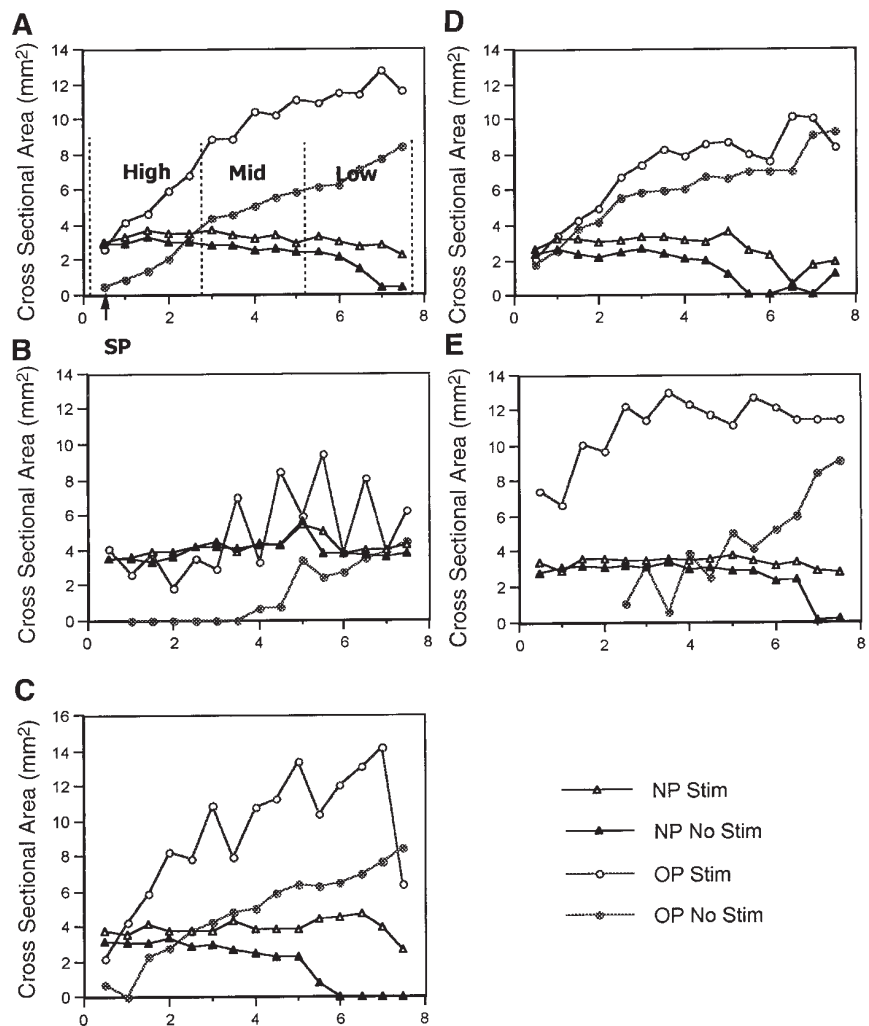


Fig. 5. CSA measurements for 5 individual rats (A–E) studied with and without medial nerve branch stimulation. See legend to Fig. 4 for more detailed explanation.

supine rat upper airway is rectilinear compared with the L-shaped upper airway in the supine human, there is considerable homology in pharyngeal muscle anatomy with humans, and the innervation and mechanical actions of the tongue muscles are similar in rats and humans (6, 9–11, 25). We studied the changes in airway CSA and regional pharyngeal volume because these measurements would help us better understand the changes in airflow mechanics previously reported during hypoglossal nerve stimulation (10).

The stimulus-gated RARE MRI protocol was used to acquire high-resolution (0.25-mm pixel), thin slice (0.5 mm) axial images of the pharyngeal airway. Other MRI protocols that were examined, such as a RARE protocol with constant stimulation or a GEFI protocol, could have been used to acquire the image data. However, in both cases image acquisition would have required several minutes, and continuous stimulation of the motor nerves for this long may have fatigued the muscles and altered their mechanical actions on the pharyngeal airway. By repeating short trains of stimulation during the image acquisition, followed by a long recovery time, we obtained high-resolution images

without continuous stimulation. The duty cycle used in the present study was 34%. Gilliam and Goldberg (11) found that the fatigue index (defined as the percentage muscle tension remaining after 2 min stimulation at 60 Hz using a duty cycle of 50%) was 67% during whole nerve and lateral branch stimulation and 76% during medial branch stimulation. It is likely that some reduction in tongue movements occurred over the course of the stimulation trials in the present study. In the results described by Gilliam and Goldberg, either whole nerve or lateral branch stimulation caused tongue retraction, and in their model the muscles contracted isometrically against the force transducer. In contrast, medial branch stimulation in their model evoked isotonic contractions, which explains why the fatigue index was higher during medial branch stimulation. In the present study, the duty cycle was shorter and the tongue was allowed to move, and both of these factors should have minimized fatigue. Nevertheless, even if the extent of tongue movement changed slightly from the beginning to the end of the protocol because of fatigue, the result would be to make the increase in

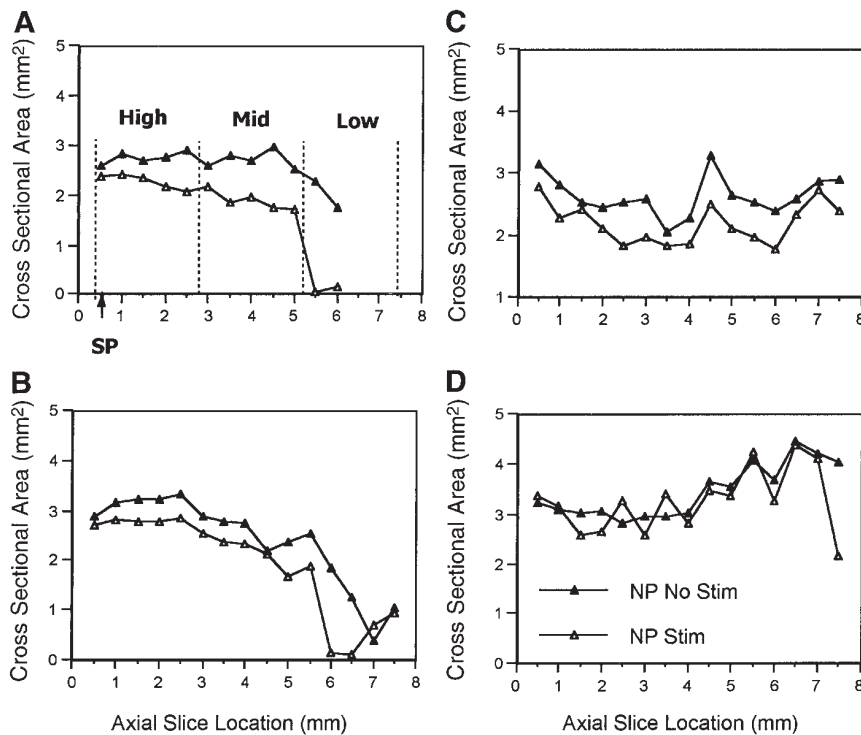


Fig. 6. CSA measurements for 4 individual rats (A–D) studied with and without lateral nerve branch stimulation. Notations are the same as those used in Figs. 3 and 4, except that the OP airway was closed during lateral branch stimulation, explaining why there are no OP airway measurements in these plots. See METHODS for a more detailed explanation of the measurement techniques and associated caveats.

measured airway volume smaller than what would occur in the absence of fatigue.

Airway baseline conditions varied depending on whether whole nerve, medial branch, or lateral branch stimulation had been applied during setup procedures and during testing in the magnet. Because of limitations related to maintaining the appropriate MRI setup parameters, the rat could not be taken out of the magnet once testing was begun. Thus once the imaging protocol was initiated we were unable to standardize tongue position either before or after stimulation. Because of this, the rats studied during lateral branch stimulation had no definable OP airway, because the latter became closed during the initial stimulation period that occurred after the animal was placed in the magnet (see Fig. 2C). Nevertheless, our primary objective was to compare the influence of whole nerve stimulation, which coactivates protrudor and retractor muscles, with the influence of medial branch stimulation, which selectively activates the genioglossus muscle and protrudes the tongue.

Another concern is that the hypoglossal nerves were bilaterally denervated in our preparation. Thus selective stimulation of the nerve branches caused unopposed tongue protrusion during medial branch stimulation and unopposed tongue retraction during lateral branch stimulation. In addition, nerve section also eliminated the spontaneous, respiratory-related activity of the tongue muscles, which is observed both in eupnea and hyperpnea in the rat (8). Moreover, muscle tone in upper airway muscles not innervated by the hypoglossal nerve was reduced by both anesthesia and the absence of upper airway pressure-related mech-

anoreflexes in these tracheotomized animals (4, 14). Together, these factors may have affected the degree to which the tongue could retain its natural position before or after motor nerve stimulation. Thus the current results describe tongue movements under conditions in which the tongue was free to move during stimulation, and while forces which might normally return the tongue to a “normal” or “standardized” baseline position were clearly absent or reduced.

Changes in Airway Geometry Evoked by Coactivation or Selective Activation of Protrudor and Retractor Muscles

An important objective of this study was to observe the changes in airway geometry and tongue position during coactivation of the hypoglossal nerve and with selective stimulation of the medial or lateral branches. In recent studies we showed that the urethane-anesthetized rat coactivates the tongue protrudor and retractor muscles during eupnea and hyperpnea (9). Subsequent studies using an isolated pharyngeal airway preparation showed that either independent stimulation of the tongue protrudor muscles or coactivation of the protrudor and retractor muscles caused an increase in maximal inspiratory airflow, whereas lateral branch stimulation reduced flow slightly (10). However, medial branch stimulation caused a greater increase in maximal flow than did stimulation of the whole hypoglossal nerve. In those experiments, both the mouth and the nose of the animal were patent, and we hypothesized that oral airflow increased markedly with medial branch stimulation, secondary to marked, un-

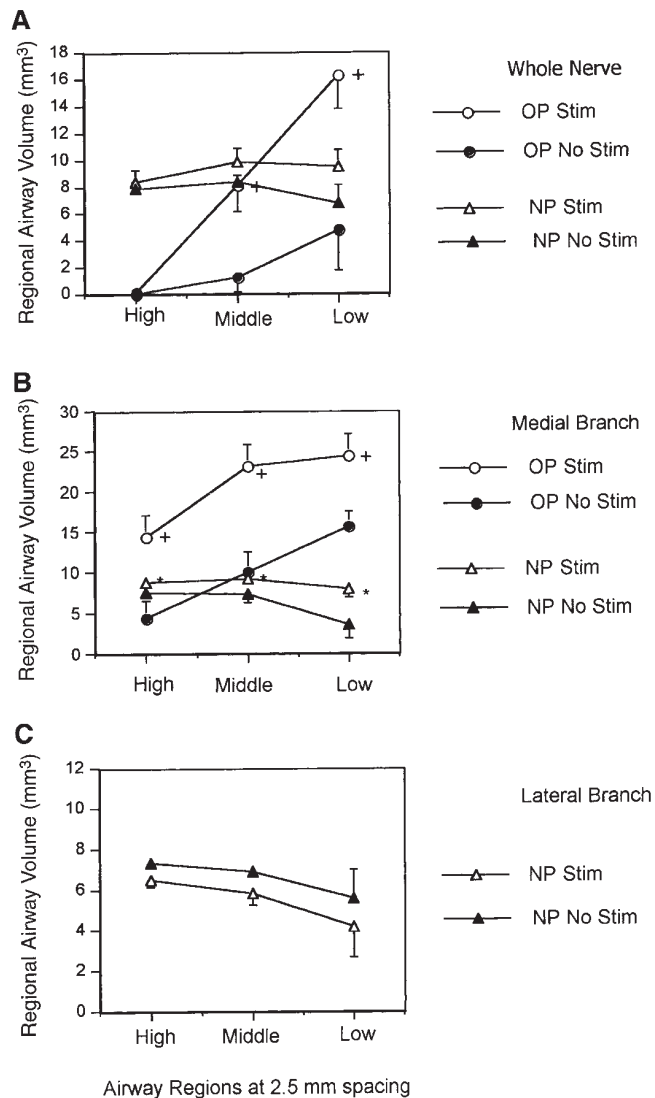


Fig. 7. Influence of hypoglossal nerve stimulation on airway volume in the high, middle, and low regions of the pharyngeal airway. The high, middle, and low regions are the same as those described in Figs. 3 and 4A. A: whole nerve. B: medial branch. C: lateral branch. *Significant increases in NP regional volume during Stim; +significant increases in OP regional volume during Stim. There are no OP values associated with lateral branch stimulation, because OP airways were closed during No Stim and Stim conditions. See legend to Fig. 4 and METHODS for details.

opposed tongue protrusion. We tested this hypothesis by comparing the change in maximal flow induced by medial branch stimulation under conditions in which the mouth was either open or sealed. We found that the change in flow was much greater when the mouth was open, suggesting that tongue protrusion dilated the OP airway, leading to the increase in flow (see Fig. 7 in Ref. 10). The present findings confirm this hypothesis by providing anatomical evidence showing that medial branch stimulation is indeed associated with more marked dilation of the OP airway compared with whole nerve stimulation.

Our prior studies also showed that whole nerve stimulation is associated with more negative collapsing pressures than medial branch stimulation, suggesting that coactivation of the protruder and retractor muscles makes the pharynx more resistant to collapse. Rowley et al. (22) have reported analogous findings in that airway stiffness increased during applied tongue and tracheal extension. The design of the present studies did not allow us to address the mechanisms of the increased airway stiffness produced by coactivation. Nevertheless, the present results and our earlier work suggest that the airway can be defended by either coactivation or independent activation of the protruder muscles, but by different mechanisms. Specifically, coactivation stiffens the airway while causing some dilation, whereas medial branch stimulation dilates the airway but does not change airway stiffness (10).

Our finding that a significant increase in the hard palate-to-submental distance occurred during stimulation of the whole hypoglossal nerve or during selective medial branch stimulation is an indication that ventral tongue movement (tongue depression) may be an important component of the overall mechanical changes that accompany airway dilation during tongue muscle activation. Ventral movement of the tongue pushes it out of the OP airway, creating a bulge in the submental region. This type of tongue movement has been noted in a previous study by Fuller et al. (10) and may be an explanation for why whole hypoglossal nerve stimulation caused an increase in airway caliber without the visible tongue protrusion that accompanies medial branch stimulation.

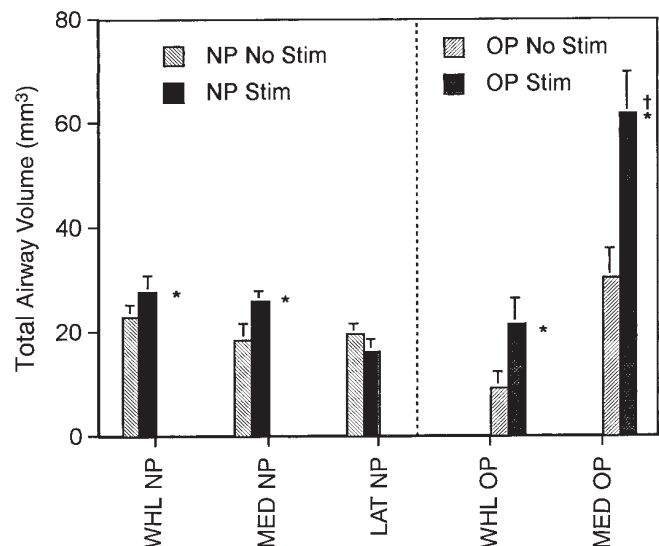


Fig. 8. Mean total airway volume in the NP (left) and OP (right) airways, during stimulation of the whole (WHL), medial (Med), and lateral (Lat) branches of the hypoglossal nerves. *Stimulation caused a significant change in NP or OP airway volume. †, The magnitude of the increase in OP airway volume with stimulation was significantly greater with medial branch compared with whole nerve stimulation (by *t*-test, with $P < 0.05$).

Contributions of Intrinsic Tongue Muscles

Both medial and lateral branches of the hypoglossal nerve contain motoneurons that innervate intrinsic and extrinsic muscles of the tongue (17). Thus in all of our experiments both intrinsic and extrinsic tongue muscles were activated during muscle nerve stimulation. Our experimental design and data interpretation focus on the changes in pharyngeal geometry that occur in response to activation of the extrinsic tongue muscles, which evoke gross tongue movements (9, 10). The intrinsic muscles control fine tongue movements including tongue shape (17), and it is likely that the latter variable changed during nerve stimulation in the present studies. However, this does not necessarily indicate a specific mechanical role for the intrinsic muscles with respect to changes in pharyngeal airway geometry. Rather, activation of the intrinsic muscles likely changes tongue stiffness. Although imaging techniques have recently begun to examine the three-dimensional shape changes in the human tongue relating to human vocalization, we did not analyze shape changes in the tongue but rather focused on the outcome variable, airway CSA during stimulation (5).

Clinical Significance

Ilomaki et al. (14) found an approximately twofold expansion of the hypopharyngeal region during whole hypoglossal nerve stimulation in five anesthetized patients, but they did not examine the differences between selective medial or whole nerve stimulation. In a study in obstructive sleep apnea (OSA) patients, Eisele et al. (7) found that either whole hypoglossal nerve stimulation or medial branch stimulation resulted in more negative collapsing pressures and a corresponding increase in maximal inspiratory airflow. It is possible that unilateral stimulation, which was used in the human studies described above, did not achieve a mechanically balanced movement of the tongue (7, 14). In fact, Eisele et al. reported that unilateral hypoglossal nerve stimulation caused ipsilateral deviation during selective medial branch stimulation that might have limited airway dilation during medial or whole nerve stimulation. However, it is not clear why airway flow was increased to a similar degree with whole or medial nerve stimulation in humans, whereas in the rat medial branch stimulation resulted in a greater increase in flow compared with whole nerve stimulation (7, 10). It is possible that the marked differences in airway shape between rats and humans are responsible for these different findings.

In conclusion, the results of this study lend support to the idea that stimulation of the tongue muscles through the hypoglossal nerve could effectively dilate the NP and OP airways and prevent airway obstruction in OSA (7, 13, 14, 16, 20). An important application of the present work may be the development of a nerve stimulation device for OSA patients who do not respond well to uvulopalatopharyngoplasty or continuous positive airway pressure (15). However, it is not yet clear whether stimulating the whole hypoglossal

nerves (which coactivates protruder and retractor muscles) or only the medial branches (selective activation of protruder muscles) results in the most patent pharyngeal airway. The present studies, which reveal anatomical changes in the airway coincident with tongue muscle activation, show that medial branch stimulation enlarges the OP airway more than does whole nerve stimulation. In contrast, biophysical data have shown that whole nerve stimulation stiffens the airway more than does medial branch stimulation (10). Accordingly, the next step is to develop a comprehensive model that incorporates both anatomical and biophysical data in an effort to predict which upper airway muscles should be stimulated to obtain the most patent pharyngeal airway.

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