Influence of tongue muscle contraction and dynamic airway pressure on velopharyngeal volume in the rat

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Fregosi RF. Influence of tongue muscle contraction and dynamic airway pressure on velopharyngeal volume in the rat. J Appl Physiol 104: 682–693, 2008. First published December 13, 2007; doi:10.1152/japplphysiol.01043.2007.—The mammalian pharynx is a collapsible tube with little skeletal support and is subject to collapse as its transmural pressure becomes negative. The velopharynx (VP), which lies posterior to the soft palate, is considered to be one of the most collapsible pharyngeal regions. I tested the hypothesis that negative transmural pressure would narrow the VP, and that electrical stimulation of extrinsic tongue muscles would reverse this effect. Pressure (−6, −3, 3, and 6 cmH2O) was applied to the isolated pharyngeal airway of anesthetized rats that were positioned in a 4.7-T MRI scanner. The volume of eight axial slices encompassing the length of the VP was computed at each level of pressure, with and without bilateral hypoglossal nerve stimulation (0.1-ms pulse, one-third maximum force, 80 Hz). Negative pressure narrowed the VP, and either whole hypoglossal nerve stimulation (coactivation of protruder and retractor muscles) or medial nerve branch stimulation (independent activation of tongue protruder muscles) reversed this effect, with the greatest impact in the caudal one-third of the VP. The dilating effects of medial branch stimulation were slightly larger than whole nerve stimulation. Positive pressure dilated the VP, but tongue muscle contraction did not cause further dilation under these conditions. I conclude that the narrowest and most collapsible segment of the rat pharynx is in the caudal VP, posterior to the tip of the soft palate. Either coactivation of protruder and retractor muscles or independent contraction of protruder muscles caused dilation of this region, but the latter was slightly more effective.

The VP and oropharynx are believed to be the regions most vulnerable to collapse in patients with obstructive sleep apnea (24–26, 37, 41, 54). However, we know little about the influence of tongue muscle contraction on VP volume changes in either human subjects or animal models, and recent studies suggest that the VP is a complex pharyngeal region that does not behave uniformly along its length in cats or rats (7, 52). For example, the VP in the adult rat is only ~10 mm long, but it shows differences in compliance between its most rostral and caudal regions (52). This observation suggests that volume changes evoked by tongue muscle contraction along the VP will not be uniform, and that there is likely a small, highly compliant region that is vulnerable to collapsing pressure, but also amenable to expansion and/or stiffening with contraction of the tongue muscles. Several investigators have hypothesized the existence of such a region (14, 20, 24, 38, 39, 43, 47–49, 51), and, although its boundaries are purported to be small, it should be amenable to precise localization with MRI.

Accordingly, the goal of the present study was to evaluate the influence of extrinsic tongue muscle contraction and dynamic changes in pharyngeal pressure on regional VP volume. VP volume changes, measured at 1-mm intervals throughout the length of the rat VP, were estimated with MRI as dynamic changes in pharyngeal pressure on regional VP volume. For both anatomic and physiological reasons, neuromuscular activation is inadequate in at least some patients with primary snoring, upper airway resistance syndrome, or obstructive sleep apnea (41, 54). These patients cannot maintain a positive pharyngeal transmural pressure during sleep, leading to periodic pharyngeal collapse with associated hypoventilation and hypoxia throughout the night.

At present, the favored treatment option for these patients is application of positive pressure to the pharyngeal lumen (continuous positive airway pressure). Although continuous positive airway pressure therapy is effective when consistently used, patient compliance is poor because it is uncomfortable, loud, and associated with disagreeable drying of the oral and nasal mucosa (53). As a consequence, there has been considerable interest in developing novel methods to alleviate obstructive apnea, including electrical stimulation of pharyngeal muscles (12, 15, 16, 27, 35–37, 42, 45, 46). The success of electrical stimulation therapy for obstructive sleep apnea requires a detailed understanding of the airway regions that are most vulnerable to collapse in a given subject and the particular muscles that must be activated to obtain optimal changes in airway dimensions and compliance.

The VP and oropharynx are believed to be the regions most vulnerable to collapse in patients with obstructive sleep apnea (24–26, 37, 41, 54). However, we know little about the influence of tongue muscle contraction on VP volume changes in either human subjects or animal models, and recent studies suggest that the VP is a complex pharyngeal region that does not behave uniformly along its length in cats or rats (7, 52). For example, the VP in the adult rat is only ~10 mm long, but it shows differences in compliance between its most rostral and caudal regions (52). This observation suggests that volume changes evoked by tongue muscle contraction along the VP will not be uniform, and that there is likely a small, highly compliant region that is vulnerable to collapsing pressure, but also amenable to expansion and/or stiffening with contraction of the tongue muscles. Several investigators have hypothesized the existence of such a region (14, 20, 24, 38, 39, 43, 47–49, 51), and, although its boundaries are purported to be small, it should be amenable to precise localization with MRI.

Accordingly, the goal of the present study was to evaluate the influence of extrinsic tongue muscle contraction and dynamic changes in pharyngeal pressure on regional VP volume. VP volume changes, measured at 1-mm intervals throughout the length of the rat VP, were estimated with MRI as dynamic pharyngeal pressure was reduced (negative, collapsing transmural pressures) or increased (positive, distending transmural pressures). Tongue protruder and retractor muscles were stimulated either independently or simultaneously at five discrete levels of pharyngeal transmural pressure. I found that the largest effects of both pressure and tongue muscle contraction act posterior to the caudal tip of the soft palate, suggesting that...
this is the narrowest and most collapsible segment of the rat pharyngeal airway.

METHODS

Animal preparation. Eighteen male Sprague-Dawley rats (324.6 ± 10.8 g, mean ± SE) were used in accordance with guidelines established by the Institutional Animal Care and Use Committee (IACUC) at the University of Arizona. All experimental procedures and manipulations were reviewed and approved by the IACUC at The University of Arizona. The experimental preparation was described in detail in recent papers (4, 52) and is shown schematically in Fig. 1. Animals were anesthetized with urethane (1.2 g/kg iv) and breathed through a tracheotomy tube. A second tracheotomy was made ~5 mm caudal to the vocal folds, through which a nonmagnetic pressure transducer (Millar Instruments) was advanced rostrally to a point ~3–5 mm caudal to the left nares, to measure nasopharyngeal pressure. The left nare remained open to allow airflow; the right nare was sealed with super glue. A catheter was advanced through the same tracheotomy to a point just rostral to the vocal folds (i.e., in the hypopharynx) and connected in series to a pressure or vacuum source and a second differential pressure transducer (Validyne, model DP45–28) that provided an estimate of the pressure applied at the hypopharynx. I studied the airway at four dynamic, nasopharyngeal pressure values, ~6, ~3, 3, and 6 cmH2O, by applying either suction (via a vacuum source) or positive pressure (compressed air). Baseline measurements under static conditions at atmospheric pressure were also made. The duration of pressure application corresponded to the duration of imaging time, ~2 min. The application of each pressure level was fully randomized, and all pressure levels were studied in each animal, once with and once without stimulation of the hypoglossal nerves or its main branches.

Wire electrodes were placed around the hypoglossal nerves bilaterally (activation of all tongue muscles), or on the medial (protruder muscle activation) or lateral (retractor muscle activation) branches. The electrode lead wires were shielded and passed outside the magnet where they were connected to a stimulator (Grass S88) via separate constant-current stimulus isolation units (Grass PSIU6). Nerves were stimulated with 0.1-ms pulses at 90 Hz. The threshold was defined as the lowest current that caused visible tongue movement (9, 52); the current above which tongue protrusion or retraction appeared to be maximal defined the maximal current level. A stimulus level of one-third to one-half maximal was used for subsequent stimulation trials (range: 50 μA to 5 mA).

MRI protocol. Rats were placed in a MRI scanner (Bruker Instruments, Billerica, MA) (Fig. 1) and positioned so that the pharyngeal airway was at the iso-center of the gradient coil (4, 9, 52). The MRI protocol involved two sequences: gradient echo fast imaging (GEFI) and rapid acquisition with relaxation enhancement (RARE), as described in detail previously (4, 9, 52). GEFI 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. (Please paste the following link into your web browser for an example of a GEFI sequence: http://www.physiology.arizona.edu/labs/mlab/GEFI.mov. Be sure to press the ‘play’ button to see how the tongue moves with stimulation.) The RARE technique was used in concert with a stimulus-gated acquisition protocol to obtain axial images of the VP during pressure application, with and without stimulation, achieved by triggering the acquisition software with the stimulator output pulse (4, 9, 52).

Data analysis. The nasal VP was defined as the region on the dorsal side of the soft palate, extending caudally from the junction of the hard and soft palate to the tip of the soft palate, as shown in Fig. 2. The oral VP was defined as the portion of the airway on the ventral side of the soft palate encompassing the oral airway lumen between the tongue and the soft palate (Fig. 2).

The cross-sectional area of eight consecutive images spanning the VP from the caudal edge of the soft palate to its rostral extent was measured (refer to Fig. 2 to visualize these anatomic landmarks). Each axial slice was 1 mm thick, so measurements of cross-sectional area and the volume of each slice are equivalent. I evaluated the influence of whole nerve (n = 6), medial branch (n = 6), and lateral branch (n = 6) stimulation at atmospheric pressure, and at positive (3 and 6 cmH2O) and negative (~3 and ~6 cmH2O) pharyngeal pressures. All pressure and stimulation/no-stimulation trials were randomized. I also estimated the regional compliance of the nasal airway at each 1-mm segment of the VP, in each of the three groups of rats (i.e., whole nerve, medial branch, and lateral branch stimulation groups). This was done by constructing linear regression curves of the volume-pressure relationship under stimulated and nonstimulated conditions for each animal. The regression analysis was based on all four dynamic pressure levels (~6, ~3, 3, and 6 cmH2O). After the curves for each animal were constructed, average values for each of three nerve stimulation groups were obtained (see Fig. 12).

Results for each measured variable were analyzed with two-way repeated-measures ANOVA, with VP segment (i.e., slices 1–8) and stimulation/no-stimulation the main factors. If the results of ANOVA were significant, differences between stimulation and no-stimulation conditions for each slice were evaluated with Bonferroni post hoc tests (Graph Pad Prism Software, San Diego, CA). A P value of ≤0.05 was considered significant for all tests.

RESULTS

Comparison of nasal and oral airway volume along the rat VP under static conditions at atmospheric pressure. Figure 3 shows nasal and oral VP volume at 1-mm intervals, starting at the tip of the soft palate caudally and extending rostrally to the junction of the hard and soft palate. The nasal airway was widely patent throughout the VP, whereas the oral air space was large near the tip of the soft palate, but narrowed significantly starting at a point 3 mm above the tip of the soft palate.
The nasal airway was significantly wider than the oral airway at all points more rostral than 3 mm above the tip of the soft palate (Fig. 3). Whole hypoglossal nerve and nerve branch stimulation with the airway under static conditions at atmospheric pressure. Representative axial images showing the influence of stimulating the whole hypoglossal nerves, or the medial or lateral nerve branches, on airway dimensions at atmospheric pressure are shown in the middle panels of Figs. 4, 5, and 6, respectively (for orientation, the arrows on the bottom right panel of Fig. 4 denote the nasal and oral airways). The average data is shown in Fig. 7, wherein the data points represent slice-by-slice data through the VP, starting 1 mm above the tip of the soft palate and extending rostrally for 8 mm. Whole hypoglossal nerve and medial branch stimulation had a negligible influence on the oral airway, with the changes confined to a modest dilation 3–4 mm above the tip of the soft palate (Fig. 7, left top and middle). Lateral branch stimulation had no effect on the oral airway at any level of the VP (Fig. 6, middle, and Fig. 7, left bottom).

The nasal airway was open throughout the length of the VP in all groups of animals (see images in Figs. 4–6, middle, and the average data in Fig. 7, right). Whole hypoglossal nerve stimulation increased nasal airway volume in the caudal VP (Fig. 7, right top), but not in more rostral regions. In contrast, medial branch stimulation increased nasal airway volume significantly at all levels of the VP (Fig. 5, and Fig. 7, right middle). Lateral hypoglossal nerve stimulation had no discernible effect on nasal airway volume at any level of the VP (Fig. 6 and Fig. 7, right bottom).

Stimulation of the whole hypoglossal nerves at negative and positive dynamic airway pressures. The influence of stimulating the whole hypoglossal nerves on VP volume at negative and positive airway pressures is shown as images in Fig. 4, with the average data in Fig. 8. Stimulation dilated the oral airway at airway pressures of −3 and 3 cmH2O (Fig. 8, left middle two panels), although this effect was significant only at a single location in the caudal VP (i.e., at a level 3 mm above the tip of the soft palate). Stimulation dilated the nasal airway at pressures of −6 and −3 cmH2O, but only in the caudal-most regions of the VP (Fig. 4, left, and Fig. 8, right top two panels). Whole hypoglossal nerve stimulation had no effect on the nasal VP at positive airway pressures (Fig. 4, right, and Fig. 8, right bottom two panels).

Stimulation of the medial hypoglossal nerve branches at negative and positive dynamic airway pressures. Images showing the influence of stimulating the medial hypoglossal nerve branches on average changes in VP volume at negative and positive airway pressures are shown in Fig. 5, with the average data in Fig. 9. Stimulation dilated the oral component of the VP at an airway pressure of −3 cmH2O, although this effect was significant only at a single level located 5 mm above the tip of the soft palate (Fig. 9, left, second from the top). Stimulation also caused significant oral airway dilation at a pressure of 6 cmH2O, with significant effects localized to the middle of the VP (Fig. 5, right, and Fig. 9, left bottom).

Stimulation dilated the nasal airway at all pressure levels, with the largest effects observed at an airway pressure of −6 cmH2O, where all but the rostral-most 1-mm segment of the VP failed to dilate with stimulation (Fig. 5, left, and Fig. 9, right top). The airway-dilating influence of medial nerve branch stimulation at −3, 3, and 6 cmH2O was confined to the caudal-most regions of the VP (Fig. 9, right).

Stimulation of the lateral hypoglossal nerve branches at negative and positive dynamic airway pressures. Lateral nerve branch stimulation had no significant effects on the oral airway, although there was a clear trend for airway narrowing at an airway pressure of 6 cmH2O (see images in Fig. 6, and average data in Fig. 10, left bottom), but, due to highly variable responses, the results of the ANOVA were not significant. Significant airway narrowing with lateral nerve branch stimulation was observed in the nasal airway at pressures of −3 and 3 cmH2O, but only in the rostral-most regions of the VP (Fig. 10, right middle two panels).

Statistical comparison of the effectiveness of whole hypoglossal nerve and medial branch stimulation on VP airway volume. Since either whole hypoglossal nerve or medial nerve branch stimulation consistently evoked nasal airway dilation,
primarily at atmospheric and negative airway pressures, we wished to compare the effectiveness of these two interventions. To do this, we computed the stimulation-induced change in nasal airway volume at airway pressures of 0, −3, and −6 cmH2O (Fig. 11). Medial nerve branch stimulation caused slightly more dilation of the nasal VP at zero airway pressure, but the difference was significant only at the midpoint of the VP (Fig. 11, top). Similarly, there was a trend for a greater dilating effect of medial branch compared with whole hypoglossal nerve stimulation at −3 cmH2O, but the only significant effect was in the caudal VP, 2 mm above the tip of the soft palate. There were no systematic differences between stimulation methods at an airway pressure of −6 cmH2O.

**Regional compliance.** The compliance of each 1-mm segment of the VP was estimated, and the results are shown in Fig. 12. In all three nerve stimulation groups, compliance was found to be highest near the tip of the soft palate and lowest at the rostral end of the VP. Stimulation did not alter compliance.
significantly in any of the groups, although there was a trend for reduced compliance with stimulation in all groups.

**DISCUSSION**

**Summary.** My major finding is that stimulation of the whole hypoglossal nerves, or of the medial branches alone, dilates the VP under both static and dynamic flow conditions. The largest effects occurred under conditions of negative pharyngeal transmural pressures, and the most consistent dilation was observed in the caudal one-third of the VP. Tongue muscle contraction consistently dilated the nasal VP, whereas effects on the oral VP were relatively small and inconsistent. Direct statistical comparison of the dilating influence of medial nerve branch stimulation (independent activation of tongue protrudor muscles) with the influence of whole hypoglossal nerve stimulation (coactivation of protrudor and retractor muscles) revealed that the dilating effects of medial branch stimulation were larger when the airway lumen was held at atmospheric pressure or at a moderately collapsing transmural pressure of $-3 \text{ cmH}_2\text{O}$. In contrast, the dilating influences of whole and medial branch stimulation were the same when airway transmural pressure was held at $-6 \text{ cmH}_2\text{O}$. Compliance was higher in the caudal compared with more rostral levels of the VP in all groups of animals. I conclude that the narrowest and most collapsible segment of the rat pharynx is in the caudal VP, posterior to the tip of the soft palate. Either coactivation of protrudor and retractor muscles or independent contraction of protrudor muscles caused dilation of this region, with the latter slightly more effective under conditions in which transmural pressure is modestly negative.

**Critique of methods.** Although the strengths and limitations of our experimental model have been addressed in detail previously (4, 9, 52), here I review some technical issues that are particularly relevant to the present experimental design. First, my goal was to measure responses to dynamic pressure changes, as opposed to the nonphysiological static changes that have been used to measure pharyngeal compliance by others (7, 23, 31) and us (3). This is an important issue because collapse of the pharyngeal airway in patients with sleep apnea is a dynamic event, occurring in the face of airflow, and collapse can occur when flow is in either the inspiratory or expiratory direction (43). Having said that, I am aware that, under dynamic conditions, fluid flow through the pharynx may lead to regional differences in pressure acting on the pharyngeal wall. However, the small variations in pressure that may exist along the pharynx are very small, compared with the large pressure levels that we are applying, and so these effects, if they indeed exist, would be very small. Moreover, I computed the cross-sectional area of each millimeter of the VP separately, so, even if there were small rostral-to-caudal variations in pressure, these would be similar under stimulated and nonstimulated conditions within each discrete 1-mm segment. It is also important to note that measurements were not taken until flow and downstream pressure had fully stabilized. This was done by adjusting the source pressure until a stable target pressure was obtained, resulting in steady-state flow conditions.

The second methodological issue is that the rat pharynx is more rectilinear than the curved human pharynx, but several observations have validated the functional similarities of the rat and human pharynx, particularly with regard to the neural control (1, 2, 5, 6, 10, 19, 33) and mechanical effects (15, 16, 27, 33, 35–37, 45, 46) of extrinsic tongue muscle contraction. The third issue is that the rat epiglottis reaches the caudal margin of the soft palate, which contrasts with the adult human, in whom the epiglottis and soft palate are separated. However, the adult rodent pharynx is similar to the human infant airway, in which the soft palate and epiglottis are in close apposition until about the second year of life. The mechanical interactions between the tongue and

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**Fig. 6.** Representative axial images through the midregion of the VP (5 mm rostral to the tip of the soft palate), showing the influence of stimulating bilaterally the lateral branches of the hypoglossal nerves (Stim) at three levels of pharyngeal pressure, under No Stim and Stim conditions. In this animal, both nasal and oral airways were patent, but stimulation had no obvious effects at any transmural pressure.
Soft palate must also be considered, especially under conditions where tongue movements are substantial, as in this study. Forward movement and depression of the tongue base, evoked by either coactivation or independent protrudor muscle contraction, take pressure off the soft palate, allowing it to move forward. Although soft palate movement was not analyzed systematically, I did not note any significant movements of the soft palate under any conditions in this isolated upper airway preparation. Finally, it is important to note that the alterations in VP geometry observed in this model are due to direct stimulation of the tongue muscles. In contrast, in intact unanesthetized subjects, spontaneous activation of multiple upper airway muscles may alter the mechanical actions of the tongue muscles and produce different effects on the VP than observed herein. Thus, although the lessons learned from studying pharyngeal physiology in animal models may not tell us exactly how the human airway will function, there are enough similarities in structure and function to make these models useful for developing hypotheses that can be tested in human subjects.

VP volume and hypoglossal nerve stimulation at atmospheric pressure. Stimulation of the whole hypoglossal nerves dilated the caudal 3 mm of the nasal VP, but effects on the oral VP were small and inconsistent. Medial hypoglossal branch stimulation also had a modest effect on the oral VP, but a strong and consistent effect on the nasal VP across its entire length. These data are consistent with our previous study in the rat (9), which showed that medial branch stimulation evoked a greater increase in oropharyngeal volume (but not nasopharyngeal volume) than whole hypoglossal nerve stimulation, under conditions in which the pharyngeal lumen was maintained at atmospheric pressure. In contrast, Kuna (30) showed a greater increase in cross-sectional area with whole nerve stimulation compared with medial branch stimulation in a decerebrate cat model, suggesting that coactivation of tongue protrudor and retractor muscles is more effective in dilating the VP in the cat (30). The reason for the different findings is uncertain, but could be due to a species-dependent difference in pharyngeal anatomy, different mechanical actions of the tongue muscles, the effects of anesthesia, or differences in stimulation protocols.
**Whole XII stimulation**

**Oral velopharynx**

**Nasal velopharynx**

Fig. 8. Average data showing the influence of stimulating the whole hypoglossal nerves (coactivation of tongue protrudor and retractor muscles) at negative and positive airway pressures, as indicated on each panel. Data for Stim (■) and No Stim control conditions (▲) are represented by different symbols, as indicated. Each graph shows the volume of each axial slice (y-axis), starting 1 mm above the tip of the soft palate and extending upwards for 8 mm (x-axis), approximating the junction of the hard and soft palate. Left: oral airway volume; right: nasal airway volume. Stim different than No Stim: *P < 0.05, **P < 0.01, ***P < 0.001.

*Tongue muscle stimulation and VP volume at negative and positive pharyngeal airway pressures: protrudor stimulation vs. coactivation of protrudor and retractor muscles.* Negative pressure application narrowed the nasal VP, and under these conditions stimulation of the whole hypoglossal nerves or the medial branches increased nasal VP volume, particularly in the caudal regions of the VP. Although either coactivation of protrudor and retractor muscles or independent activation of protrudor muscles dilated the pharynx, there were some quantitative differences. Stimulation of the medial nerve branches dilated the nasal VP along its entire length at a pressure of −6 cmH₂O, whereas at −3 cmH₂O only the caudal segment of the nasal VP dilated. Contraction of the genioglossus muscle causes both ventral advancement (protrusion) and caudal depression of the tongue base, whereas coactivation of the genioglossus with the main retractor muscles (hyoglossus and styloglossus) results in net tongue retraction and enhanced depression (13, 18, 21, 22, 32, 34). Stimulation of the genioglossus muscle alone dilates the airway, but does not cause significant changes in pharyngeal stiffness, as shown in both animal (20, 52) and human subjects (35, 37). In contrast, protrudor-retractor muscle coactivation evokes both pharyngeal dilation and increased pharyngeal stiffness, leading to a more negative critical collapsing pressure in animal models (20, 39, 50) and in human subjects (15, 27, 35–37, 45). This flexibility allows the nervous system to activate tongue mus-
cles in a manner that can emphasize either stiffness or dilation, in accordance with the adjustment that results in the biggest drop in airflow resistance under a given set of conditions.

Stimulation was much less effective under conditions in which the pharynx was exposed to positive transmural pressures. This was anticipated, given that positive pressure alone causes substantial dilation of the airway. Nevertheless, the rat pharynx is relatively rigid compared with the human pharynx. Although one might predict that stimulation of the tongue muscles would be much more effective in human subjects with positive critical collapsing pressures, there does not appear to be clear consensus. For example, Isono et al. (27) showed no effect of bilateral stimulation of the tongue surface on VP cross-sectional area at positive transmural pressures, despite a dilating effect at negative transmural pressures. In contrast, a recent study showed a significant increase in pharyngeal cross-sectional area with direct stimulation of the genioglossus muscle (35). The conflicting results are likely due to the different stimulation protocols, although in both studies it is unclear how much of the tongue musculature was stimulated: in the former, surface stimulation was applied to the tongue body, and it is likely that protruder and retractor muscles (both intrinsic and extrinsic) were activated. Also, although stimulating electrodes were inserted into the genioglossus muscle in the latter study, current spread to adjacent muscles cannot be ruled out. This is because the fibers of all seven extrinsic and intrinsic tongue muscles are very tightly intermingled within the tongue body, increasing the probability of current spread via volume conductance.

It is also clear that the position of the mandible has a major influence on pharyngeal airway size and on tongue movements. Indeed, stimulation of the tongue muscles, especially at the relatively high intensities used herein, is associated with ventral displacement of the mandible, as shown in the accompa-
nying video. Although I could not quantify the magnitude of mandibular movement, it was always observed. This marked ventral displacement of the mandible underscores the strong tongue depression that is evoked by coactivation of the tongue protruder and retractor muscles and clearly demonstrates the complex interactions between the mandible and tongue. This is consistent with the work of Isono et al. (28), who showed that manual advancement of the mandible by the investigators dilated the otherwise passive VP in human subjects with obstructive sleep apnea. It is likely that this mechanism contributed to the VP dilation observed with whole and medial nerve branch stimulation in the present experiments. It is also likely that the dilation observed with stimulation of the tongue muscles is due to ventral displacement of tissues in the lateral and ventral pharyngeal walls, as observed by Brennick et al. (8), who used a sophisticated imaging paradigm in an attempt to quantify soft tissue movements in the rat pharynx. However, the study by Brennick et al. examined medial branch simulation only, so it is not yet clear how stimulation of the whole hypoglossal nerves or the lateral branches will influence soft tissue movements in the pharynx.

**Locating the narrowest segment of the pharyngeal airway.** The present analysis indicates that the caudal region of the VP, ~2–3 mm above the tip of the soft palate, is the narrowest segment of the rat pharyngeal airway. This region also narrowed the most with negative pressure application and dilated the most with stimulation of the tongue muscles. The regional compliance data show that the caudal VP is also the most

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**Fig. 10.** Average data showing the influence of stimulating the lateral hypoglossal nerve branches (independent activation of tongue retractor muscles) at negative and positive airway pressures, as indicated on each panel. Figure conventions are as in Fig. 5. *Stim different than No Stim, \( P < 0.05.\)
compliant region, so taken together these results indicate that this region is the narrowest and most collapsible segment of the rat pharynx. This conclusion, based on direct visualization and measurement with MRI, is consistent with our previous estimates of the location of maximal pharyngeal narrowing during negative pressure application in the rat, based on dynamic measurement of maximal pharyngeal airflow and critical collapsing pressures (20). This is also consistent with data in human subjects, which suggests that the caudal VP is the narrowest and most collapsible segment of the pharyngeal airway (11, 17, 24, 25, 29, 40, 44). Thus the rat is a useful model for biophysical studies of the human pharyngeal airway.

In conclusion, the results of this study demonstrate that the VP is the most vulnerable region of the mammalian pharynx, and that electrical stimulation of tongue muscle nerves can minimize or prevent VP narrowing. However, our analysis also demonstrates that the efficacy of tongue muscle electrical stimulation to alleviate human sleep apnea cannot be judged until there are more studies that systematically vary stimulation of different hypoglossal nerve branches, mandibular position, and pharyngeal transmural pressure.

Fig. 11. Direct, statistical comparison of whole hypoglossal nerve and medial nerve branch stimulation on the change (Δ) in nasal VP volume. Each graph shows the change in nasal VP volume as a function of VP location, starting 1 mm above the tip of the soft palate and extending upward for 8 mm. *Stim different than No Stim, P < 0.05.

Fig. 12. Regional compliance (C) of the rat VP for whole nerve (top), medial nerve branch (middle), and lateral nerve branch (bottom) preparations. In each panel, the solid lines indicated the No Stim conditions, and the dashed lines the Stim condition. In all cases, compliance is highest near the tip of the soft plate, and lowest at the rostral end of the VP. There were no differences in compliance with stimulation of either whole, medial, or lateral hypoglossal branches, although there is trend for compliance to be less with stimulation in all groups. ++ P < 0.05, +++ P < 0.01, and ++++ P < 0.001: different than most caudal level of the VP (slice 1). See text for more details.
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