Effects of RLN and SLN stimulation on glottal area

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In vivo canine experiments have demonstrated that vocal fold stiffness varies proportionately with changing levels of recurrent laryngeal nerve (RLN) and superior laryngeal nerve (SLN) stimulation. This study evaluated the morphologic changes in the glottis at varying levels of nerve stimulation and the presumed effects on laryngeal air particle velocity. Stroboscopic data from the in vivo canine model of phonation were examined under varying conditions of RLN and SLN stimulation. Computerized analysis of stroboscopic images was used to reconstruct the glottal area vs. time waveforms. As RLN stimulation increased, glottal area per cycle decreased \(p < 0.05\). However, as SLN stimulation increased, glottal area per cycle increased \(p < 0.05\). These results support the hypothesis that increasing RLN stimulation at similar levels of SLN stimulation produces an increase in air particle velocity, whereas an increase in SLN stimulation causes a decrease in air particle velocity. (OTOLARYNGOL HEAD NECK SURG 1994:110:370-80.)

Area of glottal opening is a crucially important phonatory variable. The pulses of air that flow through the glottis during vocal fold vibration are dependent on the duration, amplitude, and shape of the glottal area. Abnormal vocal quality can often be explained by irregularities in glottal area, as in a vocal cord paralysis. In addition, the glottic area establishes the anatomic configuration that underlies measures of glottal resistance, an important determinant in the successful treatment of vocal fold paralysis.\(^1,2\) Despite its significance, control of glottal area during phonation is not well understood. For example, the contribution of the recurrent laryngeal nerve (RLN) and superior laryngeal nerve (SLN) to glottal area and the resulting effects on laryngeal function have not been well documented, despite much theoretical discussion.\(^3,4\)

Glottal configuration in the normal state is determined by three variables: RLN and SLN excitation and glottal air flow. Previous studies using an in vivo canine model have demonstrated that vocal fold stiffness varies proportionally with levels of RLN and SLN stimulation.\(^1\) The individual effects of RLN and SLN stimulation on fundamental frequency (F\(0\)) have been evaluated by many authors and have been correlated with measures of vocal fold stiffness. However, the contribution of each individual nerve to glottal area and the resulting effects on laryngeal function are not well understood.\(^5\) Knowledge of the individual contributions of each nerve to normal phonation is essential for understanding changes that occur in the glottal function of patients with RLN or SLN paralysis.

To address this issue, the in vivo canine model was used to assess the effects of RLN and SLN stimulation on glottal area. New measurement techniques permit analysis of glottic area per cycle of phonation from videostroboscopy.\(^6\)

These techniques have been used previously to study the effects of variations in air flow on glottal area at constant levels of RLN and SLN stimulation.\(^1\) In that study, increasing air flow produced an increase in glottal area per cycle and a decrease in glottal resistance. Our study examined the effects of RLN and SLN stimulation on glottic area at constant levels of air flow.

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Supported by the 1990 American Laryngological Association Research Award.

This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles.

Received for publication April 6, 1993; accepted Sept. 2, 1993.

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23/1/51352

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METHODS AND MATERIAL

The in vivo canine model of phonation (Fig. 1) has been described in detail previously. Four mongrel dogs (approximately 20 kg each) were premedicated with acepromazine intramuscularly. Sodium thiopental was administered intravenously to attain a level of corneal anesthesia. Additional sodium thiopental was used to maintain this level of anesthesia throughout the procedure.

Each animal was placed supine on the operating table. A midline incision was made to expose the trachea from the hyoid bone to the sternal notch. A low tracheostomy was performed at the level of the sternal notch and cannulated with an endotracheal tube for ventilation. A second tracheostomy was performed superiorly and a cuffed endotracheal tube was passed in a rostral direction with the tip positioned 10 cm below the vocal folds. The cuff was inflated and air was passed through this rostral endotracheal tube from a laboratory wall outlet. Air flow was humidified and heated by bubbling through 5 cm of heated water so that the temperature of the air was 37° C when measured at the glottal outlet. Upstream subglottal pressure was measured using a catheter-tipped pressure transducer (Millar Instruments, model SPC-330; Houston, Texas). The subglottal pressure transducer was passed rostrally through the superior tracheotomy and placed 2 cm below the glottis.

Air flow was controlled by a valve at the laboratory wall outlet and measured with a U-tube flowmeter (Gilmont Instruments, model F1500; Great Neck, N.Y.). One-centimeter segments of recurrent and superior laryngeal nerves were isolated and Harvard bipolar electrodes (South Natick, Mass.) were applied. A constant current nerve stimulator (WR Medical Electronics Co., model S2LH; St. Paul, Minn.) was used to stimulate the RLN bilaterally and a constant voltage source (Grass Instruments, model 54H; Quincy, Mass.) was used to stimulate the...
Table 1. Levels of RLN and SLN stimulation and subglottic pressure

<table>
<thead>
<tr>
<th>SLN stimulation</th>
<th>RLN stimulation</th>
<th>Subglottic pressure (cm H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off</td>
<td>Low</td>
<td>30 to 35</td>
</tr>
<tr>
<td>Off</td>
<td>High</td>
<td>50 to 60</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>30 to 35</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
<td>50 to 60</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>30 to 35</td>
</tr>
<tr>
<td>High</td>
<td>High</td>
<td>50 to 60</td>
</tr>
</tbody>
</table>

SLN, Superior laryngeal nerve; RLN, recurrent laryngeal nerve.

SLN bilaterally. These nerves were stimulated at 70 to 80 Hz, with 0.5 to 1.2 mA (RLN) or 0 to 1.5 mA (SLN) intensity for 1.5-msec pulse duration. Phonation was produced by constant air flow of 318 cc/sec for all trials.

A PGG sensor was placed on the trachea immediately inferior to the larynx. A xenon cable light source was secured in the oral cavity to provide transglottal light for excitation of the PGG sensor. An EGG sensor was placed on either side of the thyroid cartilage while the ground electrode was secured to the sternocleidomastoid muscle.

Stroboscopy. Stroboscopic videoendoscopy was performed using a Bruel and Kjaer stroboscopic light source (model 4914; Marlborough, Mass.) to illuminate the glottis through a zero degree rigid scope connected to a CCD camera (Jedmed, model 70-5110; St. Louis, Mo.). The endoscopic lens of the camera remained a constant distance from the larynx throughout each experiment. The image was recorded on a ¾-inch professional videocassette recorder (Sony, model VO9850; Park Ridge, N.J.).

Synchronization. Synchronization of PGG and video signals has been described in detail by Berke et al. A Hitachi oscilloscope (model V-1050F; Torrance, Calif.) was used to separate the vertical synchronization trace of the video signal. This vertical trace was digitized along with a five ms square wave pulse (SWP). The SWPs were also recorded simultaneously on the videotape sound track. These SWPs were used to synchronize the video frames with the digitized PGG and subglottic pressure signals.

Digitization. Subglottal pressure waveforms, EGG waveforms, PGG waveforms, SWPs, and the vertical trace of the video signal were simultaneously digitized using a 12-bit analog to digital converter. The subglottal pressure and PGG signals were low-pass filtered at 3 kHz and sampled at 20 kHz for 2.8 seconds.

Research design. Phonatory trials were performed at varying levels of RLN and SLN stimulation. Low and high RLN and SLN stimulation were provided to each subject as shown in Table 1. Levels of low and high nerve excitation were selected to maintain target levels of subglottal pressure, as shown in this table. For the low SLN stimulation condition, current was set at 0.5 mA; current for the high SLN stimulation condition was set at 1.2 mA. For the low RLN stimulation condition, current varied from 0.5 to 1.2 mA, as necessary to maintain a constant subglottal pressure of 30 to 35 cm H₂O. In the high RLN stimulation condition, current varied from 1.5 to 2.0 mA in order to maintain subglottal pressure at 50 to 60 cm H₂O.

Videoendoscopcic Image evaluation. Glottal area was measured using a mouse-driven software package (Image Pro II, Media Cybernetic; Silver Spring, Md.). Figure 1 shows the hardware necessary for area measurements in the canine. The videoendoscopic image was first digitized by the frame grabber board (Data Translation, DT-2853 60SQ; Marlborough, Mass.). After outlining the desired portion of the digitized video image (Fig. 2) with the mouse, the area of the measured trace was calculated in pixels. A standard centimeter ruler was lowered to the level of the glottis to convert pixels into mm².

Dependent variables. Five dependent variables were measured from the digitized signals just described. The F0 for each trial was calculated from the PGG signal. Peak area was measured from that video frame in a stroboscopic cycle having the largest area as measured with the Image Pro software. Open quotient (OQ), defined as the duration of the open period divided by the duration of the glottal cycle, was measured by calculating the open period from the differentiated EGG signal (dEGG) and the duration of the glottal cycle from the peak of the
PGG signal. The open period was measured from the time of the peak opening velocity spike to the time of the peak closing spike from the dEGG. Peak open time was defined as the time in msec from initiation of glottal opening to peak opening, as measured from the reconstructed videostroboscopic cycles (Fig. 3).

Finally, waveforms representing changes in glottal area over time were constructed from a sequence of area measurements. These waveforms reflect both the duration and magnitude of glottal opening. In contrast, open phase measures only the duration of opening, whereas peak glottal area assesses only the maximum magnitude of glottal opening. First, the videostroboscopic frame depicting the point of maximal glottal opening was determined from the synchronized PGG signal. The glottal areas in the remaining frames within a cycle were then measured. The relative times of successive video frames were converted into real time by measuring the duration of glottal opening from the open phase from the dEGG signal for each trial. Thus, the glottal area vs. time (GAVT) function was plotted. Time zero represents the moment of opening of the upper vocal fold margins; the last point on each curve represents the instant of closure of the lower fold margins. The relationship between upper fold motion, lower fold motion, and the measured glottal area waveform is shown in Fig. 4. In this figure, the shaded area represents the duration and magnitude of glottal opening.

A single GAVT waveform usually included 14 to 30 points. A best-fit curve to each GAVT waveform was obtained by interpolation, and the waveforms were recorded onto ¼-inch videotape. The area under the GAVT waveform was then measured using the Image Pro software and the pixel area was converted into mm²/cycle.

RESULTS

Four animals were studied using the methods described. However, recordings could not be made...
in each design cell (Table I) for every animal. In particular, only one trial of stable phonation was obtained for the RLN stimulation high/SLN stimulation off condition. Previous experience with the in vivo canine model has demonstrated difficulty achieving phonation without SLN stimulation, especially in the presence of high levels of RLN stimulation. Further, data could not be collapsed across animals because of differences in larynx sizes. Thus, results from one dog for which a complete data matrix was available were included in the analyses described later.

Figure 5 shows the GAVT waveforms for each SLN and RLN stimulation condition. Average values of F0, peak glottal area, OQ, peak open time, and the area under the GAVT waveform are given for each experimental condition in Table 2. Several of these dependent variables were significantly correlated in the dog studied (Table 3). Therefore, a multivariate analysis of variance (MANOVA) was used to examine the separate effects of RLN and SLN stimulation on these measures of vocal function.

MANOVA results showed significant effects of SLN stimulation on the combined dependent variables (Wilks' Lambda = 0.16; F[5,6] = 6.26, p < 0.05). Univariate analyses of variance (ANOVARs) showed that increasing SLN stimulation level caused increases in F0 (F[1,10] = 12.44, p < 0.05), in OQ (F(1,10) = 17.49, p < 0.05), and in the area under the GAVT waveform (F[1,10] = 43.72, p < 0.05). No significant effects of SLN stimulation condition on peak glottal area or on peak open time were observed, and these variables were dropped from the analysis that follows.

Stepdown analysis was used to determine which of these univariate effects represented unique effects of SLN stimulation, and which simply reflected the intercorrelations among the dependent measures. Because F0 is known to be correlated with SLN stimulation, F0 was treated as a covariate of stimulation levels, and a multivariate analysis of covariance (MANCOVA) was undertaken for the remaining dependent measures (OQ and area under the GAVT waveform). This analysis showed no significant effects of SLN on the remaining dependent variables once the effects of F0 were taken into account (Wilks' Lambda = 0.36; F[3,7] = 4.12, p > 0.05). Therefore, the only independent effect of SLN stimulation in this experiment was an increase in F0.

For RLN stimulation, MANOVA results also showed significant effects on the combined dependent variables (Wilks' Lambda = 0.05; F[5,6] = 23.37, p < 0.05). Univariate ANOVAs showed that increasing RLN stimulation level caused a statistically significant increase in F0 (F[1,10] = 9.40, p < 0.05), a decrease in peak area (F[1,10] = 29.68, p < 0.05), and a decrease in the area under the GAVT waveform (F[1,10] = 105.14, p < 0.05). Stepdown analysis was again performed. The effects of RLN stimulation level on peak glottal area and area under the GAVT waveform remained significant when F0 was treated as a covariate in the analysis (Wilks' Lambda = 0.10; F[2,8] = 37.47, p < 0.05; peak area: F[1,9] = 14.03, p < 0.05; area under the GAVT waveform: F[1,9] = 82.71, p < 0.05). However, no significant effect of RLN stimulation on peak glottal area was observed when both F0 and area under the GAVT waveform were treated as covariates of RLN stimulation level. This statistical analysis implies that any changes in peak area with RLN stimulation are predictable from the simultaneous changes in F0 and in overall glottic.
area. No significant effects of RLN stimulation on OQ values or on peak open time were observed. The peak open time remained constant for all levels of RLN and SLN stimulation (Fig. 6).

Significant interactions between RLN and SLN stimulation levels also occurred (Wilks' Lambda = 0.09; F[4,7] = 17.62, p < 0.05). Examination of univariate ANOVAs showed significant interactions among SLN and RLN stimulation conditions for F0 (F[1,10] = 7.89, p < 0.05), peak glottal area (F[1,10] = 33.17, p < 0.05), and area under the GAVT waveform (F[1,10] = 41.40, p < 0.05). These interactions are difficult to evaluate statistically, given the small amount of data from a single animal and the interactions among dependent variables discussed earlier. However, Table 2 suggests that RLN stimulation affected F0 and peak glottal area in the SLN off and SLN low stimulation con-

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**Table 2. Results of each experimental condition**

<table>
<thead>
<tr>
<th>SLN</th>
<th>RLN</th>
<th>Subglottic pressure</th>
<th>F0, Hz</th>
<th>Peak area (mm²)</th>
<th>Open quotient</th>
<th>Peak open time (msec)</th>
<th>Area under GAVT waveform (mm²/cycle)</th>
<th>Normalized glottal area, mm²/msec</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Low</td>
<td>35</td>
<td>165</td>
<td>4.45</td>
<td>0.259</td>
<td>0.97</td>
<td>3.16</td>
<td>0.521</td>
</tr>
<tr>
<td>None</td>
<td>High</td>
<td>50</td>
<td>215</td>
<td>3.82</td>
<td>0.247</td>
<td>0.72</td>
<td>2.27</td>
<td>0.488</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>30</td>
<td>231</td>
<td>6.90</td>
<td>0.412</td>
<td>0.98</td>
<td>5.57</td>
<td>1.287</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
<td>60</td>
<td>260</td>
<td>4.57</td>
<td>0.413</td>
<td>0.94</td>
<td>3.28</td>
<td>0.853</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>35</td>
<td>262</td>
<td>5.49</td>
<td>0.490</td>
<td>1.05</td>
<td>5.60</td>
<td>1.467</td>
</tr>
<tr>
<td>High</td>
<td>High</td>
<td>55</td>
<td>264</td>
<td>5.56</td>
<td>0.487</td>
<td>1.09</td>
<td>5.07</td>
<td>1.338</td>
</tr>
</tbody>
</table>

SLN, Superior laryngeal nerve; RLN, recurrent laryngeal nerve; GAVT, glottic area vs. time.
Fig. 5. Examples of glottal area vs. time waveform plots for each condition of the experimental paradigm. Each dot represents one digitized frame measurement.
conditions, but not in the SLN high stimulation condition. For the area under the GAVT waveform, Table 2 demonstrates a decrease in the area under the GAVT with an increase in RLN for each level of SLN stimulation. Also, as SLN stimulation increased, the area under the GAVT increased. RLN stimulation conditions differed more for the SLN low stimulation condition than for the SLN off or SLN high stimulation conditions.

To further compare the area under the GAVT for various RLN and SLN stimulation conditions, the area value in mm²/cycle was multiplied by F0 to produce a “normalized glottic area” value. By adjusting for F0 differences associated with changing levels of RLN and SLN stimulation, this value (in mm²/msec) allowed a comparison of changes in glottic area with time across stimulation conditions. Results (Fig. 7) parallel those reported in Table 2. An increase in RLN stimulation caused a decrease in the normalized glottic area, whereas an increase in SLN stimulation resulted in an increase in the normalized glottic area.

**DISCUSSION**

Measurements of glottal area during phonation were gathered using stroboscopic techniques at varying levels of SLN and RLN stimulation. Synchronous analysis of the PGG signals allowed for calculation of F0 with changing levels of stimulation. As previously reported,10 increasing levels of SLN stimulation produced a statistically significant increase in F0. Increasing RLN stimulation also caused a significant increase in F0 when SLN stimulation was low or absent, but not when the SLN was maximally stimulated. Apparently, very high levels of SLN stimulation overrode the effects of RLN stimulation on F0. These findings are consistent with other studies, including the work of Titze et al.11 They reported that F0 was influenced more by the level of cricothyroid muscle activity (i.e., SLN stimulation) than thyroarytenoid muscle activity (i.e., RLN stimulation). This finding is also consistent with the theories of Hirano,12 who proposed that F0 is primarily determined by the tension in the vocal fold cover, which is controlled in turn by altering the vocal cord length through SLN stimulation. Also, our data suggest that the effects of nerve stimulation on F0 are a result of changes in the interactions of RLN and SLN stimulation on vocal length over the course of the glottic cycle, as argued by Titze et al.13

Peak open time did not vary with either RLN or SLN stimulation levels. One explanation for this finding is found in the theoretic work of Rothenberg,14 who hypothesized that the glottal opening phase was controlled by the overall vocal tract inertia, including the glottal inductance. In this context, an invariant time of peak glottal opening suggests that the time needed to overcome the vocal tract inertia on initiation of a glottic cycle is relatively constant.

Peak glottal area decreased significantly with increased RLN stimulation when SLN stimulation was low or absent, but no differences were observed in the high SLN stimulation condition. These findings also suggest that increasing RLN stimulation resulted in a decrease in the speed of glottal opening, because peak open time remained constant and peak glottal area decreased with increasing RLN stimulation. Furthermore, the rate or slope of glottal closure may be estimated from the data. The slope of glottic closure increased with increasing levels of RLN stimulation. Faster glottic closure is associated with excitation of the higher harmonics in the acoustic spectrum.15

In this study, changes in levels of RLN stimulation did not produce significant changes in OQ, whereas increasing SLN stimulation was associated with an increase in OQ. High OQ values have been reported in breathy and female voices.5,16 Human videostroboscopic data have revealed a separation of the

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**Table 3. Correlations among dependent variables**

<table>
<thead>
<tr>
<th></th>
<th>F0</th>
<th>OQ</th>
<th>Peak area</th>
<th>Area under GAVT waveform</th>
<th>Peak open time</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OQ</td>
<td>0.891*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak area</td>
<td>0.313*</td>
<td>0.445</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area under GAVT</td>
<td>0.551</td>
<td>0.758*</td>
<td>0.832*</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Peak open time</td>
<td>0.198</td>
<td>0.555</td>
<td>0.142</td>
<td>0.406</td>
<td>1.000</td>
</tr>
</tbody>
</table>

F0; Fundamental frequency; OQ; open quotient; GAVT, glottic area vs. time.

*Denotes correlation significant at p < 0.05 (adjusted for multiple comparisons).
vocal processes of the arytenoid cartilages, especially with breathy voicing. The increased glottal area found in the current study is consistent with these previous findings, and suggests that a greater degree of SLN activation may underlie these voicing modes.

An increase in RLN stimulation produced a statistically significant decrease in the area under the

Fig. 6. Comparison of peak open times at low and high levels of SLN stimulation with increasing RLN stimulation.
GAVT waveform, regardless of SLN stimulation condition. Recall that air velocity equals the ratio of air flow to area. Although not physically measured, this relationship implies that in the present study, increases in RLN stimulation caused an increase in air velocity (because air flow remained constant). Fant has previously argued that air particle velocity should increase with higher transglottic pressures, as found with increasing RLN conditions. He postulated that subglottic pressure primarily controlled glottic air particle velocity. Because increasing RLN stimulation caused a decrease in glottic area, a probable increase in glottal resistance, and a hypothesized increase in subglottic pressure, our data support the relationship between RLN stimulation and air particle velocity proposed by Fant.

In addition, the area under the GAVT waveform increased significantly with increasing levels of SLN stimulation. Thus, an increase in SLN stimulation predicted a decrease in air particle velocity. Estimates of air velocity may be made by combining the area measures of this study with the subglottic pressure measures from another in vivo canine study. Recall the Bernoulli equation and substitute \( \frac{Q}{A} \) for velocity:

\[
P = \frac{1}{2} \frac{Q^2}{A^2}
\]

in which \( P \) = subglottic pressure, \( \rho \) = density, \( Q \) = flow, and \( A \) = area. Using this relationship, one can assess the expected changes in glottic air particle velocity with changing levels of SLN stimulation. Subglottic pressure has been shown to decrease with increasing SLN stimulation levels. Because glottic area per cycle increased with increasing SLN stimulation in the present study while flow remained constant, air velocity is predicted to decrease. Also, the increase in glottic area underlies the increased \( OQ \) values obtained with increasing SLN stimulation. Thus, increasing SLN is associated with a dilatation of the glottal configuration, whereas increasing RLN stimulation reduces the laryngeal opening during phonation.

CONCLUSION

In conclusion, fundamental frequency increased with both an increase in SLN and RLN stimulation, as found in previous reports. Despite changes in nerve stimulation, peak open time remained constant. Peak glottic area decreased with increasing RLN stimulation, and a faster closing slope was
predicted from this finding. The OQ increased with increasing SLN stimulation, whereas no significant effects were noted for an increase in RLN stimulation. Glottal area per cycle decreased with increasing RLN stimulation and increased with increasing SLN stimulation. Finally, at constant air flow, glottic air particle velocity is hypothesized to increase with increasing levels of RLN stimulation and decrease with increasing levels of SLN stimulation.

We would like to thank Ming Ye, MD, Hong-Shik Choi, MD, and Mr. Manuel Natividad for technical assistance.

REFERENCES