

The Effect of Recurrent Laryngeal Nerve Stimulation on Phonation in an In Vivo Canine Model

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The present investigation was designed to examine the effect of variation in recurrent laryngeal nerve stimulation (RLNS) on vocal fold vibration. Photoglottography (PGG), electroglottography (EGG), and subglottic pressure (P_{sub}) were measured in seven mongrel dogs using an in vivo canine model of phonation. The PGG, EGG, and P_{sub} signals were examined at three fundamental frequencies (F_0) (100 Hz, 130 Hz, and 160 Hz) for RLNS, using a constant rate of air flow. Increasing RLNS, which caused activation of the intrinsic laryngeal muscles, produced a modest increase in F_0 , a marked increase in P_{sub} , no change in the open quotient (OQ), and an increase in the closing quotient (CQ). Phase quotient (Q_p), which describes the interval between opening of the lower and upper fold margins, decreased with increasing RLNS.

INTRODUCTION

Theoretical models have been important in consolidating data and directing future investigations of phonatory control mechanisms. However, the verification of information about phonation generated by these models requires experimentation with physiologic preparations. The present investigation was designed to study the effect of change in vocal fold mass and stiffness on vocal fold vibration, by use of an in vivo canine model of phonation. The effect of variation in superior and recurrent laryngeal nerve stimulation (SLNS and RLNS), under conditions of constant air flow, was studied photoglottographically and electroglottographically, while measuring subglottic pressure. The results of the stimulation of the superior laryngeal nerve have been reported in a companion paper.¹ The present

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study reports the results of recurrent laryngeal nerve stimulation.

The myoelastic-aerodynamic theory of phonation as described by van den Berg² postulated that the driving force for vocal fold vibration is the pulmonary generated air stream. The fundamental frequency (F_0) of phonation depends on the effective mass and stiffness of the vocal folds interacting with transglottal pressure. The mass and stiffness of the folds are themselves determined by the action of the internal and external laryngeal muscles. According to this theory, control of F_0 is influenced by a number of independent physiologic parameters: 1. the effective mass of the vibrating part of the vocal folds; 2. the effective tension in the vibrating part of the vocal folds; 3. the effective area of the glottis during the vibratory cycle; 4. subglottic pressure; 5. the damping of the vocal folds. Titze³ stated in his tutorial on the myoelastic-aerodynamic theory that, under large amplitude conditions, F_0 appears to be controlled entirely by static and amplitude tissue stiffness rather than explicitly by aerodynamics. Titze concluded that F_0 control is primarily elastic with marked intonation patterns programmed centrally and implemented by major muscular contraction. Additional reflex intonation patterns appear to be controlled by peripheral feedback implemented by lesser, but significantly faster contractions. Because control of fundamental frequency is myoelastic, there are limits on control of F_0 by subglottal pressure alone. Such control is, therefore, inseparably connected with vibrational amplitude and less directly with vocal intensity.

Laryngeal Control Parameters

Determinants of the stiffness and mass of the vocal folds are the contractile state of the extrinsic and intrinsic laryngeal muscles, which include the following: cricothyroid muscle (CT), vocalis muscle

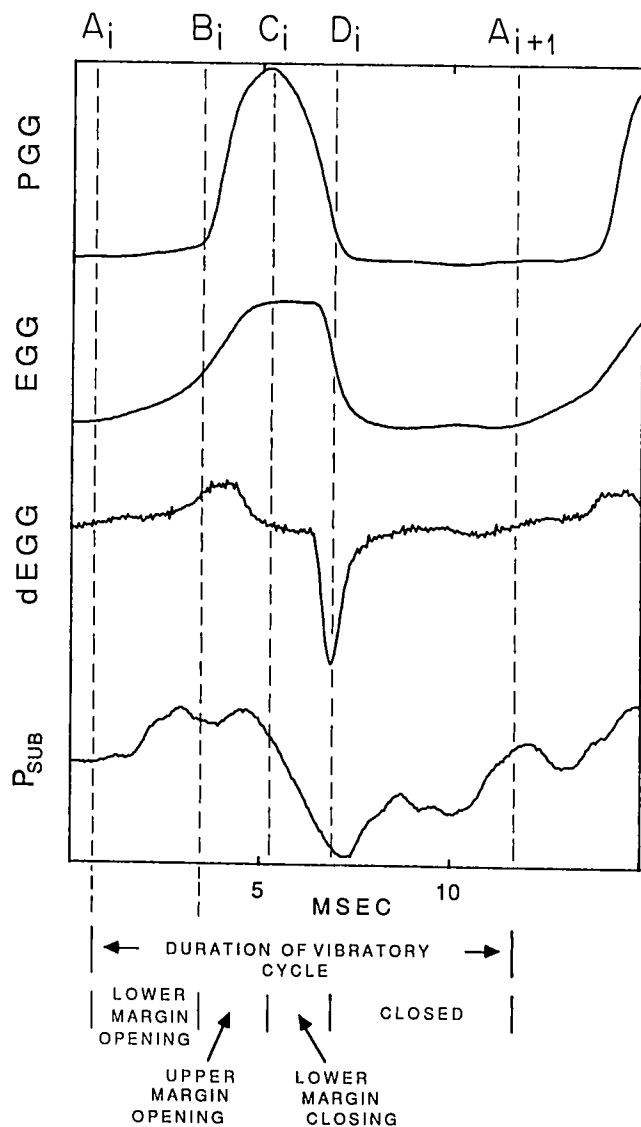


Fig. 1. PGG, EGG, dEGG, and Psub signals recorded from a canine preparation phonating in the modal register (reprinted with permission of *The Journal of the Acoustical Society of America*).

(VOC), lateral cricoarytenoid muscle (LCA), interarytenoid muscle (IA), posterior cricoarytenoid muscle (PCA), cervical strap muscles.

Function of the vocalis muscle (VOC) is considered a contributor to F0 elevation in human and canine phonation, although the effect of the VOC on laryngeal adjustment has not been fully delineated. Electromyographic studies in humans have demonstrated that VOC activity increases with increasing F0.⁴⁻⁶ The vocalis has been shown to be an antagonist to external lengthening applied by the CT muscle.⁷ Isometric contraction of the VOC increased its tension,⁸ thus elevating F0 by increasing vocal cord stiffness. Koike, *et al.*⁹ noted that stimulation of the VOC uniquely causes a medial bulging at the mid-portion of the vocal cord. Hirano^{10,11} also studied stimulation of the VOC and found that it produced a

more rounded, thickened vocal cord. Stimulation of the recurrent laryngeal nerve (RLN) in the dog produces F0 elevation,¹² although to a much lesser degree than that produced by activation of the CT muscle. RLN stimulation (RLNS) activates the VOC, LCA, IA, and PCA muscles so that, from a practical standpoint, isolated VOC stimulation in Rubin's *in vivo* canine preparation was not possible.

The degree to which the laryngeal adductors control F0 has not been determined. Hirano, *et al.*⁵ showed an increase in LCA activity in humans with rising F0. Shipp and McGlone,⁶ however, failed to show a change in either LCA or IA activity with increasing F0. Stimulation of the RLN in the dog¹² caused a mass contraction of the VOC, LCA, IA, and the PCA with a small rise in F0, but the relationship of F0 increase to LCA and IA activity was not examined.

The PCA has been shown to be a powerful abductor of the vocal cords and functions primarily to dilate the glottal orifice during respiration. Its function during phonation has not been well defined. Dedo,¹³ Gay, *et al.*¹⁴ and Baer, *et al.*¹⁵ reported increasing PCA activity at high F0. Wyke¹⁶ reported that during the prephonatory tuning phase of phonation, spontaneous PCA activity ceased only to resume once phonation ensued, especially at high F0.

Most studies of phonation cite a direct relationship between Psub and F0. Using a canine preparation, Rubin¹² showed that Psub is the result of an interplay between tracheal air flow and glottic resistance. With isolated increases in air flow, he found a modest rise in Psub without significant increase in F0. Increased glottal resistance (by stimulation of the RLNs), however, led to a concurrent increase in Psub and F0.

METHODS

Subjects

Seven adult male, mongrel dogs, each weighing 25 to 30 kg, were used in the study. Each dog was screened to assess its suitability as a subject for the experiment. Dogs with long necks were preferred for ease of preparation.

Glottographic Techniques

Glottographic techniques have shown potential as tools for studying the temporal events that occur during a vocal fold cycle. Photoglottography (PGG), introduced by Sonesson in 1959, is a technique that uses a photoelectric transducer to describe time-varying changes in glottal area. Electroglossography (EGG) is a technique measuring impedance of a small electric current across the neck. Changes in impedance are modulated by changes in lateral vocal fold contact area.¹⁷ Childers¹⁷ has shown that the differentiated EGG signal (dEGG) can provide temporal information on points of upper margin opening and lower margin closing. Baer, *et al.*¹⁸ have demonstrated that combined analysis of PGG and EGG signals give essentially the same information for peak glottal opening and glottal closure as high speed laryngeal photography. Application of glottographic techniques to physiologic laryngeal preparations should provide information

about the micromechanics of vibration to assist in verification and refinement of current theories.

Experimental Preparation

The experimental setup was the same as described in the companion paper¹ and previously.¹⁹ Warm humidified air was flowed through the glottis during independent stimulation of the recurrent laryngeal nerves to produce phonation. EGG, PGG, Psub, and videostroboscopic recordings of vocal fold vibration were obtained during phonation for subsequent analysis.

Stimulus Parameters

Two nerve stimulators were used. A Grass® (model 54H) nerve stimulator was used to provide variable voltage stimulation while a WPI (301-T) nerve stimulator was used to provide a low level of constant current stimulus. Voltages ranged from 0.5 to 0.9 volts for the Grass stimulator. Currents ranged from 0.1 to 0.15 mA for the WPI stimulator. Frequency of stimulus was 80 Hz, with a pulse duration of 1.5 msec for both the Grass and WPI units.

Data Acquisition

PGG, EGG, and Psub signals were simultaneously recorded on a Tannberg® FM tape recorder (Model 115D). The signals were also monitored on two oscilloscopes (Tektronix® 5116, Hitachi® V1050-F) to assess the adequacy of the glottographic signals.

Experimental Design

Seven animals were stimulated to phonate at "target frequencies" of 80 Hz, 100 Hz, 130 Hz, 160 Hz, and 180 Hz, while holding air flow constant at 375 cc/second. While delivering a low level of constant current stimulus to the SLNs (0.10 mA), voltage stimuli to the RLNs were varied to produce the target frequencies. This was done to demonstrate the effect on vocal cord vibration of activation of the intrinsic laryngeal muscles to increasing F0. Two trials were performed of variable RLN stimulation (RLNS) to produce the five target F0s. The target frequencies were obtained in a random order in all subjects. PGG, EGG, and subglottic pressure signals were recorded for each trial at each target frequency. Phonation at 80 Hz and 180 Hz could not be achieved in some animals, so statistical analysis was limited to the middle target frequencies of 100 Hz, 130 Hz, and 160 Hz.

Data Analysis

The recorded PGG, EGG, and Psub waveforms were low-pass-filtered at 1500 Hz and digitized at a rate of 20 kHz with an LSI 11-73 computer and stored on disk. A 0.5-second sample of stable phonation was used. A multipurpose computer software program was used for data analysis and graphic display. Figure 1 shows a representative glottic cycle. Specific points in the glottic cycle were then picked for determination of temporal events of the cycle as outlined in Appendix A of this study. Moments of glottal opening and closing were the same as those reported by Childers,¹⁷ Baer,²⁰ and Berke.²¹ Point Ai was the point of initial separation of the lower fold margins, determined by the initial rise in the EGG impedance from its minimum. Point Bi was the moment of upper margin opening as determined by the positive deflection of the differentiated EGG waveform (dEGG). Point Ci was the moment of maximal glottal area as determined by the peak of the PGG waveform. Point Di identified lower margin closure, as determined by the nadir in the dEGG waveform. Point Ai+1 was lower margin opening of the next cycle. Point Bi, Ci, and Di could be reliably determined, however point Ai during high SLNS occurred on a gradual up-slope in the EGG and was difficult to discern interactively.

Ten contiguous glottal cycles from a recording of stable phonation were analyzed. For each subject, this procedure yielded mean data on glottal quotients (see above) at target frequencies of 100 Hz, 130 Hz, and 160 Hz for trial 1 and trial 2. Measured frequency (F0) was also compared to target F0. Measurement of

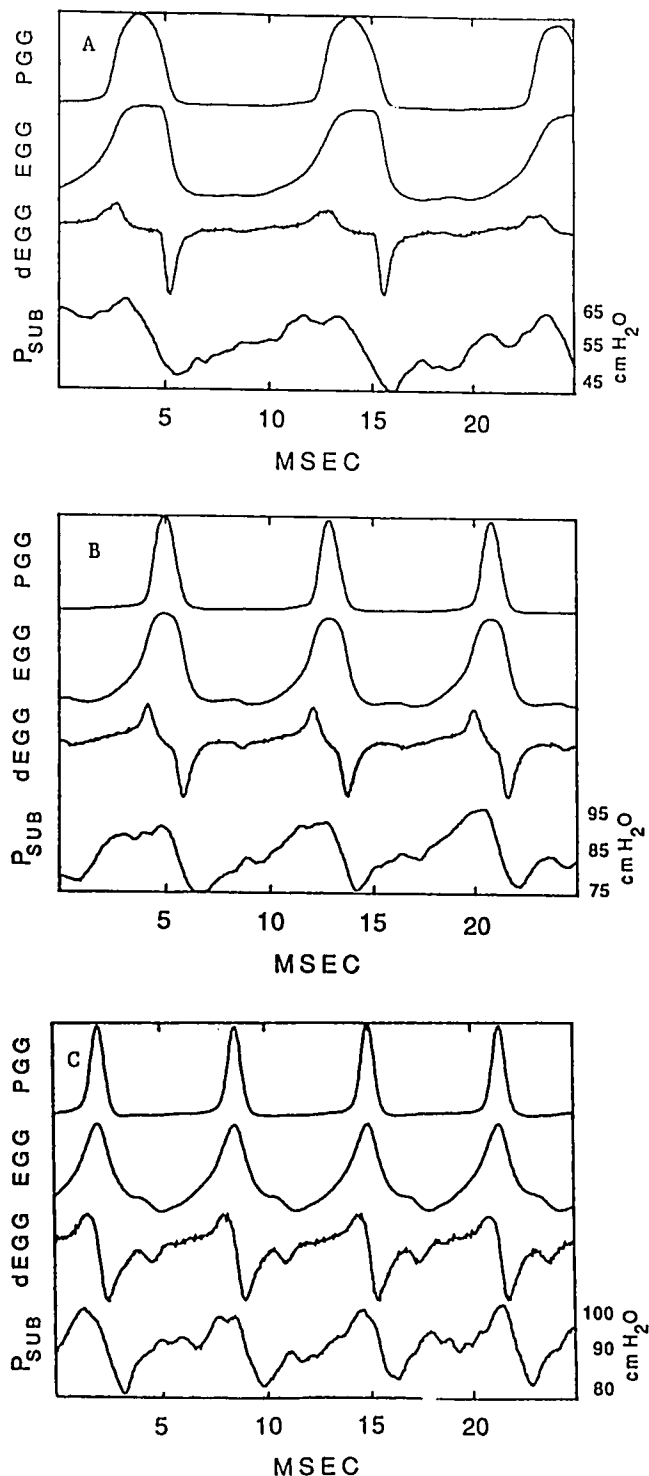


Fig. 2. Typical recordings from a subject for three levels of target frequency (TF0). A. 100 Hz, B. 130 Hz, C. 160 Hz for RLNS (reprinted with permission of *The Journal of the Acoustical Society of America*).

Psub maximum, minimum, and RMS mean were obtained for each glottic cycle analyzed.

The six glottal quotients, each representing the mean of two random trials for ten contiguous cycles for the seven subjects, were analyzed by analysis of variance (ANOVA). Separate analyses of variance were applied on quotient with target frequency

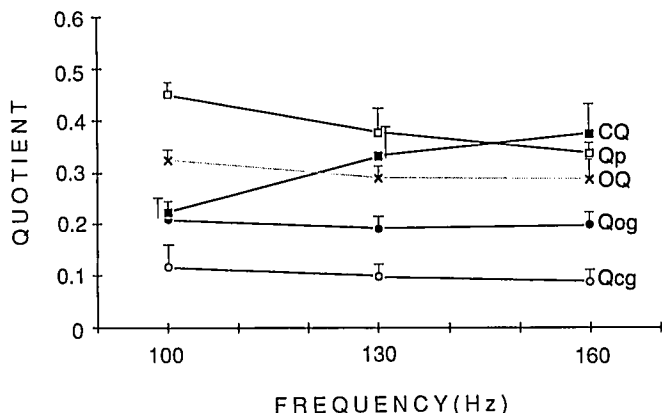


Fig. 3. Mean values (\pm S.E.M.) for Qp, OQ, Qog, CQ, and Qcg for seven subjects for RLNS at 3 TF0 (100 Hz, 130 Hz, 160 Hz) (reprinted with permission of *The Journal of the Acoustical Society of America*).

and trial as repeated measures. No difference was shown between trials 1 and 2 for any of the quotients.

RESULTS

Electrical stimulation produced F0s of 54 Hz to 340 Hz, spanning the low modal, modal, and falsetto registers judged perceptually. Figure 2 shows representative waveforms plotted for one subject at three target frequencies for the effects RLNS. As frequency increased by increasing RLNS (Fig. 2A-C) a narrowing of the EGG waveform occurred. This was associated with a decreasing open portion and a markedly increasing closed portion of the cycle. For this particular subject, mean subglottic pressure rose from 57 cm H₂O at 100 Hz to 90 cm H₂O at 160 Hz.

Examination of glottographic waveforms in Figure 2 reveals several findings with regard to subglottic pressure. Psub rises throughout the vocal fold vibratory cycle until upper margin opening (Fig. 1, Bi), at which point it drops rapidly. Upon lower margin closure (Fig. 1, Di), the Psub abruptly increases and then increases steadily until upper margin opening of the next glottic cycle. As F0 increases (Fig. 2A-C), high-frequency components appear in the Psub waveform superimposed on the mean Psub.

Figure 3 shows the effect of RLNS on temporal events of the glottal cycle for frequencies 100 Hz, 130 Hz, and 160 Hz. Qog and Qcg were not shown to change significantly as frequency increased. Because OQ is the sum of Qog and Qcg, it also did not change. SQ did not change with variation in RLNS. CQ increased markedly [$F(2,12)=11.06, p<.01$], while Qp decreased steadily as frequency increased [$F(2,12)=10.94, p<.01$].

Figure 4 displays mean Psub for five target F0s for one subject. Psub ranged from 22 to 116 cm H₂O. Whereas Psub rose minimally for SLNS as F0 increased, it rose markedly for RLNS. The alternating component of the pressure wave for this sub-

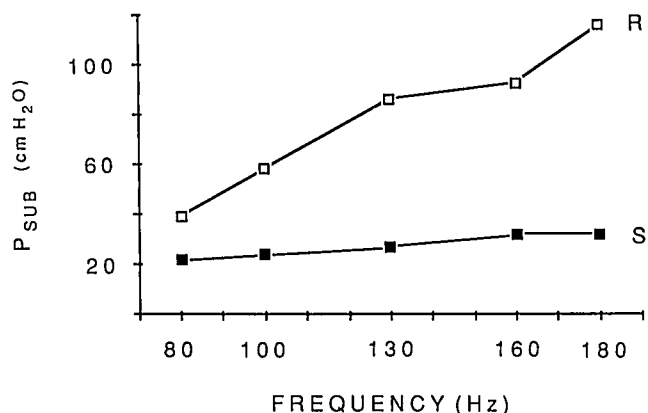


Fig. 4. Psub in cm H₂O for SLNS (S) vs. RLNS (R) for one subject (reprinted with permission of *The Journal of the Acoustical Society of America*).

ject varied between 14 to 24 cm H₂O for all levels of F0 achieved, and did not increase as Psub increased. Psub trends for RLNS and SLNS were similar for all seven subjects.

DISCUSSION

The present investigation was designed to study the effect of RLNS on temporal events of the vocal fold vibratory cycle over a frequency range of 80 Hz to 180 Hz, under conditions of constant air flow. This range was chosen to represent frequencies within the canine modal register. Data regarding frequency ranges for canine phonation do not exist. However, it appears that dogs develop both modal and falsetto registers in their normal phonation, as evidenced by the growl, the bark, and the shrill cry that dogs commonly exhibit. Hollien and Michel²² investigated the register frequencies of 12 adult human males. They found a range of 94 Hz to 287 Hz for the modal register. As the canine larynges are somewhat thicker and more massive than human larynges,¹⁰ the dog likely has a lower range of frequency for the modal register.

A modest increase in F0 resulting from an increase in RLNS was confirmed in the present investigation. All subjects developed F0 elevation to 160 Hz with increasing RLNS. However, no animals developed phonation at greater than 190 Hz by this method. OQ did not change significantly for increasing F0 by RLNS, although it tended to decrease.

Supraglottic stroboscopic imaging showed that increasing RLNS produced an increase in the amplitude of the travelling wave, but appeared to decrease the effective anterior-to-posterior vibratory fold length and lateral excursion of vocal fold movement.

Stimulation of the recurrent laryngeal nerve activates the VOC, LCA, IA, and PCA muscles. Activation of the VOC muscle increases the stiffness of the

APPENDIX A

Formulas Used in Glottographic Measurement

$$\begin{aligned} Q_p &= \text{time delay between opening of lower and upper margins / period of glottal cycle} \\ & \quad B_i - A_i / (A_{i+1} - A_i) \\ OQ &= \text{duration of open glottis / period of glottal cycle} \\ & \quad D_i - B_i / (A_{i+1} - A_i) \\ CQ &= \text{duration of complete glottal closure / period of glottal cycle} \\ & \quad (A_{i+1} - D_i) / (A_{i+1} - A_i) \\ Q_{og} &= \text{duration of glottal opening / period of glottal cycle} \\ & \quad C_i - B_i / (A_{i+1} - A_i) \\ Q_{cg} &= \text{duration of glottal closing / period of glottal cycle} \\ & \quad D_i - C_i / (A_{i+1} - A_i) \\ SQ &= \text{duration of glottal opening / duration of glottal closing} \\ & \quad C_i - B_i / D_i - C_i \end{aligned}$$

vocal cord, leading to an increased F_0 . In addition, VOC activation leads to a slackening of the mucosal cover of the vocal cord.²³ This "slackening" might have produced the relative uncoupling of the upper and lower margins that prevented an increase in OQ , as was observed when F_0 was increased due to greater SLNS.¹ Vocalis activation uniquely caused a medial bulging of the vocal fold, which contributed to an increased medial compression of the folds and thus an increased CQ . Recruitment of intrinsic adductor muscles may also have produced increased medial compression. That Q_p decreased significantly for increased F_0 by RLNS may reflect this increased "medial compression." One explanation for the decreased Q_p is that when the lower margin finally began to open during a cycle, the folds were rapidly parted by the resultant high P_{sub} . Neither Q_{og} or Q_{cg} was shown to change significantly as F_0 increased by RLNS.

The observation in the present study that the AC pressure waveform is close to its maximum before upper-margin opening and then falls to reach a nadir at closure does not correlate with similar records from humans obtained with pressure-tip transducers passed through the glottis into the trachea.^{24,25} In these records there is a sharp increase in subglottal pressure at the onset of glottal closure and a marked decrease at the onset of opening. There are a number of possibilities for these discrepancies. The pressure transducer-amplifier used in the present study might have distorted the AC waveform obtained. It is also possible that the experimental procedure gave different waveforms from those recorded in humans. Finally, some tracheal acoustic factor in humans might be different than that in the dogs used in the present investigation because of the presence of the endotracheal tube. However, because the driving force for glottic opening is subglottic pressure, a pressure rise and peak prior to opening, and a fall in pressure as flow ensues resulting in glottic closure does help to explain sustained

oscillation.

P_{sub} increased with increasing F_0 , due to RLNS, in all subjects. The increase in P_{sub} might be related to increased glottal resistance affected by RLNS. Glottographically, this is represented by an increase in the closed portion of the cycle (CQ). Subglottic pressure elevation with RLNS in this study substantiated the findings of Rubin.¹² P_{sub} for human phonation is maintained between 3 and 10 cm H₂O in normal conversational and declaratory speech, 10 to 20 cm H₂O for singing at moderate loudness, and reaches a peak of 50 to 70 cm H₂O for singing at loudest intensities.²⁶ Proctor²⁷ stated that the human phonatory system is capable of generating P_{sub} as high as 100 cm H₂O. That it does not do so may reflect feedback of subglottal mucosal mechanoreceptors. When these receptors are blocked by topical anesthesia, mean P_{sub} rises using similar pitch and intensity levels in both speech and singing.²⁸ During stimulation of the SLN and RLN in the present study, depolarization occurred in both prodromic and antidromic directions, thus effectively interrupting reflex pathways. Therefore, the high P_{sub} obtained with RLNS in both this study and that of Rubin¹² may reflect suprathreshold levels of stimulation. It is conceivable, however, that specialized functions of the canine phonatory system (*i.e.*, the bark) necessitate a high P_{sub} when abrupt, intense phonatory bursts are desirable.

CONCLUSIONS

Study of the effect of recurrent laryngeal nerve stimulation on phonation in an *in vivo* canine model yields the following conclusions: 1. Increasing RLNS, which as a net effect activates the vocalis and intrinsic adductor muscles, produced a modest increase in F_0 and a marked increase in P_{sub} . 2. No change in OQ or SQ was observed. 3. Increasing RLNS produced an increase in CQ and a decrease in Q_p .

