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

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We investigated the effect of variation in superior laryngeal nerve stimulation (SLNS) on vocal fold vibration. Photoglottography (PGG), electroglottography (EGG), and subglottic pressure (Psub) were measured in seven mongrel dogs using an in vivo canine model of phonation. The PGG, EGG, and Psub signals were examined at three SLNS frequencies (100 Hz, 130 Hz, and 160 Hz) using a constant rate of air flow. Increasing SLNS, which causes a contraction of the cricothyroid muscle, produced a marked increase in F_0, little change in Psub, an increase in the open quotient, and a decrease in the closed quotient of the glottal cycle. AM J OTOLARYNGOL 10:181–187. © 1989 by W.B. Saunders Company.

Although theoretical models have been important in providing information about phonatory control mechanisms, verification of current theories will require the use of physiologic preparations. This investigation was designed to examine the effect of changes in vocal fold mass and tension on vocal fold vibration, using an in vivo canine model of phonation. The effect of different levels of superior laryngeal nerve stimulation (SLNS) and recurrent laryngeal nerve stimulation (RLNS) under conditions of constant air flow was studied photoglottographically and electroglottographically, and subglottic pressure (Psub) was measured. This article reports the results of SLNS.

LARYNGEAL CONTROL FACTORS

The fundamental frequency (F_0) of phonation depends on the effective mass and stiffness of the vocal folds interacting with transglottal pressure. The cricothyroid muscle (CT) has been termed "the external tensor" of the vocal cord. Through action potentials carried in the external branch of the superior laryngeal nerve (SLN), CT activation produces a lengthening and thinning of the cords. Human studies using high-speed laryngeal photography during phonation have demonstrated a lengthening and thinning of the cords with rising F_0,[1] as well as an increase in electromyographic activity of the CT muscle.[2,3-5] It has been shown that stimulation of the SLN in dogs produced F_0 increases from 135 Hz to 540 Hz.[6] Lengthening and thinning the vocal folds affects F_0 by decreasing the effective vibrating mass. In addition, lengthening the vocal cord causes elongation of the vocalis muscle (VOC). It has also been shown that passive and active tension in the VOC were greatly enhanced by external stretching.[7] CT activation then elevates F_0 by lengthening the VOC, thereby increasing the stiffness and reducing the mass per unit area of the vocal folds.

IN VIVO CANINE MODEL

The dog has been the principal animal model for laryngeal studies. The canine larynx is similar to the human larynx in size and vocal fold histology; however, the upper portion of the vocal fold has a thicker and looser lamina propria than humans, resulting in increased thickness of the vocal fold.[8] The canine larynx also has a postglottic space in some animals, and, during phonation, there is a posterior V-shaped chink

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behind the arytenoids. Anastomotic fibers running between the superior and recurrent laryngeal nerves in the dog (Galen's nerve) are believed to be sensory in nature. Longitudinal elasticity curves of the epithelium, ligament, and muscle of the canine larynx have different tension-length slopes than those for humans, but their overall shape is similar. The cricoid and thyroid cartilages are more angulated and shorter in dogs; the ventricles are considerably larger and the vocal ligament is not well-defined. In spite of these differences, much information concerning the mechanics of vocal fold vibration has been derived from studies of excised canine larynges.

Some studies have suggested that excised larynges do not reproduce physiologic conditions of vocal fold tension and mass during vibration with sufficient accuracy.[9-11] The in vivo canine model appears to be a more physiologically valid preparation for studying vocal fold vibration than the excised larynges because blood flow and intrinsic laryngeal muscular tension are maintained while preventing postmortem tissue degeneration. These are critically important factors to consider when applying the results from models of vocal fold vibration to human phonation.

METHODS

Subjects

Seven adult male mongrel dogs (weighing 25 to 30 kg) were selected. Each dog was screened by direct laryngoscopy to assess its suitability as a subject for the experiment. Dogs with long necks were preferred for ease of preparation.

Glottographic Techniques

Glottography has proven useful in the study of temporal events during vocal fold movement.[12] Photoglottography (PGG) uses a photoelectric transducer to measure transillumination of light through the glottis during phonation.[13] As the vocal folds vibrate, the intensity of light transmitted through the glottis reflects the cross-sectional area of the glottis over time. Electroglottography (EGG) is a technique measuring the impedance of a small electric current across the neck in the vicinity of the vocal folds. Changes in impedance are modulated by changes in lateral vocal fold contact area, and the differentiated EGG signal (dEGG) can provide temporal information on points of upper margin opening and lower margin closing.[14] Simultaneous monitoring of PGG and EGG signals provides information for peak glottal opening and glottal closure similar to high-speed laryngeal photography.[15]

Experimental Preparation

Our experimental setup was described in a previous study and is similar to that of prior in vivo canine studies.[16,6] Each dog was anesthetized with 2 mL ketamine by intramuscular injection followed by intravenous pentobarbital until loss of the corneal reflex was achieved. The animal was then placed supine on an operating table (Fig 1) and direct laryngoscopy was performed to confirm normal laryngeal anatomy. A 7-mm oral endotracheal tube was inserted, through which the animal breathed spontaneously. A midline incision was made from the mandible to the sternum. The strap and sternocleidomastoid muscles were retracted laterally to expose the larynx and trachea. The external branch of the superior laryngeal nerves were isolated at their entrance into the CT muscle. A gauze/silver electrode was applied to the nerves and insulated from the surrounding tissue. The recurrent laryngeal nerves were isolated 5 cm inferior to the larynx. Electrodes were applied in the same fashion. Ground electrodes were sutured to the trachea and connected to the anode of the nerve stimulator. Electrical isolation between RLNS and SLNS was verified by direct observation. Maximal stimulation of the recurrent laryngeal nerves, to the point at which the strap muscles were noted to contract (approximately 9 volts), was not observed to produce contraction of the cricothyroid muscle. In addition, no lengthening or thinning of the vocal cords occurred during maximal RLNS. Isolated maximal stimulation of the superior laryngeal nerves to the point at which the strap muscles were observed to contract did not demonstrate tensing or bulging of the vocalis muscle on direct laryngoscopic observation. No arytenoid adduction or phonation could be elicited by maximal SLNS. EGG electrodes (Synchrovoice, Briarcliff Manor, NY) were placed in direct contact with the thyroid cartilage while the reference electrode was sutured to the skin. A 1.0-cm button was placed to suspend the epiglottis anteriorly through the thyrohyoid membrane to improve visualization of the vocal folds. A distal tracheotomy was performed and an endotracheal tube passed to permit the animal to breathe spontaneously. A more proximal tracheotomy was performed, through which a cuffed tracheotomy

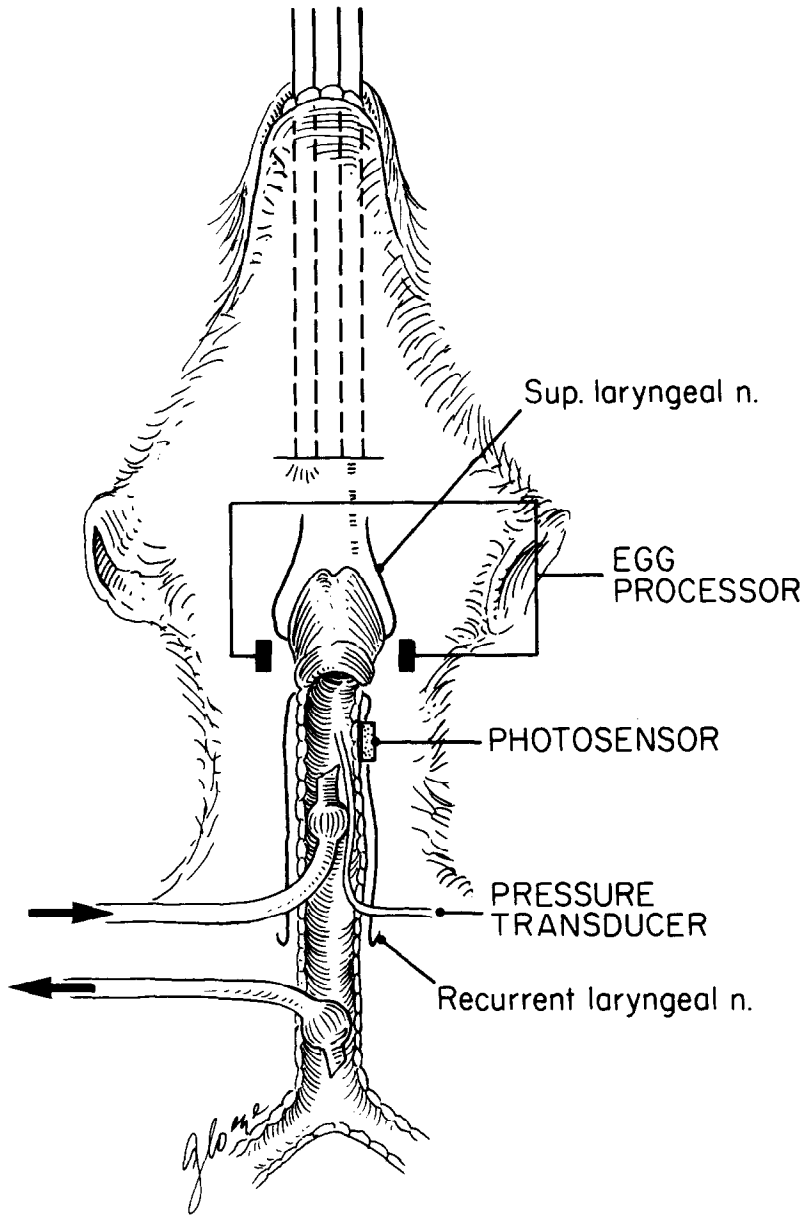

Figure 1. Diagrammatic representation of experimental preparation. (Reprinted with permission.[17])

tube was placed with its tip resting 10 cm below the glottis. A catheter-tipped pressure transducer was inserted through this upper tracheotomy. The cuff on the superiorly directed tube was inflated to just seal the trachea. Air flow, obtained from the UCLA physical plant, was passed through the cephalad tracheotomy tube. The rate of air flow was measured with a flowmeter (Gilmont Instruments, model F1500, Great Neck, NY) and kept at a constant rate of 375 cc/sec throughout the study. Unlike human phonation, which can be induced with Psub in the range of 6 to 10 cm H_2O, canine phonation requires at least 20 cm of water pressure for sustained oscillation. Because of the canine's posterior glottic chink, which allows a significant DC (constant) escape of air flow during phonation, an air flow of approximately 375 cc/sec is required to develop a Psub of at least 20 cm H_2O, and to match target frequencies of from 80 to 160 Hz. The air was bubbled through 5 cm H_2O for warming and humidification, and the temperature in the animal's trachea was measured at 15-minute intervals to assure a constant air flow temperature of 37°C. The PGG light sensor (Centronics OSD 50-2, Mountainside, NJ) was placed on the animal's trachea approximately 3 cm below the larynx. A xenon light source and fiberoptic cable provided supraglottic illumination for the PGG. A microphone (Sennheiser, Culver City, CA) was placed 15 cm from the vocal folds and connected to a Storz model 8000 laryngostroboscope (Culver City, CA) for frequency analysis of the phonatory sound. In addition, strobo-

scopic video imaging was obtained using the Storz stroboscope unit connected to a Storz 0 degree telescope via a fluid-filled light cable. The xenon light source for the PGG was connected to the other light port of the telescope. The image from the 0 degree scope was recorded by a Circon CCD video camera and a Sony ¾-inch video tape recorder (Teaneck, NJ). Although a low level of constant xenon light source was present during stroboscopic video recording, excellent stroboscopic video imaging was obtained.[18] The system was not used for objective measures; however, it was useful for the interpretation of vibratory events recorded concurrently with the PGG and EGG signals.

A catheter-tipped pressure transducer (Medical Instruments DCE-1, Hackensack, NJ) was calibrated at 37°C by submerging it in a water bath in 37°C to a depth just covering the sensor (0.5 cm). The catheter was then calibrated against a Hg manometer from 0 to 120 cm H_2O pressure.

Stimulus

A Grass (model 54H; Quincy, MA) nerve stimulator was used to provide variable voltage stimulation, while a WPI (301-T; New Haven, CT) nerve stimulator was used to provide a low level of constant current stimulus. Voltages ranged from 0.5 to 0.9 V for the Grass stimulator, and currents ranged from 0.1 to 0.15 mA for the WPI stimulator. The frequency of stimulus was 80 Hz, with a pulse duration of 1.5 ms for both units.

Data Acquisition

PGG, EGG, and Psub signals were simultaneously recorded on a four-channel Tannberg FM tape recorder (model 115D; Armonk, NY). The signals were also monitored on two oscilloscopes (Tektronix 5116, Beaverton, OR; and Hitachi V1050-F, Carson, CA) to assess the adequacy of the glottographic signals.

Experimental Design

Seven animals were stimulated to phonate at target frequencies of 80, 100, 130, 160, and 180 Hz, while maintaining a constant air flow of 375 cc/sec. While delivering a low level of constant current stimulus to the recurrent laryngeal nerves (0.10 mA), voltage stimuli to the SLNs were varied to produce phonation at the target frequencies. This was done to examine the effect of CT muscle activation on F_0. Two trials of SLN stimulation were performed to achieve the five target frequencies, which were obtained in a random order in all subjects. At 80 and 180 Hz, phonation could not be achieved in some animals, so statistical analysis was limited to the middle target frequencies of 100, 130, and 160 Hz.

Data Analysis

The recorded PGG, EGG, and Psub waveforms were low pass filtered at a corner frequency of 1,500 Hz and digitized at a rate of 20 kHz. A 0.5-second sample of stable phonation was used in the analysis. Figure 2 shows a representative glottic cycle. Moments of glottal opening and closing were the same as those that have been reported previously.[14-16] Point A_i marks the initial separation of the lower vocal fold margins,

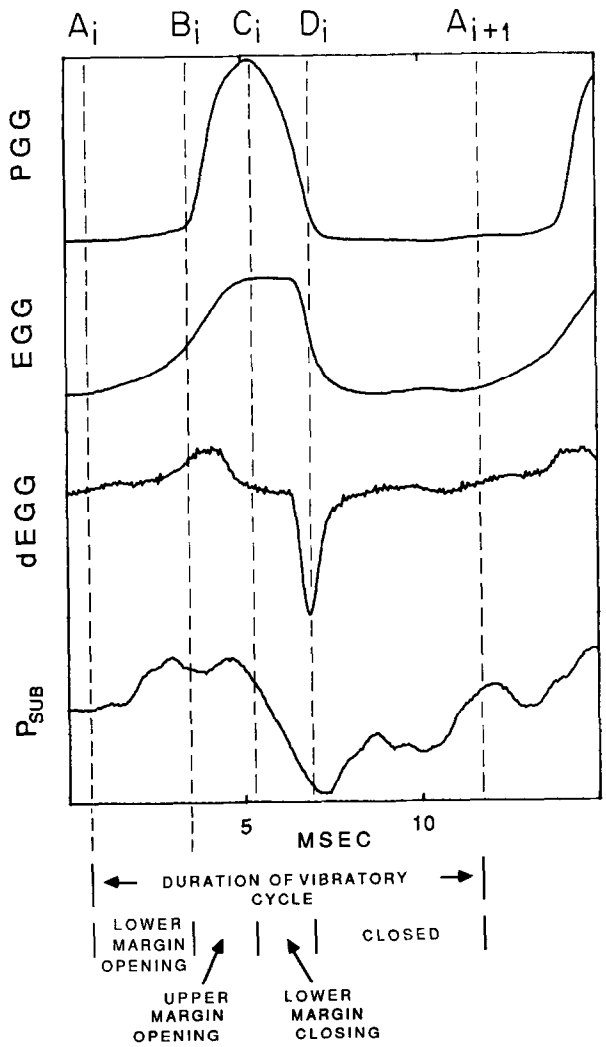

Figure 2. PGG, EGG, dEGG, and Psub signals recorded from a canine preparation phonating in the modal register. (Reprinted with permission.[17])

determined by the initial rise in the EGG impedance from its minimum. Point B_i represents the moment of upper margin opening as determined by the positive deflection of the dEGG waveform. Point C_i identifies the moment of maximal glottal area as determined by the peak of the PGG waveform. Point D_i marks lower margin closure, as determined by the lowest point in the dEGG waveform. Point A_{i+1} is the lower margin opening of the next cycle. Points B_i, C_i, and D_i could be reliably determined; however, point A_i, during high SLNS, occurred on a gradual increase in the EGG waveform and was difficult to determine. These periods occurring within the glottal cycle were divided by the total period of the vibratory cycle to determine the quotients of vocal fold vibration as described in the Appendix.

Ten contiguous glottal cycles were analyzed and the mean of these cycles was calculated. This procedure yielded means of glottal quotients as the five target frequencies for two random trials. Measured F_0 was also compared to target F_0. Mean Psub maximum/minimum and root mean squared were obtained for each glottic cycle analyzed.

The six glottal quotients were analyzed using analysis of variance (ANOVA). Separate analyses of variance were applied for each quotient with trial as a repeated measure. Each trial was a set of recordings for each five target frequencies. No significant difference was shown between trials 1 and 2 at $P = .05$ for any of the quotients, so both trials were combined for another set of ANOVAs with F_0 as a repeated measure.

RESULTS

Table 1 displays mean F_0 values obtained by SLNS for the seven subjects' target frequencies. The experimentally measured F_0s closely approximated the desired target frequencies.

Figure 3 displays temporal events in the glottal cycle as glottal quotients by target frequency

*TABLE 1. Target Frequency Versus Measured Frequency for Seven Subjects**

SLNS	
Target F_0 (Hz)	*Measured F_0 (Hz)*
80	80.2 (1.1)
100	101.8 (1.7)
130	130.1 (4.3)
160	157.1 (3.7)
180	178.8 (4.2)

* Value is mean of two trials for seven subjects over ten contiguous glottal cycles. Numbers in parentheses are standard deviation.

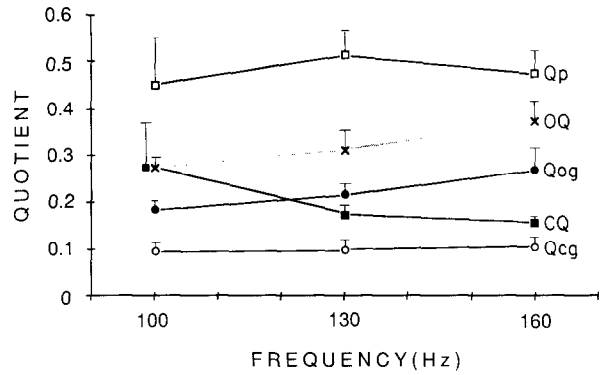

Figure 3. Mean values (±SEM) for Qp, OQ, Qog, CQ, and Qcg for seven subjects for SLNS at 3 TF_0 (100, 130, and 160 Hz). (Reprinted with permission.[17])

for SLNS. With ascending F_0, the Qog increased [$F(2,12) = 4.74$; $P < .05$] in parallel with the open quotient (OQ), whereas the closed quotient (CQ) decreased markedly [$F(2,12) = 4.50$; $P < .05$]. Newman-Keul's post-hoc multiple comparisons revealed significant differences among the three F_0 levels for these three quotients. Neither Qcg, SQ, nor Qp were shown to change significantly at $P = .05$ as F_0 increased.

Figure 4 shows representative waveforms plotted for one subject at three target frequencies. As the frequency increased with the increasing level of SLNS (Fig 4), the width ($B_i - D_i$) of the EGG waveform remained nearly constant, despite a diminishing period. This was reflected by an increasing open portion ($B_i - C_i$) and a decreasing closed portion ($C_i - D_i$) of the cycle. In this particular subject, mean Psub increased from 22 cm H_2O at 100 Hz to only 32 cm H_2O at 160 Hz for SLNS (Fig 4).

DISCUSSION

This investigation studied the effect of SLNS on physiologic events within the vocal vibratory cycle over a frequency range of 80 to 180 Hz under conditions of constant air flow. This range was chosen to represent frequencies within the canine modal register. In a previous study of adult human males, a range of 94 to 287 Hz was found for the modal register of 12 subjects.[19] The dog likely has a lower range of frequency for the modal register than the human, because the canine larynx is somewhat thicker and more massive.[20]

The profound capacity of SLNS to increase F_0 was confirmed by this study. Frequencies as high as 340 Hz were recorded during activation of the CT muscle. F_0 elevation by increasing SLNS was not accompanied by a significant in-

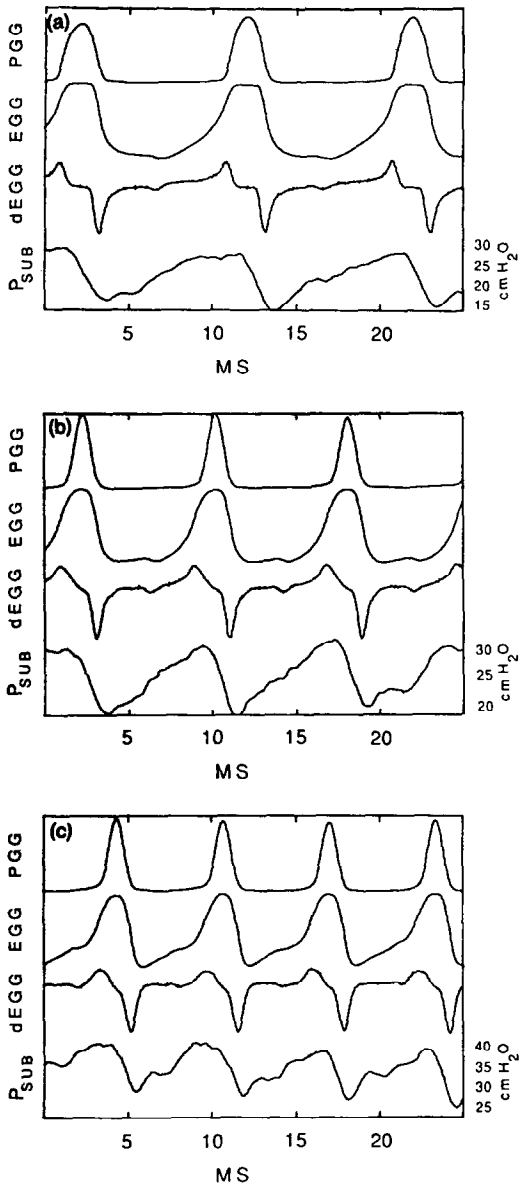

Figure 4. Typical recordings from a subject for three levels of target frequency (TF_0). A, B, and C have TF_0 of 100, 130, and 160 Hz for SLNS, respectively. (Reprinted with permission.[17])

crease in Psub, indicating that SLNS has little effect on laryngeal resistance. Increasing OQ with increased F_0 under SLNS agrees with studies of increased F_0 in human speech.[21] This may be explained by the two-mass model with increased coupling between the lower and upper margins of the vocal cords. The thinning of the vocal cords produces a shorter vertical distance between the lower and upper margins and causes a more convergent glottis.[22] Conversely, CQ decreased markedly as F_0 was increased by SLNS. This may also be explained by increased coupling between the lower and upper margins. An initial rise in Qp with increased SLNS was offset by a decline at 160 Hz so that no net change in Qp was observed. The difficulty in determining the point A_i with increasing SLNS may have contributed to an error in the calculation of Qp at 160 Hz, thus leading to the observation of no net change.

The close association between Qog and OQ for SLNS (Fig 4) was another interesting finding. Most of the change in OQ was the result of upper margin opening, rather than of lower margin closing (Qcg). As F_0 increases with increased CT activity, the opening phase of the vibratory cycle increases while the closing phase remains unchanged. This substantiates the hypothesis that as CT activity is increased in the modal register, a more convergent glottis is produced such that the upper margin governs changes in the open portion of the cycle.

Stroboscopic video imaging during increasing SLNS revealed reduced lateral excursion of the vocal cords, but this was associated with an apparent reduction in the amplitude of the traveling wave. As SLNS was increased beyond modal voice, the two-margin system was replaced by a one-margin system; that is, the lower and upper margins were observed to fuse into a vibration of one mass. Both RLNS and SLNS demonstrated the ability of the CT muscle to thin and stretch the folds for any given level of RLNS.

As shown in Fig 3, SLNS produced little increase in Psub with increased F_0. Considering that air flow was constant, CT activation had little effect on glottal resistance.

Our observation that the alternating current pressure waveform is near its maximum before upper margin opening and falls to its minimum at closure does not agree with human data obtained with pressure-tip transducers passed through the glottis into the trachea.[23,24] In those recordings, a great increase in Psub occurs at the onset of glottal closure and a marked decrease occurs at the beginning of glottal opening. These discrepancies are attributable to a low-pass filter (300 Hz) in the pressure transducer amplifier used in this study, which had the effect of delaying the actual pressure signal by several milliseconds.

CONCLUSIONS

(1) Increasing SLNS activated the cricothyroid muscle, causing a marked increase in F_0 with an increase in OQ and a small increase in Psub. The opening segment of the open phase was most affected, as evidenced by a significant increase in Qog.

(2) CQ decreased markedly with increasing SLNS.

(3) Qp was not shown to change significantly with increasing SLNS.

(4) Speed quotient was not shown to be significantly altered by F_0 elevation.

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APPENDIX A. Formulas Used in Glottographic Measurement

Qp = time delay between opening of lower and upper margins/period of glottal cycle:

$$B_i - A_i/(A_{i+1}) - A_i$$

OQ = duration of open glottis/period of glottal cycle:

$$D_i - B_i/(A_{i+1}) - A_i$$

CQ = duration of complete glottal closure/period of glottal cycle:

$$(A_{i+1}) - D_i/(A_{i+1}) - A_i$$

Qog = duration of glottal opening/period of glottal cycle:

$$C_i - B_i/(A_{i+1}) - A_i$$

Qcg = duration of glottal closing/period of glottal cycle:

$$D_i - C_i/(A_{i+1}) - A_i$$

SQ = duration of glottal opening/duration of glottal closing:

$$C_i - B_i/D_i - C_i$$

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