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Graded activation of the intrinsic laryngeal muscles for vocal fold posturing

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Abstract: Previous investigations using *in vivo* models to study the role of intrinsic laryngeal muscles in phonation have used neuromuscular stimulation to study voice parameters. However, these studies used coarse stimulation techniques using limited levels of neuromuscular stimulation. In the current investigation, a technique for fine control of laryngeal posturing was developed using graded stimulation of the laryngeal nerves. Vocal fold strain history to graded stimulation and a methodology for establishing symmetric laryngeal activation is presented. This methodology has immediate applications for the study of laryngeal paralysis and paresis, as well as general questions of neuromuscular control of the larynx.

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1. Introduction

Study of voice production and control mechanisms of the larynx is critical to furthering our understanding of human speech, communication, and phonatory pathology. Neuromuscular control of the larynx plays a critical role in phonation and has been investigated in animal (Choi *et al.*, 1993a, 1993b, 1995), human (Kempster *et al.*, 1988; Atkinson 1978; Faaborg-Andersen 1965), and computer models (Farley 1994; Story and Titze 1995). However, such studies in animals and humans have been limited, due to the difficulty in using laryngeal stimulation techniques to obtain fine control of laryngeal posturing. Animal studies have primarily used *in vivo* canine models to provide neuromuscular stimulation to laryngeal nerves. Unfortunately, however, only coarse "on/off" or "low/medium/high" settings have been utilized in these studies (Choi *et al.*, 1993a, 1993b, 1995).

The work of Nasri *et al.* (1995) shows the difficulty encountered in achieving fine control of laryngeal posturing (Choi *et al.*, 1993a, 1993b, 1995). Although Nasri *et al.* (1995) did not explicitly study laryngeal posturing, they showed that the compound nerve action potential and electromyographic signals of the thyroarytenoid (TA) muscle were sigmoid functions of the recurrent laryngeal nerve (RLN) stimulation current (or voltage). Similarly, Gorman and Mortimer (1983) measured muscle force upon electrical stimulation of the nerve to the medial gastrocnemius muscle in cats, observed a sigmoidal force response, and concluded these sigmoidal responses to be a consequence of muscle recruitment responses, first from activation of large then small diameter nerve fibers. They noted that "the current level between threshold excitation (I_0) and maximal recruitment (I_{max}) is not large." However, they also noted a number of variables, which could assist in maximizing the difference in current levels between I_0 and I_{max}, including a reduction in the pulse width of the electrical stimulus. Consequently, any method that attempts to achieve fine control of laryngeal posturing must take in account these known sigmoidal responses of muscles to the stimulation current.

Human studies have measured laryngeal electromyographic signals to correlate with the fundamental frequency (Atkinson 1978; Faaborg-Andersen 1965). However, multiple la-

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ryngeal muscles are concurrently activated in human voice production, and thus, the effect of individual muscle could not be evaluated. Kempster *et al.* (1988) applied direct stimulation to the intrinsic muscles via electromyographic electrodes while the subject phonated. However, fine control of laryngeal muscles was not attempted in this experiment. Finally, while computer modeling would be useful in conceptualizing laryngeal control mechanisms, neuromuscular data must first be obtained, on which to base such computational models.

Controlled activation of the intrinsic laryngeal muscles in a graded fashion is crucial for a variety of investigations in voice physiology, including investigations of asymmetric laryngeal pathologies (e.g., laryngeal paralysis and paresis), glottal posturing, and control of voice parameters such as fundamental frequency, phonation onset, and laryngeal aerodynamics. In order to facilitate such future investigations, the purpose of the present study is to develop a methodology, which uses graded stimulation of laryngeal nerves to achieve fine control of both symmetric and asymmetric laryngeal posturing.

2. Methods

An *in vivo* canine phonation model was used. The canine larynx is a close match to the human larynx in terms of its gross, microscopic, and histologic anatomy, and the utility of this model in voice research is well established (Berke *et al.*, 1987; Garrett *et al.*, 2000). The Institutional Animal Research Committee approved the experimental protocol. The animal was anesthetized and placed on an operating table. A vertical midline skin incision was then made on the anterior neck to widely expose the larynx and the trachea. Bilateral RLNs and superior laryngeal nerves (SLNs) were isolated. A low tracheotomy was performed for intra-operative ventilation, and the oral endotracheal tube was removed. The larynx was exteriorized into the neck by first performing an infrahyoid pharyngotomy and then by dividing the pharynx circumferentially at this level. A supraglottic laryngectomy was performed, removing bilateral false vocal folds, epiglottis, and thyroid cartilage above the level of the ventricles. This allowed the larynx to be slightly lifted off the neck and improved the exposure and access to the larynx for experimental manipulation and measurements. The nerve branch to the posterior cricoarytenoid (PCA) muscles was divided bilaterally to remove the effects of PCA contraction during RLN stimulation. Knots of fine diameter nylon sutures were placed at various locations on the thyroid cartilage, arytenoids, and vocal folds for automated tracking of laryngeal landmarks such as the vocal process.

The four laryngeal nerves were electrically stimulated independently using cathodal stimulation pulse trains applied by monopolar, flexible, carbon-elastomer electrodes. The common ground electrodes were connected to surrounding tissue. The nerve stimulation pulse trains were generated with a C computer program that programmed two peripheral component interconnect (PCI) bus-based AD/DA boards (PowerDAQ PD2-MFS-8–500/16, United Electronics Industries, Walpole, Massachusetts) to generate four different stimulation pulse trains simultaneously. The digital timer of one board (accuracy of 1 μs) was used as a reference clock to simultaneously trigger the pulse train generation, analog signal acquisition, and a high-speed digital camera. The computer-generated voltage pulse trains were transformed into current pulse trains with a constant current stimulus isolator (A-M Systems Analog Stimulus Isolator Model M 2200, A-M Systems, Sequim, Washington).

The threshold current for neuromuscular activation of laryngeal muscles was established by visually observing the onset of vocal fold twitches as pulse train amplitudes (stimulation levels) increased. A high-speed camera was used to display and record a superior view of the larynx. Care was needed to either electrically insulate the monopolar stimulation electrodes from the surrounding tissue or to decrease the electrode-nerve contact impedance. This critically affected the activation threshold. For electrical insulation, small strips of plastic foil were placed between the monopolar electrode and the surrounding tissue, thus preventing shunting of the stimulation current to the surrounding tissue. Decreasing the electrode-nerve contact impedance by filling the gap between electrode and nerve with water based lubricating jelly as an electrical conductor (Surgilube, Fougera, Melville, New York) also decreased the relative impedance of the nerve tissue versus the surrounding tissue.

Each stimulation pulse train consisted of rectangular monophasic pulses, with constant amplitude, pulse width of 0.1 ms, in contrast with a pulse width of 1.5 ms used previously in Choi *et al.* (1993a, 1993b, 1995), and pulse repetition rate of 200 Hz for both the RLN and SLN stimulation. These stimulation pulse parameters were based on preliminary tests to achieve a fused muscle contraction above stimulation threshold. The stimulation duration per pulse train was 1500 ms, which contained 300 rectangular pulses. To allow muscle recovery, each pulse train was followed by 3.5 s pause, prior to the next pulse train. The high-speed camera was typically triggered 2 ms after the onset of each pulse train to capture the geometric change in the larynx, due to intrinsic muscle activation. All four nerves were concurrently stimulated with different pulse train amplitudes.

With the AD/DA boards, analog signals could be recorded simultaneously, along with the generation of the stimulation pulse trains at a sampling rate of 125 kHz. Typical signals recorded include subglottal mean pressure, subglottal mean flow rate, subglottal acoustic pressure fluctuations, and acoustic pressure fluctuations downstream of the exposed canine larynx. The same C program that generated the pulse train sequences triggered the high-speed camera (Photron FASTCAM Ultima APX, Photron, San Diego, California). All camera settings were controlled using the DaVis software (LaVision Inc., Goettingen, Germany). The camera frame rate for these geometric posturing studies was 100 or 500 frames per second (fps) at a spatial resolution of 512×512 or 1024×1024 pixels per image.

Strain histories as a function of graded nerve stimulation were measured from high-speed digital frames using cross-correlation image processing (MATLAB image processing toolbox) that tracked laryngeal suture landmarks. The suture positions identified by MATLAB program were visually verified for accuracy. Time to maximum vocal fold length change was measured from the strain histories. To calculate vocal fold strain, the suture landmarks at the anterior commissure and the vocal processes were used. The reference length L_0 was measured at zero stimulation. Strain was calculated as $(L_i - L_0)/L_0$, where L_i is vocal fold length upon graded stimulation. This digital frame analysis also allowed for observation of the trajectories of vocal fold movement to graded stimulation.

3. Results and discussion

In vivo experiments were performed in three canines, and data were similar in all animals. Therefore, representative examples are presented. Figure 1 shows the strain of the left and right vocal folds to (a) 23 levels of graded stimulation of the left RLN, and (b) 26 levels of graded stimulation of the left SLN. For low levels of stimulation, a linear change was observed, which demonstrates graded laryngeal muscle activation to graded nerve stimulation. For higher levels of stimulation, the strain reached a plateau, which likely results from geometric constraints of the laryngeal muscles and framework. Figure 1 also demonstrates the mechanical connection of both vocal folds, as activation of the left vocal fold also resulted in similar length change in the non-stimulated right vocal fold. However, the trajectories of the vocal processes were different (see high-speed digital image inserts in Fig. 1). Results were similar between RLNs and SLNs on either side of the larynx.

The onset of vocal fold movement to stimulation could be observed within the first few high-speed frames. The maximal vocal fold strain magnitude was reached around 60 ms for RLN stimulation and around 150 ms after SLN stimulation. These results were similar for the three larynges and are similar to the results of Titze *et al.* (1997). Maximum vocal fold strain for the three larynges ranged from −20% to −30% for RLN stimulation, and +10% to +15% for SLN stimulation. These values are similar to those reported by Titze *et al.* (1997) for RLN, but not for SLN stimulation, where they reported maximal strain range from +26% to +71%. The reason for this discrepancy is unclear. During stimulation of the SLN, some lateral flaring of the thyroid cartilage occurred that may have reduced the lengthening stress of the cricothyroid muscle [see high-

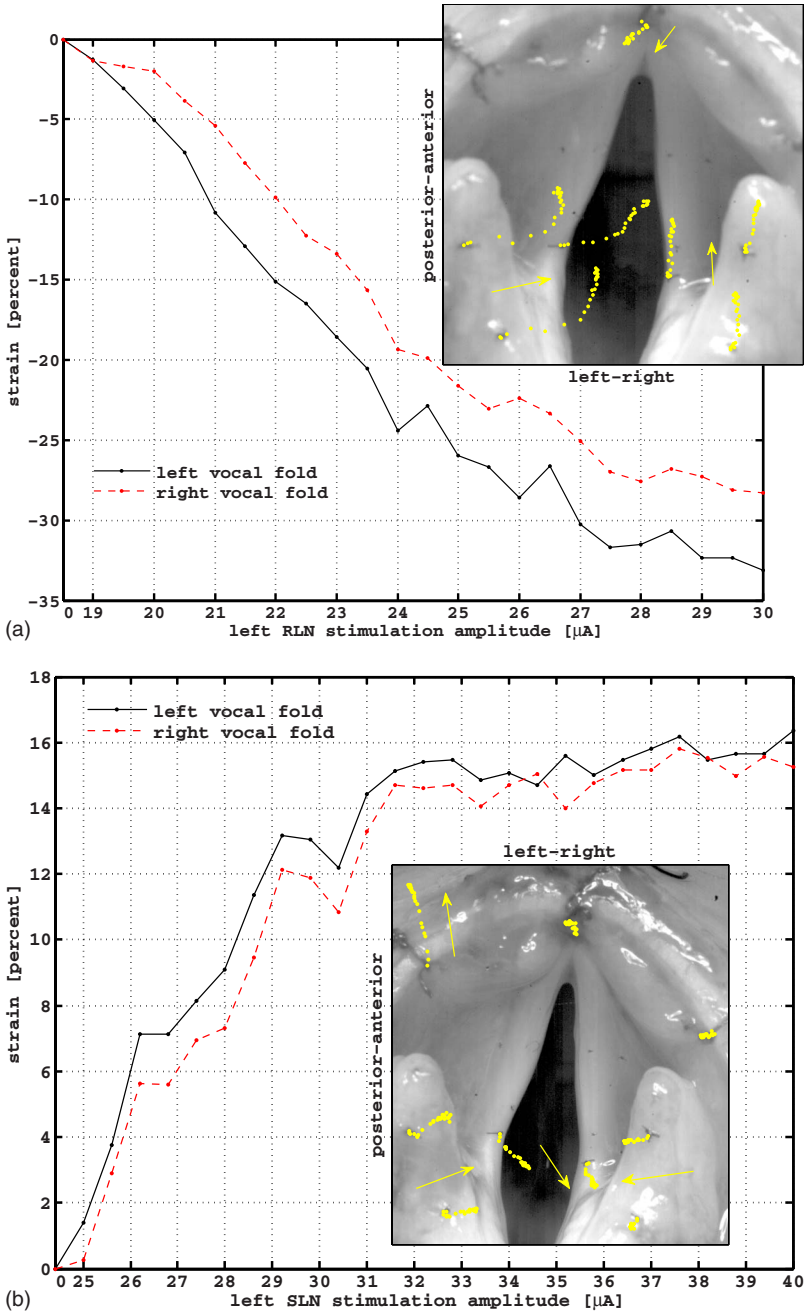

Fig. 1. (Color online) (a) Strain of the left and right vocal folds to 23 levels of graded stimulation of the *left* RLN and (b) 26 levels of graded stimulation of the left SLN. Reference length (L_0) was at zero stimulation amplitude (baseline). The strain values (percentage change in length) were measured at 1.0 s after the onset of the stimulation pulse train (pulse train duration 1.5 s, pulse width 0.1 ms, repetition rate 100 Hz). The figure insert shows individual changes in position of the suture landmarks at each level of graded stimulation (dots) and the overall direction of movement (arrows) plotted on the digital high-speed video frame obtained at baseline. Note the difference in trajectories between the stimulated *left* vocal fold and the non-stimulated *right* vocal fold, as well as the as the flaring movement of the left thyroid cartilage with ipsilateral SLN stimulation.

speed digital image insert in Fig. 1(b)]. The flaring of the thyroid cartilage was likely due to larynx preparation (exteriorization of the larynx in the neck, division of the strap muscles, and supraglottic laryngectomy) for improved visualization and measurements. However, our surgical technique appears similar to that of Titze *et al.* (1997). The effect of thyroid cartilage flaring on maximal strain of the vocal fold during SLN stimulation will need to be evaluated in future studies.

For investigations of laryngeal asymmetries, parameter values for symmetric muscle activation must first be established. Because laryngeal muscles exhibit a known sigmoidal response to stimulation current or voltage (Gorman and Mortimer, 1983; Nasri *et al.*, 1995), the range of threshold excitation current (I_0) to maximal recruitment current (I_{max}) was determined for each nerve (left RLN, right RLN, left SLN, right SLN) of each larynx. More specifically, I_0 was determined as the minimum stimulation current at which weak vocal fold twitches were observed. This was a reliable observation, and I_0 values were similar between the left and right vocal folds when electrical isolation of the electrode-nerve contact was carefully maintained during the experiment. A non-conducting material isolated the electrode-nerve contact area from touching adjacent tissue, and fluid build-up around the electrode-nerve contact points was prevented. I_{max} was determined as the minimum input current at which no further change in strain was observed.

Once I_0 and I_{max} were established for each nerve, graded stimulation was performed within that range. Symmetry of stimulation parameters was determined from the vocal fold strain and the distance between the vocal processes as a function of graded stimulation. Figures 2(a)–2(c) illustrate the left vocal fold strain [Fig. 2(a)], right vocal fold strain [Fig. 2(b)], and distance between the vocal processes [Fig. 2(c)] as a function of 11 levels of graded stimulation of the left and right RLNs (total 121 left/right conditions). The symmetry of the isocontour lines with respect to the diagonal reveals the symmetric response of vocal fold strain and vocal process distance to graded stimulation, implying symmetric graded activation of the larynx. Figures 2(d)–2(f) demonstrate the effect of adding low levels of bilaterally symmetric SLN stimulation. The results on the left vocal fold strain [Fig. 2(d)], right vocal fold strain [Fig. 2(e)], and distance between vocal processes [Fig. 2(f)] are similar to Figs. 2(a)–2(c), although there is a more abrupt transition in the measured variables at mid levels of graded stimulation of RLNs. Interestingly, the abrupt transition from a gradual change in strain to final strain levels occurred when strain had reached zero strain (resting length) as the lengthening effect on the vocal fold (positive strain) of SLN stimulation was countered by the shortening effect on the vocal fold by the gradual increase in RLN stimulation levels. The mechanism for this transition may be explained by muscle length versus force characteristics, but is a topic for further investigation.

The effect of TA stimulation is medial bulging of the mid-portions of the vocal folds (Choi *et al.*, 1993a). Figure 3 is photomontage of vocal fold postural change to symmetric graded stimulation [along the diagonal of Fig. 2(c)] of the left and right RLNs. With increasing stimulation, more medial bulging is observed until glottic closure is achieved in the mid-membranous area. This medial bulging effect to TA contraction has not been replicated in *ex vivo* or current computer models. The effects of medial bulging of the vocal folds on phonatory parameters, such as fundamental frequency, phonation onset pressures, and glottal geometry, are subjects of further research and can be studied using graded stimulation.

4. Conclusions

A methodology for graded stimulation of laryngeal nerves (left and right RLNs and SLNs) was developed, which yielded fine control of both symmetric and asymmetric laryngeal posturing. Once the threshold excitation current and maximal recruitment current were established for each nerve, a symmetric postural response of the larynx was established. Similarly, a wide range of asymmetric states was also established. In the future, this methodology will be used to study laryngeal paralysis and paresis, as well as more general questions of neuromuscular control of the larynx.

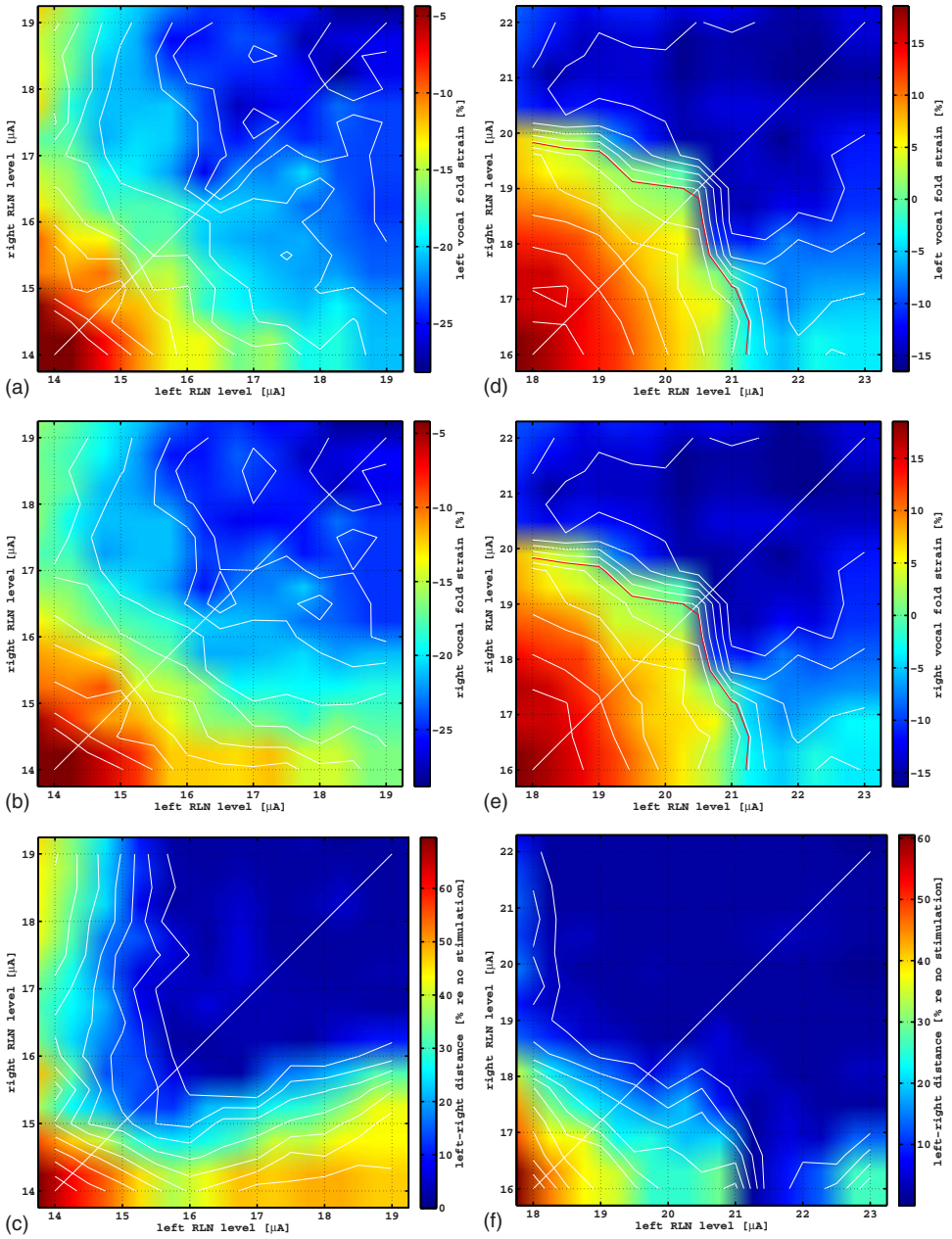

Fig. 2. (Color online) Symmetry of stimulation parameters was determined from analysis of left vocal fold strain (a),(d), right vocal fold strain (b),(e), and distance between vocal processes (c),(f), as a function of 11 levels of graded stimulation of the right and left RLNs (total 121 conditions). Zero SLN stimulation was applied in (a),(b),(c), and in (d),(e),(f) a low level of SLN stimulation amplitude was applied that was close to the excitation threshold (left SLN 24 μA, right SLN 34 μA). The symmetry of the isocontour lines with respect to the diagonal (straight diagonal white line) demonstrates symmetric response of the vocal folds, implying symmetric graded activation of the larynx. However, in presence of SLN activation (d)–(f) there was an abrupt transition in strain of the vocal folds when zero strain was reached. The percentage strain values at the stimulated conditions are with respect to reference length measured at zero stimulation. (Measurements for stimulated conditions taken at 404 ms after onset of stimulation pulse train; pulse train repetition rate 200 Hz; camera frame rate 500 fps with resolution 512 $\times$512 pixels.)

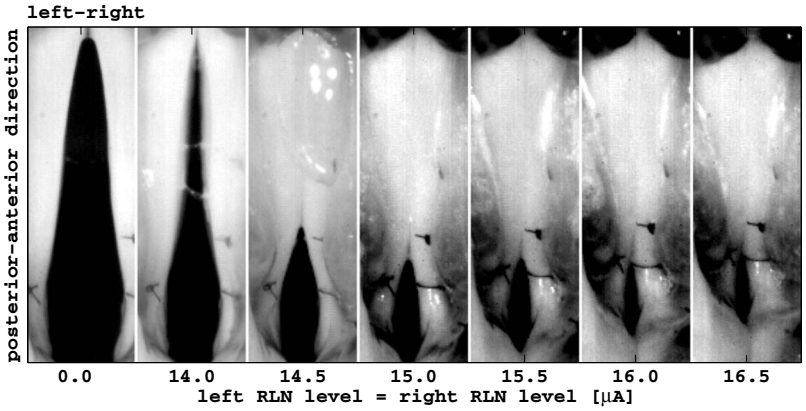

Fig. 3. Photomontage of vocal fold postural change to symmetric graded stimulation. The experimental conditions are identical to Fig. 2 and the photomontage is taken from images along the diagonal in Fig. 2(c). The medial bulging effect of the vocal folds is seen first, followed by closure of the posterior commissure.

Acknowledgments

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