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# Relation of Recurrent Laryngeal Nerve Compound Action Potential to Laryngeal Biomechanics

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This study was designed to investigate the compound action potential (CAP) of the recurrent laryngeal nerve (RLN) and to correlate this electrophysiologic signal to laryngeal biomechanics and phonatory function. Four adult mongrel canines were anesthetized. The RLN was isolated and stimulated, and recording electrodes were applied. The electromyographic (EMG) electrode was placed in the thyroarytenoid (TA) muscle. The RLN CAP and the EMG of the TA muscle were recorded and compared to the stimulation intensity, subglottic pressure ( $P_{\text{sub}}$ ), and each other. The CAP peak-to-peak and EMG peak-to-peak amplitudes demonstrated a sigmoidal relation to stimulus intensity and a linear relation to  $P_{\text{sub}}$  and to each other. On the basis of these findings, the RLN CAP appears to be a reliable physiologic measure of laryngeal function.

## INTRODUCTION

While much has been published regarding recurrent laryngeal nerve (RLN) denervation and reinnervation, surprisingly little work has focused on the relation of nerve electrophysiology to muscular activity and resulting laryngeal biomechanics. The most accepted electrophysiologic measure of neuronal properties is the compound action potential (CAP), an extracellular field potential generated as current propagates along a nerve.<sup>1-3</sup> For muscle contraction to occur, action potentials activate muscles through a chemical transmission step. Stronger muscle contractions are achieved mainly by recruiting additional neuromuscular units.

Previous studies have demonstrated that CAP behaves in a graded fashion in activating neuromuscular units.<sup>4</sup> The motoneuron discharge patterns resulting in this graded response are explained by the "size principle" postulate: the size of motoneurons correlates with their susceptibility to discharge.<sup>5</sup> The larger motoneurons fire more rapidly. With increasing excitatory input the smaller units, which are less susceptible to discharge, fire. At very high stimulation levels, all motoneurons fire simultaneously. Further increase in stimulus intensity does not result in any change in the peak-to-peak amplitude of the CAP signal.

While electrical stimulation applied to the neuronal surface first activates the larger axons, the opposite is true in the physiological contraction, and small motor neurons are activated initially. With increased strength of contraction, larger motoneurons are recruited.<sup>4,5</sup> Although during central activation there appears to be an inverse relation of excitability of motoneurons and neuronal size, an orderly recruitment of motor units still exists.

Compound action potentials have been successfully recorded from many nerves.<sup>1-7</sup> However, to our knowledge, the CAP of the RLN has not been recorded, nor has there been a correlation to laryngeal biomechanics and phonatory function. In this study, we measured the RLN CAP and correlated this signal to a measure of muscular activity of the thyroarytenoid (TA) muscle (electromyographic [EMG] amplitude) and to subglottic pressure ( $P_{\text{sub}}$ ). The relation between nerve stimulus intensity and CAP will also be studied.

The CAP was correlated with  $P_{\text{sub}}$  because prior *in vivo* experiments have demonstrated that air pressure variation in the vocal tract is one of the most important physiological variables related to phonation.<sup>8</sup> The  $P_{\text{sub}}$  is an essential component in the control of basic features of phonation such as fundamental frequency and intensity.<sup>9,10</sup> Modulation of  $P_{\text{sub}}$  is achieved through the biomechanical interactions of the intrinsic laryngeal musculature, which in turn are determined by the pattern of electrical signals con-

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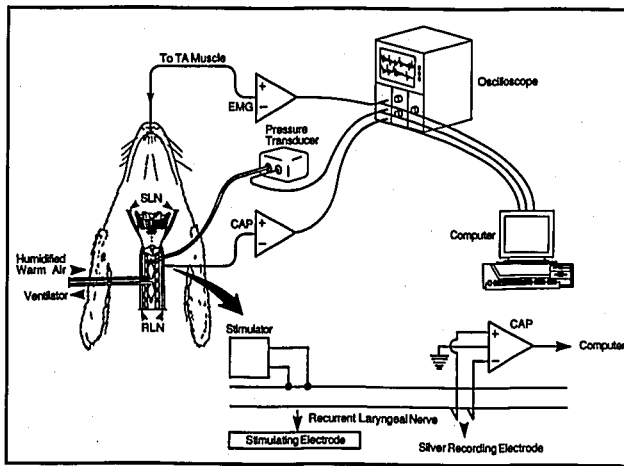


Fig. 1. Schematic representation of the experimental setup.

ducted by the RLN.

The RLN CAP  $P_{sub}$ , and TA EMG signals are measured simultaneously. The electrical activity of a muscle as measured by EMG is proportional to the force generated by its contraction.<sup>11-15</sup> Therefore, intrinsic laryngeal muscular activity will be assessed by recording the EMG from the TA muscle, an easily accessible muscle.

The primary hypothesis of this research is that the CAP of the RLN is correlated with nerve stimulus intensity and muscle contraction. If the CAP is a reliable physiologic measure of nerve function, the signal should translate proportionately into an increased  $P_{sub}$ . As a direct reflection of electrical stimulus intensity in the RLN,  $P_{sub}$  should also vary directly with the CAP and the EMG of the TA.

## MATERIALS AND METHODS

This study was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the National Institutes of Health *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 at Los Angeles.

This experiment was performed according to the in vivo canine model of phonation previously described by Berke, *et al.*<sup>16-18</sup> Four 25-kg adult mongrel canines were anesthetized with 3 mL of acepromazine maleate by intramuscular injection followed by intravenous pentobarbital sodium until loss of the corneal reflex was achieved. Additional amounts were used to maintain this level of anesthesia throughout the experiment. A 7-mm endotracheal tube was inserted, and each animal was given assisted ventilation with 95% oxygen.

A midline neck incision was made from the hyoid to the sternum. The strap and sternocleidomastoid muscles were retracted laterally to expose the trachea and larynx. A low tracheotomy was performed at the level of the suprasternal notch; an endotracheal tube was passed through to allow ventilator-assisted respiration. A second tracheotomy was

performed in a more superior location; a cuffed endotracheal tube was passed through in a rostral direction and positioned with the tip resting 10 cm below the glottis. The cuff of this tube was inflated until it just sealed the trachea. Room air from a wall source was bubbled through 5 cm of water at 37° C for warming and humidification and passed through this rostral tube.

The experimental setup is shown in Figure 1. The RLN was isolated and stimulated with current varying from 1 to 5 mA with Harvard bipolar electrodes (South Natick, Mass.). Phonation was produced with an airflow of 256 to 532 mL/s applied through the rostral endotracheal tube. The flow was controlled with a valve and measured with a pneumotachograph (model f1500, Gilmont Instruments, Great Neck, N.Y.). A Millar Mikro-Tip catheter pressure transducer (model SCP-330, Size 3F, Houston, Tex.) was passed through the upper tracheotomy to a level 2 cm inferior to the vocal folds to measure  $P_{sub}$ .

The CAP was recorded with a custom-made bipolar silver electrode placed 4 cm distal to the stimulating electrode. The recording electrode was positioned to raise gently and isolate the RLN from the surrounding tissue. A bipolar concentric needle electrode (Teca Corp., Pleasantville, N.Y.) was inserted into the ipsilateral TA muscle transorally to record the stimulus-evoked EMG. The signals from the CAP and EMG electrodes were differentially amplified by 50,000 and 10,000, respectively. The pressure, flow, CAP, and EMG signals were then filtered, digitized, and stored on a computer hard drive with commercially available software (C-Speech, Paul Milenkovic, University of Wisconsin, Madison).

Data collection and analysis were performed with the speech analysis system C-Speech. As the dog phonated, EMG, CAP, and  $P_{sub}$  were filtered, digitized, and stored for 2 seconds. The three signals were displayed on a video monitor and visually divided into four 500-msec segments. In each segment, four values each for CAP peak-to-peak ( $CAP_{pp}$ ) and EMG peak-to-peak ( $EMG_{pp}$ ) amplitudes and  $P_{sub}$  were averaged (a total of 20 for each 2-second recording) to determine the characteristic CAP, EMG, and  $P_{sub}$  for each recording.  $CAP_{pp}$  and  $EMG_{pp}$  were computed by taking the value of the sum of the largest negative peak and the next positive one. For each dog, the relation between these parameters and stimulus intensity were investigated via multiple regression. Plots comparing stimulation levels,  $CAP_{pp}$ ,  $EMG_{pp}$ , and  $P_{sub}$  were examined and linear or nonlinear models of curve fitting applied as appropriate.

## RESULTS

Figure 2 illustrates the temporal relation of typical CAP and EMG waveforms reproduced from the C-Speech speech analysis software. The vertical dotted lines represent the stimulus artifact, with the CAP signals following after a short delay. The EMG signal from the TA muscle is indicated on the lower waveform. The overall shape of each trace is similar to previously published CAP and EMG recordings.<sup>11,12,19</sup> The first negative peak in the CAP recording occurred at 0.8 msec. Given an interelectrode distance of 4 cm, this implies an action potential conduction velocity of 50 m/s, which is consistent with the results of a previous study on conduction velocity in the canine RLN.<sup>20</sup>

Several experimental manipulations were ap-

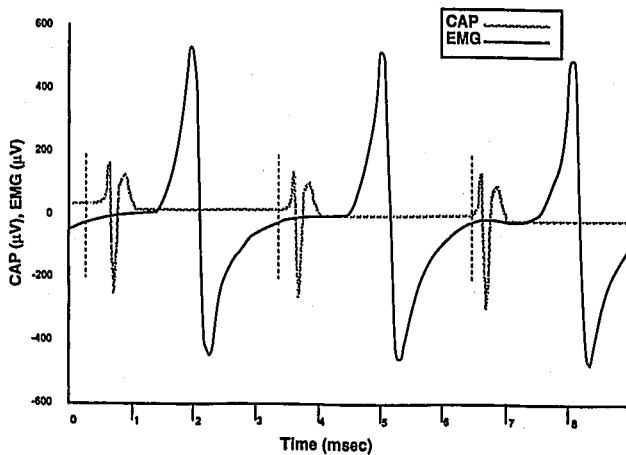
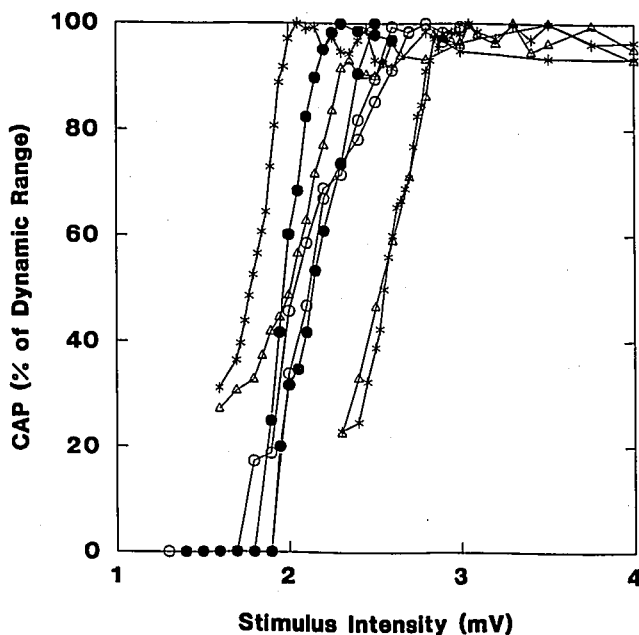


Fig. 2. Typical CAP and EMG profiles as a function of time. Traces are the result of three single electrical pulses applied to the RLN.

plied to confirm the physiologic nature of the recorded signals. Inversion of the polarity of the stimulus resulted in inversion of the stimulus artifact but did not affect the CAP. Transection of the RLN distal to the recording electrode caused the disappearance of the EMG signal with no effect on the CAP. Transection of the RLN between stimulating and recording electrodes resulted in the disappearance of both CAP and EMG. These findings indicate that CAP is a physiological property of the RLN and not an artifact of the electrical stimulus.

Figure 3, left, shows the relation between stimulation intensity and CAP for each dog and trial. CAP values in this figure, which were normalized for differences in overall levels of response, ranged from mini-



um to maximum (0%–100%). Note that all statistical analyses were performed on the unnormalized data. For each trial, CAP increased with increasing stimulation until a plateau was reached. After that plateau, further increases in stimulation intensity produced no further increases in CAP. Simple linear regression showed that a straight line fit the data for each trial ( $r^2 = 0.88-0.99$ ,  $P < .01$ ; mean = 0.96). Similarly, EMG levels increased linearly with stimulation ( $r^2 = 0.82-0.98$ ;  $P < .01$ , mean = 0.95) until a plateau was reached (Fig. 3, right). This pattern reflects the correlation between CAP values and EMG levels across dogs and trials demonstrated in Figure 4 ( $F(1,174) = 2903.45$ ,  $P < .01$ ,  $r^2 = 0.96$ ).

$P_{sub}$  increased significantly with both CAP (Fig. 5, left) and EMG (Fig. 5, right) values, again reflecting the strong correlations among these variables. For CAP versus  $P_{sub}$  across dogs and trials,  $r^2$  ranged from 0.95 to 0.99 ( $P < .01$ ), with a mean of 0.96. The squared correlation between EMG and  $P_{sub}$  values ranged from 0.92 to 0.99 ( $P < .01$ ), with a mean  $r^2$  of 0.97.

## DISCUSSION

This study measured RLN CAP and correlated this electrophysiological signal to physiological measures of phonatory function, specifically muscular activity and  $P_{sub}$ . The relation of the CAP with stimulus intensity was also studied. The  $CAP_{pp}$  demonstrated a sigmoidal relation to stimulus intensity. Similar experiments with single-fiber recordings in the cat facial nerve<sup>21,22</sup> suggest that more than one population of motoneurons may be present. These populations exhibit different excitation thresholds and different action potential conduction velocities.

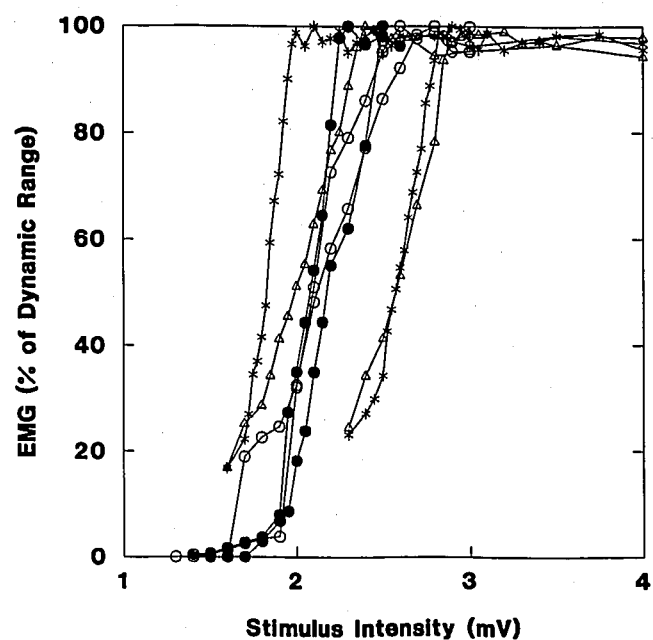


Fig. 3. Normalized  $CAP_{pp}$  (left) and  $EMG_{pp}$  (right) as a function of stimulus intensity in all animal preparations.

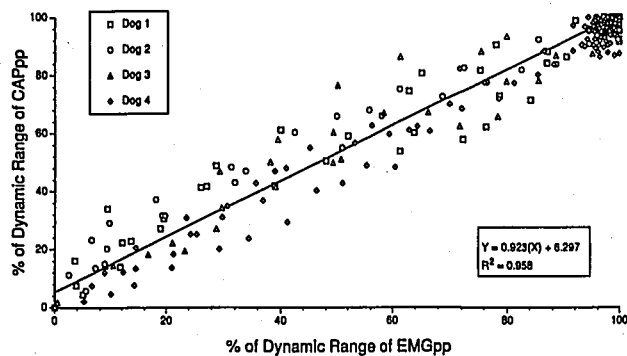


Fig. 4. Graph of percentage of dynamic range of CAP<sub>pp</sub> versus percentage of dynamic range of EMG<sub>pp</sub> for all animals studied.

The sigmoidal nature of the CAP<sub>pp</sub> versus stimulus intensity curve can be explained by Henemann's "size principle." According to this postulate, motoneuron size is correlated with susceptibility to discharge. As excitatory nervous stimulus to a muscle increases, increasingly greater numbers of motoneuron units are recruited, providing a controlled, graded generation of muscle tension and contractile force. The initial rise in CAP<sub>pp</sub> curve is secondary to an increasing number of motoneurons recruited. When all motor units are recruited, further increase in stimulus intensity does not result in a change in the CAP<sub>pp</sub>. This finding is demonstrated by the plateau seen in these curves.

As mentioned before, while it is true that the excitability of peripheral nerve fibers to electrical stimulation is proportional to fiber size, during central activation of the nerve this excitability is inversely proportional to neuron size. Regardless of the type of neuronal stimulation, there is an orderly recruitment of motor units. Therefore, it is safe to assume that a similar rise and plateau in a spontaneous CAP curve would be present.

This study also revealed that the TA EMG<sub>pp</sub> exhibited a sigmoidal relation to stimulus intensity, implying a sequential recruitment of motoneuron units with increasing electrical stimulation. At low levels of

stimulus intensity, the recruited units have large fiber diameters and low thresholds. They generate large EMGs with small variations in electrical stimulation as seen by the large changes in EMG<sub>pp</sub> at low stimulation intensities (steep part of the curve). At progressively higher stimulus levels, recruited units have smaller fiber diameters and higher electrical stimulation thresholds. They generate small EMGs with large variations in electrical stimulation. Ultimately, no further increase in EMG<sub>pp</sub> is seen with the highest levels of electrical stimulation because all of the motoneuron units have been recruited (plateau of curve).

The CAP and EMG exhibited a linear relation to each other and to P<sub>sub</sub>. These linear relations are to be expected if CAP is a reliable indicator of RLN function. Increased RLN CAP<sub>pp</sub> translated proportionately into an increased TA EMG<sub>pp</sub> and thus into an increased P<sub>sub</sub>. This relation is important because it provides an electrophysiological measurement with which to quantify laryngeal biomechanics.

In terms of the subglottic pressure, the RLN CAP accurately reflects RLN neuromuscular activity. If this principle is to hold true, there should also be a similar relation between the amplitude of the CAP and the stiffness of laryngeal muscles. The mucosal wave velocity is related to Young's modulus, which in turn is related to vocal fold stiffness.<sup>23</sup> The mucosal wave, a moving alteration in the mucosal surface of the vocal fold, can be visualized with a laryngeal stroboscope. The wave is primarily responsible for modulation of the glottal air stream during phonation. The characteristics of mucosal vibration affect the puff of air released during each glottal cycle and therefore have a significant effect on the quality and amplitude of the voice.<sup>23-25</sup> Given the relation between the wave velocity and underlying muscular contraction, a correlation between velocity and CAP would supply additional evidence that the CAP reliably reflects RLN activity.

Measurement of the graded response of RLN to electrical stimulation may potentially provide detailed information about neurobiomechanical organization of the larynx. However, most electrophysiologic

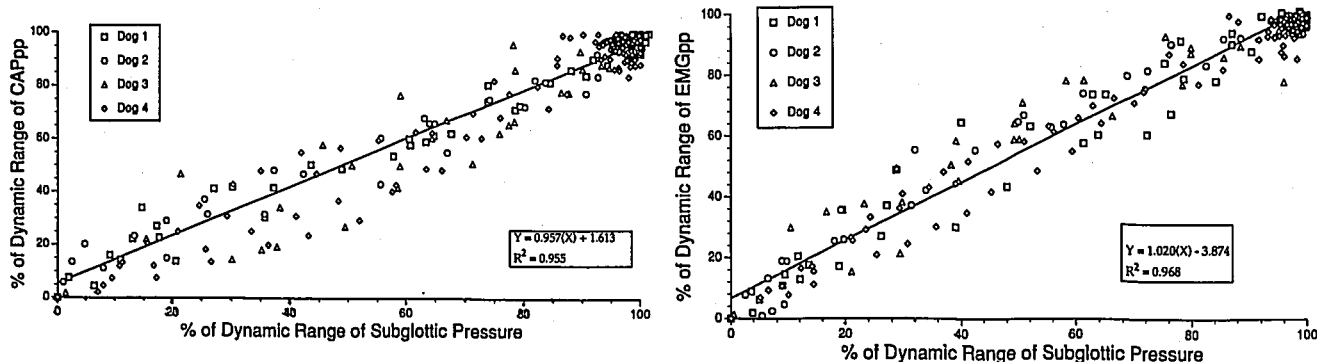


Fig. 5. Graph of normalized (percentage of dynamic range of) CAP<sub>pp</sub> (left) and EMG<sub>pp</sub> (right) versus normalized (percentage dynamic range of) subglottic pressure for all animals studied.

studies of the larynx use a variety of neuromechanical stimuli. Electrical stimulus intensity can vary depending on the type of stimulator used, as well as from trial to trial when the same device is used. CAP, in contrast, is a quantifiable measure of neuronal activity that can be measured and standardized. By measuring the range of RLN CAP<sub>pp</sub> in the in vivo model of phonation, a normal distribution can be acquired and compared across subjects for this physiologic measure. By comparing the RLN CAP with muscular activity and vocal cord vibratory physiology, improved insight into how these variables interact to govern laryngeal function will be gained. These functional investigations may have significant implications for studies of laryngeal reinnervation, fabrication of a neolarynx, laryngeal transplantation, and treatment of laryngeal hyperadductory disorders such as spasmodic dysphonia and laryngospasm.

## CONCLUSION

We have attempted to characterize the RLN CAP and to correlate this signal with the stimulus intensity, TA EMG, and P<sub>sub</sub>. The CAP<sub>pp</sub> and EMG<sub>pp</sub> demonstrated a sigmoidal relation to stimulus intensity and a linear relation to each other and to P<sub>sub</sub>. Further work is necessary to characterize how pathological disturbances in the larynx may alter this relation.

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