Ann Otol Rhinol Laryngol 111:2002

ELECTRONIC ANALYSIS OF INTRINSIC LARYNGEAL MUSCLES IN CANINE SOUND PRODUCTION

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This study explores the relationship between voice production and intrinsic laryngeal muscle (ILM) activities as expressed by orderly recruitment of their specific motor units. In 5 dogs, both the recurrent laryngeal nerve (RLN) and the vagus nerve (cranial nerve X) were stimulated via tripolar electrodes with stimulating frequencies (Fs) of 10 to 60 Hz and 0 to 7 mA during application of symmetric 600 Hz, 7 to 0 mA blocking currents. The fundamental frequency (F0) and the intensity (I) of sounds generated by tracheal insufflation of humidified air were recorded while electromyograms of the cricothyroideus (CT), thyroarytenoideus (TA), and posterior cricoarytenoideus (PCA) were obtained via surface electrodes. Contractions of the CT were concurrently induced by stimulating the superior laryngeal nerve (SLN). The recruitment rates were highly specific and were affected by which nerve was stimulated. For the RLN, PCA ramping was lowest for Fs of ≤50 Hz. For Fs of 10 to 30 Hz, the recruitment rate of the TA was significantly steeper than that for the other ILMs, and the CT had the highest rate for Fs of 40 to 50 Hz. Conversely, for the vagus nerve, PCA recruitment was highest for Fs of ≥30 Hz. The average F0 was significantly higher with the RLN than with the vagus nerve. When the TA recruited faster than the CT (ie, via the RLN, but not the vagus nerve), the F0 was higher. While only CT ramping was significantly related to changes in sound intensity, there was a trend toward a decrease when PCA ramping was higher than CT ramping, as occurred when only the vagus nerve was stimulated. Stimulation of the SLN always increased F0 and loudness. We conclude that changes in F0 occur mainly through RLN-mediated CT and TA contraction. Loudness is controlled by the CT. The PCA exerts reciprocal coupling on both functions via the vagus nerve, and they are boosted across the board by the SLN. These findings may allow artificial manipulation of voice.

KEY WORDS — artificial voice production, frequency analysis, loudness analysis, orderly recruitment, selective intrinsic laryngeal muscle contraction.

BACKGROUND AND JUSTIFICATION

Voice production results from complex interactions between intrinsic laryngeal muscles (ILMs) acting in combination to effect vocal fold adduction, abduction, and tension to vary the resistance of the glottis over the expiratory air column originating in the lungs (myoelastic theory[1]). Adduction is primarily due to contraction of the thyroarytenoideus (TA), whereas abduction is exclusively controlled by the posterior cricoarytenoideus (PCA). The cricothyroideus (CT) generates tension and a certain degree of additional adduction over the narrowed glottis.[2] The process of vocal modulation is a result of the fine-tuned antagonistic relationships between the TA and the PCA,[3] which are both innervated by the recurrent laryngeal nerve (RLN). Although the CT is the only muscle innervated by the superior laryngeal nerve (SLN), both the RLN and the SLN stem from the vagus nerve (cranial nerve X), which thus ultimately controls all ILMs. Unfortunately, little is known about the fine distribution of larynx-bound neurofibers to the individual ILMs or to their anastomoses within the larynx.

Individual ILM contributions to phonation have been difficult to outline. Simple vocal fold observation contributes little in analyzing the complexities of laryngeal muscle interplay, which responds to poorly defined servomechanisms in the brain stem.[4] In vivo electromyographic (EMG) assessments may offer useful information, but lack statistical power because of the scarcity of volunteers and difficulties in needle electrode placement during simultaneous recording of data.[5-8] Artificially induced phonation in animals under general anesthesia does not have the benefit of voluntary input.[9,10] Theoretically, each ILM could be directly assessed by stimulating peripherally

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Presented in part at the 4th European Congress of Oto-Rhino-Laryngology, Berlin, Germany, May 16, 2000.

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located branches of the RLN,[11] but concomitant excitation of anastomotic fibers serving other effectors can never be excluded.

One possible way to answer these critiques and to properly appraise nerve-muscle functional continuity is to consider individual motor unit architecture, since it varies from one unit to another. In a previous study, we showed that each group of ILMs could be specifically defined by means of an electronic circuit specially designed to produce orderly, physiological recruitment.[12] In the current research, we hypothesized that the individual roles of key glottic muscles in the production of sound could be defined on the basis of the unique attributes of different recruitment rates. We thus artificially reproduced sound by transtracheal injection of a constant flow of humidified air forced cranially through the variable laryngeal resistor during the natural, orderly recruitment of its constituent ILMs. We recognize that this situation may differ from normal physiological voice production, in which airflow is variable, and that canine data may not necessarily be extrapolated to the human situation, in which in vivo verification is in any case difficult. This phase I study compares the rates of individual EMG compound muscle action potential (CMAP) progressions to the fundamental frequency (F0) and intensity (I) of the sound recorded at the animals' mouths.

It is ironic that paralyzed ILMs have been easier to study than their healthy counterparts, possibly because they may be specifically targeted with foreign motor implants borrowed from other, noninvolved territories. We previously achieved voice manipulation in dogs with sectioned RLNs by stimulating 2 distinct conduits respectively reinnervating the TA and PCA while airflow was artificially produced to mimic pulmonary expiration.[13] Sound envelopes typical for canine barks (330 to 600 Hz[13,14]) were induced by antagonistic TA-PCA coupling primed over a background of restored baseline tone levels. A return to normal intermuscular relationships abolished by the paralytic process is logical and, indeed, a prerequisite[15] for the restoration of the dynamic interplay typical of the rehabilitation processes of any striated muscle system. This concept is a corollary to the artificial restoration of missing myotatic reflexes that ordinary nerve-muscle implantations have been unable to achieve.[16] While the "reinnervated laryngeal option" may offer promise after complete paralysis, it behooves the practitioner to consider that most laryngeal dystonias involve an organ system that remains anatomically and functionally (albeit imperfectly) connected to its neural grid. Thus, deliberately induced paralysis for the rehabilitation of such dystonias appears excessive in light of the difficulties that may arise from potentially unsatisfactory reinnervation, the necessity for separate agonistic drives to achieve reciprocal coupling, and the cumbersome nature of multiple perineural electrode placements.

Histochemical studies show that fast muscles such as the TA are composed mainly of type II myofibers (with only a few type I) as part of small motor units, while the contrary is true for slow muscles such as the PCA.[17] Interestingly, there is a direct correspondence between axonal diameters and the number of myofibers subtended within the constituent motor units.[18] It has also been established that larger axons are more excitable than their thinner counterparts.[19] Thus, standard bipolar stimulation of a nerve composed of axons of various diameters such as the RLN or the vagus nerve should result in premature contractions of the more excitable muscles (such as the PCA), since they contain higher numbers of larger motor units and axons. Conversely, the less excitable, faster muscles subtended by thinner axons (eg, the TA) cannot be expressed under standard stimulating conditions. In fact, the bipolar stimulation paradigm is in contradistinction to natural muscular activation patterns, in which muscles composed of smaller motor units are normally activated before their larger counterparts (the size principle of Henneman et al[20]).

With the above difficulties in mind and in order to study the muscles in their natural state, we previously reproduced orderly recruitment patterns using a specially designed electronic circuit.[21] The recorded EMG ramping curves were expressed as averaged CMAPs (in millivolts) via surface electrodes. By avoiding direct muscle implantation, surface electrodes preclude fragmentary evaluation and provide the observer with a global assessment of the whole muscle's activities. The CMAP values were plotted over time as the intact RLN was stimulated via a tripolar electrode cued by a dual circuit. Contractions elicited by the first (bipolar, stimulating) circuit via the lone cathode and the proximal anode were blocked through high (600 Hz) frequencies[21] by a second circuit involving the distal anode, thus achieving tripolar stimulation as originally described by Zhou et al[22] and Baratta et al[23] in feline gastrocnemius preparations. Complete muscular blockage was expressed as flat EMG CMAP lines for each ILM. Orderly recruitment progressions were graphically revealed as blocking currents were lifted from these baselines. The diverse ramping slopes thus obtained measured EMG CMAP (in millivolts) progression quotients over time (milliseconds). As intuitively expected, the TA was found to have a steeper rate of recruitment as compared to the PCA, while the lateral cricoarytenoideus (LCA) displayed intermediate values.[12] This method

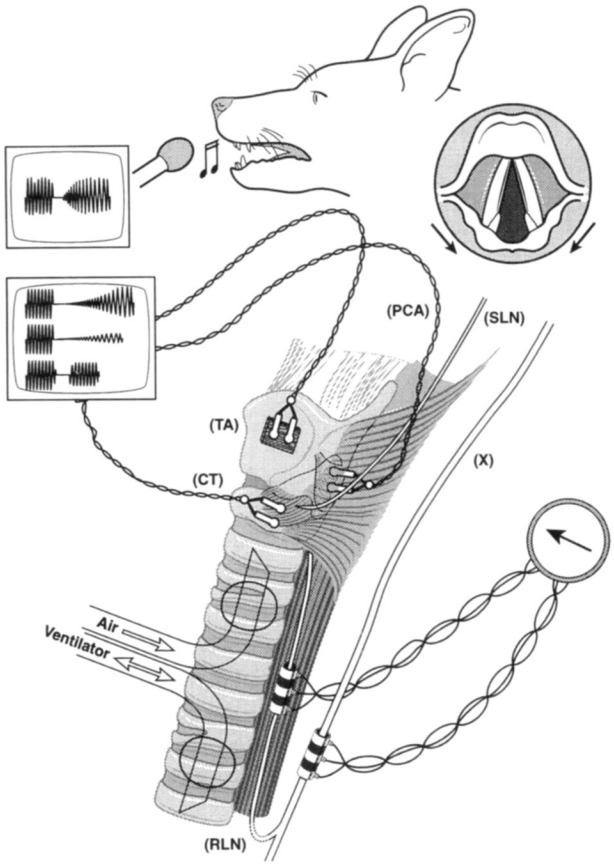

Fig 1. Block diagram of study. Larynx and trachea are exposed under general anesthesia. Recurrent laryngeal nerve (RLN), superior laryngeal nerve (SLN), and vagus nerve (X) are isolated bilaterally. RLN and X (but not SLN) are fitted with tripolar electrodes linked to dual stimulation/blocking circuit (circled arrow) producing distal anodal blockage of currents generated upstream via proximal anode and medial cathode. Spherical-shaped contact electromyographic (EMG) surface electrodes pick up resulting compound muscle action potentials (CMAPs). Vocal fold adduction (**inset**) acts as variable resistor while humidified air is blown cephalad via second endotracheal tube. Microphone at mouth records sound envelope thus produced. Recruitment rates of individual muscles (lower screen) and sound pressure levels (upper screen) are recorded and compared. PCA — posterior cricoarytenoideus, TA — thyroarytenoideus, CT — cricothyroideus.

secured for the larynx complete independence in the control of the motor unit firing rates based on their orderly recruitments.[23]

While the muscular actions obtained via orderly recruitment are an approximation of what occurs in vivo, the latter has never been observed, because standard electrodes do not recruit motor units in a physiological fashion. The use of the blocking circuit thus appears to the investigators to be the clearest way to separate the actions of the individual muscles in a living preparation. Therefore, the current research was designed to elucidate the relative participation of key ILMs and their relevant neural circuits in phonation with a view toward the eventual use of this information for the dynamic management of various laryngeal dystonias. The dynamic management of voice proposed in this study is in contradistinction to traditional static treatments of the impaired glottic resistor, in which paralysis (ie, the absence of tone) of the vocal fold may only be compensated for by medialization, and spastic states (ie, increased tone) may only abate via either RLN section or direct muscular injection of botulinum toxin. These interventions violate an otherwise intact nerve or the larynx itself, offer at best only temporary relief, may be irreversible, and are fraught with potential complications.[24]

MATERIALS AND EXPERIMENTAL METHOD

Five mongrel dogs weighing 15 to 20 kg were used. This number was determined by using a repeated-measures analysis of variance (ANOVA) approach for the various independent variables under consideration. By adherence to statistical assumptions of the tests performed and use of Bonferroni adjusted p values to examine the significance of multiple testings, this number of animals was set to detect whether independent variables accounted for 20% of the variability of the response variable with at least 90% power, as based on results of a previous study.[12]

After intravenous anesthesia with pentobarbital sodium, halothane and oxygen were administered through an endotracheal tube in the supine animals (Fig 1). Through a midline cervical incision, the dogs' artificial airways were converted to a low tracheostomy with the distal aspect of the tube facing downward well above the carina for effective bilateral pulmonary ventilation. A second endotracheal tube, destined to channel humidified air and placed above the first tracheostomy site, was directed superiorly. The RLN, SLN, and vagus nerve were then anatomically isolated on both sides and verified as eliciting muscular contraction in the larynx by contact electrical stimulation with a disposable stimulator (0.5 mA, Varistim, Clearwater, Florida). The tongue was then suspended from a frame and the epiglottis was sutured to its base to expose the glottic chink without need to use direct laryngoscopy for appropriate observation. After exposure of the TAs through windows cut on each side of the thyroid cartilage, the PCAs and CTs were also identified. The larynx was rotated alternately to one side and maintained in position via double-prong hooks under the thyroid lamina while suspended to the frame. This maneuver allowed excellent ILM exposure on the opposite side.

A tripolar electrode (Axon Engineering Company, Chesterland, Ohio) was then placed around each RLN and vagus nerve (on both sides). In addition, a 30

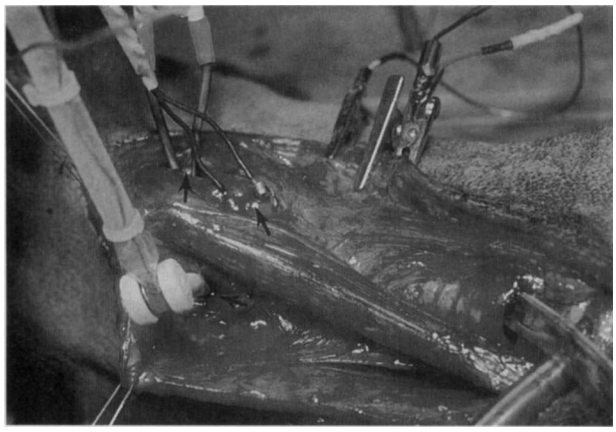

Fig 2. Spherical EMG electrodes placed over surface of TA (upper arrow), CT (middle arrow), and PCA (lower arrow).

gauge platinum wire was looped around both SLNs. Pairs of spherical surface EMG electrodes (N = 3) similar to those used in a previous study[12] were placed to effect direct contact over each of the 3 muscles (TA, PCA, CT; Fig 2). The stimulation module of the circuit (Biomedical Corporation of America, Cleveland, Ohio) produced 10 to 100 Hz, 0 to 7,000 μA, 0 to 1,000 ms currents with a 600 Hz, 7,000 to 0 μA blocking envelope injected into the RLNs and vagus nerves. Unilateral and bilateral SLN stimulations (0.5 to 2 mA) were superimposed in selected cases. The EMG probes were positioned over the surface of the exposed TA, PCA, and CT and linked to an Astromed MT95K2 multitask recorder. A low-impedance (600 Ω) Labtec DM-20SL dynamic cardioid microphone was placed at each animal's mouth and connected to a personal computer programmed with CoolEdit version 96 (Syntrillium Software Corporation; Phoenix, Arizona) and a SciCon PC Quirer sound analyzer (Los Angeles, California).

Specifically, the initial protocol called for bipolar currents respectively stimulating both RLNs and vagus nerves ("S," in milliamperes) at 10 to 100 Hz in 10 Hz increments (10 measurements). The intensities (milliamperes) were increased until saturation (ie, the amplitude level in millivolts on the graph at which no further increase in EMG CMAPs occurred for each ILM). The intensity of the blocking current ("B," in milliamperes) was then determined by trial and error as the amount necessary and sufficient to reduce S-generated EMG CMAP amplitudes to a flat line. The blocking "B" current (milliamperes) was then linearly reduced to zero by 50 μA decrements until saturation recurred in each and every 3 ILMs. The S/B balance varied from nerve to nerve (Table 1). Since SLN innervation is traditionally believed to be solely directed to the CT, this particular nerve was not submitted to a recruiting envelope. Rather,

TABLE 1. STIMULATION/BLOCKING PARAMETERS

Data Group	*Nerve*	*Firing (mA)*	*Recruitment (mA)*	*Side*
Animal 1				
A	RLN	2.0	2.0	R
B	X	2.0	2.0	R
C	X	2.0	2.0	L
E	SLN	0.5-2.0	N/A	R/L
Animal 2				
A	X	2.8	2.3	R
B	RLN	4.0	4.0	R
C	RLN	4.1	4.0	L
D	X	5.6	4.1	L
Animal 3				
A	X	3.0	3.0	R
B	RLN 10-20 Hz	1.1	1.1	R
	RLN > 30 Hz	2.5	2.5	
C	RLN	2.3	2.3	L
D	X	5.0	5.0	L
Animal 4				
A	RLN	3.4	3.4	R
B	RLN	5.0	5.0	L
C	X	7.0	7.0	L
D	X	4.7	4.7	R
Animal 5				
A	RLN	1.5	3.0	R
B	RLN	1.0	2.0	L
C	X	2.7	3.7	L
D	X	2.3	3.0	R
E	SLN	0.5-2.0	N/A	R

Groups A to D are blinded in terms of nerve-muscle combinations. Group E relates to superior laryngeal nerve (SLN) stimulation.

RLN — recurrent laryngeal nerve, X — vagus nerve (nerve X), N/A — not applicable.

the effects of CT contractions first verified in isolation were applied over a background of overall ILM saturation obtained from either RLN or vagus nerve stimulation. The humidified air output was set to a steady 600 mL/min rate, in the general range followed by other authors[9,10] to produce sound appropriately audible by the human ear.

The chart recorder speed was set at 100 mm/s (except for SLN stimulation, for which it was set at 25 mm/s, since CT ramping did not need to be evaluated). Thus, 1 cm on the graph corresponded to 100 ms in real time. Recording of data for analysis was blinded. The EMG CMAP amplitude peaks (millivolts) were measured with an electronic Mitutoyo caliper (Tokyo, Japan) at 25 ms intervals (40 data points per second). As an example, this rate allowed, within an overall stimulation period of 5 seconds, gathering of 200 data points per stimulation frequency (Fs) run (10 to 100 Hz). The data were then entered into spreadsheets for the purpose of analysis. After completion of the spreadsheets, attention fo-

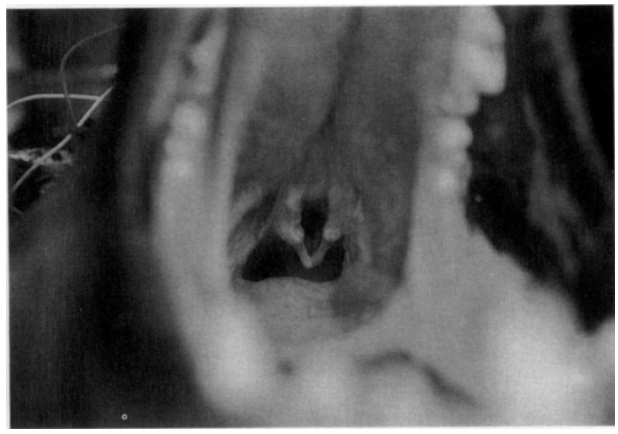

Fig 3. Tongue suspension allows direct glottic view and sound recording without endoscope.

cused on the lowest EMG CMAP amplitude (millivolts) immediately preceding ramping takeoff, as initiated by lifting the blocking current, until saturation returned to original, bipolar stimulation levels. This phase usually lasted 3 seconds, which roughly corresponded to 180 data points for a given stimulation run. With 5 dogs having their RLN and vagus nerve stimulated from 10 to 100 Hz on each side, 200 stimulation runs were planned for orderly recruitment. The data thus collected were lumped into several cohorts comprising individual groupings of CT, TA, and PCA amplitudes, either from RLN or vagus nerve stimulation. The sound envelope corresponding to variations in CMAP EMG amplitudes was recorded via the low-impedance microphone and the CoolEdit program. The sample rate (number of sound samples recorded per second) was set at 4,800 bits per second to provide optimum quality of the audio spectrum.

The data sound curves (N = 50) involving 10 (10 to 100 Hz) frequency measurements (Fs) per animal[1-5] were stored on floppy disk. The data were analyzed by use of time periods (seconds) coincident to EMG ramping, starting with initiation, until saturation. In this manner, only the window of data during the orderly recruitment process was considered for sound analyses. The F0 (hertz) and loudness or intensity (decibels) were calculated from sound curves with PC Quirer. The F0 from fast Fourier transforms within the time windows was calculated at 10 ms intervals by setting PC Quirer for optimal display, considering window length settings (5 to 30 ms), step sizes (5 to 15 ms), frequency tracking threshold (±2%), calculation ranges (50 to 300 Hz), display ranges (50 to 300 Hz), frequency grid displays (20 to 50 Hz), and running time grid (0 to 300 ms). The F0 was graphed to appear as a series of data points superimposed over the original, generic sound curve. Intensity (I; decibels) was calculated by the PC Quirer program every 10 ms.

The times for initiation of ramping EMG CMAP data were then aligned with frequency and intensity values simultaneously recorded from the evoked sound. Graphically, time "zero" was defined as the beginning of EMG CMAP ramping. The derivative of the time envelope was then displayed and quantified for each ILM. The TA, PCA, and CT ramping slopes were grouped in 2 cohorts depending on the stimulated nerve (RLN or vagus) as TARLN and TAX, PCARLN and PCAX, and CTRLN and CTX. These data were then individually plotted against evoked F0 and I to achieve 3-dimensional curves.

Repeated-measures ANOVA was used to determine differences over the range of stimulation applied. If significant differences existed, pairwise comparison of muscles was performed and p values were adjusted by Bonferroni's method. Next, the relationship between the 3 muscles and 2 nerves with both changes in intensity and frequency was evaluated with repeated-measures ANOVA. To determine the amount of variability accounted for by these factors, R2 analyses, the percentage of variability explained, were computed. R2 is a measure of the percentage of variation accounted for solely by the statistical model. To contrast p values and R2, the significance of the p values indicate 95% certainty that certain nerves and muscles are related to the measurements, and the R2 values assess the degree to which they explain the outcomes. The remaining unexplained variation may be due to uncontrollable experimental design factors, or perhaps other physiological parameters yet to be examined such as "nerve" and "muscle" data for intra-dog or inter-dog variability. The R2 values do not indicate the significance that is found with p values, but only that a certain percentage of the variability has yet to be quantified. All calculations were performed with SAS version 8 software (SAS Institute Inc, Cary, North Carolina).

At the completion of these experiments, the animals were painlessly sacrificed with intravenous Pentothal.

RESULTS

With the exception of a total lack of response on one side following RLN stimulation in dog 1, all EMG CMAPs were evaluable and verified on the video monitor (Fig 3). Stimulation/blocking (S/B) parameters are shown in Table 1. Of 19 nerve-ILM sides addressed, a total of 570 EMG sheets were created and distributed as follows: 9 RLN-ILM and 10 vagus-ILM cohorts. The groups of experiments thus totaled 57 (19 × 3) for all 5 dogs. Since, however, only 10 to 60 Hz (6 Fs groups) could be retained for analysis because of poor ramping differentials above 60

TABLE 2. MULTIVARIATE ANALYSIS OF VARIANCE RESULTS FOR EMG AMPLITUDE RAMPINGS

Variable	*p*
Time since blockage	<.001
Muscle	.002
Nerve	<.001
Stimulating frequency	<.001
Nerve-muscle interaction	<.001
Muscle–stimulating frequency interaction	<.001

EMG — electromyographic.

Hz, the number of evaluable data sheets totaled only 162 ($9 \times 3 \times 6$). They further comprised 4 data points per 100 ms for an arbitrarily designated time interval of 10 seconds, ie, a total of 64,800 data points for RLN-ILMs, and ($10 \times 3 \times 6$) or 180 sheets and a total (with also 400 data points) of 72,000 data points for vagus-ILMs. These numbers were further reduced as ramping analysis specifically focused on the 3-second intervals separating lifting of blockage from saturation. This setup ultimately resulted in an envelope of 120 data points per ILM per stimulating frequency for the 3 seconds (ie, 19,440 for RLN-ILMs) distributed as 120 EMG peak measurements (4 per 100 ms for 3 seconds) on 6 frequencies (10 to 60 Hz) for 9 runs, and 21,600 for vagus-ILMs in which all the nerves (10 runs) were productively stimulated. These multiple measures increased the power of the study design.

The ramping of individual nerve-muscles as expressed after lifting of the blocking currents was highly specific to each group. Four variables were evaluated for each experiment by repeated-measures ANOVA for their effect on EMG recruitment slopes (Table 2). They were 1) muscle types (TA, PCA, CT); 2) nerve types (RLN, X); 3) stimulation frequencies (Fs, 10 to 60 Hz); and 4) ramping windows (0 to 3,000 ms). Analyses indicated that the recruitment slopes were independently and significantly affected by all of those variables. The effect of Fs on EMG ramping was significantly different for each ILM (TA, PCA, CT; $p < .001$) and independent of stimulation frequencies (Tables 2 and 3).

On the basis of these results, ILM muscle types were compared for each combination of stimulating frequencies and nerve types used (Table 4). When the RLN was stimulated with 10 Hz currents, the PCA recruitment slopes were significantly lower than those displayed by both the TA and the CT. With 10 to 30 Hz, the TA recruitment curves were significantly steeper than those for the PCA, but in the range of 40 to 50 Hz, the CT had significantly higher recruitment rates than the other ILMs. For the vagus nerve, no significant differences were observed between the 3 muscles under 10 to 20 Hz Fs (Table 5 and Figs 4 and 5). However, for 30 Hz or higher, vagal stimulation produced significantly greater recruitment rates for the PCA as compared to the CT and TA. The PCA's higher recruitment rates produced under vagal stimulation must, therefore, be contrasted with the low progression figures obtained with the RLN.

The amount of CT ramping was the only significant variable related to a change in intensity ($p = .03$; Table 5). The relationship is best illustrated in Fig 5, which shows that no other ILMs were involved. The CT accounts for 33% (R2) of the variability of intensity; therefore, 67% is created by other sources, such as inter-dog and intra-dog variability or possibly variable proportions of dominant motor units within the ILMs. Variability in response may also be understood as reflecting trends falling, by nature, short of complete architectural and histochemical homogeneity.

The average F0 produced was significantly related to the stimulated nerve ($p = .02$). The F0 was higher for the RLN than for the vagus nerve (average, 209.8 ± 14.9 Hz versus 148.6 ± 18.2 Hz). Since the R2 for this model was 55%, more than half of the variability in frequency could be explained by which nerve was stimulated. We also found that the F0 was significantly correlated with the difference in recruitment between the TA and the CT ($p = .003$). When the former muscle recruited faster than its counterpart, higher F0 values (200.0 ± 73.3 Hz) were re-

TABLE 3. DIFFERENCES IN EMG AMPLITUDE RAMPING BASED ON NERVE AND STIMULATING FREQUENCY

	Vagus Nerve		*Recurrent Laryngeal Nerve*	
Stimulating Frequency (Hz)	*Differences Between Muscles (p)*	*Pairwise Muscle Differences*	*Differences Between Muscles (p)*	*Pairwise Muscle Differences*
10	.37	No differences	<.001	TA > PCA; CT > PCA
20	.33	No differences	<.001	TA > PCA; TA > CT
30	.005	PCA > TA; PCA > CT	.005	TA > PCA
40	.001	PCA > TA	.008	CT > PCA
50	<.001	PCA > TA; PCA > CT	.004	CT > PCA; CT > TA
60	<.001	PCA > TA; PCA > CT	.003	PCA > TA; PCA > CT

CT — cricothyroideus, TA — thyroarytenoideus, PCA — posterior cricoarytenoideus.

TABLE 4. COMPARISONS OF RECRUITMENT SLOPES ACCORDING TO STIMULATED NERVES

		Pairwise p Values		
Muscle	*Mean Slope*	*CT*	*TA*	*PCA*
RLN				
CT	0.057		.225	.162
TA	0.031	.225		.786
PCA	0.026	.162	.786	
X				
CT	0.044		.716	.048*
TA	0.039	.716		.019*
PCA	0.073	.048*		.019*

CT — cricothyroideus, TA — thyroarytenoideus, PCA — posterior cricoarytenoideus.
*Statistically significant.

TABLE 5. COMPARISONS OF SLOPES FOR VAGUS NERVE AT HIGH AND LOW FREQUENCIES

		Pairwise p Values		
Muscle	*Mean Slope*	*CT*	*TA*	*PCA*
Low frequencies				
CT	.044		.770	.722
TA	.040	.770		.937
PCA	.040	.722	.937	
High frequencies				
CT	.044		.794	.007*
TA	.038	.794		.003*
PCA	.111	.007*	.003*	

*Statistically significant.

corded as compared to when the reverse (CT > TA) was true (164.4 ± 54.8 Hz). Since overall TA ramping exceeds that of the CT when the RLN is stimulated and there are no significant differences between the muscles with the vagus nerve, the RLN can be considered as instrumental in effecting F0 changes (Table 5).

We next investigated whether the role of the PCA was also related to frequency (F0 in hertz) and intensity (decibels), as compared to other muscles. We did find a trend toward decreased loudness for vagus stimulation when PCA recruitment rates were greater than CT recruitment rates (p = .07). As we have observed, when the vagus nerve is used, the recruitment rate for the PCA tends to be greater than that for either the CT or the TA. Therefore, the resulting lower intensity when the vagus nerve is used may be created by the recruitment rate of the PCA exceeding that of the other muscles (Table 6). Sound pressure level (normalized), intensity (decibels), and F0 (hertz) curves are depicted in Figs 6 and 7.

Although isolated SLN stimulation was illustrated by lone vocal fold elongation, no changes in the sound envelope occurred. By contrast, both frequency (F0) and intensity (decibels) were markedly boosted with SLN stimulation over prior ILM saturation levels via either the RLN or the vagus nerve. The resultant sound pressure increases were so high that their maxima were off the chart.

DISCUSSION

This research constitutes the first attempt to separately define the muscular interactions of the inde-

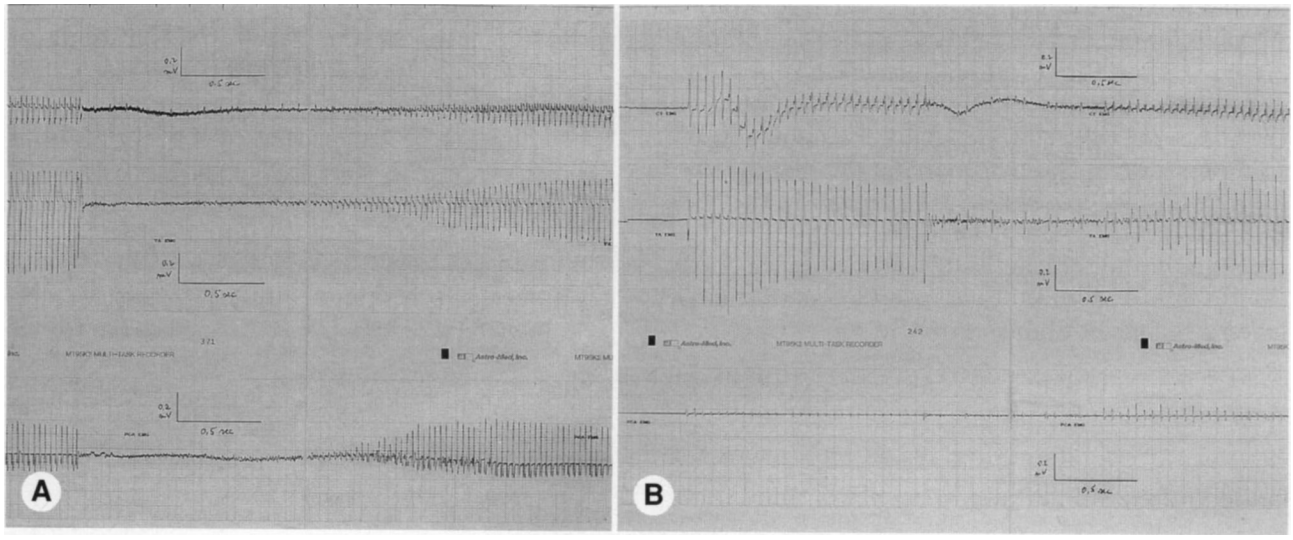

Fig 4. EMG CMAP tracings (in millivolts) of (top to bottom) CT, TA, and PCA for **A)** RLN and **B)** nerve X stimulation/blocking envelopes. Stimulation via perineural electrode in bipolar mode (30 Hz, 2 mA for RLN; 20 Hz, 2.1 mA for nerve X) produces maximal amplitudes (saturation, left side of picture) in all 3 intrinsic laryngeal muscles. Superimposed anodal block (600 Hz, 2 mA for RLN; 600 Hz, 2.1 mA for nerve X) arrests neural conduction, resulting in flat EMG configuration. Progressive lifting of blocking charges (2 to 0 mA for RLN; 2.1 to 0 mA for nerve X) allows return of EMG CMAPs to their original saturation levels. Note that recruitment rates vary from muscle to muscle (differential rampings) and between stimulated nerves. Paper speed is 100 mm/s. Calibration is in millivolts.

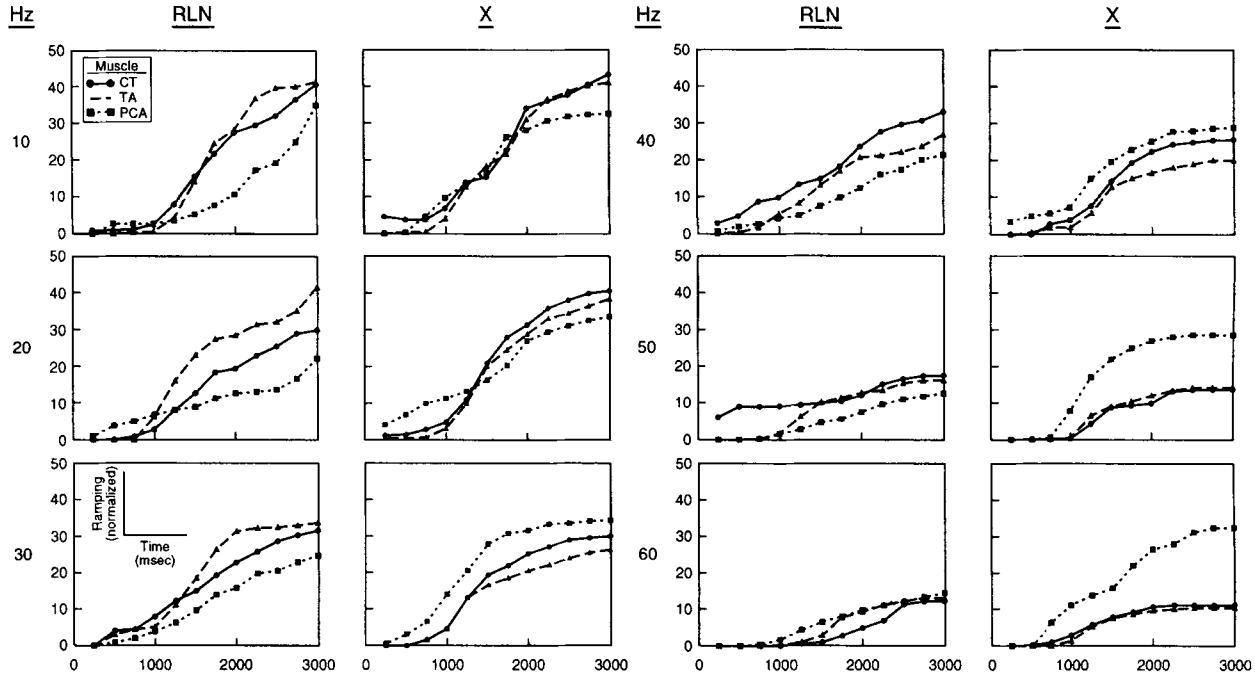

Fig 5. EMG CMAP progressions (normalized values, abscissa) over time (milliseconds, ordinate) for 10 to 60 Hz. When RLN is stimulated (stimulation/blocking), TA ramping is highest up to 30 Hz, and CT predominates for 40 to 50 Hz. PCA is lowest for all frequencies except 60 Hz. Conversely, this muscle's recruitment is highest with nerve X stimulation (stimulation/blocking) under 20 to 60 Hz. CT and TA follow grossly identical progressions under those conditions.

pendent anatomicophysiological units involved in voice production via artificially induced recruitment strategies. While this approach may not necessarily allow extrapolation of canine data to humans, there currently appears to be no clearer approach to securing effective laryngeal control.

Muscles are specialized biological machines that fulfill specific task-oriented functions dependent upon a number of characteristics such as vectorial anatomy, mass, stiffness, interactions with companion effectors, distinct motor neuron architectures, etc. Encasement within a tight fibrocartilaginous capsule makes ILM and related neurologic pathway contributions to voice production especially cumbersome. Laryngeal mutually exclusive functions have traditionally been thought to be supported by the RLN, if SLN input to the CT (which is arguably of an extrinsic nature) is set aside, but both nerves carry afferent and efferent fibers to both nerves within the vagus. Fortunately, voice may be conveniently defined by its F0 and intensity (I). Such numerical interface for voice represents an opportunity compared to other laryngeal functions of earlier phylogenetic appearance (eg, swallowing) that may not be so conveniently defined.

The current protocol involved RLN, vagus, and SLN stimulation, since the 3 nerves collectively channel all afferent and efferent fibers to and from the larynx. Whereas the SLN is specific to the CT, anatomically distinct nerve bundles within the RLN are not sufficiently defined, until late division close to

TABLE 6. RELATIONSHIP WITH INTENSITY AND FREQUENCY

	p Values	
Variable	*Changes in Intensity*	*Average F0*
Stimulating frequency	.41	.10
Nerve (RLN versus X)	.54	.02*
PCA EMG results	.96	.65
CT EMG results	.03*	.12
TA EMG results	.87	.83

F0 — fundamental frequency.
*Statistically significant.

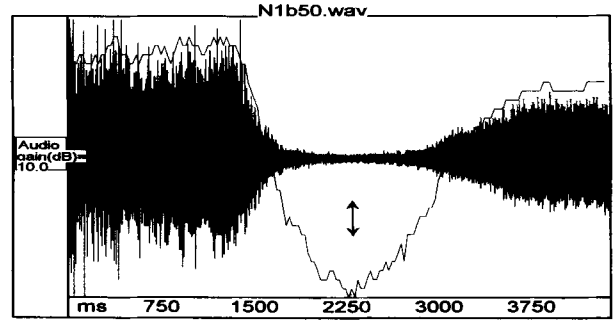

Fig 6. Maximal sound pressure levels (normalized) and intensity (decibels) curves recorded during standard bipolar stimulation (50 Hz, 2 mA, left side) give way, respectively, to flat line and drop (arrows) with 600 Hz anodal blockage (2 to 0 mA). Progressive lifting of blocking currents allows controlled return toward original values with time (abscissa, milliseconds), as seen on right side. Paper speed is 100 mm/s.

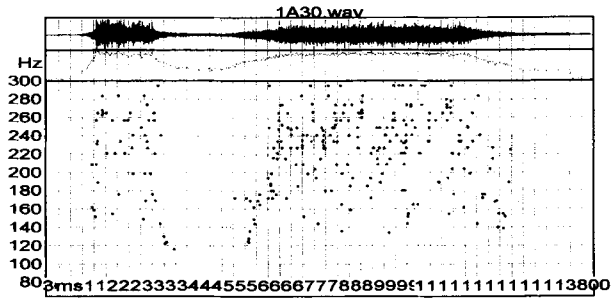

Fig 7. Fundamental frequencies (dots, hertz in ordinate) of sound envelope appearing in upper section for 30 Hz bipolar stimulation and 600 Hz anodal blockage of 2 to 0 mA (see also Fig 6). Lower paper speed (25 mm/s) precludes more explicit display of time (milliseconds, abscissa) on graph.

or within the larynx,[25] to allow selective ILM stimulation. Also, the precise intralaryngeal neurofiber distribution is not entirely known.[26] Therefore, ILMs were targeted to power the variable glottic resistor via specific orderly recruitment according to the pioneering contributions of Zhou et al[22] and Baratta et al[23] in the feline hind-limb musculature.

The significant differences in ramping values found in our earlier study with RLN stimulation[12] are confirmed in the current study. Not surprisingly, data were equally significant when the vagus nerve was involved (Fig 5). Armed with these patterns of independent ILM behavior, we could proceed to the second stage of the current research, allowing ramping EMG CMAPs to be plotted against sound data (F0 and I) from the acoustic spectrum. Our setup did not allow recording EMG CMAPs and sound curves on the same graph, and starting times had to be manually adjusted. However, potential discrepancies remained small and inconsequential because of the large number of data points, and potential flaws associated with raw intersubject or experiment variations were avoided, because EMG amplitude comparisons involved series of ratios.

A detailed discussion of the clinical aspects of human voice production is clearly beyond the scope of this research. To avoid adding variables to an already complex analysis, we have deliberately ignored the importance of subglottic pressure variations in the genesis of sound, either experimental or voluntarily induced. Additional concerns about the true sum of multivectorial forces within individual ILMs[27-30] should also be eventually considered.

Although nearly all ILM contractions, including those of the interarytenoid muscles,[11,31] boost F0, the increase is generally considered to be a result of unique cooperation between the TA and the CT.[5,31,32] Conversely, whereas it has been debated whether a low F0 may be explained on the basis of an individual decrease in CT contraction,[33,34] the TA could either raise or lower the frequency, depending on CT tension levels.[5] Active TA tension generates increases (as we showed; Fig 5) as long as CT contraction levels allow the amplitude of vocal fold vibration to be sufficiently large. Conversely, increased CT activity lowers the frequencies, because resulting tension in the vocal folds[27] outweighs the small gains the TA may achieve.[35] The resulting voice modulation[3,5] may thus compare with interactions between the TA and other ILMs, such as the LCA, for swallowing,[11] even though the outputs of this most effective adductor and the fine-tuned TA do not necessarily coincide nor follow similar timing schedules.[36]

There has been surprisingly little written on the PCA's contribution to voice production, possibly because of its indirect role. Although vocal fold abduction from isolated stimulation of the PCA via its terminal branch not unexpectedly results in frequency and intensity drops,[7] the pivotal function of this muscle is mutual balance with its antagonist, the TA. This balance helps stabilize the cricoarytenoid joint, while also opposing increased adduction from a strong CT pull, thus bracing the arytenoids and further stiffening the vocal folds.[3,5,7,13]

Our findings of high TA ramping rates from RLN stimulation (at least up to 30 Hz) come as no surprise, since the muscle is predominantly composed of small motor units with fast-contracting myofibers. Conversely, the PCA's lower recruitment rates observed under similar experimental conditions are related to prevailing slow motor units. Interestingly, these progressions are inverse when the vagus nerve is stimulated. On the basis of these observations, mutual reciprocal coupling could follow 2 modalities. First, an antagonistic RLN-mediated, TA-bound "strong" adduction balances against PCA-bound "weak" abduction; an alternate view is that the "strong" vagus-activated, PCA-bound abduction balances against the TA's relative "weakness." This idea is further supported by the presence of larger contingents of adductory fibers in the RLN as compared to the vagus nerve, in which abductory pathways would predominate. Phylogeny further suggests, at least for voice, a more ancient role for vagus-mediated laryngeal abduction (via the PCA, primed to counter air hunger), as compared to RLN-mediated (less vital) adduction via the TA.

The F0 increases observed in this study depend on CT-TA coupling when the RLN is stimulated. Such a relationship is intriguing, considering that in contrast to the TA, the arguably extrinsic CT is solely innervated by the SLN.[34] Whatever the responsible mechanism (eg, increase in subglottic pressure regard-

less of experimental conditions of constant flow[14]), CT activation under RLN stimulation could be explained by linkage within the endolarynx via the ansa Galeni, intramuscular terminal branching patterns,[26,37,38] reflexogenic pathways originating in ILM proprioceptors,[39] or an antidromic route to the vagus nerve. The nonsignificant trend in loudness decrease related to PCA contraction only when the vagus nerve (but not the RLN) was stimulated suggests the presence of abductory fibers within the vagus nerve. Obviously, conduction studies would help further elucidate this matter.

On the basis of our data, we submit that voice manipulation may become possible in the future. Levels of loudness may be controlled by RLN stimulation to achieve CT ramping, coupled with reciprocal curbing of PCA activities through the vagus nerve. For F0 boosting, the TA may be instrumental, as long as the CT does not take over, but the moderator functions of the PCA still assume its recognized reciprocal function. In all cases, however, direct SLN stimulation increases F0 and I across the board, assuming that prior tension is present in the other ILMs.

CONCLUSION

This research has correlated voice frequency and intensity in a canine model with recruitment patterns of key ILMs stimulated under conditions of subglottic insufflation. The ramping levels differed from muscle to muscle and also varied depending on whether the RLN or the vagus nerve was stimulated. The TA was found to be instrumental in F0 modeling at low intensity levels when balanced with the PCA under vagus nerve control. The CT enhances both F0 and I, ideally through TA-PCA coupling, a cooperation also observed for fine tuning at lower intensities. This study offers the first statistically significant analysis of laryngeal function in vivo under conditions of variable recruitment of motor units. The technique may further allow electronic manipulation of voice for neurologic conditions that fall short of complete laryngeal paralysis.

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