Dynamics of Intrinsic Laryngeal Muscle Contraction

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Objectives: Laryngeal function requires neuromuscular activation of the intrinsic laryngeal muscles (ILMs). Rapid activation of the ILMs occurs in cough, laughter, and voice-unvoiced-voiced segments in speech and singing. Abnormal activation is observed in hyperfunctional disorders such as vocal tremor and dystonia. In this study, we evaluate the dynamics of ILM contraction.

Study/Design: Basic science study in an in vivo canine model.

Methods: The following ILMs were stimulated: thyroarytenoid (TA), lateral cricoarytenoid/interarytenoid (LCA/IA), cricothyroid (CT), all laryngeal adductors (LCA/IA/TA), and the posterior cricoarytenoid (PCA). Neuromuscular stimulation was performed via the respective nerves at current levels needed to achieve maximum vocal fold posture change. Muscle contraction and posture changes were recorded with high speed video (HSV). HSV frames were then analyzed to measure response times required from the onset of muscle contraction to the time the vocal folds achieved maximum posture change.

Results: In all muscles, the onset of posture change occurred within 10 to 12 milliseconds after neuromuscular stimulation. The average times (± standard deviation) to achieve final posture were as follows: TA 34.5 ± 6 ms (N = 15), LCA/IA 55 ± 12 ms (N = 14), recurrent laryngeal nerve 43 ± 8 ms (N = 18), CT 100.8 ± 17 ms (N = 26), and PCA 91.2 ± 8 ms (N = 3). Data distribution appeared normal.

Conclusion: Results showed a difference in muscle activation time between different ILMs consistent with reported differences in muscle fiber composition. These data also provide an estimate of the limits of laryngeal contraction frequency in physiologic and pathologic laryngeal states.

Key Words: Intrinsic laryngeal muscles, in vivo canine, muscle activation times, prephonatory posture.

Level of Evidence: NA

INTRODUCTION

Activation of the intrinsic laryngeal muscles (ILMs) is essential in nearly all laryngeal functions, including voice production, airway protection, respiration, and effective cough reflex. Many neurolaryngologic pathologies such as vocal tremor, spasmodic dysphonia, and laryngospasm result in hyperfunctional movements of the laryngeal muscles. Thus, measurement of ILM dynamics is useful to understand neuromuscular function and the limitations in normal physiology as well as pathologic states with laryngeal hyperfunction.

The ILMs consist of four paired muscles and one unpaired muscle. Each muscle has a unique trajectory and role in shaping the glottis for various laryngeal functions.[1,2] Previous investigations of ILM function have primarily focused on ILM role in voice production and in the control of laryngeal posture, but the dynamics of ILM movement has received little attention. Laryngeal muscle dynamics reflect the muscle fiber composition, which is reported as a percentage of type 1 (slow) and type 2 (fast) fibers (Table I).[6,8,22–30]

Laryngeal adductor muscles include the paired thyroarytenoid (TA) and lateral cricoarytenoid (LCA) and the unpaired interarytenoid (IA). The TA moves the vocal process medially and inferiorly during vocal fold adduction.[3] The TA is composed primarily of fast-twitch type II fibers. The fast isoforms (types IIA and IIX) compose 53.8% of the TA.[4] In our previous investigation on ILM function using an in vivo canine neuromuscular model, the onset of TA activation was noted as an inferomedial bulging of the vocal fold around 14 milliseconds (ms) after nerve stimulation. The midmembranous region was near fully adducted by 22 ms, and the entire membranous fold adducted by 47 ms.[5] The LCA is composed primarily of type IIB fibers.[6] Much like the TA, the LCA exhibits high succinic dehydrogenase and low NADH2 tetrazolium reductase, suggesting greater fatigue resistance than limb muscle phenotypes.[6] LCA/IA activation was noted to begin adducting the posterosuperior vocal fold by 16 to 22 ms and achieved maximal posture change by 47 ms.[5]

The paired posterior cricoarytenoid (PCA) muscle abducts the vocal fold by moving the vocal process laterally and superiorly.[7] In the human PCA, the vertical belly

Muscle Fiber Type Composition for Each Intrinsic Laryngeal Muscle

Muscle	Animal	Type 1 (%)	Type 2 (%)	Reference No.
PCA	Human	52–67	37–48	25–27
	Vertical belly	61	36–44.7	8,24
	Horizontal belly	75	23–28.3	8,24
TA	Human	26–53.3	46.6–65	6,23,24,26
	Canine	5–11.36	95	22,27
	Feline	9.2	90.8	30
CT	Human	38.3–47	61.6	6,25–27
	Canine	40–45	60	22
	Feline	44.6	55.4	30
LCA	Human	41.6	58.3	26
	Canine	16.3	83.7	27–29
	Feline	11.4	88.6	30

Percentage of type 1 (slow) and type 2 (fast) muscle fiber types is shown for each ILM in humans, canines and felines. References provided.

CT = cricothyroid; ILM = intrinsic laryngeal muscle; LCA = lateral cricoarytenoid; PCA = posterior cricoarytenoid; TA = thyroarytenoid.

is composed of 61% type I (slow-twitch) and 36% type II (fast-twitch) fibers, compared to 75% and 23%, respectively, in the horizontal belly.[8] The slight difference in fiber composition is consistent with the sphincter function of the vertical belly in phonation and swallowing, which requires a relatively short response time; and the more dominant role in respiration of the horizontal belly, which is active during inspiration.[8] During in vivo neuromuscular stimulation of a canine larynx, the PCA has a slow time course of activation, with changes first appreciable by 16 to 33 ms and maximum posture change achieved by 100 ms.[5] The cricothyroid (CT) muscle is composed of two bellies, the pars recta and pars oblique. Together these two components pull the anterior part of the cricoid cartilage backward and upward to lengthen vocal fold, constrict the glottis, and dilate the pharynx.[3,9–11] If the vocal folds are already adducted, the CT can be activated to increase vocal fold tension leading to increased pitch.[3,6] In ex vivo human larynges, the CT is composed of fast MyHC isoforms (i.e., IIA, IIB, and IIX) and type I and beta slow fibers.[4,6] Changes in glottal posture from CT activation in the in vivo canine model are first appreciable by 16 to 33 ms, with maximum posture change by 100 ms.[5]

It is generally understood that the TA and the LCA muscles are the fastest ILMs, whereas the CT and PCA are the slowest. However, the variability of ILM dynamics between different larynges is unknown. Thus, we investigated ILM dynamics across 26 unique vocal folds (left and right) from 15 canine subjects. We evaluated the activation times for TA, LCA/IA, CT, PCA, and the combined laryngeal adductors (recurrent laryngeal nerve [RLN], TA/LCA/IA). Although we expect to find the TA and LCA/IA to be faster muscles compared to the PCA and CT within larynges, we hypothesize that there will be some variability of muscle dynamics between larynges that reflects the reported variability in fiber type ratios among ILMs. We also seek to better understand the

biomechanics and limits of laryngeal muscle activation as it pertains to both normal and pathologic laryngeal function.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at the University of California, Los Angeles. An in vivo canine selective laryngeal activation model was utilized as described previously.[12,13] Data was collected from 15 Mongrel canines prospectively. All ILMs from each larynx could not be evaluated because each canine experiment had a unique focus on a subset of muscles. As such, the number of unique vocal folds analyzed for each muscle varied. There were 15 unique vocal folds for TA, 14 for LCA, 18 for the combined adductors (RLN), 26 for CT, and three for PCA.

Surgical exposure of the larynges and laryngeal nerves was performed as previously described.[12,13] Surgery was performed by the same surgeon in all cases.[12,13] In brief, under general endotracheal anesthesia a vertical midline incision was made in the neck to expose the larynx and trachea. A low tracheostomy was then performed and used for ventilation. The RLN was identified in the neck; followed distally; and the nerve branches to the TA, LCA/IA, and PCA were identified. The external branch of the superior laryngeal nerve (SLN) was identified close to its insertion to the CT muscle. An infrahyoid pharyngotomy was performed, and the larynx was exteriorized in the neck for high-speed video recording. A supraglottic laryngectomy was also performed to fully expose and optimize the superior view of the true vocal folds. India ink was used to mark 0.5 mm flesh points on the superior surface of the vocal folds in three locations: the vocal process, midmembranous vocal fold, and just posterior to the anterior commissure.

ILMs were activated as follows: to activate the TA, the TA branch was divided from the RLN and the distal stump (TA branch) stimulated using a cuff electrode. The LCA/IA was stimulated together because the IA branch is small and exposing it can damage surrounding muscles and nerves. To stimulate the LCA/IA, the TA and PCA branches were divided and the intact RLN was stimulated. To simulate all adductors (TA/LCA/IA), the PCA branch was divided and the RLN was stimulated. The location of RLN stimulation in each experiment was consistent at about 2 cm caudal to the cricopharyngeus muscle. To stimulate the CT muscle, the external branch of the superior laryngeal nerve (eSLN) was stimulated. The internal branch of the SLN was always divided during the larynx exteriorization. The location of eSLN stimulation was also consistent in all experiments at about 2 cm from the insertion of the nerve to the CT muscle. To stimulate the PCA muscle, the RLN branch to the PCA was identified and the distal stump (PCA branch) was stimulated.

Neuromuscular stimulation of each ILM was performed for 1,500 ms with uniform 0.1 ms cathodic pulses at 100 Hz. The lowest stimulation level that achieved the maximal posture change was selected for analysis of muscle dynamics for this study. This stimulation level was determined by gradually increasing the stimulation current from threshold muscle activation, for which just a hint of muscle movement was seen, to maximal activation, for which no additional posture change to stimulation was seen. Each 1,500 ms pulse train was followed by a 3.5 second pause to allow for muscle recovery. From our previous experience, we needed about 8 to 10 steps of increasing stimulation current to reach the level that led to maximal posture change. Vocal fold motion and deformation during neuromuscular stimulation was captured with a high-speed digital camera at 3 thousand frames per second at a resolution of 512 × 512 pixels (Phantom v210, Vision Research Inc., Wayne, NJ).
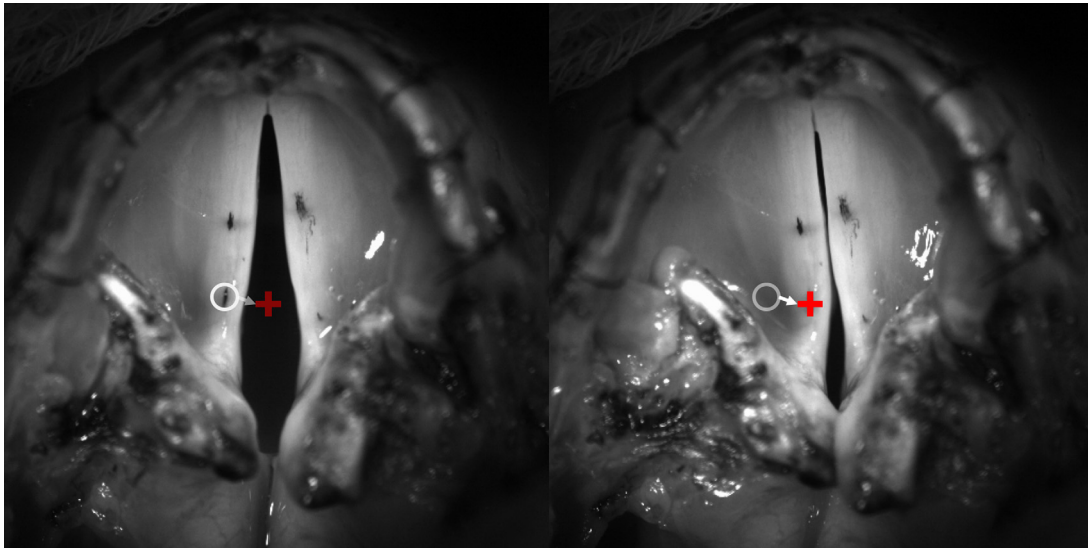
Fig. 1. Experimental glottal view of vocal fold adduction. Superior view of glottis with India ink flesh points seen on the left and right mid- and posterior vocal folds. Left frame is resting posture just at the onset of left lateral cricoarytenoid stimulation. Right frame is final fully activated posture showing full adduction of the left vocal fold at the vocal process. Open white circle denotes point of reference along the posterior vocal fold at rest just before motion onset. White arrow denotes trajectory of vocal fold adduction. Red + denotes final adducted posture. The time it takes to go from the white circle to the red + is taken as the activation time.

Movement of the India ink landmark was used to assess the onset and completion of all visible vocal fold motion and posture change from muscle activation. Onset time for measurement of muscle dynamics was marked at the first visible sign of movement of the ink mark. The ink mark at the vocal process was tracked for assessment of PCA, LCA/IA, all adductors (RLN), and CT muscle dynamics; and the mark at the midmembranous vocal fold was tracked for TA muscle dynamics. Measurement of the duration of postural change was assessed manually using Phantom Camera Control Application software, version 1.3 (Vision Research Inc.), which allows frame-by-frame analysis of landmark trajectory and measurement of distance traveled. The muscle activation time, defined as the time required for displacement from onset of movement to final maximum posture, was calculated based on the number of video frames needed to travel this distance (three video frames per ms) (Figure 1).

Statistical analysis included one-way analysis of variance (ANOVA) and post hoc least significant differences (LSD) tests to compare across ILMs, Students t test to compare left and right sides for a given ILM, measurement of kurtosis, and the nonparametric one-sample Kolmogorov-Smirnov test to evaluate the distribution of activation times for each ILM. This was performed using SPSS Statistics, version 24 (IBM Corp., Armonk, NY).

RESULTS

In all muscles, the onset of posture change (vocal fold movement) consistently occurred within 10 to 12 ms after the start of neuromuscular stimulation. Table II details the muscle activation times for all larynges for each muscle group both left and right sided. The average muscle activation times (± standard deviation) to achieve final prephonatory posture were as follows: TA 34.5 ± 6 ms, LCA/IA 55 ± 12 ms, RLN 43 ± 8 ms, CT 100.8 ± 17 ms, and PCA 91.2 ± 8 ms. Figure 2 plots the muscle activation times for all larynges for each muscle group to provide an idea of the data distribution. Furthermore, the kurtosis was computed for each muscle group to get an

idea of normalcy of the data distribution. The kurtosis was near zero for TA, LCA/IA, and CT, whereas the RLN demonstrated a more positive kurtosis and thus a more peaked distribution (RLN kurtosis of 2.47). Kolmogorov-Smirnov testing further demonstrated that all ILMs exhibited a uniform distribution, with exception of the combined adductors. Students t tests were performed to compare left- versus right-sided muscles for each ILM. Left versus right TA, LCA/IA, and RLN were not significantly different, whereas left- versus right-sided CT muscle activation times differed significantly ($P = 0.012$). One-way ANOVA confirmed a significant intergroup difference among ILMs, with post hoc LSD tests demonstrating a significant difference of the means of all ILMs except when comparing the CT and PCA muscle groups ($P = 0.20$).

DISCUSSION

In this study, we evaluated ILM dynamics over a large number of canine larynges. As expected, the TA and LCA/IA had fast activation times of around 50 ms or less, whereas the CT and PCA had activation times of around 100 ms. This is also consistent with our previous findings from ILM activation in an in vivo canine hemilarynx model.[5,14] As detailed in Table I, these stark differences in activation times are related to differences in muscle fiber type composition, with the laryngeal adductors having a greater percentage of type 2 (fast) fibers. We now demonstrate that there also is interlaryngeal variation within each muscle, as illustrated in Figure 2. This is consistent with fiber type ratio variations within muscle groups between larynges as well. The phonetogram, or voice range profile, of human individuals may vary greatly depending on age, sex, time of day, experience, respiratory volume, mouth opening, medical treatment,

TABLE II.
Average Time From Onset of Vocal Fold Movement to Achieving Final Posture

N	TA	LCA/IA	RLN	CT	PCA
1	23.7	43	42	85.7	90
2	44.3	70	41	96	83.7
3	30.7	59.3	34	97	100
4	36	61	34	100	
5	33.7	62.7	43	103.3	
6	35.3	45.7	32.7	114.7	
7	29.3	69	49	85	
8	31	32.7	42.7	98.3	
9	33.3	58.7	42.3	113	
10	32	63.3	42	76.3	
11	32	37.7	47.7	100	
12	42	46.3	37.3	65.7	
13	43.7	59.7	37.7	91	
14	31	61.3	41.7	76.7	
15	39.7		42.3	120	
16			44	94.7	
17			64	121	
18			55.7	113.3	
19				129.7	
20				74.7	
21				115.7	
22				115.7	
23				82.7	
24				110.7	
25				122.7	
26				116.3	
Average (ms)	34.5	55	43	100.8	91.2
SD	6	12	8	17	8

All times are in milliseconds.
CT = cricothyroid; IA = interarytenoid; LCA = lateral cricoarytenoid; ms = millisecond; PCA = posterior cricoarytenoid; RLN = recurrent laryngeal nerve; SD = standard deviation; TA = thyroarytenoid.

abduction cycle on average is about 8 Hz. Note that this estimation of 8 Hz is based on one cycle of full adduction and full abduction, which is not often found in most laryngeal tasks except cough. Thus, the possible frequency for tasks requiring limited excursion of adduction/abduction could be significantly higher.

However, there are always additional biomechanical constraints for a given muscle motion task beyond muscular contraction. In voice and speech production, the physiologic constraints for vocal fold motion include the time to initiate joint motion from rest, directional constraints of joint mobility, and tissue viscoelasticity because the entire vocal fold needs to move regardless of the ILM activated. Vocal fold motion also requires neuromuscular coordination of agonist–antagonist muscles; that is, when adductor muscles contract, the abductor muscle has to relax and stretch and vice versa. For a given vocalization task, stabilizing the prephonatory posture to ensure the correct posture before phonation also requires time. Finally, muscles have to adjust posture along a continuum (one posture is the direct product of the preceding posture) and cannot change position in a discontinuous fashion. Literature on appendicular skeletal muscle tells us that muscle performance may be tied to preactivation length and/or joint angle, as has been shown with the hamstring muscles.[20,21]

In addition, control of the laryngeal muscles is complex. The primary motor neurons controlling the intrinsic laryngeal muscles are located in the nucleus ambiguous (NA). The NA receives both excitatory and inhibitory input from the brainstem central pattern generators controlling respiration, cough, and swallowing. For volitional movement, such as for phonation, there is direct innervation from the cerebral cortex. For emotional vocalizations,

and many other potential independent variables.[15,16] Variations in individual muscle activation times, as we capture here, may further contribute to variability in voice production across subjects.

Many laryngeal tasks require rapid adduction/abduction of ILMs. These include glottal stops (e.g., *uh-oh!*), voice-unvoiced-voiced segments in speech, laughing, and coughing. Neurologic vocal dysfunctions such as vocal tremor also manifest as rapid rhythmic activation of the ILMs. Laughter is a series of repeated vocalizations during exhalation, requiring rapid adduction followed by relaxation of the vocal folds. Interestingly, the typical frequency of both vocal tremor and laughter is about 5 to 6 Hz.[17–19] In the present study, we find that the average combined activation times of all laryngeal adductors (44 ms) and the PCA (91 ms) is 131 ms. If we consider the TA (35 ms) and the PCA, this cumulative activation time is 127 ms on average, or about 8 Hz, and well within the findings in tremor and laughter. One could even surmise that the limits for the frequency of an adduction/
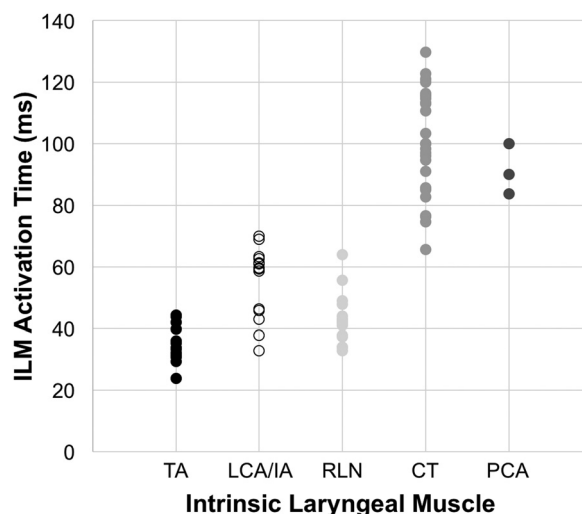

Fig. 2. Intrinsic laryngeal muscle activation times. Activation times plotted for each muscle for each subject provides an idea of the data distribution. Post hoc least significant difference test demonstrated significant differences of the means of all ILMs except when comparing CT and PCA muscle groups ($P = 0.20$). CT = cricothyroid; IA = interarytenoid; ILM = intrinsic laryngeal muscle; LCA = lateral cricoarytenoid; PCA = posterior cricoarytenoid; RLN = recurrent laryngeal nerve; TA = thyroarytenoid.

there are additional connections from the limbic system. Therefore, despite the rapidity at which the laryngeal adductors and abductors can contract in relative isolation, the maximum frequency at which these motions can be coordinated is limited by these biomechanical and neurophysiologic constraints.

CONCLUSION

The intrinsic laryngeal muscles have unique muscle fiber type compositions that contribute to their dynamics. The laryngeal adductors have more type 2 (fast) fibers and demonstrated rapid activation times from onset of movement to final posture of around 50 ms or less in our study. The CT and PCA have proportionally more type 1 (slow) muscle fibers, consistent with the slower activation times of around 100 ms. Activation times for each ILM maintained a uniform distribution across all 26 unique vocal folds. These activation times would theoretically allow for more rapid laryngeal posture changes than what is actually observed in vocal tremor or laughing. This discrepancy is most likely related to biomechanical and neurophysiologic constraints. Future studies aimed to address these questions are warranted.

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