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Non-Stimulated Rabbit Phonation Model: Cricothyroid Approximation

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Abstract

Objective—To describe a non-stimulated *in vivo* rabbit phonation model using an Isshiki type IV thyroplasty and uninterrupted humidified glottal airflow to produce sustained audible phonation.

Study Design—Prospective animal study.

Methods—Six New Zealand white breeder rabbits underwent a surgical procedure involving an Isshiki type IV thyroplasty and continuous airflow delivered to the glottis. Phonatory parameters were examined using high-speed laryngeal imaging, acoustic, and aerodynamic analysis. Following the procedure, airflow was discontinued and sutures remained in place to maintain the phonatory glottal configuration for microimaging using a 9.4 Tesla imaging system.

Results—High-speed laryngeal imaging revealed sustained vocal fold oscillation throughout the experimental procedure. Analysis of acoustic signals revealed a mean vocal intensity of 61 dB and fundamental frequency of 590 Hz. Aerodynamic analysis revealed a mean airflow rate of 85.91 mL/s and subglottal pressure of 9 cm H₂O. Following the procedure, microimaging revealed that the *in vivo* phonatory glottal configuration was maintained, providing consistency between the experimental and post-experimental laryngeal geometry. The latter provides a significant milestone that is necessary for geometric reconstruction and to allow for validation of computational simulations against the *in vivo* rabbit preparation.

Conclusion—We demonstrate a non-stimulated *in vivo* phonation preparation using an Isshiki type IV thyroplasty and continuous humidified glottal airflow in a rabbit animal model. This

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preparation elicits sustained vocal fold vibration and phonatory measures that are consistent with our laboratory's prior work using direct neuromuscular stimulation for evoked phonation.

Keywords

rabbit model; phonation; vocal fold

Introduction

The objective of thyroplasty surgery is to modify the phonatory position of the vocal folds through changes to the laryngeal cartilage framework. Thyroplasty procedures include four primary classifications of medialization and augmentation, lateralization, relaxation, and tensing¹. Bilateral Isshiki type IV thyroplasty, or cricothyroid approximation, is a technique in which the thyroid cartilage and cricoid cartilage are approximated. This action results in increased lengthening and tension, similar to engaging the cricothyroid muscle². Type IV thyroplasty is a common procedure for altering the vocal pitch of male-to-female transsexual patients³ and in lengthening and tensing the paralyzed vocal fold⁴.

Investigators have used various approaches to examine the effects of suture tension, force, and direction on medialization thyroplasty,⁵ arytenoid adduction,^{6–8} and to modify glottal configuration in *ex vivo* models^{9–14}. For example, Alipour and Karnell¹² described a method of suturing the muscular processes of the arytenoid cartilages to adduct the vocal folds in excised canine larynges. To vary the degree of glottal gap, Alipour and Karnell¹² tensed and released sutures attached to the epiglottis. Khosla and colleagues¹⁰ simulated vocal fold adduction by placing sutures through the vocal processes of the arytenoid cartilages. To demonstrate the precision of this technique, the sutures were placed symmetrically in the anterior-posterior and inferior-superior positions.

Using a canine model, Gray and Titze¹⁵ applied sutures and clamps and continuous airflow to elicit phonation from animals *in vivo*. The posterior vocal process was sutured and the arytenoid cartilages clamped together to approximate the vocal folds. The purpose of the present study was to describe a non-stimulated *in vivo* rabbit phonation model using an Isshiki type IV thyroplasty and uninterrupted humidified glottal airflow to produce sustained audible phonation. High-speed laryngeal imaging, acoustic, and aerodynamic assessment was used to assess phonatory output parameters and laryngeal microimaging performed following the procedure to examine post-experimental glottal configuration. These experiments represent a critical milestone in our ability to build subject specific computational simulations using a realistic, as opposed to idealized laryngeal geometry. Computational simulations derived from these physical reconstructions can then be validated against the known output parameters obtained during *in vivo* experimentation.

Materials and Methods

Animals

This study was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, National Institutes of Health Guide for the Care and

Use of Laboratory Animals, and Animal Welfare Act (7 U.S.C. et seq.). The animal protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee. Six male New Zealand white breeder rabbits weighing 3–4 kg were used. Animals were anesthetized via intramuscular injections of ketamine (35 mg/kg), xylazine (5 mg/kg), and acepromazine (0.75 mg/kg). To maintain anesthetic effects, ketamine (17.5 mg/kg) and acepromazine (0.375 mg/kg) were subsequently administered as needed. Heart rate, oxygen saturation level, and temperature were monitored throughout the procedure to assess state of anesthesia and general wellbeing.

Phonation Procedure

Animals were placed on an operating platform in the supine position. To prepare for surgery, the neck was shaved from the submentum to the chest. Local anesthesia (0.2% lidocaine) was administered at the surgical site and a midline incision was made from the hyoid bone to the sternal notch to expose the larynx and trachea. A tracheostomy was then created to provide a stable airway. The trachea was transected just proximal to the sternum and the lower portion of the trachea was suspended to the sternal fascia. A 3.5 cuffed endotracheal tube (Willy Rusch GmbH, Kernen, Germany) was inserted into the upper portion of the bisected trachea and positioned 2 cm below the glottal opening. The cuff of the endotracheal tube was inflated to seal off the trachea and deliver airflow. Continuous humidified airflow was delivered to the glottis heated at 37° C using a Gilmont Instruments flowmeter (GF-8522, Barrington, IL) and Conch Therm III humidifier (Hudson, RCI, Temecula, CA). A bilateral Isshiki type IV thyroplasty² was performed to simulate the action of the cricothyroid muscle. The thyroid cartilage and cricoid cartilage were sutured together using 3–0 Webcryl sutures (Patterson Veterinary, Saint Paul, MN) to approximate one another. Suture positioning provided increased lengthening and tension to the vocal folds, providing medial movement of the arytenoid cartilages and vocal fold approximation. Tenseness of the sutures was adjusted until there was audible phonation. Figure 1 is an image demonstrating the *in vivo* suture placement and approximation of the thyroid and cricoid cartilages.

The larynx was suspended using an 11-cm Hollinger-Tucker pediatric anterior commissure side-slotted laryngoscope (Karl Storz Endoscopy-America, Inc., El Segundo, CA). Three trials of vocal fold vibration were recorded using a 0-degree, 4.0-mm rigid endoscope coupled to a FASTCAM MC 2.1 high-speed camera (KayPENTAX, Montvale, NJ). The images were captured in black and white with 512 × 96 pixel resolution at a rate of 10,000 frames per second. To obtain acoustic measurements, three trials of phonation were recorded using a Perception 170 Condenser microphone (AKG, Vienna, Austria) positioned 10 cm from the opening of the laryngoscope. Uninterrupted, continuous phonation was recorded for approximately 10–20 seconds. Recordings were digitized using Computerized Speech Lab Model 4500 (KayPENTAX, Montvale, NJ). The most stable 1-second portion of the acoustic waveform was selected and analyzed to obtain mean vocal intensity sound pressure level (dB) and mean fundamental frequency (Hz) values. Following acoustic analyses, sound waveforms were edited using *Cool Edit Pro* v. 2.1 (Syntrillium Software, Phoenix, AZ, 2003). To improve visualization of the waveforms, signals were amplified and excessive noise was removed. Sound spectrograms were created for spectrographic feature analysis. In addition, three trials of airflow rate (ml/s) and subglottal pressure (cm H₂O) were

documented. At the end of the phonation procedure, humidified airflow was discontinued and the sutures between the thyroid and cricoid cartilages remained in place. The animals were sacrificed and larynges were harvested.

Magnetic Resonance Imaging Procedure

Excised laryngeal specimens were secured in a 12 mL syringe with Fomblin 06/6 perfluoropolyether (Solvay Solexis, Thorofare, NJ) and placed in a 38-mm inner diameter radiofrequency coil. Scanning sequences were performed using a Varian 9.4 Tesla horizontal bore imaging system (Varian Inc., Palo Alto, CA) to obtain multislice scout images in the axial, coronal, and sagittal imaging planes. Acquired data were reconstructed using Matlab 2012a (Mathworks Inc., Natick, MA) using an inverse Fourier transform. Immediately following excision, the first laryngeal sample was immersed in 10% formalin for 48 hours for tissue fixation. The sample was then transferred and soaked in 3 mM Magnevist gadolinium contrast agent (Baylor HealthCare Pharmaceuticals Inc., Wayne, NJ) in 30 mL phosphate buffer saline (PBS) for 36 hours. A 3D spin echo T2-weighted scanning sequence was used with an effective echo time of 15 ms, repetition time of 100 ms, and a field of view of $40.96 \times 22.4 \times 22.4$ mm. The number of excitations was 4 and the matrix size was $512 \times 320 \times 320$. Total scanning time was 11 hours and 24 minutes.

Results

Suture tenseness between the thyroid and cricoid cartilages was modified until phonation was audibly perceived. The vibratory properties were captured using high-speed laryngeal imaging. Figure 2 shows a representative high-speed montage of one glottal cycle, revealing both the open and closed phases of vibration. The predominant mode of contact occurred along the middle one-third portion of the vocal folds.

Acoustic signals of rabbit elicited phonations were recorded. As shown in Figure 3, 1-second central portions of the sound waveforms were selected for analysis. Mean vocal intensity was 61.39 dB sound pressure level ($SD = 4.03$). Table 1 displays the means and standard deviations for vocal intensity across all animals for each trial. Closer examination of each trial revealed a vocal intensity range of 56.15–68.52 dB. Analysis of the acoustic trials for frequency revealed a mean fundamental frequency of 590.25 Hz ($SD = 80.79$), with a frequency range of 419–728 Hz (Table 2).

Sound spectrograms of the same 1-second central portions of rabbit elicited phonations were generated (Figure 4). Spectrographic features included strong, distinctive harmonic content. Smooth, continuous variation in frequency was also observed. Lastly, aerodynamic measurements of airflow rate and subglottal pressure were documented for all trials (Table 3). Mean airflow rate was maintained at 85.91 mL/s ($SD = 15.19$) across rabbits. Mean subglottal pressure was 9.00 cm H₂O ($SD = 1.54$). After each phonation procedure, the laryngeal tissues were harvested, with cricothyroid sutures in place to maintain the *in vivo* phonatory glottal configuration. Several specimen preparations and scanning sequences were used to optimize overall image resolution, contrast between tissues, and to minimize tissue deformation. Figure 5a shows a representative *ex vivo* larynx with cricothyroid sutures in

place, while Figure 5b displays a representative magnetic resonance image obtained in the axial viewing plane (80 microns).

Discussion

Our group has previously described an evoked rabbit phonation model using neuromuscular input to the laryngeal musculature and controlled airflow to the glottis¹⁶. In the present study, we describe a non-stimulated *in vivo* rabbit phonation model using an Isshiki type IV thyroplasty procedure for cricothyroid approximation, and continuous humidified airflow delivered to the glottis. Elicited phonation samples were analyzed using acoustic, aerodynamic, and high-speed video analyses. Cricothyroid approximation resulted in an adequate amount of vocal fold lengthening and tensing, that when combined with glottal airflow, produced a sustained and audible phonation. Acoustic analysis of elicited phonation samples revealed vocal intensity and fundamental frequency values that are consistent with modal intensity phonation using pulsed electrical stimulation^{17, 18}. Sound spectrograms from the present study revealed modal intensity elicited phonations, characterized by strong harmonics and rare occurrences of aperiodicity, as described previously¹⁹. Additionally, the aerodynamic properties of elicited phonations, notably airflow rate and subglottal pressure, are in congruence with expectations for rabbit evoked modal intensity phonation.

A variety of theoretical, physical, and computational models have been developed to investigate the interactions between the aerodynamic and vibratory components of phonation^{20–27}. In addition to providing a useful tool for optimization of phonosurgical procedures, the model described herein may provide a useful approach for reconstruction of the realistic laryngeal geometry and glottal configuration for computational modeling. Validation of the phonation simulations against the known phonatory outputs from the experimental *in vivo* model may yield a more accurate representation of vocal fold mechanics and enhanced insight into the study of normal and abnormal voice production.

The non-stimulated *in vivo* phonation model described provides a useful adjunct to computational and theoretical models derived from experimental studies of excised larynges. Following the phonation procedure, the freshly harvested larynx is prepared for microimaging with the vocal folds maintained in the adducted phonatory position. The *in vivo* elicited phonations provide for precise phonatory output parameters that can be quantified against computer simulations using a physical reconstruction of the actual laryngeal geometry, as opposed to an idealized geometry. Despite these notable advantages, it is noted that the non-stimulated *in vivo* preparation, similar to the excised larynx preparation, will have limitations in the representation of physiology, due to the lack of thyroarytenoid input. However, the major advantage is that precise features of glottal configuration and vocal fold vibration can be captured within the native three-dimensional environment of a living animal with an intact vocal tract. An additional advantage to this approach is that it produces a consistent glottal configuration between the *in vivo* phonation experiments and the data derived from laryngeal microimaging, which allows the computational model to better represent actual positioning of the vocal folds during the *in vivo* experiment. It is also worthwhile to note that vocal fold adduction may be incorporated into the computational model after the scan, either through artificial manipulation of the

laryngeal geometry or through a physics-based simulation of the tissue deformation; however, either approach can introduce additional modeling uncertainties^{28–29}.

Because of the need to preserve tissue geometry and minimize post-harvest deformation of the tissues for magnetic resonance imaging, *in vivo* and *excised* laryngeal experiments were not combined in the present study. However, the concept of direct comparison of the *in vivo* intact larynx with the *excised* larynx from the same animal after harvest is an interesting idea that may be worth pursuing in the future. This combined approach may allow for the modeling of pharyngeal contributions to the acoustic signal. This direct comparison of the *in vivo* and *ex vivo* larynx would allow for improved modeling of the three dimensional relationships of the native upper airway.

Conclusion

In the present study, we describe a non-stimulated *in vivo* phonation preparation using an Isshiki type IV thyroplasty and continuous humidified glottal airflow in a rabbit model. This preparation elicits sustained vocal fold oscillation and phonation that is consistent with modal intensity phonation evoked using direct neuromuscular stimulation. Despite the obvious disadvantage of lack of neuromuscular input, this model provides several advantages for computational modeling of tissues. The ability to validate derived simulations using precise phonatory output parameters and physical reconstruction of the subject-specific laryngeal geometry from the *in vivo* animal experiments is possible. Further, glottal configuration from the *in vivo* experiments can be maintained by leaving the Isshiki type IV thyroplasty sutures in place. This allows for microimaging of the larynx and reconstruction of the laryngeal tissues using a realistic, as opposed to an idealized geometry for computational modeling. The consistency in laryngeal geometry and glottal configuration, between the *in vivo* phonation and microimaging experiments on which the reconstruction is based, also allows for subject-specific validation of the computational simulations against the known output parameters obtained during *in vivo* experimentation.

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References

1. Isshiki N. Mechanical and dynamic aspects of voice production as related to voice therapy and phonosurgery. *Otolaryngol–Head Neck Surg.* 2000; 122(6):782–793. [PubMed: 10828787]
2. Isshiki N, Morita H, Okamura H, Hiramoto M. Thyroplasty as a new phonosurgical technique. *Acta Otolaryngol.* 1974; 78(5–6):451–457. [PubMed: 4451096]
3. Neumann K, Welzel C. The importance of the voice in male-to-female transsexualism. *Journal of Voice.* 2004; 18(1):153–167. [PubMed: 15070236]
4. Gibbins N, Bray D, Harries ML. Long-term quantitative results of an Isshiki type 4 thyroplasty: A case study. *Journal of Voice.* 2011; 25(3):283–287. [PubMed: 20335003]

5. Czerwonka L, Ford CN, Machi AT, Levenson GE, Jiang JJ. A-P positioning of medialization thyroplasty in an excised larynx model. *The Laryngoscope*. 2009; 119(3):591–596. [PubMed: 19235760]
6. Noordzij JP, Perrault DF Jr, Woo P. Biomechanics of arytenoid adduction surgery in an ex vivo canine model. *Ann Otol Rhinol Laryngol*. 1998; 107(6):454–461. [PubMed: 9635454]
7. Inagi K, Connor NP, Suzuki T, Ford CN, Bless DM, Nakajima M. Glottal configuration, acoustic, and aerodynamic changes induced by variation in suture direction in arytenoid adduction procedures. *Ann Otol Rhinol Laryngol*. 2002; 111(10):861–870. [PubMed: 12389852]
8. Hoffman MR, Witt RE, Chapin WJ, McCulloch TM, Jiang JJ. Multiparameter comparison of injection laryngoplasty, medialization laryngoplasty, and arytenoid adduction in an excised larynx model. *The Laryngoscope*. 2010; 120(4):769–776. [PubMed: 20213797]
9. Alipour F, Jaiswal S, Finnegan E. Aerodynamic and acoustic effects of false vocal folds and epiglottis in excised larynx models. *Ann Otol Rhinol Laryngol*. 2007; 116(2):135–144. [PubMed: 17388238]
10. Khosla S, Murugappan S, Lakhamraju R, Gutmark E. Using particle imaging velocimetry to measure anterior-posterior velocity gradients in the excised canine larynx model. *Ann Otol Rhinol Laryngol*. 2008; 117(2):134–144. [PubMed: 18357838]
11. Finnegan EM, Alipour F. Phonatory effects of supraglottic structures in excised canine larynges. *J Voice*. 2009; 23(1):51–61. [PubMed: 17400425]
12. Alipour F, Karnell M. Aerodynamic and acoustic effects of ventricular gap. *J Voice*. 2013; 28(2):154–160. [PubMed: 24321590]
13. Alipour F, Finnegan EM, Jaiswal S. Phonatory characteristics of the excised human larynx in comparison to other species. *J Voice*. 2013; 27(4):441–447. [PubMed: 23809568]
14. Alipour F, Finnegan E. On the acoustic effects of the supraglottic structures in excised larynges. *J Acoust Soc Am*. 2013; 133(5):2984–2992. [PubMed: 23654402]
15. Gray S, Titze I. Histologic investigation of hyperphonated canine vocal cords. *Ann Otol Rhinol Laryngol*. 1988; 97(4 Pt 1):381–388. [PubMed: 3408113]
16. Ge PJ, French LC, Ohno T, Zeale DL, Rousseau B. Model of evoked rabbit phonation. *Ann Otol Rhinol Laryngol*. 2009; 118(1):51–55. [PubMed: 19244964]
17. Kojima T, Van Deusen M, Jerome WG, Garrett CG, Sivasankar MP, Novaleski CK, Rousseau B. Quantification of acute vocal fold epithelial surface damage with increasing time and magnitude doses of vibration exposure. *PLoS ONE*. 2014; 9(3):e91615.doi: 10.1371/journal.pone.0091615 [PubMed: 24626217]
18. Kojima T, Valenzuela CV, Novaleski CK, Van Deusen M, Mitchell JR, Garrett CG, Sivasankar MP, Rousseau B. Effects of phonation time and magnitude dose on vocal fold epithelial genes, barrier integrity, and function. *The Laryngoscope*. 2014; [Epub ahead of print]. doi: 10.1002/lary.24827
19. Awan SN, Novaleski CK, Rousseau B. Nonlinear analyses of elicited modal, raised, and pressed rabbit phonation. *J Voice*. 2014; 28(5):538–547. [PubMed: 24836360]
20. Hirano M. Morphological structure of the vocal cord as a vibrator and its variations. *Folia Phoniatr (Basel)*. 1974; 26(2):89–94. [PubMed: 4845615]
21. Jiang JJ, Titze IR. Measurement of vocal fold intraglottal pressure and impact stress. *J Voice*. 1994; 8(2):132–144. [PubMed: 8061769]
22. Khosla S, Murugappan S, Gutmark E, Scherer R. Vortical flow field during phonation in an excised canine larynx model. *Ann Otol Rhinol Laryngol*. 2007; 116(3):217–228. [PubMed: 17419527]
23. Bakhshaei H, Young J, Yang JCW, Mongeau L, Miri AK. Determination of strain field on the superior surface of excised larynx vocal folds using DIC. *J Voice*. 2013; 27(6):659–667. [PubMed: 24070590]
24. Gunter HE. A mechanical model of vocal-fold collision with high spatial and temporal resolution. *J Acoust Soc Am*. 2003; 113(2):994–1000. [PubMed: 12597193]
25. Van den Berg J. Myoelastic-aerodynamic theory of voice production. *J Speech Hear Res*. 1958; 1(3):227–244. [PubMed: 13576514]
26. Chang S, Tian F-B, Luo H, Doyle JF, Rousseau B. The role of finite displacements in vocal fold modeling. *J Biomech Eng*. 2013; 135(11):111008. [PubMed: 24008392]

27. Tao C, Jiang JJ, Zhang Y. Simulation of vocal fold impact pressures with a self-oscillating finite-element model. *J Acoust Soc Am*. 2006; 119(6):3987–3994. [PubMed: 16838541]
28. Xue Q, Zheng X, Mittal R, Bielamowicz S. Subject-specific computational modeling of human phonation. *J Acoust Soc Am*. 2014; 135(3):1445–56. [PubMed: 24606281]
29. Hunter EJ, Titze IR, Alipour F. A three-dimensional model of vocal fold abduction/adduction. *J Acoust Soc Am*. 2004; 115(4):1747–59. [PubMed: 15101653]

Figure 1.
Isshiki type IV thyroplasty. Suture placement is shown between the cricoid and thyroid cartilages bilaterally to produce cricothyroid approximation.

Figure 2.
Glottal cycle montage of the closing and opening phases of vocal fold vibration captured with high-speed digital imaging.

Figure 3.
Representative acoustic waveform of a 1-second central portion of evoked rabbit phonation.
Sound signal was amplified and excessive noise was eliminated.

Figure 4.
Representative sound spectrogram of a 1-second central portion of evoked rabbit phonation demonstrating strong harmonic content.

Figure 5.
Representative images of: a) glottal configuration from cricothyroid approximation using an Isshiki type IV thyroplasty and b) magnetic resonance imaging of the larynx in the axial viewing plane, immediately following the *in vivo* phonation procedure.

Table 1

Mean Vocal Intensity in dB Sound Pressure Level and Standard Deviations (in Parentheses) of Rabbit Phonation across Three Trials.

<u>Animal</u>	<u>Trial 1</u>	<u>Trial 2</u>	<u>Trial 3</u>	<u>Averaged Trials</u>
1	59.23 (1.43)	62.20 (0.36)	61.11 (1.38)	60.85 (1.50)
2	56.76 (1.39)	58.20 (1.74)	58.60 (1.27)	57.85 (0.97)
3	64.94 (1.10)	57.65 (3.15)	...	61.30 (5.15)
4	57.91 (1.48)	56.15 (2.78)	57.26 (2.00)	57.11 (0.89)
5	65.98 (0.86)	63.32 (1.49)	63.25 (1.47)	64.18 (1.56)
6	64.83 (1.14)	68.52 (2.82)	67.78 (2.07)	67.04 (1.95)

Table 2

Mean Fundamental Frequency in Hz and Standard Deviations (in Parentheses) of Rabbit Phonation across Three Trials.

<u>Animal</u>	<u>Trial 1</u>	<u>Trial 2</u>	<u>Trial 3</u>	<u>Averaged Trials</u>
1	618.81 (4.23)	564.83 (5.87)	608.24 (1.81)	597.29 (28.61)
2	553.64 (7.94)	560.75 (7.97)	563.15 (11.50)	559.18 (4.95)
3	432.70 (10.93)	419.56 (2.65)	...	426.13 (9.29)
4	665.57 (17.30)	613.40 (92.91)	683.76 (20.06)	654.24 (36.52)
5	683.15 (11.25)	603.45 (12.52)	605.92 (12.83)	630.84 (45.32)
6	728.61 (24.14)	539.20 (179.66)	589.56 (177.67)	619.12 (98.10)

Table 3

Mean Airflow Rate in mL/s, Mean Subglottal Pressure in cm H₂O, and Standard Deviations (in Parentheses) of Rabbit Phonation.

<u>Animal</u>	<u>Mean Airflow Rate</u>	<u>Mean Subglottal Pressure</u>
1	69.46 (13.48)	10.67 (1.15)
2	85.03 (0.00)	8.00 (0.00)
3	73.79 (0.00)	...
4	85.03 (0.00)	7.33 (0.58)
5	85.03 (0.00)	...
6	113.08 (0.00)	10.00 (0.00)