Parameters From the Complete Phonatory Range of an Excised Rabbit Larynx

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Summary: Objective. This study aims to collect data throughout the complete phonatory range using rabbit larynges.
Study Design. This is a methodological excised rabbit larynx study.
Methods. Seven rabbit larynges were dissected and mounted on a modified excised laryngeal apparatus. Phonation was initiated at phonation threshold pressure (PTP) and airflow was increased by consistent increments until phonation instability pressure (PIP) was reached. At each airflow level, aerodynamic measurements, acoustic recordings, and high-speed videos were recorded. This procedure was repeated at multiple elongation conditions to further explore the parameters. Data were then compared across subjects and elongation conditions.
Results. At PTP, subglottal pressure, fundamental frequency, and sound pressure level were found to increase significantly as elongation was increased. As elongation was increased at PIP, airflow was found to significantly decrease, whereas fundamental frequency was found to significantly increase. Vibratory amplitude decreased at both PTP and PIP as elongation increased. Also, as elongation increased, the range of all parameters was found to decrease significantly.
Conclusions. The results obtained, combined with the similarities of the histologic structure of the vocal fold lamina propria between rabbits and humans, validate the rabbit larynx as an effective and reliable model for tissue inflammation studies.
Key Words: Excised larynx–Rabbit larynx–Tissue inflammation–Phonation threshold–Phonation instability.

INTRODUCTION

Excised larynges have been used to study vocal fold mechanics and pathologies for centuries.[1] Although larynges of different animals, such as rats, cows, pigs, and sheep, have been examined, the most commonly studied is the excised canine larynx.[2,3] Excised canine larynges are feasible to study in the excised booth and are similar to human larynges in size, structure, and ability to phonate with mucosal wave patterns.[2] Although these models are viable in most vocal fold research, canine larynges are not ideal representations for studies involving vocal fold histology such as inflammation and wound healing studies of the vocal fold lamina propria.[3] There are innate histologic differences between canine and human larynges that limit canine model use for these types of studies. Canines exhibit sheets of collagen, elastin, and a loose ground substance in the superficial lamina propria, whereas a human superficial lamina propria layer contains only loose ground substance.[3] This difference in tissue structure leads to discrepancies in tissue response between canines and humans. Thus, another animal larynx with more comparable vocal fold histology, such as the rabbit, is warranted for studies of vocal fold inflammation and healing.[4] Unlike the canine, the superficial lamina propria of the rabbit larynx consists of only loose ground substance, similar to that of a human.[4] Therefore, as a histologic model for human larynges, the rabbit larynx proves superior to the canine larynx.[4,5]

A number of studies have been conducted examining the histologic properties of rabbit larynges. However, little research on the vocal fold mechanics of the rabbit larynx has been reported and excised booth studies of rabbit larynges are even more rare.[6–8] In 2013, Maytag et al successfully dissected and mounted five rabbit larynges on an excised booth apparatus.[6] Maytag et al recorded reliable acoustic, aerodynamic, videokymographic, and electroglottographic data and found that rabbit acoustic results were similar in intralarynx variability to canine laryngeal data. These preliminary results represent reliable excised data obtained from a healthy *ex vivo* rabbit larynx model.

The vocal fold mechanisms of canine larynges are well documented and proven useful in examining the tissues' ability to phonate or change phonation patterns when affected pathologically or during a healing process with vocal fold scarring.[9,10] Despite excised canine larynges' extensive use in studying vocal fold dynamics, a model more histologically similar to human tissue is warranted to study tissue response and changes. Rabbit larynges are the ideal model for vocal fold inflammation studies due to the feasibility of mounting them on an excised booth setup similar to that of canines and their closer resemblance to the human tissue layers in the vocal folds.[11–16] Therefore, the vocal fold mechanisms of rabbit larynges should be investigated further so this model can be used extensively within this field.

The purpose of the present study was to make adaptations to an excise booth primarily used for canine larynges, to allow for reliable data collection of the complete range of phonation in rabbit larynges. Parameters were measured at consistent flow increments from phonation threshold pressure (PTP) to phonation instability pressure (PIP). The effect of elongation was also of interest, and so measurements were repeated on various elongation conditions. The parameters that were chosen to be

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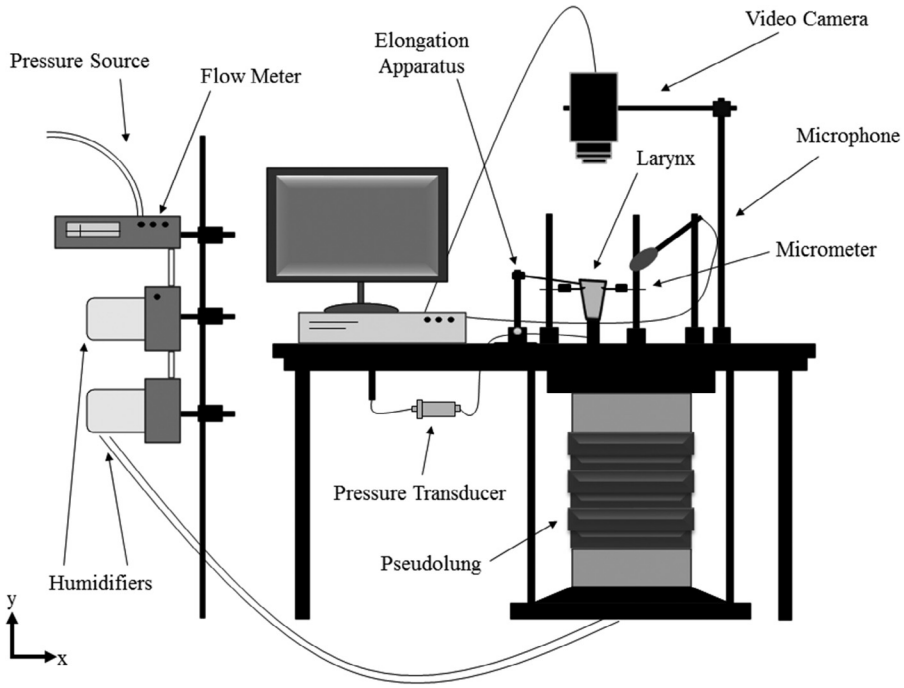

FIGURE 1. Schematic of the excised larynx booth setup.

measured were airflow, subglottal pressure, fundamental frequency (F_0), sound pressure level (SPL), and vibratory amplitude (VA). These parameters were chosen to examine common physiological relationships observed in human larynges.[17–19] It is hypothesized that as elongation of the vocal folds increases, the phonatory range will shorten. Therefore, the difference between the measurements at the level of phonation threshold to phonation instability will be examined for each parameter as well. Also, by further exploring these parameters in rabbit larynges, the utility of rabbit larynges as tissue inflammation models can be assessed.

METHODS

Excision and dissection

Seven rabbit larynges were harvested from white New Zealand rabbits. All larynges were euthanized for research purposed unrelated to the present study and donated by Covance, Inc. (Madison, WI). No animals were killed expressly for the purpose of this study. The rabbit larynges were excised and stored in 0.9% saline solution and frozen in a −20°C freezer until they were dissected according to the procedure described by Maytag et al.[6] Before dissection, each larynx was thawed individually in a room temperature water bath.

Mounting

Each larynx was mounted on the apparatus shown in Figure 1 as specified by Jiang and Titze.[20] An adapter was placed on the outlet tube of the pseudolung so that the rabbit larynx trachea, which has a smaller diameter than a canine's, could be securely mounted. A metal clamp fastened the trachea to the outlet to prevent air leakage during phonation. Needles were used as single pronged micrometers to adduct the arytenoids, which resulted in the adduction of the vocal folds. A suture was placed through the thyroid cartilage and attached to a device that created tension that could be manipulated to control elongation of the vocal folds. To limit dehydration after the larynx was mounted, 0.9% saline solution was applied to the vocal folds between trials. Figure 2 shows one of the rabbit larynges, mounted as described, during phonation at a point with maximal glottal opening.

Data collection

For each larynx, data were collected over the complete phonation pressure range (PPR), which is defined as the range from PTP to PIP. To complete data collection across this range, flow

FIGURE 2. Image of a mounted rabbit larynx on the excise booth setup. Note the rods used to adduct the arytenoids, the suture used for controlling elongation, and the zip tie used to create a tight seal with the outlet of the pseudolung.