Published in final edited form as:

Curr Bioinform. 2011 ; 6(3): 286–304. doi:10.2174/157489311796904673.

Experiments on Analysing Voice Production: Excised (Human, Animal) and *In Vivo* (Animal) Approaches

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Abstract

Experiments on human and on animal excised specimens as well as *in vivo* animal preparations are so far the most realistic approaches to simulate the *in vivo* process of human phonation. These experiments do not have the disadvantage of limited space within the neck and enable studies of the actual organ necessary for phonation, i.e., the larynx. The studies additionally allow the analysis of flow, vocal fold dynamics, and resulting acoustics in relation to well-defined laryngeal alterations.

Purpose of Review—This paper provides an overview of the applications and usefulness of excised (human/animal) specimen and *in vivo* animal experiments in voice research. These experiments have enabled visualization and analysis of dehydration effects, vocal fold scarring, bifurcation and chaotic vibrations, three-dimensional vibrations, aerodynamic effects, and mucosal wave propagation along the medial surface. Quantitative data will be shown to give an overview of measured laryngeal parameter values. As yet, a full understanding of all existing interactions in voice production has not been achieved, and thus, where possible, we try to indicate areas needing further study.

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Recent Findings—A further motivation behind this review is to highlight recent findings and technologies related to the study of vocal fold dynamics and its applications. For example, studies of interactions between vocal tract airflow and generation of acoustics have recently shown that airflow superior to the glottis is governed by not only vocal fold dynamics but also by subglottal and supraglottal structures. In addition, promising new methods to investigate kinematics and dynamics have been reported recently, including dynamic optical coherence tomography, X-ray stroboscopy and three-dimensional reconstruction with laser projection systems. Finally, we touch on the relevance of vocal fold dynamics to clinical laryngology and to clinically-oriented research.

Keywords

Larynx; hemilarynx; vocal fold; dynamics; laryngeal flow; acoustics

1. INTRODUCTION

At first glance, the vocal folds may appear to be relatively simple structures; yet, the variety of sounds they produce is remarkable. So is the sheer number of physiological systems called into play for voice production. Vocal sounds vary in frequency, intensity and spectral features, and these parameters typically have complex temporal patterns, particularly during speech or singing. At a basic level, voice is a result of interaction between a largely passive vocal fold mucosa vibrated by airflow and a sophisticated laryngeal neuromuscular system that controls the position, tension and shape of the vocal folds. Despite the complexity of the system as a whole, the final common pathway of voice production is the motion of the vocal folds interacting with air to make sound. The role of the larynx as a sound generator that can be activated even in a cadaver has been known for a long time: Leonardo da Vinci, for example, showed that a cadaver can generate a voice if the larynx is squeezed and the lungs are compressed [1]. How airflow animates the vocal folds, even in the absence of muscular control, is a critical starting point in studies of voice production and an active area of investigation. Physical models, excised animal or human larynges and *in vivo* animal models have provided convenient systems for study and a large body of information has evolved.

A key problem uniting speech science, laryngology and speech language pathology is the relationship between vocal fold dynamics and sound production. Vocal fold dynamics refers to the description, analysis and understanding of vocal fold motion. Considering the vast variety of vocal sounds that humans (and other species) can produce, it is intuitive that vocal fold dynamics must be complex, highly variable and exquisitely controlled. The study of vocal fold dynamics is also challenging due to the small size of the vocal folds, their rapid movements, the complex neuromuscular/cartilaginous superstructure that supports and controls them and the relative inaccessibility of the larynx deep in the throat.

At least 7 biomechanical factors can influence vocal fold dynamics: (1) the aerodynamics of the driving airstream, (2) vocal fold shape, (3) the material properties and coupling of the vocal fold layers, (4) the actions of muscles that can alter the length, tension and shape of the vocal folds, (5) the symmetry of the paired vocal folds, (6) the collisions between the two vocal folds, and (7) configurations of the subglottal and supraglottal vocal tracts. These factors interact in complex and non-linear ways. Each factor is also vulnerable to

pathological changes resulting from injury or disease, with possible consequences for vocal function.

Vocal fold dynamics have been studied using direct and indirect measures of vocal fold motion [2]. As in other areas of research, progress in understanding has gone hand-in-hand with progress in developing new ways of acquiring and visualizing data. Modeling has also been a useful tool for identifying salient parameters, organizing and understanding empirical data, and generating and testing hypotheses. In turn, models must be continually re-formulated to incorporate novel observations of tissue motion, structure and rheology [3,4].

Studying the voice using *in vivo* human subjects for basic and clinical research has greatly increased our understanding of the human phonation process [2]. The clinical endoscopic method of investigation is depicted in Fig. (1). However, visual access and invasiveness of experimentation are limited in humans due to many considerations, including the constricted space of the supraglottal vocal tract. Airflow and subglottal pressure are difficult to measure in human subjects, as the point of interest for these parameters often lies below the vocal folds. Additionally, selective neural activation of muscles that control arytenoid position (adduction/abduction) and vocal fold elongation/tension cannot be controlled or quantified easily in humans. These shortcomings have been overcome in part by innovative excised human and animal preparations. *In vivo* animal phonatory preparations (where phonation is evoked in anesthetized animals) have also contributed to new insights into the aerodynamic-structure-acoustic interactions during phonation. Experiments on the different models have focused on many different interrelations. Some studies describe only one of the three important components of flow, dynamics and acoustic outcome. Others investigate the interrelations of two out of the three components, such as flow and acoustics. Another approach has been to alter laryngeal parameters to investigate their influence on all three components. Most of the studies have focused largely on the vocal folds, but the influences of false vocal folds and other supraglottal structures are also recognized as important Fig. (2). Due to the sheer number of publications in the topic, this review focuses mainly on studies within the last decade. The review of *in vivo* work spans a broader time range because much of the work was performed before the year 2000.

2. VOCAL FOLD DYNAMICS AS STUDIED IN ANIMALS IN VIVO

The study of vocal fold dynamics using live, anesthetized, non-human mammals has presented many opportunities and challenges. The *in vivo* larynx is alive, perfused with blood and the neuromuscular and sensory components are intact, functional and accessible for stimulation. Tracheal cannulation provides access for controlling glottal airflow or subglottal pressure and simultaneous measures of acoustics, physiological variables and vocal fold tissue motion are possible. The *in vivo* model has allowed researchers to study the roles of laryngeal muscles, the effects of muscle contraction on mechanical properties of vocal fold tissue, the mucosal wave and to simulate clinical scenarios, including disease states (such as nerve paralyses) and surgical manipulations (such as treatments for glottal insufficiency). There have been recent advances in the use of these *in vivo* models, such as developing new muscle activation strategies, imaging vocal fold dynamics in three

dimensions using an *in vivo* hemilarynx preparation and correlating vocal fold dynamics with cellular physiology by controlling phonation ‘dose’ in live rabbits.

2.1. Action of the Laryngeal Muscles on Normal Vocal Fold Dynamics

Hast [5] used an *in vivo* canine model in an elegant and landmark study in 1961, proving that vocal fold adduction caused by nerve stimulation is too slow to support generation of voice via oscillations in muscle action. This provided direct evidence against the ill-fated neurochronaxic theory (which proposed that vocal fold vibration resulted from rapid muscular contractions) and supported the myoelastic-aerodynamic theory proposed by Van den Berg [6], a theory that is now universally accepted.

Core features of the *in vivo* model as used by Hast and updated by subsequent users include (1) cannulation of the distal trachea for respiration and proximal trachea for phonation, (2) use of warm, humidified air for flow- or pressure-controlled phonation, (3) nerve stimulation for control of vocal fold adduction, tension and length and (3) stroboscopy or high-speed imaging (HSI) to observe mucosal wave features. Although the canine has been the most valuable model species for studies pertinent to human laryngeal function, there are some significant differences between canine and human larynges that should be kept in mind. The canine has larger arytenoid cartilages, larger cricothyroid muscles [7], a larger posterior ‘chink’ during phonation, anastomotic fibers between recurrent (RLN) and superior laryngeal nerves (SLN), a less well defined vocal ligament [8], a thicker lamina propria with a more vertically oriented free edge and some shape differences in the laryngeal cartilages [3]. Differences in the microstructure of the lamina propria have also been noted [9-18]. And of course there are marked differences in the sounds voiced by humans and canines [16].

Beginning in the late 1980s, the canine model has been very productively employed by Berke and colleagues at University of California, Los Angeles [17,18]. The preparation they developed is shown in Fig. (3) in an early configuration. One of the first observations from this group was that recurrent laryngeal nerve (RLN) stimulation causes a marked medial bulging of the vocal folds through thyroarytenoid muscle (TA) activation [19]. This change in shape, with concomitant changes in tension, is clearly difficult to reproduce when simulating physiological adduction in inanimate larynx preparations by simply approximating the vocal processes.

Nerve stimulation was used to explore relations between subglottal pressure (P_{sub}), fundamental frequency (F_0) and phases in the glottal cycle [20-22]. With constant airflow, increasing RLN stimulation increased P_{sub} , decreased the amplitude of the traveling wave, decreased vocal fold excursion, decreased the anterior-posterior extent of the mucosal wave and caused small increases in F_0 . Superior laryngeal nerve (SLN) stimulation, on the other hand, profoundly increased F_0 with little effect on subglottal pressure (P_{sub}), while open quotient increased and closed quotient decreased. Among other conclusions, they surmised that SLN stimulation changes the shape of the vocal folds to a thinner, more convergent profile such that the upper vocal fold margin becomes more important in determining the dynamics. Measures of glottal area for different levels of RLN and SLN stimulation were subsequently reported [23,24].

In 1990 Berke *et al.* from the UCLA group examined the effects of different levels of RLN stimulation on medial compression, glottal opening, airflow, phonation intensity and frequency [25]. They found a relatively small (5-10 dB) increase in intensity when airflow was increased during constant compression. Conversely, a large increase in intensity (30 dB) occurred when compression was increased during constant airflow. They also made the interesting observation that subglottal pressure changed little as airflow was increased, suggesting that the resistance of the larynx was varying as a function of flow rate. A collapsible tube model of vocal fold vibration was proposed based on this observation of a 'flow-controlled non-linear resistance' [26].

Experiments from the UCLA group progressed from stimulation of the RLN as a whole to more selective activation of different muscles, including studies of the thyroarytenoid (TA) and posterior cricoarytenoid (PCA) muscles [19,27], the two bellies of the cricothyroid (CT) muscle [28], the interarytenoid (IA) muscle [29,30], and a comparative study of adductory forces generated by the IA, TA and lateral cricoarytenoid (LCA) muscles [31]. Taken together, these studies provide a more detailed appreciation of synergies between the different muscles in the control of vocal fold tension, arytenoid position and vocal fold length.

Hsiao *et al.* used direct stimulation of the CT muscle combined with midbrain stimulation in the canine *in vivo* model [32] to study the relationship between subglottal pressure and vocal fold vibration frequency [32,33]. The results of the first study extended our understanding of how vocal fold length, which is largely controlled by the CT muscle, influences the modulation of F_0 by subglottal pressure. Titze [34] had likened the vocal fold to a ukulele string because the short length of the vocal fold relative to the amplitude of vibration could cause dynamic fluctuations in tension and hence pitch. The increased excursion due to increased subglottal driving pressure was proposed to cause an increase in dynamic tension and a rise in F_0 . However, it has to be noted that the term dynamic refers to changes in the tension during a glottal cycle. It was shown, that this tension is a function of the vibratory amplitude [35]. The validity of this concept has been borne out by experiments of Hsiao and others.

A related study of the effects of CT activation on register changes by Hsiao *et al.* [36] is of methodological interest because of the way mid-brain stimulation was used to induce phonation, which was then combined with more direct muscle stimulation. The phonation produced by brain stimulation is arguably more natural than that produced by artificial nerve stimulation and regulated, sustained airflow as employed in the UCLA model. Hsiao *et al.* observed that small increments in CT stimulation could induce mode changes during mid-brain stimulated phonation. This approach may eventually help tease apart vocal fold dynamics in the context of species-specific vocalizations where sustained steady phonation is rare.

A residual problem has been the steep recruitment function for electrical excitation of motor axons [37]. This causes limited dynamic range and repeatability of the stimulation. Progress has been made recently by Chhetri *et al.* in developing stimulation methods that can yield

better gradation of contraction [37]. Hopefully this will enable future studies where more natural patterns of laryngeal muscle activity can be generated.

2.2. Effects of Muscle Contraction on Static Mechanical Properties

Yumoto and Kadota measured mucosal pliability using an *in vivo* canine hemilarynx model [38]. Using a ‘sucking method’, they measured the medial deformation of the vocal fold surface when imposing a force of about 2 g to punctuate areas via a tube. The deformation was assessed with and without direct stimulation of the TA muscle and also after euthanasia. TA stimulation resulted in increased deformability, indicating decreasing mucosal stiffness. This is consistent with other observations of the effects of TA stimulation on F_0 and with the cover-body hypothesis proposed by Hirano [8]. The complex interplay of cover and body stiffness with vocal fold length and the resulting effects on F_0 have been elucidated clearly by Titze [39].

Titze *et al.* (1997) also used an *in vivo* canine model to measure the range, speed and time course of changes in vocal fold length in response to RLN and SLN stimulation [40]. Microsutures were placed in the mucosa to track changes in distance between vocal fold fleshpoints. The thyroid cartilage was transected horizontally to allow for direct imaging of the superior surfaces of the vocal folds. The mean strain for SLN stimulation was 45 % (elongation), while it was –17 % for RLN stimulation (shortening). Co-stimulation gave the intermediate value of 26 % elongation. The time constant for elongation averaged 30 ms, which was judged to be in reasonable agreement with measures of maximum pitch modulation rates in humans.

2.3. Detailed Observations of Vocal Fold Oscillation (Mucosal Wave)

Berke *et al.* [17] used the *in vivo* canine model and observed that the lateral motion of the lower vocal fold edge ceased its lateral movement as the superior edge opened, suggesting a simultaneous drop in the subglottal driving pressure at this point. Similarly, the lateral spread of the mucosal wave from the upper edge diminished when the inferior edges re-established contact. In this study both supraglottal and subglottal imaging was performed, leading to the interesting observation that the ‘closing’ phase, as defined from photoglottography (PGG) and electroglottography (EGG) waveforms, is underestimated because the closing of the lower margin cannot be seen from above, if it is in a less medial position than the upper margin and is not captured by PGG or EGG signals.

The velocity of the mucosal wave was measured by Sloan *et al.* [41,42]. Positive correlations between velocity and vocal fold stiffness (as modulated by stimulation of the RLN) were observed. Direct measurements of vocal fold stiffness were made with a mechanical ‘tensionometer’.

2.4. Simulating Clinical Scenarios

Moore *et al.* [18] made videostroboscopic observations of simulated states of vocal fold paralysis. They observed a diminished excursion of the lower margin of the normal fold, presumably due to abnormal apposition by the paralyzed side. SLN asymmetries, on the other hand, led to shifts along the superior/inferior dimension and unusual patterns of

vibration. An earlier study by Isshiki *et al.* [43] made the interesting observation of a correlation between periodic entrained oscillation when the gap between normal and paralyzed folds was small, and the presence of less periodic modes when the gap was wider. This is an example where small differences in a single parameter can lead to drastically different modes of vibration, with implications for understanding hoarseness.

Other clinical issues that have been studied using *in vivo* animal models include hemilaryngectomy [44], simulated spasmodic dysphonia [45], SLN paralysis [46], medialization procedures [47], scar treatment with autologous fibroblasts [48] or Hylan-B [49], and injection laryngoplasty [50]. In a variation on previous methods of tracheal cannulation in acute experiments, Garrett and colleagues used a removable tracheal needle for serial measures of phonation in canines to assess effects of steroids and mitomycin-C on wound healing [51,52]. Paniello and Dahm [53] also created a chronic phonation model where electrodes were implanted for repeated laryngeal nerve stimulation over a period of 12 weeks. Thus this model has been an important pre-clinical test-bed for clinical procedures. In a very recent study, Karajanagi *et al.* [54] developed a chronic phonation model using canine specimens to investigate structural and functional effects of an injected biogel that has the potential to alleviate deficiencies in vocal fold pliability. Periodic assessments of HSI-derived maximum vocal fold vibratory amplitude, mucosal wave presence, and phonation threshold pressure were obtained over the course of one to four months. Results indicated that, in cases where the biogel effectively acted as a superficial lamina propria layer, similar amplitudes of vibration and mucosal wave activity were observed relative to contralateral vocal folds that acted as controls [55].

A common denominator in many of the clinically-oriented studies is the focus on mucosal wave properties as key indicators of vocal function. Often this has been done by having blinded observers rate ‘presence’, ‘absence’ or ‘reduction’ of the mucosal wave from videostroboscopic recordings. More quantitative measures of mucosal wave dynamics would enhance this important aspect of the assessment. Hopefully, in the future more quantitative data can be extracted from high-speed video sequences [56] and stereoscopic methods [57] and/or dynamic optical coherence tomography [58] to make these assessments more objective and sensitive.

2.5. Correlating Tissue and Cellular Dynamics

Recently, a very promising *in vivo* rabbit phonation model was developed by the Rousseau group at Vanderbilt University [59,60]. This model has been used to study changes in gene expression associated with phonation, which builds on Gray’s hyper-phonated *in vivo* canine model [61]. It was shown in the rabbit model that the expression of some genes related to extracellular matrix homeostasis can be altered by inducing phonation [59]. Little is known about vocal fold dynamics in the rabbit, so these will be important data to obtain for correlation with cellular responses.

3. EXCISED HUMAN AND ANIMAL LARYNX EXPERIMENTS

This chapter covers the motivations, goals and results of various experimental setups. Publications listed in this chapter present results which were generally obtained with a full

larynx, i.e. both vocal folds still being present. In contrast, Chapter 4 covers experiments undertaken with a so-called “hemilarynx”, where one vocal fold was removed. For the full larynx experiments, most commonly air is insufflated from the area inferior to the vocal folds by the remains of the trachea to achieve a self-sustained oscillation. As the larynx is kept as a whole, information of the medial vocal fold surface cannot be obtained by visual inspection.

Beforehand it must be mentioned that excised experiments come with disadvantages, unavailable muscle contraction and decay of the tissue and thus a limited experimental time are two major points. These disadvantages are weighed in the major advantages of and ease of access, visually but also for physical inspection. Moreover, life-like surroundings of the vocal folds, including muscles, ligaments and cartilages are an added benefit compared to other models. The purpose of such experiments is to produce more life-like results, considering vocal fold dynamics, air flow and produced acoustic signal.

3.1. Fluid Analysis

The analysis of the airflow, considering fluid dynamics, encompasses several aspects: the amount of volume flow necessary to obtain oscillation, the pressure and velocity distributions along the path from the subglottis through the glottis to the supraglottal area and the relations of pressure and volume flow to the output acoustic signal. An example is the investigation of turbulences along this path, which are theorized to be partly the cause for broadband noise in the primary voice signal.

3.1.1. Velocity Flow Field—Airflow from the subglottal area through the glottis into the supraglottal area is important to the phonation process. The interaction of dynamic air pressure and air velocity with the surrounding structures is believed to play a major part in sustaining vocal fold oscillations. In one experimental and theoretic study by Alipour *et al.* the spatial distributions of velocity and pressure were studied using a Plexiglas model of a human larynx, excised canine larynges (to study pulsatile flow), and a computational model [62]. They found evidence for a parabolic laminar velocity profile upstream of the glottal constriction and turbulent and asymmetric velocity profile downstream of the glottal constriction.

When airflow from the lungs is obstructed by vocal fold motion into a phonatory posture, the vocal folds begin to oscillate. In turn, the oscillating vocal folds create turbulences in the exiting air stream [63]. The amount of turbulence in the glottal jet during phonation was investigated by Alipour and Scherer [63] using the excised canine larynx model. Three methods (smoothing, wavelet de-noising, and ensemble averaging) were examined for analysis of both the deterministic signal and the residual turbulence portion. Smoothing appeared to be the most successful method because it preserved gross cyclic variations important to perturbations and modulations, while extracting turbulence at reasonable levels.

Airflow at the superior edge of vocal folds can be laminar, even if the tracheal airflow is predominantly turbulent. Turbulences are regarded as a possible cause of an irregular or rough-sounding voice. Oren *et al.* [64] hypothesized that the smooth converging shape of the subglottis reduces the majority of the potential turbulence. Three excised canine larynges

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were used to investigate the turbulence intensity below the cricoid cartilage and 2 to 3 mm above the superior vocal fold edge via hot-wire anemometry. The authors derived their hypothesis from wind tunnel design practice, thus the vocal fold oscillation was not desired. Results showed that for the centre of the jet, there was moderate turbulence below the cricoid cartilage and laminar flow at the 2 to 3 mm level above the vocal fold edge. For the shear layer, there was very high turbulence below the cricoid cartilage and low turbulence 2 to 3 mm above the folds. Therefore, the authors concluded that the smooth converging shape of the subglottis reduces turbulence significantly. Furthermore, such findings may have important implications for surgeries that affect the subglottal area.

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In 2007 Khosla *et al.* [65] investigated supraglottal velocity flow fields. Particle image velocimetry (PIV) data from three excised canine larynges were obtained. The authors found that vortices occurred immediately above the vocal folds. The location and shape of the vortices was dependent on the phase of the phonation cycle. Also, during specific phases of the glottal cycle vertical structures were consistently found, including starting vortices, Kelvin-Helmholtz vortices and entrainment vortices. Using the same method, Khosla *et al.* [66] investigated the anterior-posterior velocity gradients in the excised canine larynx model evaluating the vortical structures in their dimensional expansion. For this purpose, they measured supraglottal velocity flow fields in both midcoronal and midsagittal planes. Results showed that there was no significant anterior-posterior velocity gradient immediately above the vocal folds. However, the laryngeal jet was found to narrow in width and skew towards the anterior commissure downstream. Also, vortices were observed at the anterior and posterior edges of the flow. The authors concluded that downstream narrowing in the midsagittal plane was correlated with a phenomenon known as axis switching, and that this applied to the vortices in both sagittal and coronal planes. The same group investigated intraglottal vortices, which can generate negative pressure between the vocal folds [67]. Negative pressure lead to a suction force, which contributes to a sudden, rapid closing of both vocal folds. Such rapid closing can produce increased acoustic intensity and increased higher harmonics. Six excised canine larynges were investigated: three with symmetric and three with asymmetric but periodic motions. Additionally, they theorized that decreasing the closing speed of vocal folds can reduce loudness and energy in higher frequency harmonics and thus, results in reduced voice quality. Furthermore, they reported that the amplitudes of higher frequencies were reduced during periodic asymmetric motion.

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To determine whether intraglottal vortices and resulting acoustics are changed by scarring, five unilaterally scarred excised canine larynges were compared to five normal larynges via PIV and high-speed imaging (HSI) by Murugappan *et al.* [68]. PTP was increased in the scarred larynges. Vortex strength and higher harmonics consistently increased as subglottal pressure was increased. However, increasing the maximum displacement of scarred fold did not consistently increase the higher harmonics. The authors suggested that use of higher subglottal pressures may excite higher harmonics and improve intensity for patients with unilateral vocal fold scarring.

3.1.2. Pressure-Flow, Pressure-Frequency—In 1997 Alipour *et al.* [69] analysed pressure-flow relationships using five excised canine larynges. A linear relationship within the experimental range of phonation for each adduction level was detected. Differential

glottal resistance increased as the “vocal process gap” was reduced. The authors emphasized that these conclusions can be used in computer simulations of speech production and benefit clinical insight into the aerodynamic function of the human larynx.

Alipour and Scherer [70] studied pressure-frequency relations in the excised canine larynx. Glottal adduction was accomplished either by pressing the arytenoids together or by simulating lateral cricoarytenoid muscle activation with a suture through the muscular process. A series of pressure-flow sweep experiments revealed that the pressure-frequency relation was nonlinear for set adduction and elongation levels, and was highly influenced by the adduction and elongation. The authors observed that, for the first phonatory mode the average rate of change of frequency with pressure was $(2.9 \pm 0.7) \text{ Hz/(cm H}_2\text{O)}$, and that for the higher mode the rates were $(5.3 \pm 0.5) \text{ Hz/(cm H}_2\text{O)}$ for adduction changes and $(8.2 \pm 4.4) \text{ Hz/(cm H}_2\text{O)}$ for elongation changes.

Additionally, Alipour and Jaiswal investigated phonatory characteristics [71] and the glottal flow resistance [72] in excised pig, sheep, and cow larynges. Each excised larynx was subject to a series of pressure-flow sweeps, with adduction and flow rate as independent variables. The subglottal pressure, F_0 , and glottal flow resistance were treated as dependent variables. They found that the pressure-frequency relations were nonlinear for these species as well. They found that the pig had the highest average oscillation frequency ($220 \pm 57 \text{ Hz}$) and the highest average phonation threshold pressure ($7.4 \pm 2.0 \text{ cm H}_2\text{O}$), while the cow had the lowest average frequency ($73 \pm 10 \text{ Hz}$) and the lowest phonation threshold pressure ($4.4 \pm 2.3 \text{ cm H}_2\text{O}$). Their detailed results also indicated a nonlinear behaviour in the pressure-flow relations of these larynges with increasing glottal resistance due to increases in adduction.

3.1.3. Phonation Threshold Flow—The phonation threshold flow describes the minimum flow rate that is necessary to obtain self-oscillating vocal fold dynamics and thus a primary voice signal. Hottinger *et al.* [73] studied phonation threshold flow (PTF) along with pressure (PTP) as a function of pre-phonatory glottal width in ten canine excised larynges. Metal shims were used to produce glottal gaps ranging from 0.0 to 4.0 mm. For each glottal gap, airflow was increased until the vocal folds started vibrating. Significant differences in the aggregate mean PTF values over a large range of the widths tested (1.0–4.0 mm) were found. PTF increased with increasing glottal width in a linear fashion. They also observed that PTF was more sensitive to posterior glottal width than PTP and suggested that PTF may be a more effective indicator for vocal diseases related to abduction compared to PTP.

In another study using canine larynges, Jiang *et al.* found a significant correlation between PTF and vocal fold elongation [74]. No statistically significant relationship was found between the size of larynx and the measured PTF. The authors concluded that PTF was correlated with vocal fold elongation and with PTP for small magnitudes of elongation and that PTP may be a useful indicator of the biomechanical status of vocal folds.

Witt *et al.* [75] investigated the effect of dehydration on PTF in eleven excised canine larynges. It was suggested that a critical period of dehydration may exist after which

phonation can no longer be initiated. PTF was measured with and without saline application to maintain hydration. Results showed that PTF increased significantly with dehydration. These results support clinical observations regarding hydration and also point out the importance of controlling for hydration in experimental studies.

3.2. Structure Vibration Analysis and Acoustics

3.2.1. Dynamics—Propagation of the mucosal wave has been theorized to be important for phonation and has been subject of several investigations. Yumoto *et al.* [76] investigated the location of mucosal upheaval in response to variations in vocal fold tension and mean airflow rate (MFR) in twelve excised canine larynges. The vocal fold tension was controlled by cricothyroid approximation, i.e. by lengthening the vocal fold and thus increasing longitudinal tension. Subsequently, the vocal folds were processed and examined histologically to correlate the location of the upheaval with underlying tissue structure. The term ‘mucosal upheaval’ refers to an observable region of medial displacement on the subglottal surface of the vocal folds that is correlated with initiation of the mucosal wave. The authors found that for a constant vocal fold tension, the mucosal upheaval appeared more laterally, but its position on the mucosa did not change, when the mean air flow rate was increased. For an increase in vocal fold tension, it appeared more medially. The anatomic correlations showed that the mucosal upheaval was located where the thyroarytenoid muscle comes close to the epithelium, which is inferior to the main bulk of the lamina propria.

Kusuyama *et al.* [77] analyzed vocal fold vibration using x-ray stroboscopy with multiple markers to derive a more accurate description of tissue motion than is possible with conventional imaging at the time of the study. Results from experimental phonation of excised canine larynges showed that the onset of regular waves were first observed just above the lowest point of the lamina propria, and that this zone shifted upward with increasing F0, but downward with increasing intensity. The starting point of the mucosal wave was confirmed on the lower surface of the vocal fold.

In 2008 Jiang *et al.* [78] proposed a least squares method to quantify mucosal waves via videokymography (VKG) [2]. An algorithm for automated mucosal wave measurement was used to examine vocal fold vibrations in 17 excised larynges for different subglottal pressures and line-scan positions. The results revealed that the highest amplitude was measured at the midpoint of the vocal fold. The second highest amplitude was identified in the anterior portion, followed by the posterior portion. In contrast, frequency and phase delay of the vocal fold dynamics did not vary significantly. The authors concluded that this method allowed for easy examination of biomechanical properties of the vocal folds.

Tsai *et al.* [79] used ultrasound imaging to study the vocal fold vibration during phonation in excised human larynges. Echo-particle image velocimetry (Echo-PIV) analysis was used to trace the tissue particles in the motion pictures. Results showed a quasi-longitudinal wave along the body of vocal folds in the coronal plane of the vocal ligament, with a propagation direction equal to that of the mucosal wave. This finding varies from the currently popular vocal fold models.

Zhang *et al.* [80] identified three different vocal fold vibratory patterns in excised canine larynges using digital kymography (DKG). The type 1 pattern showed a periodic time-series of glottal edges and a distinct frequency spectrum. The type 2 pattern showed a time-series of alternating high- and low-amplitude waves and a frequency spectrum including a subharmonic frequency component. Both types displayed regular and symmetric vibrations. The type 3 pattern showed an aperiodic time-series of glottal edges, a broadband frequency spectrum, and irregular as well as asymmetric vibratory patterns. The authors suggested that imaging with DKG allows the categorization of various laryngeal vibrations.

Kataoka and Kitajima [81] investigated the relationship of F_0 and the change rate of F_0 in relation to the transglottal pressure (dF/dP). Working from the hypothesis that change rate is influenced by the vibrating depth as well as the vibrating length of vocal folds the authors examined three excised canine larynges. The goal of the experiments was to produce data that would help understand the adjustments of length and depth of vibration when regulating F_0 . The authors made the finding that a positive correlation was found between dF/dP and F_0 , given that the length of vibration showed a negative correlation. However, the authors only could notice that for a negative relation between dF/dP and F_0 , the length of the vibration seemed to be relevant.

Chung *et al.* [82] assessed the effects of a unique pitch-raising surgical technique designated “upper displacement of the anterior commissure” (UDAC) and compared the results with those obtained through cricothyroid approximation (CTA). The authors used 20 excised human larynges to determine vocal fold length, F_0 , and videokymography parameters preoperatively, post-CTA, and post-UDAC. Results showed that the amplitudes of the post-CTA and post-UDAC vibrations were significantly lower than the preoperative values. Also, vocal fold length and F_0 increased significantly after UDAC and even more after CTA.

3.2.2. Bifurcation Analysis—Configurations of laryngeal parameters (e.g. sub glottal pressure or tension), where a minimal variation yields an abrupt (unsteady) change in dynamic behaviour or acoustic signal (e.g. frequency jump), are called bifurcation points. Analysing bifurcation points is a valuable procedure for revealing the mechanisms behind the PTP, the phonation instability pressure (PIP), and phonation pressure range (PPR). In 1996, Berry *et al.* [83] applied bifurcation analysis in excised larynx experiments. Phonation onset and vocal instabilities were studied in relation to subglottal pressure and to adduction and elongation asymmetries. Results showed that even though geometric asymmetries are more obvious compared to elongation asymmetries, the latter have significant influence on vocal fold dynamics and acoustic output. Furthermore, in general the asymmetries provoked jumps between phonation registers (“chest-like”, “falsetto-like”, and “flute-like”). These registers are defined by different vibratory patterns of the vocal folds and acoustic signatures.

In 2007 Zhang *et al.* [84] studied mechanisms of PIP using bifurcation analysis in ten excised larynges. They found that PTP, PIP, and PPR were effectively identified using the bifurcation analysis. Elongation led to a significant increase in PTP, no significant change in PIP, and a significant decrease in PPR. The authors concluded that PIP and PPR represent important parameters when assessing phonation instability.

Using excised larynx experiments, Tokuda *et al.* [85] analyzed bifurcation and chaos in register transitions by varying longitudinal tension. They took advantage of two distinct vibration patterns obtainable in the excised preparation, which closely resemble chest and falsetto registers of the human voice. Nonlinear predictive modelling and biomechanical modelling were used for analysis. Chest and falsetto vibrations were found to correspond to two coexisting limit cycles. Irregular vibrations observed at the register jumps were due to chaos that exists near the two limit cycles. The authors concluded that longitudinal tension provided an alternative mechanism to generate chaotic vibrations in excised larynx experiment other than strong lateral asymmetry or excessively high subglottal pressure.

Švec *et al.* [86] studied abrupt chest-falsetto register transitions (jumps) based on the theory of nonlinear dynamics. The investigations were performed using an excised human larynx and three living subjects (one female and two male). Results from the excised larynx revealed that a small, gradual change in tension of the vocal folds can cause an abrupt change of register and pitch. Hysteresis was also observed: the upward register jump occurred at higher pitches and tensions than the downward jump. Thus, the authors concluded that the chest and falsetto registers can be produced with practically identical laryngeal configurations. The magnitude of the frequency jump was measured as the “leap interval”.

In 2009 Alipour *et al.* [87] investigated the aerodynamic effects of abrupt frequency changes. They hypothesized that change in vibratory mode in excised canine larynges could be triggered by a continuous change in subglottal pressure and flow rate, and did observe changes in F_0 and mode of vibration during the pressure-flow sweep. A lower F_0 mode had relatively large amplitude mucosal waves, significant vocal fold contact, a rich spectral content and a relatively intense acoustic signal. On the other hand, the higher F_0 mode had little or no vocal fold contact and a dominant first partial.

3.2.3. Unilateral Pathologies and Asymmetric Vibrations—Vocal fold pathology is often correlated with observations of asymmetric vibration. Hence, it is essential to understand the cause-and-effect bases for asymmetric behaviour. In 2006 Maunsell *et al.* [88] studied asymmetry in vocal fold dynamics due to asymmetric cricothyroid muscle action. For this purpose, vocal fold oscillation in excised porcine larynges was measured with EGG and an optical measurement method, the latter giving information about the vertical displacement. Cricothyroid muscle contraction was simulated by using a suture in each vocal fold, to which an individual force was applied. The results showed that periodicity of vibration was maintained, but phase shifts in vertical displacements between the two vocal folds were observed. Subharmonics and biphonation were also consistently observed. The authors concluded that lax vocal folds are more susceptible to developing aberrant spectral changes with increasing airflow, and that consideration of the mass, tension, and position asymmetries of vocal folds is important for diagnosing and treating dysphonic patients.

Coupling or entrainment effects between the two vocal folds have also been shown to influence vocal fold dynamics. To better understand coupling as seen in clinical situations, Ouaknine *et al.* [89] investigated coupling using chaos analysis and Lyapunov’s coefficients.

They demonstrated the effects of coupling using an excised larynx that was made experimentally asymmetric. Similarly, Giovanni *et al.* [90] studied the nonlinear behaviour of vocal folds in special cases of laryngeal pathology. An excised porcine larynx model was used to create asymmetries in tension between the two vocal folds. Resulting vocal signals were characterized for irregularities such as diplophonia. Spectral and phase domain results showed evidence of nonlinear behaviour in 85 % of experimental signals. The authors concluded that coupling and subsequent nonlinear effects result in synchronization of vocal fold vibration. In turn, methods of nonlinear dynamics may be used for objective voice analysis.

This was further confirmed by Kobayashi *et al.* [91]. They assessed effects of unilateral vocal fold atrophy on phonation in canines. One RLN was severed to cause vocal fold atrophy in four of seven animals. Vocal fold dynamics were measured in the post-mortem excised larynges using laser-Doppler vibrometry for the vertical displacement and photoglottography for the lateral displacement. Vibration became periodic and phase became symmetrical when the thyroid ala on the atrophied side was pressed medially. Amplitudes on the atrophied side were significantly greater than on the normal side. They concluded that closing the prephonatory glottal gap through interventions may have a beneficial effect on hoarseness [91] and indeed this is the basis of commonly performed medialization procedures.

Another study related to improving coupling of the vocal folds is that of Witt *et al.* [92], who evaluated the efficacy of a titanium vocal fold medializing implant (TVFMI) for the treatment of unilateral vocal fold paralysis on the basis of acoustic, aerodynamic, and mucosal wave measurements in eight excised canine larynges. The authors reported that the phonation threshold flow, phonation threshold power, jitter, and shimmer decreased significantly after medialization. The phonation threshold pressure also decreased, but the difference was not significant. The phase difference between the normal and paralyzed vocal folds and the amplitude of paralyzed vocal folds decreased. The authors finally suggested that TVFMI was effective in achieving vocal fold medialization, and significantly improved aerodynamics and acoustic characteristics of phonation.

Tsuji *et al.* [93] compared the medialization of vocal folds with type I thyroplasty (TPI) to arytenoid rotation using ten excised human larynges. Each vocal fold was treated with either TPI or arytenoid rotation (AR), so that direct comparison was possible. Mean vibratory amplitude in the posterior vocal fold on the TPI side was significantly larger than on the arytenoid rotation side. Phase asymmetry was noted in 90 % of the cases and in 70 % of the cases the mucosal wave on the TPI side was decreased. Moreover, the TPI lead to an increased vocal fold thickness and stretching of the mucosa, causing more alterations in the vibration parameters compared to the AR method.

Many researchers have used excised larynx testing to evaluate the success of procedures designed to repair an experimentally injured vocal fold. For example, Kishimoto *et al.* [94] tested the therapeutic potential of hydrogel encapsulated hepatocyte growth factor (HGF) for the management of acute-phase vocal fold scarring in 8 canines. Four unilaterally scarred canines were injected with hydrogels (0.5 mL) containing 1 µg of HGF, while other canine

larynges were injected with hydrogels containing saline solution. The results were analyzed via excised larynx testing and demonstrated significantly better vibrations in the HGF-treated group as assessed by judgements of mucosal wave amplitude. In addition, PTP was significantly lower in the HGF-treated group.

3.2.4. Irregular Phonation—Regular vibrations display spatial symmetry, temporal periodicity and well-defined frequency spectra. In contrast, irregular vibrations show complex spatiotemporal plots, aperiodic time series and broadband spectra [95]. As found in prior studies, the global entropy, correlation length from spatiotemporal analysis, and correlation dimension from nonlinear dynamic analysis can reflect the difference between regular and irregular vibrations, which have been applied as follows:

In 2007 Zhang *et al.* [95] investigated the biomechanical applications of spatiotemporal analysis and nonlinear dynamic analysis, to quantitatively describe regular and irregular vibrations of twelve excised larynges from HSI recordings. With respect to irregular vibrations, the authors reported statistically higher global entropy and correlation dimensions of irregular vibrations and a significantly lower correlation length. Their findings suggested that spatiotemporal analysis and nonlinear dynamic analysis are capable of describing the complex dynamics of vocal fold vibrations recorded by HSI. A similar method was applied to quantitatively analyze phonation in nine excised canine larynx experiments by Jiang *et al.* [96]. The authors reported that the correlation dimension and maximal Lyapunov exponent indicated a significant difference between irregular and normal phonation. However, such difference was not detectable in jitter, shimmer, and peak prominence ratio. Thus, these parameters were not suitable to distinguish irregular from normal phonation. The authors finally concluded that these findings assist investigators in understanding rough phonation and developing methodologies for voice disorder diagnosis.

Zhang and Jiang [97] investigated spatiotemporal chaos in excised larynx vibrations using HSI. Results demonstrated spatiotemporal chaos with decreased spatiotemporal correlation and increased entropy in the vocal fold vibrations.

Zhang and Jiang [98] studied asymmetric spatiotemporal chaos induced by a polypoid mass using HSI in an excised canine larynx. As found in prior studies, normal vocal folds show spatiotemporal correlation and symmetry. Their vibrations are dominated mainly by the first vibratory eigenmode (i.e. main direction of dynamics). However, the pathological vocal folds displayed asymmetry and spatiotemporal irregularity with decreased spatial correlation. Moreover, the energy was spread across a large number of eigenmodes. The authors concluded that spatiotemporal analysis represented a valuable clinical tool for detection of laryngeal mass lesions.

Murugappan *et al.* [99], using excised porcine larynges, investigated the acoustic characteristics of phonation when liquid material is present on the vocal folds. The presence of liquid on the folds during phonation was generally found to suppress the higher frequency harmonics and generate intermittent additional frequencies in the low and high end of the acoustic spectrum. Perturbation measures showed a higher percentage of jitter and shimmer when liquid material was present on the folds during phonation. The finite correlation

dimension and positive Lyapunov exponent measures revealed that the presence of materials on vocal folds excited a chaotic system.

3.2.5. Physiological Vocal Fold Parameters—Structural dynamics are governed by the underlying material parameters (e.g., stiffness and elasticity). The frequency dependency and the influence of tension on these parameters are also investigated in excised larynx models. Berry *et al.* [100] investigated rotational and translational stiffness of arytenoid motion about the cricoarytenoid joint using five excised human larynges. A external force was applied to the arytenoid cartilage to simulate posterior cricoarytenoid (PCA) muscle activation. Translational stiffness was obtained by plotting force versus displacement, while rotational stiffness was computed by plotting torque versus angular rotation. Results showed that translational stiffness along the anterior-posterior direction was three times greater than translational stiffness in the other two directions, showing anisotropy of the vocal folds.

An experimental setup was used to assess the relevance and accuracy of low-order vocal fold models by Rutty *et al.* [101]. A classical two-mass model was implemented with a fixed delay in the coupling between the masses. Validation of theoretical model predictions to experimental data showed that the model allowed for qualitative understanding of phonation. However, quantitative discrepancies remained large due to an inaccurate estimation of the model parameters and the crudeness in either flow or mechanical model descriptions.

In 2007 Tao *et al.* [102] proposed a new method to extract the physiologically relevant parameters of a vocal fold mathematic model (masses, spring constants and damper constants) from high-speed video image series. A genetic algorithm was used to optimize model parameters until the model and dynamics measured from excised larynges were well correlated. The physiologically relevant parameters from high-speed videos of an excised larynx model were extracted using this method. The authors demonstrated that the proposed parameter estimation method can successfully detect the increase of longitudinal tension due to the vocal fold elongation from the glottal area signal. They concluded that the method offered potential clinical application to the inspection of vocal fold tissue properties.

3.2.6. New Analysis Technologies with Applications in Vocal Fold Dynamics—

Typical acoustic and aerodynamic measures may fail to adequately inform clinicians how each fold individually contributes to phonation and how each fold should therefore be optimally treated. To address this limitation, Heaton *et al.* [103] developed the aerodynamic vocal fold driver (AVFD) for intraoperative functional assessment of each vocal fold. The AVFD is a hand-held probe that can be introduced through a glottiscope during phonosurgery and positioned to drive a vocal fold subglottally (akin to hemilarynx phonatory testing described in the next section). Similar positive correlations between driving air pressures and phonation frequency and amplitude were found in AVFD-driven phonation of individual vocal folds versus whole-mount phonation in 14 canine excised larynges. The AVFD method could also measure improvements in the function of damaged vocal folds after they were treated by injection of cross-linked hyaluronic acid gel, enabling the detection of unilateral tissue changes not readily apparent from acoustic and aerodynamic measures during whole-mount phonation.

Luegmair *et al.* [104] presented an optical reconstruction method for the 3D measurement of vocal fold dynamics, Fig. (4). The method uses a high-speed camera and a laser-projection system. The method allows for high-accuracy in space (error < 15µm) and time (rates up to 4000 frames per second). Additionally, information over the entire superior vocal fold surface is obtained. The authors concluded that a miniaturization of the laser-projection system will allow for *in vivo* 3D clinical studies of the vocal fold dynamics to be undertaken.

Triggered optical coherence tomography was recently developed by Kobler *et al.* as a tool for studying vocal fold dynamics [58]. In this study a Fourier-domain optical coherence tomography (OCT) system was triggered in strobe-like fashion to capture coronal cross-sections of vocal fold structure at different phases in the cycle of vibration. The imaging depth was 1.0-2.0 mm with a spatial resolution of 10-15 µm in X, Y and Z planes and a temporal resolution of 0.1 ms. After compiling data acquired over multiple cycles of vibration, the oscillations of epithelium and lamina propria were displayed in movie sequences. The shape, amplitude, and velocity of vocal fold mucosal waves could be measured, including ripples of the mucosal wave as small as 100 µm in vertical height. Internal strain was observed in both normal and implanted vocal folds. An example of an OCT data set is shown in Fig. (5) (Kobler, unpublished data), where 18 closely spaced planes were sampled sequentially during sustained phonation, resulting in a rich anatomical and functional data set. This method may be useful for relating biomechanics to anatomy and disease and for assessing the functional rheology of implants in the context of real tissue carriage. Ouaknine *et al.* [105] studied biphonation on excised porcine larynges via a novel system allowing the noncontact measurement of each vocal fold vibration, based on optorelectrometry.

4. HEMILARYNX EXPERIMENTAL APPROACHES AND ANALYSIS

While analyzing excised full larynges may provide quantitative information regarding vocal fold vibration, the superior view has its limitations. For example, from a superior aspect, it is impossible to quantify the medial surface dynamics of the vocal folds, which captures the details of the opening and closing of the glottis, which is known to be a critical component of voice production. From a superior aspect, it is also difficult to quantify the propagation of the mucosal wave along the medial surface of the vocal folds Fig. (1). Because of these limitations, clinicians usually refer to mucosal wave propagation along the superior surface of the folds, where they see the wave most clearly. Unfortunately, the mucosal wave attenuates quickly upon reaching the superior surface of the folds.

The hemilarynx methodology Fig. (6) was developed to facilitate quantitative measurements of the medial surface dynamics of the vocal folds [106-110]. Knowledge of such quantitative dynamics is essential to help clinicians identify the sources of vibratory irregularities in patients with voice disorders. In terms of its influence upon voice production, the most critical region of mucosal wave propagation occurs on the medial surface of the vocal folds, where it originates. Before reaching the superior surface, the wave travels a relatively long distance long the medial surface, it generates significant tissue vibrations exhibiting geometric and viscoelastic nonlinearities, and it couples nonlinearly with several other sub-systems, including the nearfield glottal airflow, the acoustic

resonances of the sub- and supra-glottal systems, and the opposite vocal fold during glottal closure (if collision occurs). Indeed, mucosal wave propagation along the medial surface of the vocal fold is governed by a very complex set of dynamics that is only beginning to be understood. The modeling of such complexity is still in its infancy, and requires further quantitative data for validation. Hence, quantification of the medial surface of the vocal folds using hemilarynx experiments holds promise for increasing our understanding of physical mechanisms of regular and irregular voice production.

Once quantitative information regarding medial surface dynamics of the vocal folds has been obtained, it is also imperative to implement appropriate analysis techniques to closely scrutinize possible physical mechanisms of regular and irregular vocal fold vibration.

Spatio-temporal analysis techniques, such as the method of empirical eigenfunctions, have proven well-suited for this task [57,108,110,111]. This technique has its roots in modal analysis, a tool which has been exploited extensively in the field of speech science for the specification of formants (resonances or eigenfrequencies of the vocal tract) to differentiate vowels [112]. Similar to the vocal tract, the vocal folds can be characterized by their eigenmodes and eigenfrequencies. Indeed, spatio-temporal decomposition techniques, such as the method of empirical eigenfunctions, have been utilized to disclose specific eigenmodes and synchronization patterns for a variety of phonation types, encompassing periodic, quasi-periodic and chaotic phonatory utterances [57,97,108-111,113,114]. Such tools have facilitated the study of physical mechanisms of regular and irregular vocal fold vibration.

4.1. The Excised Canine Hemilarynx

While computational models may lack the realism of physiologic models, the study of vocal fold vibrations in a finite element model provided the initial motivation for current studies of medial surface dynamics in hemilarynx experiments [111]. First, the vibrations of a finite element model of the vocal folds were used to compute empirical eigenfunctions.

Motivated by the results of this computational model, quantitative imaging of the medial surface of the vocal folds moved to a laboratory setting to document the vibrations of actual vibrating vocal fold tissues [107]. These studies built upon a hemilarynx methodology previously developed by Jiang and Titze [106]. Canine larynges were harvested from canines sacrificed for other purposes, such as cardiac research. To create a hemilarynx, the right vocal fold and portions of the surrounding cricoid and thyroid cartilages were removed. So that specific vocal fold fleshpoints could be tracked, 9-0 black monofilament nylon microsutures were placed along the medial surface of a mid-coronal cross-section of the left vocal fold, with a spacing of about between microsutures of about 1 mm. The left vocal fold was mounted against a glass plate, and a prism was placed on the opposite side of the glass plate, yielding two oblique views of the medial surface of the left vocal fold. Airflow was passed between the left vocal fold and the glass plate to induce vocal fold vibration. HSI then captured vocal fold vibrations through the two oblique views supplied by the prism. Using a direct linear transform, image coordinates were converted to physical coordinates. Significantly, the results of this first excised canine hemilarynx experiment confirmed the two primary conclusions of the previous finite element study: two spatial eigenmodes

effectively captured normal periodic vibrations, and an analysis of the spatio-temporal eigenmodes disclosed a physical mechanism for self-sustained oscillation of the vocal folds [107].

4.2. The Excised Human Hemilarynx

Later, the excised human hemilarynx was also investigated. Because it better represented the natural, layered tissue morphology of the human vocal folds, it was considered superior to the excised canine larynx as a model of human phonation [108-110]. These subsequent experiments also provided the following enhancements: (1) using a larger prism, the full medial surface of the vocal fold was imaged (as well as part of the superior surface), rather than just one coronal plane of the medial surface [108,109], (2) three-dimensional vibrations were imaged and computed, rather than just two-dimensional vibrations (e.g., anterior-posterior vibrations were no longer neglected [108,109]), (3) vibration patterns were studied as a function of forces applied to elongate and adduct the vocal folds, and as a function of glottal airflow [110]. Although greater complexity was observed in tracking the 3D vibrations of the entire medial surface of a human vocal fold, the essential dynamics of the vibration patterns were captured by two dominant eigenmodes, which assisted in disclosing physical mechanisms of self-sustained vocal fold oscillation, vocal efficiency and vocal fold impact stress [108-110].

4.3. The *In Vivo* Canine Hemilarynx

Next, the *in vivo* canine hemilarynx was also investigated, because it is the only laboratory model of phonation which can simultaneously contract, stiffen, and bulge the thyroarytenoid (TA) muscle. Thus, it was considered important to investigate a fully intact neuromuscular model of phonation [57,113]. In these investigations, TA muscle activity was varied to produce various types of chest-like and vocal fry-like vibration patterns. Although the hemilarynx procedure was implemented in the same way as in the excised human larynx, accessibility was more limited for the *in vivo* canine because of surrounding anatomical features. This resulted in fewer sutures and greater spacing between sutures on the medial surface of the vocal folds. Thus, the spatial eigenmodes were not computed with as great precision as in the excised human hemilarynx experiments. Nevertheless, chest-like and fry-like vibration patterns and F_0 variations were observed as a function of TA muscle activity [57,113]. In the future, the *ex vivo*, perfused larynx (a larynx which is kept neuromuscularly intact after being removed from its host body using perfusion techniques) may allow greater spatial resolution in imaging the medial surface of fully intact neuromuscular laboratory models of phonation [115]. Moreover, newly-developed graded stimulation techniques [37] may also facilitate more systematic and comprehensive studies of neuromuscular control of vocal fold vibration.

5. EXCISED LARYNGES INCLUDING SUPRAGLOTTAL STRUCTURES

As discussed above, typical excised larynx experiments involve the use of a whole-mount or hemilarynx setup that provides a framework for self-sustained vocal fold oscillations with control over parameters such as vocal fold tension and subglottal pressure. In the *in vivo* situation, voice production mechanisms involve complex interactions among vocal fold

tissue motion, airflow volume velocity, and sound pressure [116]. Nonlinear source-filter coupling theory justifies the need for understanding the roles of subglottal and supraglottal structures (filters) on vocal fold oscillations (source) to generate the radiated acoustic pressure waveform. This section explores the roles of supraglottal structures—the epiglottis, ventricular vocal folds, resonant cavities—in sustained vocal fold oscillations of both whole-mount and hemilarynx preparations. Effects are cast in terms of aerodynamic, acoustic, and electroglottographic changes.

The effects of the presence or absence of a supraglottal cavity was first investigated by Alipour *et al.* [117] using canine and cadaver hemilarynx preparations. Fig. (7) schematically depicts the setup in which the vocal tract was represented by a hard rubber tube that was 2.5 cm in diameter (cross-sectional area of 4.9 cm²), 0.25 cm in wall thickness, and 15 cm in length. With control over a subglottal airflow source, the primary output measures were the mean subglottal pressure and mean transglottal airflow during sustained oscillations at a particular subglottal pressure level. The phonation threshold pressure indicated the minimum pressure necessary for vocal fold oscillation with and without a supraglottal tract, and the laryngeal resistance (derived as the slope of the pressure/airflow relationship) quantified the efficiency of the system at transferring pressure into airflow energy.

Results showed that the phonation threshold pressure of a cadaver larynx changed from 5 cm H₂O (490 Pa) in the no-tract condition to 3 cm H₂O (294 Pa) when the supraglottal tract was included. In contrast, the phonation threshold pressure of a canine larynx was observed to be slightly *higher* with the supraglottal tract (9 cm H₂O versus 8 cm H₂O), pointing to inter-species differences that necessitate further investigation. Laryngeal resistance (in units of aerodynamic ohms, or cm H₂O · s/L), averaged over four adductory conditions, was observed to increase with the addition of the supraglottal tube. In the cadaver larynges, average laryngeal resistance increased from 13.2 ohms without the supraglottal tract to 20.7 ohms with the supraglottal tract. Similar results held across two cadaver and two canine larynx preparations. Increased laryngeal resistance translates to a system with increased aerodynamic efficiency, leading these results to suggest that the vocal tract system facilitates more efficient vocal fold oscillation *in vivo*.

To yield additional insight into the presence of a supraglottal cavity, high-speed imaging with calibrated units in three-dimensional space has been obtained in a cadaver hemilarynx preparation [118]. The study recognizes not only potential effects of the presence of a supraglottal cavity but also of variations in the cross-sectional area of the epilarynx region just superior to the vocal folds. Fig. (8) displays the dimensions of the supraglottal structures. The area of an axial cross section of the epilarynx segment was varied using hard rubber rectangular prisms. A grid of microsutures on the vocal fold epithelium allowed for the three-dimensional tracking of tissue motion to estimate vocal fold displacement and velocity changes along the anterior-posterior and superior-inferior axes.

Results showed that a decrease in the cross-sectional area of the epilarynx resulted in decreases in phonation threshold pressure, mean glottal airflow, sound pressure level, and vocal fold lateral displacement and velocity. F_0 was observed to generally decrease with

decreasing epilarynx area. In agreement with Alipour *et al.* data [117], phonation threshold pressure decreased from 2.35 kPa in the no-tract condition to 2.0 kPa, 1.27 kPa, and 0.96 kPa for conditions with epilarynx cross-sectional areas of 205.9 mm², 71.0 mm², and 28.4 mm², respectively. At the tissue level, general trends suggest that decreases in epilarynx area reduce the maximum displacements of the vocal folds along the medial-lateral and superior-inferior directions. In contrast, maximum anterior-posterior vocal fold tissue motion is affected to a smaller degree by changes in epilarynx area. Detailed recordings of the tissue motion at different locations along the vocal fold surface have the potential to guide improvements to synthetic models, mathematical models, and phonosurgery.

Whereas the effects of supraglottal structures external to the excised hemilarynx were investigated above, two subsequent studies investigated the role of the presence and position of the intrinsic laryngeal structures of the epiglottis and ventricular vocal folds in canine whole-mount excised larynges [4,119].

In the first study, controlled parameters were the presence and degree of medial compression of the ventricular vocal folds and presence and degree of anterior-posterior compression of the epiglottis [119]. The absence of both the epiglottis and ventricular vocal fold structures resulted in decreased laryngeal resistance, slightly decreased F_0 , and decreased sound pressure level (at a particular subglottal pressure). The successive inclusion of the ventricular vocal folds and the epiglottis resulted in increases in the laryngeal resistance, which was derived from the slope of the linear pressure-flow relationship. The subglottal pressure was varied from a minimum threshold at approximately 7 cm H₂O to 24 cm H₂O with no supraglottal structures intact. Although the inclusion of the ventricular vocal folds did not yield an appreciable change in the phonation threshold pressure, the presence of both the ventricular vocal folds and epiglottis increased the phonation threshold pressure to approximately 20 cm H₂O (allowing further increases to 39 cm H₂O). Anterior-posterior compression, due to manipulation of the epiglottis, did not have a significant effect on laryngeal resistance. Medial compression of the ventricular vocal folds, however, increased the laryngeal flow resistance. With the epiglottis removed and ventricular vocal folds intact, lower fundamental frequencies and instabilities were achievable during vocal fold vibration. The presence of both supraglottal structures contributed to an elevated acoustic sound pressure level, particularly in the low-frequency harmonic region.

In addition to overall sound pressure level changes, Finnegan and Alipour [4] describe changes to the frequency spectra of the acoustic pressure signal due to the presence or absence of the ventricular vocal folds and the epiglottis (see Fig. (2) for larynx photographs). With no supraglottal structures, the sound spectrum exhibited harmonics whose amplitudes decreased monotonically with increasing frequency. With the addition of the ventricular vocal folds, the spectrum became non-monotonic due to the second harmonic (as opposed to the first harmonic) exhibiting the peak amplitude in the spectrum, and the F_0 decreased. This result provides evidence for the contribution of a resonance. With the presence of both the ventricular vocal folds and the epiglottis, a similar resonance was observed around 200 Hz, accompanied by a further decrease in F_0 . Fig. (9) displays the frequency spectra of the three experimental conditions. The source of this resonance and

why it seems to peak around 200 Hz is not known and requires further theoretical and empirical studies.

Low-frequency noise components increased with the absence of the ventricular vocal folds and epiglottis [4]. Further quantitative assessments of this noise energy for each supraglottal condition over multiple larynx preparations would aid in addressing this qualitative observation. In addition, changes in the position of the ventricular folds and the epiglottis were induced to investigate their aerodynamic and acoustic influences. The epiglottis was positioned horizontally by pulling on a suture placed on the anterior wall of the epiglottis. The epiglottis in the upright (natural) position exhibited decreased laryngeal resistance and decreased F_0 relative to corresponding values during phonation with a horizontally positioned epiglottis. In one case in which the epiglottis was removed, medial compression of the ventricular vocal folds created an irregular acoustic sound pattern that seemed to arise from an engagement of the ventricular vocal folds during simultaneous phonation of the true vocal folds. The irregularity largely disappeared when the ventricular vocal folds were abducted.

6. SUMMARY

The data obtained by excised and *in vivo* experiments have proven to be valuable in several ways. First, the *in vivo* approach allows for an analysis of neuromuscular function through the use of electrical stimulation. This results in a deeper insight into muscle contraction achieved by nerve stimulation and the resulting changes in vocal fold dynamics. Furthermore new clinically oriented studies have indicated new approaches for the treatments of phonation disorders due to asymmetric vibratory patterns.

Second, excised larynx experiments have generated valuable data, considering the interrelation of several phonation parameters, e.g. the linear relation between airflow and subglottal pressure. Additionally, the increasing amount of data considering fluid dynamics (e.g. vector flow fields) allows for more precise statements to be made about the interaction between structure and airflow. In particular, the influence of the subglottal and laryngeal geometry on airflow was investigated. The dynamics of vocal folds and the governing underlying physiological properties were the subject of several investigations. Phenomena related to bifurcations, asymmetries and irregular dynamics were measured and quantified.

Third, hemilarynx experiments revealed new insights into mucosal wave propagation. Due to the free visibility of the medial surface in these experiments, the vertical displacement component could be extracted and determined.

Finally, evidence shows that the presence of supraglottal tracts and structures facilitates sustained vocal fold oscillation and adds additional resonance characteristics that affect the spectral content of the sound source. Taken as a whole, this review suggests that future experiments with excised larynx preparations should take into account potential nonlinear acoustic, aerodynamic and tissue interactions that arise during vocal fold oscillation.

7. OUTLOOK

The presented methods produced promising data yielding insight in to primary voice signal production. However, the findings are not based on a significant amount of data. Hence, widespread studies have to be undertaken to confirm current findings. Challenges in the future lie in the further refinement of existing models towards more realistic behavior. For numerical models, this means the consideration of all three dimensions in Finite Volume or Finite Element Models. Also the fluid-structure-acoustic interaction must be considered. However, the resulting high computational costs as well as still unsolved mathematical difficulties (e.g. contact problem during vocal fold collision) pose a long term goal. Regarding synthetic models, improvements have to be performed by considering realistic vocal tract and vocal fold shapes. Additionally, the vocal fold build-up has to consider realistic material parameters and vocal fold activation (e.g. CT and TA muscle activation). Finally, dependencies between voice production and neurologic activities have been neglected so far.

ACKNOWLEDGEMENTS

This work was made possible by Deutsche Forschungsgemeinschaft (DFG) grant no. FOR 894/2 “Strömungsphysikalische Grundlagen der Menschlichen Stimmgebung”. Dr. Berry’s contributions to this investigation were supported by NIH grant no. R01 DC03072. Drs. Kobler and Mehta acknowledge support from the Institute for Laryngology and Voice Restoration, the Eugene B. Casey Foundation and NIH grant no. R01 DC007640. We thank all colleagues for providing the figures.

ABBREVIATIONS

F₀	Fundamental frequency
HIS	High Speed Imaging
PIP	Phonation
PPR	Phonation pressure range
PTF	Phonation threshold flow
PTP	Phonation threshold pressure
Psub	Subglottal pressure
RLN	Recurrent laryngeal nerve
SLN	Superior laryngeal nerve

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Fig. (1). Schematic sketch of the human head. The larynx and sub- and supra-glottal tracts are depicted. In clinics, by coupling the endoscope to a digital high-speed camera or stroboscopy system, the oscillating vocal folds can be visualized in a video format. On the right, one frame of such a video is shown. Note that the visibility is restricted to the superior aspect of the vocal folds.

Fig. (2).

Photographs of a canine excised larynx preparation in which (top) the epiglottis and ventricular vocal folds are intact, (middle) the epiglottis is removed and the ventricular vocal folds are intact, and (bottom) both epiglottis and ventricular vocal folds are removed. B and C offer views from the anterior and posterior position, respectively. From Fig. 1 in [4].

Fig. (3).

In vivo canine preparation allowing for phonation of anesthetized animal with concurrent stimulation of laryngeal nerves, recording of physiological measures and stroboscopic imaging of vocal fold vibration [17]. (Reprinted by permission of The Laryngoscope, Wiley & Sons, Inc.)

Fig. (4).

Single frame of a high speed video of an excised porcine larynx. The projected laser grid is clearly visible. S1, S2, and S3 represent the lines, where the contours on the right side are taken from. The lower left figure shows the extracted surface of the visible superior vocal folds.

Fig. (5).

Example data set obtained using triggered dynamic optical coherence tomography as applied to a vibrating calf hemilarynx specimen. A. View of the medial surface of the bisected calf larynx. The approximate locations of the 18 coronal sections below are indicated between the two arrows. B. Coronal OCT images at 8 equally-spaced phases of the periodic cycle of vibration during a sustained phonation.

Fig. (6). Human hemilarynx: One vocal fold is cut off to expose the medial surface of the opposite one. A glass plate serves then as replacement of the missing vocal fold. The displacements of the medial surface can then be observed through the glass plate.

Fig. (7).
Schematic of a hemilarynx setup with a supraglottal tract: superior (left) and medial (right) perspectives shown. From Fig. (2) in [117].

Fig. (8).
Depiction of the supraglottal cavities attached to a cadaver hemilarynx preparation. From Fig. 1 in [118].

Fig. (9).

The acoustic sound spectra due to the presence for the following experimental conditions in a canine excised whole-mount preparation: (top) presence of both epiglottis and ventricular vocal folds, (middle) presence of ventricular vocal fold and no epiglottis, and (bottom) absence of both epiglottis and ventricular vocal folds. From Fig. 9 in [4].